IV International Conference on Environmental, Industrial and Applied Microbiology

BioMicroWorld2011

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BioMicroWorld2011 Conference is a multi-disciplinary forum that brings together active researches involved in environmental, industrial and applied microbiology with the aim of communicating current research priorities and progress in the field, and identifying new approaches and research opportunities in applied microbiology.

BioMicroWorld consolidates a series of conferences whose current edition has received about 950 abstracts to be considered for presentation and has attracted nearly 575 researchers from more than 60 countries.

TOPICS

• Agriculture, Soil, Forest Microbiology
• Environmental, Marine, Aquatic Microbiology. Geomicrobiology
• Food Microbiology
• Industrial Microbiology - Future Bioindustries
• Medical Microbiology - Pharmaceutical Microbiology - Antimicrobial agents and chemotherapy
• Methods - Quantitative Models and Bioinformatics in Microbiology - Technology development
• Microbial Physiology, Metabolism and Gene Expression
• Biodegradation and Bioremediation
• Biotechnologically Relevant Enzymes and Proteins
• Biofilms

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Agriculture, Soil, Forest Microbiology

16S rRNA gene pyrosequencing reveal that oxygen intrusion has no effect on the anodic microbial community in air-cathode MFCs

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The exhaustion of fossil fuel resources and the destructive risks of global climate change caused by the net increase in atmospheric CO_2 are impelling countries to search for renewable, carbon-neutral energy sources. The complex organic matter in residual biomass produced from human activities (e.g. municipal wastewater) is particularly attractive in this context because it is a renewable source of energy and is carbon neutral. Microbial fuel cells (MFCs) are a novel biotechnology that hold great promise for the simultaneous treatment and electricity generation from wastewater. In MFCs, microorganisms known as exoelectrogens (i.e., can transfer electrons outside the cells) catalyze the direct conversion of organic matter found in wastewater to electricity. Currently, there is a lack of knowledge on the microbial ecology of exoelectrogens in MFCs in general, and in air-cathode MFCs in particular.

An important parameter that affects the microbial community of exoelectrogens in air-cathode MFCs is oxygen intrusion through the cathode. Therefore, the objective of this study was to develop a better understanding of the effect of oxygen intrusion through the cathode on the composition of the microbial community that develops on the anode. To achieve this objective six cube-shaped, single-chambered, air-cathode MFCs were constructed with three different configurations: closed circuit, open circuit, and with the cathode sealed off from air. Duplicate MFCs were used for each configuration. The voltage was monitored every 30 minutes across a fixed resistance of 1,000 Ω using a multimeter with a data acquisition system. The microbial community structure and composition on the anode was monitored by 16S rRNA gene pyrosequencing.

In the closed circuit MFCs, the maximum voltage and power density were 541 ± 17 mV and 31.7 ± 2.1 W/m^3. The removal of chemical oxygen demand was similar for the open circuit and closed circuit MFCs, but was different from the sealed off reactor. The predominant anode community in the closed circuit MFCs was *Geobacteraceae*, which is well known for its ability to transfer electrons outside the cell. In the open circuit MFCs, the dominant organism was *Rhodocyclaceae*. *Rhodocyclaceae* and *Hyphomicrobiaceae* were dominant in the sealed off anode.

A new clade of *Mesorhizobium* nodulating chickpea

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The chickpea-rhizobia symbiosis is one of the least studied legume-rhizobia associations, nevertheless genotypic diversity within rhizobia able to nodulate chickpea has been addressed. Two species were described to specifically nodulate chickpea, namely *Mesorhizobium ciceri* and *M. mediterraneum*. Recently, this view has been changing and chickpea rhizobia have been shown to belong to several species within the genus *Mesorhizobium* (1). The main aim of the present study was to confirm the existence of two new chickpea nodulating genospecies, proposed before by Laranjo *et al.* based on the 16S rRNA gene phylogeny (2). Moreover, this work intended to infer a high resolution phylogeny within the *Mesorhizobium* genus. One convenient approach to infer species phylogeny is to combine sequence of several unlinked and neutrally evolving loci, which are shown in advance to yield congruent topologies by appropriate statistical tests. The phylogenetic structure of the *Mesorhizobium* clade was evaluated by comparison of 16S rRNA gene, ITS, *atpD* (ATP synthase F1, beta subunit), *dnaJ* (DnaJ chaperone) (3), *glnA* (glutamine synthetase I), *gyrB* (DNA gyrase beta subunit), and *recA* (recombinase A) sequence analysis. The congruence between classifications of mesorhizobia based on five core genes was estimated and the phylogenies obtained with the different genes are in overall good agreement. Furthermore, a well-supported, almost fully resolved phylogenetic tree was obtained, when the combined data were analysed. Although the majority of *Mesorhizobium* species show relatively low sequence divergence at core loci, there are certain species groups that are consistently recovered in phylogenies of different core loci: *M. mediterraneum*/*M. temperatum*, *M. tarimense*/*M. tianshanense*, *M. loti*/*M. ciceri*, *M. amorphae*/*M. septentrionale*. This implies that there is little horizontal gene transfer of core loci between these groups; therefore their core genomes form independent evolutionary lineages, which is an important principle for defining species. Our analyses of ITS, *atpD*, *dnaJ*, *glnA*, *gyrB* and *recA* sequences fully support the *Mesorhizobium* clade as well as its placement in the rhizobial phylogeny, but not all species and isolates relationships within the genus. Relationships among species are not the same for all genes, which suggests that there may be recombination of chromosomal genes between *Mesorhizobium* species. These five housekeeping genes agree with the 16S rRNA and ITS based phylogenies. Moreover, ITS sequences provide discrimination at the intraspecific level. This enlarged phylogenetic accordance is only true for the core genome and not the accessory or symbiosis genes, such as *nifH* and *nodC*, which are more subjected to lateral gene transfer (4). The studied chickpea mesorhizobia fell into different groups that represent two new genospecies, which form a new clade within the genus *Mesorhizobium*.

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Keywords *Mesorhizobium*; chickpea; phylogeny; core gene; accessory gene; symbiosis

A New Report of Griseofulvin Producing *Nigrospora oryzae* from the *Emblica officinalis* Gaertn. and Its Antimicrobial Activity Against Human Pathogenic Bacteria

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Griseofulvin is a nontoxic antifungal antimitotic drug derived from several species of *Penicillium.* It has been used for the treatment of dermatophytic infections. The main aim of this work was to identify Griseofulvin-producing endophytic fungus from *Emblica officinalis* and evaluate its activity against human pathogenic bacteria. Based on morphological and ITS rDNA sequence analysis, the endophyte was confirmed as *Nigrospora oryzae* (DBT-150). Antimicrobial activity of crude extract was evaluated against human pathogenic bacteria *P. aeruginosa* (ATCC -13388), *S. choleraesuis* (ATCC-10708), *S. aureus* (ATCC-6538), *E.* coli (ATCC-11775). MIC results of the extracts which showed antibacterial activity up to 1, 0 mg/mL. This is the first report of production of Griseofulvin from Endophytic *Nigrospora oryzae* isolated from *E. officinalis*.

Keywords: Endophyte, Griseofulvin, Human pathogenic microbes, *Nigrospora*

A new strains of endophytic and rhizobacteia for plant protection and growth stimulation under condition of high concentration of sodium chloride and heavy metals

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In this study we selected the strains of nonpathogenic endophytic and rhizobacteria which can protect the agricultural plants and stimulate their growth under condition of high concentration of sodium chloride and heavy metal. It was shown what two strains of *Bacillus subtilis* with high antagonistic activity against phytopathogenic fungi and bacteria can growth under 10% of sodium chloride. Also ability of selected bacterial strains to growth on medium with different concentration of Cd^{2+} and $Pb2^{+}$ it has been studied. The experiments using gnotobiotic systems with wheat showed that plants treated with strains of endophytic and rhizobacteria were more resistant to sodium chloride and heavy metal stress. Ability of selected endophytic and rhizobacteria strains to promote plant growth in the presence of NaCl, Cd^{2+} and Pb^{2+} has been demonstrated. These selected strains will be studied in future greenhouse and field experiments for ability to minimize salinization and heavy metal pollution stresses.

Keywords: endophytic bacteria, biocontrol, PGPR, gnotobiotic system, sodium chloride, cadmium, lead.

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A novel approach to determine the nitrogen fixation ability of cyanobacterial strains

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For the determination of nitrogen fixation ability of cyanobacterial strains a novel approach was developed where cells were grown in BG 11 media where $[^{15}N]$-$NaNO_3$ was the only supplied nitrogen source and thus any organic ^{14}N found in the cells would by necessity come from fixation from the air. The ratio of $^{14}N/^{15}N$ was determined in the extracted amino acids after different time periods (after 15, 30, 40, 50 and 60 days of inoculation) using GC-MS. It was observed that after 15 days all amino acids were highly labeled with $[^{15}N]$, whereas after 30 days the reduction in $[^{15}N]$ was noticed in all strains and this reduction was continuously recorded up till 40 days. After 50 days, GC-MS analysis showed that most of the amino acids again become enriched with $[^{15}N]$ in few strains, suggesting that at higher density growth stages that fixation no longer supplied significant nitrogen. We are quite excited about this finding in that it provides now a detailed method to evaluate not only the nitrogen fixing potential of the cyanobacteria in culture, but suggests novel approaches to evaluation of the ability of the strains to provide nitrogen enrichment to plants under co-cultivation conditions.

Actively N_2O Emitting β-Proteobacteria from South East Asian Tropical Peatland Soils – Their Physicochemical Responses, Genetic Traits and Probable Origins –

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In terrestrial ecosystem, reclaimed tropical peatlands are one of the most active N_2O flux sources, which are called "hot spot" for N_2O flux.[1)] The reclaimed peatlands accelerated soil degradation, leading to releasing a massive N_2O along with a constant ratio of CO_2, both known as important greenhouse gases. In laboratory experiments using a gellan gum soft gel medium mimicking the acidic farm soils, N_2O emitting bacteria in the reclaimed peatland soils in three different regions (Central Kalimantan and Sarawak in Borneo Island, and Riau in Sumatra Island) were screened, and consequently 8 strains were isolated as culturable, dominant N_2O emitters bacteria from the soils, all of which showed potent activity to produce N_2O from excessive amounts of NO_3^- (0.6-3 μg N_2O ml^{-1} sugarless medium d^{-1}). By means of sequence determination of 16S rRNA genes, they were identified as families *Burkholderia* (frequent N_2O emitters in the peatland soil) and *Janthinobacterium* (a minor N_2O emitter in the soil),[2)] both of which belong to subclass of β-Proteobacteria.

N_2O emission of these β-Proteobacteria was pH-dependent, and highly active at acidic regions close to soil pH ranging from pH 4.0 to 4.5. In addition, all the N_2O emitters responded to supplemented carbon source (as 0.5% sucrose) to accelerate N_2O production allowing 25-560 fold higher levels of N_2O emission than those without the supplemental carbon source. Activities of their nitrous oxide reductase (N_2OR) in these N_2O emitters were also measured by acetylene inhibition method. In this assay, 10% acetylene gas injected to headspace of the gas-chromatography vial culturing an N_2O emitter inhibits N_2OR of denitrifier tested, leading to further accumulation of N_2O due to inhibition of N_2 production. Therefore, high N_2OR activities of the N_2O emitters in acetylene inhibition assay would allow further N_2O accumulation in the headspace. However, results were all negative at both pH 4.5 and 6.5. Besides, detection of N_2OR gene (*nosZ*) by PCR using a universal primer set for *nosZ* was unsuccessful, although the target gene fragments were detected from positive controls, *Pseudomonas nitroreducens* NBRC 12694T, *Paracoccus denitrificans* NBRC 102528T and *P. denitrificans* NBRC 12442.

Draft sequence of 6.7 Mbp genomic DNA from the most active N_2O emitting strain, *Burkholderia thailandensis* 112-B30-5S, showed the presence of denitrification-associating *narGH*, *nirB*, and *norB* genes on the genome but not *norZ* gene. As one of the key enzyme of unusually active N_2O emission is nitrate reductase, amino acid sequence (AA) homology of the nitrate reductase large subunit translated from *narG* gene of the strain 112-B30-5S was compared to those of 50 denitrifying bacteria. The AA of nitrate reductase large subunit showed a high similarity with those of *Burkholderia* group in phylogenetic analysis (Fig. 1), suggesting horizontal gene transmission from a genuine denitrifiying *Burkholderia* in pristine peat swamp forest soil before reclaimation. Another key enzyme, nitric oxide reductase, large subunit also showed a similar result. Key process of the active N_2O emission in reclaimed peat soil is thus estimated to be emergence of *nosZ* gene-missing *Burkholderia* spp. or similar β-Proteobacteria that had been adapted to acidic peat soil and acquired capability of nitrate respiration in wet peat soil. This finding would link to new approach to repressing active N_2O flux from the reclaimed peatland by land and microbial managements.

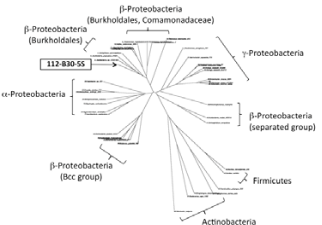

Fig. 1 Dendrogram for AA sequences of nitrate reductase large subunit

Keywords nitrous oxide; N_2O emission; tropical peat soil; *Burkholderia* spp.; *nosZ*;

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An individual-based model for dealing with organic amendments and mineral nitrogen soil fertilizations

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In the northeast of Spain, the production of large amounts of pig slurry that exceed the fertilization requirements of local field crops is contributing to the deterioration of the soil environment. In recent years, the construction of treatment facilities has allowed the exportation of nutrient surplus as thermally dried pig slurry (TDPS). Even though it is already used as an amendment, little is known about its effect on soil N availability. An adaptation of Stanford and Smith (1972) long-term aerobic incubation test was used to monitor C and N apparent mineralization after the addition of TDPS at different rates. A set of experimental trials was performed to assess the amounts of added N and organic C that mineralize during incubations and to monitor NO_3^-, N availability and CO_2 emissions (Ramirez et al. 2011).

A simulator model well-known as INDISIM (INdividual DIScrete SIMulation) and its different versions have been used successfully to study diverse biological systems where microorganisms are the main agents driving the behaviour of these systems (Ferrer et al. 2008). The systems modelled with INDISIM are those built up by biotics and abiotic elements, and the difference between the microorganisms and their metabolic rules, as well as the available resources of the environment, can be controlled. INDISIM-SOM encompasses a wide range of physical, chemical, and microbiological processes that control the short-term dynamics of soil C and N, namely, decomposition, mineralization or immobilization of C and N, and humification (Ginovart et al. 2005, Gras et al. 2010, 2011). In particular, in terms of microbiology, INDISIM-SOM explicitly describes the uptake, metabolism, reproduction, death, and lysis of microbial cells belonging to two broad metabolic groups, heterotrophic microorganisms (ammonifiers or decomposers) and nitrifying bacteria (autotrophic).

The main goals proposed in this work are: i) to model the addition of slurry and mineral N into soils and develop a new version of INDISIM-SOM, called INDISIM-SOM_{FERTL} that allows the study of the variables linked to turnover of soil C and N, ii) to verify that this simulator is able to reproduce the conceptual approach designed, and, iii) to compare the simulation results with the previously mentioned set of experimental trials.

The parameterization and initialization of the new simulator was changed with respect to INDISIM-SOM, together with some of the sub-models involved, mainly those linked to ammonia adsorption and desorption and the use of labile N or labile C as energetic sources for microbial maintenance. New values for some parameters were obtained in order to fit the simulations to the above mentioned experimental data, according to the sensitivity analysis carried out in a previous work (Gras et al. 2011). Successful results were achieved with the new simulator INDISIM-SOM_{FERTL}. This confirms that this simulator is an interesting tool to investigate and improve the understanding of the processes that take place with organic amendments and mineral nitrogen soil fertilizations.

Keywords Fertilization of soils; Modelling and simulation

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Anti-oxidative stress enzymes in golden chanterelle (*Cantharellus cibarius*)

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Chanterelle mushrooms are ectomycorrhizal (EMC) fungi and grow in symbiotic association with the root tips of live forest trees. While forests are essential for survival and productivity of chanterelle mushrooms, these, in turn, contribute to the trees nutrition and defense against various environmental stresses. The EMC association with forest trees promotes fine roots development and produces antibiotics, hormones and vitamins useful to plants [1]. On the other hand, the mushrooms receive up to ~ 19 times more carbohydrate from their hosts than normal leakage of the root system would cause, stimulating plants to do more photosynthesis [2]. Moreover, EMC fungi improve growth conditions by protecting plant roots from pathogens, by moderating the effect of heavy metal toxins, by promoting soil structure [3]. Chanterelles are also edible mushrooms, globally renowned as a delicacy quite beneficial for the health; among 10 recognized species in Europe, the golden chanterelle (*Cantharellus cibarius*), also known as girolle, is the primary commercial one. Unfortunately, a decrease in EMC mushrooms polulation has been threatening the blooming chanterelle market. A causal relation between the decline of EMC fungi and tree vitality has been observed in European forests for instance [4], emphasizing the role played by these fungi in environmental issues. EMC mushrooms are known to secrete antioxidant enzymes in the environment, as part of their bioremediation properties; in addition, part of the health benefits provided by chanterelle mushrooms result from their antioxidant properties. Thus the purpose of this research was to investigate the activity of anti-oxidative stress enzymes in the golden chanterelle (girolle) mushroom.

Golden chanterelles (girolles) were homogenized in phosphate buffer 0.1 M, pH 7.0, containing protease inhibitor; the homogenate was centrifuged at 3,000 g for 10 min, then at 35,000 g for 30 min. The supernatant, called "crude extract" was used for our studies. Superoxide dismutase (SOD) activity was measured by three different spectrophotometric methods, based on, respectively, the inhibition of pyrogallol autooxidation in alkaline solution, the inhibition of cytochrome *c* reduction, and the inhibition of nitro blue tetrazolium reduction. Catalase activity was assayed spectrophotometrically by following H_2O_2 dismutation at 240 nm. Peroxidase activity was measured by following spectrophotometrically the H_2O_2-mediated oxidation of o-dianisidine at 460 nm, of guaiacol at 470 nm, and of ferulic acid at 310 nm. Gel electrophoresis of the extract was performed under non-denaturing conditions and followed by activity staining for the enzymes tested.

Results showed that activity was detectable for all enzymes assayed, reflecting the various anti-oxidative stress activities fulfilled by the organism in response to the environmental challenges of its natural habitat. pH activity profiles determined for catalase and peroxidase revealed a peak at pH 8 with a shoulder at pH 6 for catalase and a single peak at pH 4, regardless of the reducing substrate, for peroxidase. Expressed per mg protein in the extract, 0.11 units peroxidase were detectable with o-dianisidine as the reducing substrate, 0.011 units with guaiacol and 0.016 units with ferulic acid; the activity was inhibited by KCN with IC_{50} that varied depending on the substrate. Activity staining for peroxidase after non-denaturing gel electrophoresis of girolle extract revealed at least one band with estimated molecular weight of 45 kD. For catalase, up to 10 units were detectable at pH 8 and the activity was inhibited by KCN with an IC_{50} of 0.15 mM. Activity staining for catalase after non-denaturing gel electrophoresis revealed at least two bands with estimated molecular weights of 350 kD and 280 kD, respectively. Up to 18 units SOD were detectable per mg extract protein; activity staining for SOD after non-denaturing gel electrophoresis revealed a single band with an estimated molecular weight of 30.2 kD. Thus in this research, we characterized the activity of key intracellular anti-oxidative stress enzymes, namely superoxide dismutase, catalase, and peroxidase, present in the golden chanterelle (girolle) mushroom.

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Keywords *Cantharellus cibarius*; anti-oxidative stress enzymes; ectomycorrhizal fungi; catalase; superoxide dismutase; peroxidase

Arbuscular mycorrhizal fungal colonisation might change the composition of *Coleus blumei* root extracts. Preliminary results

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The roots of *Coleus* sp. are rich with polyphenols and diterpenes. Among the most important compounds found in the extracts of plants of this genus are forskholine (diterpene) and rosmarinic acid (polyphenol). Forskholine activates the enzyme adenyl ciclase thus increasing the intracellular levels of cyclic AMP (cAMP). It is commonly used in cell physiology research. Potential applications as an inhibitor of colon cancer cell growth and as an adjuvant in the treatment of neuronal diseases is being studied. Rosmarinic acid is a natural and powerful antioxidant that has been used in the cosmetics and pharmaceutical industry is now increasingly used in the food industry. The presence of both compounds in *Coleus blumei*, a widespread, stress resistant garden plant is not well established. To determine their presence samples of plant roots inoculated and not inoculated with *Glomus intraradices* BEG 72 were analyzed by liquid chromatography associated with mass spectrometry (LC-MS) ion trap. The results show that rosmarinic acid was found in plant roots of both inoculated and non inoculated *Coleus blumei*, whilst forskholine was only found in roots where the symbiosis was established. This result show that the presence of the symbiosis can alter the biosynthesis of secondary metabolites and thus influence the composition of medicinal plants root extracts.

Keywords mycorrhizal fungi; essential oils; *Glomus intraradices*

Auxin producing Cyanobacterial strains and their impact on the growth of *Triticum aestivum* var Uqab 2000

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In the present study cyanobacterial strains were isolated from the rhizosphere of different plants crops. They were identified by 16S rDNA gene sequencing and evaluated for in vitro auxin production as well as growth stimulation of *Triticum aestivum* var Uqab 2000. In the initial studies a preliminary screening of isolated cyanobacteria was done using a well established colorimetric method to evaluate the strains for auxin production. Most of the strains, as expected, showed a high colorimetric response only when medium was supplemented with L-tryptophan. It was also noted that at very high concentrations of tryptophan, a few strains do not showed further increment in auxin production. These colorimetric results required confirmation my more rigorous physical measurements, and the widely accepted exacting method for such analysis is obtained by GC–MS, thus analysis of cyanobacterial culture media was conducted. For the quantitative GC-MS analysis, strains were grown in 100 ml of BG 11 media, either supplemented with 250 μgml^{-1} tryptophan or without tryptophan addition. GC-MS analysis demonstrated that all strains had the potential to produce varying amounts of auxin in the presence and absence of tryptophan. However, the more precise GC-MS analyses showed a lower amount of auxin in strains as compared to colorimetric method. From GC–MS analysis it was shown that IAA concentrations ranged from 0.49 to 5.39 $\mu g \cdot ml^{-1}$ in 30 day cultures and that the filamentous strains were more efficient at producing IAA as compared to unicellular strains. Plant microbe experiments showed the positive correlation between auxin production by cyanobacterial strains and endogenous IAA content of *T. aestivum*. Cyanobacterial strains have the ability to increase the endogenous IAA content and growth of *Triticum aestivum* var Uqab 2000. The Significance and Impact of the current Study was that these cyanobacterial strains can be effectively used to enhance the growth and yield of agronomically important crops.

Bacterial metabolic activities in composting. Evolution throughout the process

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Composting has been considered as one of the worthiest methodologies for the recycling and valorization of organic wastes. Since ancient times, human beings have produced variable amounts of residual materials which, to a greater or a lesser extent, were treated. Nevertheless, it was until the beginning of the 20th century that scientific rules were applied to composting, when Sir Albert Howard developed the Indore system. Since then, knowledge about composting has positively evolved and we understand the process in a greater extension now. However, some aspects remain unsolved and further investigations are still needed for a full understanding of the whole process.

Since composting is a biological process, organisms, namely microorganisms play a key role in the evolution of the organic materials and in the transformations they suffer from wastes to safe organic amendments (compost). The presence of different microbial populations according to the prevalent environmental conditions and their metabolic activities determine the undergoing reactions and the correct modification of the organic substrates. In this sense, the quantitative monitoring of main enzymatic activities may provide valuable information for establishing the timing of the biological modifications throughout the process.

The present study is focused on the isolation of mesophilic bacterial population through the different stages of the process and the determination of metabolic activities associated to this population. Three piles were constituted using the woody fraction of tomato plants. The C:N ratio was adjusted to 25 by adding chips from a sawmill facility. A total of 19 samples were extracted according to the thermal profile of the piles and 308 mesophilic bacterial strains were isolated. All of them were analyzed regarding catalase and oxidase activities, their ammonifying and phosphatase capacity and their ability for degrading starch, fatty acids, pectins, proteins and lignocellulose (cellulose, hemicellulose and lignin).

Most of the isolates showed positive catalase and ammonifying activities, while cellulolytic capacity were not detected and just one isolate showed ability for degrading lignin. On the contrary, the capability for hemicellulose modification was detected in 114 strains, which means 37% of the total isolates. The majority of the hemicellulolytic isolates were detected at the end of the process, during the maturation phase. From a quantitative point of view, the intensity of this activity was also higher at the maturation stage, since more than 60% of the detected microorganisms during this cooling phase showed the ability for degrading this polymer. A similar pattern was observed for lipolytic and proteolytic activities, since the higher values for fatty acids and proteins degradation were detected in the samples extracted during the final part of the process, always over 50%. Nevertheless, the distribution of isolates throughout the different phases did not show a clear trend, with maximal number of lipolytic strains at the middle of the process and proteolytic isolates at the initial phases. Amilolytic activity showed an irregular pattern with phases displaying high levels of activity following by phases with low levels. These low levels coincided with the end of the bio-oxidative phase of the process, while the preceding stages, as well as the cooling and maturation phases, were characterized by higher levels of amylase activity. In the case of the phosphatase activity, the lower levels (under 10% of activity) were mostly detected following the thermophilic stages during the bio-oxidative phase, while values between 25% and 45% were observed in most of the other samples extracted during this bio-oxidative, as well as during the cooling and maturation stages. Moreover, the maximal number of isolates showing phosphatase activity was obtained from the termophilic samples. The last of the metabolic activities associated to the transformation of carbonated macromolecules was the pectinolytic activity. This activity was only detected in 5 microorganisms (less than 2% of the total isolates), with higher levels of activity (lower than 10%) at the end of the cooling stage.

The monitoring of the metabolic capabilities of the microbiota associated to the different stages of a composting process provide valuable information for the understanding of the transformations that organic matter undergoes and may be a powerful tool for the development of strategies leading to more efficient process by means of the inoculation of microorganisms showing proper activities at specific stages.

Keywords composting; metabolic activities; biotransformation

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Beneficial symbiotic system of pea (*Pisum sativum L.)*: plant - arbuscular mycorrhizal fungi – rhizosphere/nodule bacteria

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Existence of common legume plant genes implicated in interactions with both rhizosphere bacteria (including nodule bacteria) and arbuscular mycorrhizal fungi and common structural-developmental traits for nitrogen-fixing symbiosis and arbuscular mycorrhiza posed a question about evolution of such plant-microbe systems. Also it demonstrates the necessity of exploitation of such a tripartite symbiosis (legume plant + arbuscular mycorrhizal fungi + rhizosphere bacteria (including nodule bacteria)) in sustainable agriculture.

Great genetic variability by effectiveness of such a system, was demonstrated for pea. This, in its turn, posed a question of development of new types of complex inoculants to select highly symbiotically effective plants during breeding process. The field trials (performed during years 2000-04) demonstrated highly beneficial effect of such a kind of inoculation on plant biomass production and protein content, and therefore, necessity of breeding to improve symbiotic potential of legume crops.

The methodology of breeding and development of complex inoculants will be discussed.

Key words: beneficial plant-microbe systems, arbuscular mycorrhiza, beneficial rhizosphere bacteria, nodule bacteria,

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Biofertilizer with diazotrophic bacteria and fungi chitosan improving cowpea biomass yield, nutrient uptake and some soil attributes

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A greenhouse experiment was carried out to evaluate the agronomic efficiency of the biofertilizer produced from phosphate and potash rocks in mixture with earthworm compound enriched in N by free living diazotrophic bacteria and bioprotector with fungi chitosan by inoculation with *Cunninghamella elegans*. The mixed biofertilizer with addition of chitosan from shrimps and from fungi (*C. elegans*) were tested on cowpea nodulation, biomass yield and nutrient uptake, and also in changes in some chemical attributes of an acidic soil. Cowpea (cv. IPA 206) was cropped and applied the treatments: (a) mineral fertilizer (FNPK), (b) mixed Biofertilizer (BNPK), and (c) Biofertilizer-Bioprotector with fungi chitosan from *C. elegans* with addition of shrimps chitosan produced chemical processes. A control treatment with cow manure and without NPK fertilization was added. After forty five days of sowing the plants were harvested and determined nodules biomass, (NB), shoot biomass (SB) and total N, P and K. Soil samples were collected and analyzed pH, total N, available P and available K. Biofertilizers-Bioprotector increased NB, SB, total N, P, and K accumulated in shoots. Furthermore, biofertilizers reduced soil pH and increased total N available P and available K. The result focuses the great potential of rock biofertilizer inoculated with free living diazotrophic bacteria and *C.* elegans as alternative to NPK fertilization in acidic soils.

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Biofertilizer-Protect with Free Living Diazotrophic Bacteria and Fungi Chitosan as Affecting Green Pepper Growth and Some Soil Properties

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In a field experiment the agronomic effectivity of biofertilizer from P and K rocks in mixture with earthworm compound enriched in N by free living diazotrophic bacteria and bioprotector with fungi chitosan produced by *Cunninghamella elegans* were studied. In laboratory assays (Petri dishes) the effect of fungi chitosan and shrimps chitosan against plant pathogenic fungi were tested. In the field experiment the effectiveness of mixed biofertilizer and of the Bioprotector were evaluated on green pepper (*Capsicum annuum*) yield and nutrient uptake, and also observed the changes in some soil properties. The fertilization treatments were: conventional mineral fertilizer (FNPK) in recommended rate; mixed Biofertilizer (BNPK) in three rates and (c) Biofertilizer-Bioprotector (BNPK + *C. elegans*) in three rates; (d) a control treatment without NPK fertilization and applied cow manure. In Petri dishes assays fungi chitosan (*C. elegans*) and shrimp chitosan inhibited fungi pathogen growth. In field experiment the BNPK and BNPK + *C. elegans* treatments showed the highest green pepper yields and PK uptake in fruits, compared to fertilizer treatment. On leaves were not observed significant. In soil biofertilizer (BNPK) reduced pH and increased total N available P and available K in soil. The results focuses the great potential of rock biofertilizer with free living diazotrophic bacteria and biofertilizer with *C.* elegans as alternative to NPK fertilization.

Changes in Arabidopsis Root Architecture by Auxin Producing Rhizobacteria in MS Media and Sand System

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Soil Microorganisms play an important role in plant growth and development by Direct and Indirect ways in relation to health nutrition and development. Plant root development can be largely affected through the association of roots with plant growth-promoting rhizobacteria. In this study, *Arabidopsis Thaliana (Col- 0)* showed contrasting responsiveness in root development to inoculation with the rhizospheric auxin producing rhizobacteria *PNS-1, PNS-2and PNS-3* in MS media and Sand system in invitro condition by increasing primary root length, lateral root length and number of lateral roots. *A. thaliana* reporter plants for auxin (DR5) responded similarly with increased root branching in the presence of these bacteria.

Keywords: Rhizobacteria, *Arabidopsis, Auxin*

Characterization of *Ochrobactrum* spp. obtained from rhizosphere of cactus species growing in Mexican xerophytic highlands

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TheTehuacan-Cuicatlan nature reserve in central Mexico, is an arid area of unique plant biodiversity constituted mainly by xerophytes, with exeptional occurence of rare and endemic species. In this area thera are 75 registered species of Cactaceae, and 21 of them are endemic. This natural diversity is being threatened by several human activities; however, uncontroled recolection of plants is probably the main hazard to various cacti species. Management and propagation of cactus plants normally do not take into account the microbial community associated to their roots, which probably are related to plant important processes (i.e. nutrient solubilisation, N_2 fixation). Based on their growth type 3 cactus species were chosen; an herbaceous *Mammillaria carnea (Mc)*, a shrub *Opuntia pilifera (Op)* and a species with tree-like growth; *Stenocereus stellatus* (Ss), and the bacterial communities in their respective rhizospheres were assessed.

The objective of this work was to characterize *Ochrobactrum* strains obtained from cactus rhizospheric samples, by means of 16S rDNA sequence analysis, API systems, antibiotic resistance profile and production of indol acetic acid (IAA).

Rhizospheric isolates obtained inTy and N_2 free (Nf) media were grouped by PCR-DGGE of the V6-V8 regions of the 16S rDNA. Selected members of each group were used for almost full length 16S rDNA sequence analysis. Members of the collection assigned to the genus *Ochrobactrum* were subject to API 20E and API 20NE biochemical profiling tests. Antibiotic resistance was tested against Polymixin (300UI), Colistin (30µg/ml) and Chloramphenicol (60 µg/ml). IAA production was meassured by means of Salkowski reaction.

Based on colony morphology 82 isolates were obtained; 42 in TY and 40 in Nf media. After DGGE-based profiling, we obtained 21 ribotypes for TY and 19 ribotypes for Nf media. Selected ribotype-colonies from each group were characterized by 16S rDNA sequencing. From the 40 ribotype groups created, 12 corresponded to different members of the genus *Ochrobactrum,* the most abundant species was *O. thiophenivorans* represented by 10 ribotypes, and O. *lupini* only by 2. Biochemical profiling allowed to form 5 API groups with no relation to those formed by molecular methods. Antibiotic resistance tests were applied to representative mebers of the 12 ribotype-groups and only 3 of those groups (F10, F15, F22) were resistant to all antibiotics used. Production of IAA was positive for all ribotype groups, the production levels were broad ranging from 13 to 49 µg/ml medium.

The abundance of *Ochrobactrum* strains in all samples point them as important members of the rhizosphere bacterial communities in the three species of cacti. Further experiments are trying to elucidate the possible role of *Ochrobactrum* spp as a plant growth promoting bacteria related to cacti species, and to establish their possible N-fixing abilities

Keywords: Ochrobactrum; cactus rhizosphere

Characterization of the microbial community of Paricutín volcano, Michoacán, México

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Microorganisms capable of growing in extreme environments such as high pressure, acidic or alkaline pH, and high or low temperatures are called extremophyles (Rossi *et al,* 2002). It is possible that these organisms can be found in the soil of volcanic vents as well as in fumaroles. Paricutin Volcano, located in Michoacán Mexico, had its birth and major period of activity in the decade 1940-1950, although continues producing sulfurous emanations .To date, there have been few reports on botanical, zoological and geological characteristics from the Paricutin volcano zone, and none regarding the microbial communities present in the vents of the volcano.

In this work we describe the bacterial and fungal populations present in the Paricutin volcano vents using conventional culture techniques and culture-independent molecular methods for the analysis of environmental DNA.

Soil samples were collected from different vents, located in the cone of Paricutin volcano. Chemical analyses of the samples were carried out by flame atomic absorption (FLAA) using standard methods (EPA, 1996). A procedure for DNA isolation was implemented based on previously described protocols. Bacterial population analysis was performed by T-RFLP based on 16S rRNA gene (Osborn, 2002; Blackwood, 2003) and by ARISA (Ranjard, 2001).

The physicochemical analysis of the samples indicated that the pH varied in the range 6-8.5 and that temperature was in the range 45-75 ^{o}C; the major element found was iron followed by phosphorus and sulphur.

The bacterial diversity in the samples was evaluated by T-RFLP, which according to the profiles observed indicated that no more than 5 Operational Taxonomic Units (OTUs) are present in the samples; probably, this results as a consequence of the extreme conditions of the samples. Statistical treatment of the data was done to determine similarities or differences between all samples. These data provide the bases to determine how many independent clones should be obtained as part of the 16S rDNA gene libraries required for identity analysis.

We also performed gene bank analysis (ARDRA) for bacterial populations of the samples and partially characterized some culturable isolates regarding their resistance to hexavalent chromium.

Keywords: Paricutín volcano, extremophyles, chromatereducer, T-RFLP, bioremediation.

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Chickpea growth promotion ability of a mesorhizobia strain expressing an exogenous ACC deaminase gene

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Bacteria that express ACC deaminase can uptake and convert ACC into α-ketobutyrate and ammonia, thus, reducing plant ethylene levels. The use of bacteria that express high levels of ACC deaminase has been reported to help plants to overcome various stresses, including waterlogging. The main goal of the study reported herein was to assess the nodulation performance of a chickpea mesorhizobia strain transformed with an exogenous ACC deaminase gene (*acdS*), and its subsequent ability to increase plant growth under normal and waterlogging conditions. Chickpea mesorhizobia strain LMS-1 was transformed with the *acdS* gene of *Pseudomonas putida* UW4 by triparental conjugation using plasmid pRKACC. A plant growth assay was conducted to evaluate the plant growth promotion ability of the LMS-1 wild-type strain and LMS-1 (pRKACC) transformed strain, under normal and waterlogging conditions. Bacterial ACC deaminase and nitrogenase activities were also determined.

By expressing the exogenous *acdS* gene, the transformed strain LMS-1 showed a 127% increased ability to nodulate chickpea and a 125% promotion of the growth of chickpea compared to the wild-type strain, under normal conditions. Under waterlogging stress conditions, plants inoculated with either LMS-1 (pRKACC) or the LMS-1 wild-type strain showed a similar nodule number, suggesting that waterlogging conditions do not affect the nodulation of chickpea. No significant relationship was found between ACC deaminase and nitrogenase activity. The results obtained in this study show that the use of rhizobial strains with improved ACC deaminase activity might be very important for developing microbial inocula for agricultural purposes.

Keywords rhizobia, chickpea, ACC deaminase, waterlogging, nodulation

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Degradation of tannins by *Cronobacter sakazakii* isolated from goat

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Tannins, present in various foods, feeds and forages, have anti-nutritional activity; however, presence of tannase (tannin acyl hydrolase) in microorganisms inhabiting rumen and gastrointestinal tract of ruminants overcome the toxic effects of tannins. The degradation studies on tannic acid were conducted using tannin degraders isolated from rumen and faecal samples of animals. Out of 36 isolates, *Cronobacter sakazakii* was observed to degrade tannic acid (0.1% in minimal media) anaerobically to gallic acid, pyrogallol as determined by Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). A tannase activity of 21.23 ± 0.132U/ml was observed for the isolate. The organism was tolerant up to 4% of tannic acid in the medium and resulted in lag phase extended to 6 hrs at 3% and 4% tannic acid concentration. The tannic acid was completely metabolized after 72 h of incubation with pyrogallol as the main product. The rate of degradation of tannic acid was faster in case of *Cronobacter* compared to other isolates. Further degradation of pyrogallol to resorcinol was not observed when incubated upto 96 h. A combined activity of tannase (converting tannic acid to gallic acid) and gallic acid decarboxylase (metabolizing gallic acid to pyrogallol) in a single isolate has potential in synthesizing useful pyrogallol from tannins present in agrowastes in an environmentally acceptable manner. Further work is in progress to identify the key gene responsible for this activity.

Keywords Tannin; Cronobacter; gallic acid; pyrogallol;

Development of a typing scheme for the characterization of the bacterial population associated to summer squash (*Cucurbita pepo)* cultivated in green-houses in Almeria (Spain)

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A preliminar typing scheme has been developed for the characterization of the bacterial population present in plant and fruit tissue of summer squash. The objective of this work is to stablish a systematic method to identify and differenciate among different saprophitic or pathogen bacterial strains isolated from plant or fruit tissues in order to know about the origin and possible routs of contamination.

Tissue phragments were cultivated directly or previously macerated on buffered peptone water and then streaked on nutrient agar plates. A total of 26 isolates were investigated using morfological, biochemical and pathogenicity tests showing at least 13 biotypes. Colony appearance, cellular morphology, gram staining and a total of eleven biochemical tests were used to differenciate amongst different isolates, showing a good discrimination ability. Two of the isolates were positive for tobacco sensitivity test in *Nicotiana xanthi* leaves.

More research is needed to identify at the specie, subespecie and patovar level using molecular tools in order to determine the possible presence of pathogen bacteria leves.

Keywords: summer squash; *Cucurbita pepo*; plant pathogens; biochemical typing

Difference of a peptide pheromone and a genomic sequence between *Streptococcus bovis* and *Streptococcus gallolyticus*

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Backgrand

Streptococcus bovis is usually a major lactate-producing bacterium in the rumen, and often proliferates when ruminants are fed diets containing large amounts of readily fermentable carbohydrates, such as starch. Thus, *S. bovis* sometimes causes rapid acidification in the rumen as a consequence of excessive lactate production, and is thought to contribute to the progress of rumen acidosis. Therefore, it is desirable to control the growth of *S. bovis*. In addition, *S. bovis* has also been detected in the human intestine, and may be associated with general bacteremia, endocarditis, heart valve infections, and deterioration of the colon wall in colon cancer. Therefore, it is desirable to suppress the overgrowth of *S. bovis* in the human colon, as well as in the rumen. Recently, *S. bovis* is redivided into four DNA clusters according to the 16S rDNA sequences in detail, and two of them, biotype II/1 [*S. bovis*] and biotype I [*S. gallolyticus*], were isolated from the rumen and human colon. Thus, it is also important to control the growth of *S. gallolyticus*. It has been proposed that the peptide pheromone signaling system is a streptococcal intercellular communication mechanism. Previously, we sequenced the genes of the ComC peptide and its two components system (AB284382). In order to control the growth of *S. bovis* via the ComC peptide pheromone signaling system, we compared the function of this system between *S. bovis* and *S. gallolyticus*. Furthermore, we show the draft genome sequence of *S. bovis* and compare to that of *S. gallolyticus*.

Resalts

The nucleotide sequence of the gene encoding ComC peptide was found in *S. gallolyticus*. The amino acid sequence identity of the *S. gallolyticus* precursor ComC was similar to that of *S. bovis*, however, that of the mature ComC (region downstream from the Gly-Gly cleavage site) was different, suggesting that the mature ComC is specific to each species. Addition of *S. gallolyticus* mature ComC peptide to the medium of *S. gallolyticus* increased the growth rate and transformation frequency. However, neither the growth rate nor transformation frequency were unaffected by the addition of *S. bovis* mature ComC peptide in *S. gallolyticus*. On the contrary, *S. gallolyticus* mature ComC peptide had no effect in *S. bovis*. A single shotgun pyrosequencing run of *S. bovis* genomic DNA using a Genome Sequencer FLX system (454 Life Sciences) resulted in 220,000 high-quality reads (mean read length 230 bp) that were assembled using Newbler soft ware (454 Life Sciences) into approximately 40 contigs >500 bp long. Paired-end sequencing produced approximately 106,000 reads. Assembly of the paired-end reads produced 13 scaffolds containing 40 large contigs. The sequence was annotated using Annotation Engine (J. Craig Venter Institute) and manually curated using Manatee (http://manatee.sourceforge.net/). The draft genome comprises approximately 2,000,000 bases including approximately 2000 predicted CDSs. A functional classification of CDS was categorized into 20. *S. bovis* genome has putative genes for complete glycolysis and Entner-Doudoroff pathways. *S. bovis* has predicted genes for the synthesis of most essential amino acids and some vitamins and cofactors. These properties were similar to those in *S. gallolyticus*. However, *S. bovis* seems to be less able to degrade polysaccharides derived from the plant cell wall than *S. gallolyticus*, because *S. bovis* has less polysaccharide hydrolases. *S. gallolyticus* might be more advantageous to thrive than *S. bovis*, when these bacteria competes for polysaccharide in the rumen or large intestine.

Acknowledgments

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Keywords genome sequence; peptide pheromone; rumen bacteria; ruminal acidosis; *Streptococcus bovis*

Diversity and antagonistic properties of microorganisms isolated from ‘fired plots’ under shifting cultivation in North East India

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Shifting cultivation, locally known as *jhum*, is an age-old agricultural practice in hills of northeast India. It refers to ‘slash and burn’, a cyclic process that involves (1) clearing land through the burning of natural vegetation, (2) clearing the burnt material, (3) the sowing of crops, and (4) leaving the land fallow for several years after a period of crop cultivation. In the present study, soil samples collected from the fired and fallow plots under shifting cultivation have been analysed for their physico-chemical and microbial characteristics. Significant differences in pH and electrical conductivity were recorded in soil of fired and fallow plots. Significantly higher amounts of total organic carbon and nitrogen were estimated in fallow plots as compared to the fired plots. The fire operations, however, resulted in stimulation of microbial communities. Bacterial and actinomycetes counts were significantly higher in fired plots as compared to the fallow plots[1]. Based on 16S rRNA analysis the representative bacterial species recovered from the ‘fired plots’ were found to belong to the genus *Bacillus*, *Pseudomonas* and *Streptomyces*. Most of these species were found to be positive for phosphate solubilization, and antagonism against plant pathogenic fungi. The antifungal property was demonstrated due to the production of diffusible and volatile substances. Several isolates produced ammonia, salicylic acid, HCN and siderophores and many were found to be positive for the production of hydrolytic enzymes. Recovery of these species after the fire operations is indicative of the “microbiological merit” of shifting cultivation.

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Diversity of entomopathogenic bacteria for the control of damage caused by white grubs (Coleoptera:Scarabaeidae)

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Biocontrol by the use of entomopathogenic bacteria has been a promising option to the use of chemicals for the management of insect pests according to the principles of sustainable agriculture.

In Mexico and other countries the larvae of insects of the scarab family known as white grubs (Coleoptera:Scarabaeidae) are soil pests of many crops including Graminae, Leguminosae, vegetables and ornamentals. The roots of plants are the source of food for larvae mainly in soils with low content of organic matter. There is no effective biological control agent in Mexico and there are about 68 different species of white grubs (*Phyllophaga* spp) reported as potential pests. The aim of our research is the search and characterization of pathogenic bacteria active against *Phyllophaga* spp larvae to be used in a future program for Integrated Pest Management.

Bacteria have been collected for several years, isolated from the haemocoel of dead larvae previously showing disease symptoms. The larvae have been collected from cornfields located at different regions of the country. The isolates were propagated at 30° C on nutrient broth-agar plates. The isolates showing homogeneous and good yields were selected for oral bioassays. Pathogenic bacteria were selected by their ability to cause anti-feeding effect (AFE) and mortality (M) by at least two rounds of oral bioassays. For this purpose, healthy larvae of *Phyllophaga blanchardi* were fed with small pieces of carrot coated with the selected isolates. Uncoated carrot was used to feed control larvae. Differences in the percentage of consumed carrot among control and experimental groups were evaluated by ANOVA. Mortality was evaluated by the statistical test X Square.

The results of our investigation have rendered the identification of 18 isolates able to cause significant AFE, since larvae consumed only 6-46 % of their food. Significant mortality was observed after 35 days from the beginning of the bioassays. Sequencing of the gene for 16S rRNA, showed that the species of the selected bacteria were *Serratia marcesens* (6 isolates), *Enterobacter agglomerans* (2 isolates), *Lycinybacillus sphaericus* (3 isolates), *Enterobacter cloacae* (1 isolate), *Pseudomona fluorescens* (1 isolate), *Acinetobacter calcoaceticus* (1 isolate), *Bacillus thuringiensis* (2 isolates*)* and *Serratia entomophila Mor4.1* (1 isolate). The isolates were selected as pathogenic strains. We think that some of these strains could be selected to develop biocontrol agents to prevent crop damage caused by white grubs. The strains are also a source of genes to be used in biotechnological applications in agriculture.

Keywords: entomopathogenic bacteria, Coleoptera:Scarabaeidae, biocontrol

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Diversity of rhizospheric halotolerant bacteria associated to chenopod plants *Atriplex* and *Suaeda*

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Plants growing in saline soils are exposed to various levels of moisture and salinity stress during their life cycle. Plant associated microbes may help mediate such stress. We analyzed rhizospheric, soil and leaf litter microbial communities associated with two saline-adapted chenopod plants, *Suaeda mexicana*, from central Mexico and *Atriplex canescens*, from the Chihuahuan Desert region of the United States. In order to characterize the cultivable microbial community, soil and leaf litter samples were processed and analyzed by traditional surface spread plating methods. The samples were plated on sixteen different culture media: modified R2A; Casamino acids; BHAP; PDA; TYA and YCED. Each medium contained either 4% or 10% NaCl (w/v), and was adjusted to either neutral or basic pH.

Cells of 43 strains, selected as representatives of the cultivated isolates, were lysed by freeze-boiling and directly applied to PCR mixtures. Amplification of 16S rDNA fragments was carried out using the primer pairs F984GC-R1378 for bacteria and ITS1F-ITS4 for the sole fungal isolate.

Sequences obtained from PCR products obtained from Atriplex isolates were homologous to sequences of the bacterial genera *Penibacillus, Streptomyces, Promicromonospora, Rhodococcus, Bacillus* and *Pseudomonas*, and the fungal genus *Aspergillum*. *S*equences homologous to the genera *Artrhobacter, Streptomyces, Nocardia, Cellulosimicrobium, Pseudomona*s and *Bacillus* were amplified from *Suaeda* isolates.

Total DNA was extracted from samples of soil and leaf litter in different seasons (winter and spring). Using a Universal Bacterial 16S Primer, Tag-Encoded FLX Amplicon Pyrosequencing (TEFAP) was used to assess microbial diversity .

Table 1. Percentages of TEFAP amplicons from Atriplex and Suaeda rhozospheres and litter with homology to indicated phylum in Winter, 2010 and spring of 2011.

	Rhizosphere Suaeda		Rhizosphere Atriplex		Litter Suaeda		Litter Atriplex	
Phylum	S Winter	S Spring	A Winter	A Spring	S Winter	S Spring	A Winter	A Spring
Actinobacteria	34.35	20.48	47.15	46.59	27.19	30.31	27.82	35.19
Proteobacteria	32.32	36.90	30.15	26.24	48.53	44.65	62.30	51.90
Chloroflexi	12.32	6.24	7.94	4.91	3.09	4.46	0.08	0.51
Bacteroidetes	6.34	2.70	1.23	1.04	8.47	6.49	6.03	7.56
Firmicutes	4.88	1.94	8.64	15.02	2.44	2.26	0.50	0.92
Gemmatimonadetes	3.78	20.10	1.16	1.63	0.18	0.74	0.17	0.17
Verrucomicrobia	1.67	6.43	0.89	0.23	3.66	2.84	0.11	0.10
Planctomycetes	0.85	0.52	0.39	0.70	0.36	0.55	0.17	0.27

Each of the isolates were used for the inoculation of axenically grown *Amaranth* spp seedlings under different concentration of NaCl (0-1%) and plant growth promotion was evaluated.

Keywords ; halotolerant microbial diversity Atriplex, Suaeda, pyrosequencing.

dnaK and *groESL* are highly induced in heat-tolerant rhizobia

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In free-living state or during symbiosis with leguminous plants, rhizobia are affected by environmental factors, such as temperature or pH. The ability of these bacteria to endure stress is very important in order to achieve high efficiency in the atmospheric nitrogen fixation process, especially in legume-rhizobia symbiosis under suboptimal conditions. The objectives of the present work were to evaluate the temperature stress tolerance of native chickpea rhizobia and to investigate if tolerance is related to isolates' species or origin site. Another aim was to study the molecular bases of temperature stress tolerance in rhizobia, by comparing the expression levels of major chaperone genes, in thermotolerant and thermosensitive isolates.

Fifty three chickpea mesorhizobia previously isolated from several provinces of Portugal, and assigned to different species, were used (1). Temperature tolerance was evaluated under cold (15ºC), heat (37ºC) and heat shock stress (48ºC, 15 min). Mesohizobia isolates showed high diversity in their ability to grow under temperature stress; nevertheless most isolates tolerate heat shock or cold stress better than the heat stress. Isolates from distinct species groups differed significantly in their ability to tolerate temperature stress and an association was found between some provinces of origin and stress tolerance of the isolates. For the study of the molecular bases of temperature stress tolerance, the mRNA levels of the major chaperones were analysed upon stress using a set of tolerant and sensitive chickpea rhizobia, belonging to different Mesorhizobium species. The analysis of the *dnaK* and *groESL* expression by northern hybridisation showed an increase in the transcripts levels with heat but not with cold stress. Interestingly, after temperature upshifts, a higher induction of chaperone genes was detected in tolerant isolates compared to that of sensitive isolates, from the same species (2). The present study suggests a relationship between higher transcriptional induction of the major chaperone genes and higher tolerance to heat in rhizobia.

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Keywords: Temperature stress; *dnaK*; *groESL*; rhizobia

Dynamics of symbiotic bacteria populations elicited by their co-migration with the host plants into the novel soil environments

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Symbiotic microorganisms interacting with terrestrial plants (N_2-fixing bacteria, mycorrhizal fungi, endophytic, rhizospheric and epiphytic microbes) are of a great importance for adaptations of their plant hosts towards the natural environments and for the crop productivity in the sustainable agricultural systems. The beneficial (nutritional, defensive, growth regulatory) impacts of these microorganisms on their host development depend on the microbial population dynamics in the endosymbiotic (*in planta*) and soil niches which is elicited by the partners' co-adaptation and results in the rapid evolution of the microbial genomes [1]. In this co-adaptation, crucial role belongs to the partners' joint migrations (invasions) into the novel ecological zones including the artificial introductions of cultured plants into the novel cropping areas. Using a variety of molecular methods (RAPD-fingerprinting, PCR-RFLP analysis of chromosomal and plasmid loci, plasmid profiling) we demonstrated that migration of the rhizobia-legume (*Rhizobium leguminosarum* bv. *trifolii* – clover, *R.l.* bv. *viceae* – vetch) symbiotic systems into the novel soil-climatic zones may be accompanied by the decreased microsymbiont diversity [2]. However, this diversity may be restored rapidly or even extended due to the accumulation of mutations ensuring the adaptations of the invaded strains for the novel edaphic conditions accompanied by the gene exchange between the introduced and local microbial genotypes. We developed the mathematical simulation approach [3] which allows us to demonstrate that the panmictic structures may evolve swiftly in the introduced rhizobia population based on the host-induced frequency-dependent selection in favor of the rare recombinants formed due to horizontal transfer of symbiotically essential (involved in host nodulation, or in symbiotic N_2 fixation) genes from the invaded rhizobia strains into the local soil bacteria. The resulted recombinants may be converted into the regular plant symbionts due to the combined impacts of individual (Darwinian) and group (kin, inter-deme) selective pressures induced by the host plants in the microbial populations which occupy the rhizospheric and endophytic niches. Under the natural conditions, these pressures may result in the enhanced ecological efficiency of mutualistic partners' interactions based on the "reciprocal altruism" directed from the irreversibly differentiated N_2-fixing bacteroids to their plant hosts [4]. The microbial gene systems encoding for the symbiotic traits (e.g., nodulation and N_2 fixation gene networks in nodule bacteria) evolved rapidly due to the combination of horizontal gene transfer, intra-genome rearrangements and the host-induced selective pressures elicited in the microbial populations occupying the endosymbiotic (*in planta*) niches. We proposed the multi-stage evolutionary scenario for the evolution of plant-microbial symbioses [5] which may be used for the computer simulation of the ecological and genetic consequences of introduction of the novel (e.g., genetically modified) symbiotically effective microbial genotypes into the sustainable agricultural systems. Supported by RFBR grant 09-04-00907a.

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Keywords population dynamics; plant-microbe beneficial interactions; horizontal gene transfer; invasions of symbiotic systems into novel ecological zones; natural selection; mathematical simulation; sustainable agriculture.

Ecology of *Bacillus* sp. and *Lysinibacillus* sp. in rice field soils from Southern Brazil

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In Brazil, the Rio Grande do Sul state is responsible for approximately 64% of rice production, which is a worldwide consumed food. In agroecosystems bacteria develop important functions in the soils. This study aimed the analysis of a possible impact of different cultivation systems on the soil bacterial community. The field work was developed at the EEA/IRGA station (Cachoeirinha, RS), evaluating the influence of the irrigated rice fields into the soil microbiota, since the latter plays an important role maintaining the soil conditions, consequently affecting the rice production. Bacteria were isolated from soil from four different cultivation systems (pre-germinated, conventional plantation, direct plantation and minimum cultivation), in the 2009/10 agricultural year. Bacteria selected as spore-forming Gram-positive were submitted to growth in selective media, as well as morphocitochemical analysis. Test t, ANOVA one way, Principal Components Analysis (PCA) and Simple Linear Regression were applied in order to evaluate the averages values and compare them with physicochemical parameters. The results obtained showed that Gram-positive bacteria were more abundant than the Gram-negative group ($t = 6.9$; $gl = 44.9$; $p<0.001$), but apparently the cultivation systems did not influence their distribution ($F = 0.13$; $gl = 3$; $p = 0.9$). Spore-forming Gram-positive bacteria were also significantly more abundant compared to non-spore-forming bacteria ($t = 11.76$; $gl =3$; $p<0.001$). Rice phenological phases influenced spore-forming bacteria distribution in the pre-germinated system ($F = 7.15$; $gl = 3$; $P < 0.001$), direct plantation ($F =3.57$; $gl = 3$; $P < 0.001$), minimum cultivation ($F = 7.34$; $gl = 3$; $P < 0.001$) and conventional plantation ($F = 19.04$; $gl = 3$; $P < 0.001$), but there was no significant difference among the cultivation systems ($F = 0.854$; $gl = 3$; $p = 0.467$). The species presented different frequencies according to the rice phase, while *Bacillus thuringiensis* was the only one found in the maturation phase, representing in average 5% of the spore-forming species. In the vegetative phase, *B. cereus* correspondend to 23% of the spore-forming species, and the frequencies varied between the two species in the offseason and the reproductive phase. *Lysinibacillus sphaericus* was found only in the offseason. The physycochemical parameters that had the major influence upon the bacterial species were Al, O.M., H + Al and pH related to the axis 1 from the PCA, which explains 36%; and P and Mg, related to the axis 2, which explains 29%. However, the simple linear regression analysis showed only two parameters correlated to the bacterial groups, H+Al, which positively influenced the spore-forming bacteria ($Y = 6.959 + 3.676X$; $R^2 = 0.467$; $gl_{1.7} = 0.042$; $p<0.05$), and Mg, which positively influenced *B. thuringiensis* ($Y = 1.500 + 5.000X$; $R^2 = 0.460$; $gl_{1.7} = 5.963$; $p<0.05$). Phosphorus, potassium, magnesium and sodium are known as promoters of changes in the soil microbial community since some of these nutrients limit the development of microorganisms, suggesting that the bacterial community composition might vary according to the mineral element available in the soil, thus altering the diversity. Therefore, this process promotes the variation of microenvironments and the spatial variation of bacterial communities. Bacteria promote plant growth due to many factors, and the P solubilization realized by microorganisms is considered one of the most important when related to plant nutrition by P, since chemical fertilizers promote negative impacts to the environment and increase the agricultural production cost. Some *Bacillus* species increase N, P and K assimilation or can even promote the release of Ca, Mg, Al and Si in the soil, thus favoring plant development through nutrient contribution. Besides helping to understand the interactions between microorganisms and the environment, a better knowledge about the diversity can also light up new species with biotechnological potential applied to the agroecosystems or to ecosystems with high anthropogenic impact, which thereby might recover and maintain the biotic parameters equilibrium in the environment.

Keywords: spore-forming bacteria; soil; rice

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Effect of *Bacillus SP.* Isolated from the Soil against some Fusarium Strains

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Polypeptide antibiotics or antimicrobial compounds which constitute the Bacillus bacteria have been gaining importance as a result of studies. The Bacillus species that produce antibiotics are *B. subtilis, B. polymyxa, B. brevis, B. licheniformis, B. circulans, B. cereus*. Polypeptide antibiotics produced by *Bacillus* that are used in medical treatments are bacitracin, gramycidin S, polymyxin, tyrotricidin. However only limited data exist on antibiotics or antimicrobial compounds from *Bacillus spp*, therefore presents an interesting genus to investigate since it produces diverse array of antimicrobial peptides representing several different basic chemical structures. In this study we aimed to determine antimicrobial activity of *Bacillus* strains against six *Fusarium* strains which are pathogen microfungi of sugar beet.

In this study a total of 16 *Bacillus* strains isolated from the some soil examples in Turkey. Also fungi were isolated from diseased sugar beets by using Rose Bengal Chloramphenicol (RBC) Agar and incubated at 25 °C for 5-7 days. Identification of grown isolated bacteria colonies was based on morphologic, biochemical and culturing characteristics. However, macroscopic and microscopic properties of the fungi isolates are examined and they were identified microscopically to genus level with traditional methods using diagnostic literatures. Antifungal activity of isolated bacteria strains were determined against *Fusarium oxysporum, Fusarium solani, Fusarium culmorum, Fusarium moniliforme standart* strains and our 2 Fusarium sp. isolates.

Isolated 16 bacteria strains were Gram positive rods and were identified as *Bacillus sp*. and our 2 fungal isolates were identified as *Fusarium sp.* As a result of antimicrobial activity studies, 6 *Bacillus* spp. isolates had antibacterial activity against at least two or more *Fusarium* strains. The zones of inhibition ranged between 1.5±0.000 -7.6±0.000 cm.

Key words: *Bacillus, Fusarium*, sugar beet

Effect of AM fungus *Glomus mosseae* on the growth and physiology of *Erythrina variegata* Linn., grown in different kinds of soil

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Arbuscular mycorrhizal (AM) fungi occur almost in all kinds of soil. The effect of AM fungal association to crops and forest tree seedlings grown in sandy loam soil has been well documented, but association of AM fungi to arid zone plants has received little attention. The present study investigated the effects of AM fungus, *Glomus mosseae* on the growth and physiology state of *Erythrina variegata* Linn., grown in four different soils *viz*., Sandy loam soil, Organic manure enriched soil, Red soil and Sandy soil in a completely randomized design. After 90 days of growth under nursery conditions plants were harvested. Microbiological parameters (percentage of mycorrhizal infection); growth parameters (root & shoot dry weight and leaf area) and physiological parameters (chlorophyll content, carotenoids, protein, soluble starch and sugar in leaves) were determined. AM fungal plants had higher plant biomass, higher chlorophyll content (chlorophyll a and b), carotenoids, protein content and significantly declined soluble sugar and starch in leaves than in non-AM fungal plants.AM inoculation enhanced the growth and physiology of *Erythrina variegata* tree seedlings grown in all kinds of soil used except sandy soil.. In sandy soil, even though colonization was observed, the AM fungus did not show much growth and nutrient uptake when compared with the other types of soils. This might be due to the poor status and poor nutritional soil structure. The influence of AM fungus on the plants growth was more in organic manure enriched soil than in sandy loam soil, red soil and sandy soil.

Keywords: Arbuscular mycorrhizal fungi, Plant physiology, *Erythrina variegata*, *Glomus mosseae,* Kinds of soil.

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Effect of biofumigation and repeated biosolarization on soil fungal communities in pepper crops

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Sweet pepper (*Capsicum anuum* L.) is a high-value crop grown as as monoculture in greenhouses on which hundred of farmers depend in Southeast Spain. Soil disinfestation is a standard practice to control soil-borne diseases an to prevent soil fatigue effect that occurs when soil disinfestation is not carried out between two consecutive crops in the same soil. This effect seems to be associated with a build-up of soil fungal populations at the end oft he crop cycle.

A broad spectrum of non-chemical alternatives to methyl bromide (MB) has been deeply studied since its banning in 2005. In this work, the effect of manure amendments alone (biofumigation, B), in combination with solarization (biosolarization, BS) and the reiteration of BS with a gradual decrease in the amount of applied manure were compared with MB.

The extent of disinfestation was measured from the density of soil fungi isolated from the soil before and after the respective treatments. *Aspergillus* spp. and *Fusarium* spp, followed by *Penicillium* spp. and *Rhizopus* spp., were systematically present in the soils and were clearly reduced after soil fumigation. B and BS reduced fungal density, tot he same extent as MB, although no treatment eliminated them. Moreover, the progressive reduction of manure applied did not lead to a lower efficacy than MB. Therefore, the use of B or BS had a fumigant effect on soil fungal communities of pepper crops comparable to MB.

Keywords soil fungal communities, manure amendments, soil fatigue effect

Effect of drought on Alfalfa-rhizobia symbiosis

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As a major contributor to the reduced nitrogen pool in the biosphere, symbiotic nitrogen fixation by legumes plays a critical role in a sustainable production system. However, this legume contribution is actually influenced by many environmental constraints as water deficit and salinity. Indeed, water deficit constitutes one of the significant problems facing agricultural production in many areas of the world and limits legume productivity. The present study aims to evaluate the symbiotic combinations behaviors of two Moroccan alfalfa (*Medicago sativa* L.) populations and five rhizobial strains, isolated form five Moroccan regions, under drought conditions. Seed of two alfalfa populations were allowed to germinate in pots filled with sterile sand (previously rinsed with distilled water) and sterile peat at 1 and 1/10 ratio respectively. After the emergence of the first true leaves, the number of plants was adjusted to 5 per pot and the plants were inoculated with five rhziobial strains isolated from five Moroccan different areas. One week after inoculation, plants were subjected to two water treatments: optimal irrigation (75% of field capacity, FC) or drought (25% FC). The experiment was conducted in a greenhouse at 32/22 ºC d/n, 50-80% of RH and a photoperiod of 16h:8 (17000 Lux). At 30 days of stress, plants were harvested and assessed for their performances using plant growth and nodulation parameters with focus on some physiological criteria that could be involved in symbiosis tolerance. Results mentioned that the water stress reduced plants and nodules biomasses, nodule number, plants length, leaves relative water content, nodule membrane permeability and stomatal conductance. The behaviors of considered symbiotic combinations were significantly different for all measured parameters.

Keywords: Alfalfa populations; rhizobia; drought; nodule membrane permeability; stomatal conductance.

Effect of inoculation with fungal pellets of *Anthracophyllum discolor* in a biobed contaminated with atrazine

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One source of contamination is the use of pesticides in agriculture, especially when filling spraying equipment, a typical point source of contamination. Several strategies have been suggested that the use of biobeds provide a matrix to absorb and facilitate pesticide biodegradation.

Much attention has been directed toward the use of white–rot fungi (WRF) for bioaugmentation to improve the biodegradation of contaminants. One of the barriers to successful implementation of fungal bioaugmentation is the development of inexpensive and high quality fungal inoculum.

The effect of fungal pellets on microbial community in biobeds is scarcely known. Therefore, the aim of this study was to evaluate the effect of bioaugmentation with fungal pellets in a biobed contaminated with atrazine (Fig. 1). The fungal pellets of *Anthracophyllum discolor* were formulated with 3 different supports, based on lignocellulosic, oligosaccharides and salt (F1, F2, F3) materials. The biomix of the biobed was prepared by mixing an allophanic top soil (Andisol), commercial peat and wheat straw in a volumetric proportion of 1:1:2, respectively and was inoculated with the fungal pellets (10% w/w). The biobed was contaminated with 60 mg/Kg of atrazine. After 30 days of incubation at 20ºC, different biological parameters were studied (total ligninolytic enzyme activity, fluorescein diacetate activity (FDA) and respiratory activity). The biodegradation of atrazine process was monitored by HPLC. The microbial community was monitored by 16SrRNA; 18 SrRNA; 26SrRNA gene and ITS DGGE techniques.

The biodegradation of atrazine was 99% for F1, F2, F3 supports being slightly higher than the biobed non-inoculated with fungal pellets. After 30 days of incubation, the biological parameters were the highest for the fungal pellet F1 (FDA= 1209 ± 134 µg FDA/g; 3018 ± 178.2 ug CO_2 g^{-1}; 1.63± 0.1 U /kg-1). No significant differences were found between F2 and F3 pellets respect to the biological activities. As well as, the result of DGGE showed an interesting interaction between native microbial and fungal population during the bioaugmentation process.

Figure 1. Bioaugmentation of fungal pellets of *Anthracophyllum discolor* after 10 days of inoculation at 20°C in a biobed contaminated with atrazine.

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Keywords Bioaugmentation; biobed; fungal pellets, *Anthracophyllum discolor*.

Effect of *Nigella sp.* alkaloids on bacterial and fungal phytopathogens

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Currently, the research of the means of fight against phytopathogens turns to the use of bioactive natural substances. Because of the long period of storage, mode of vegetative propagation of the plant, the gravity of the symptoms of plant pathologies such soft rot in the cultures of consumption can be a serious problem which affects agro alimentary industry. Consequently, it is important to minimize the risks of disease occurred in the cultures of consumption by taking care that the plants are free from disease-causing agents by the use of process of fight biological effective. Our work fits accordingly, to test the antimicrobial activity of two species the Nigella seeds secondary metabolites (phenols, alkaloids, flavonoides.) on plant pathogens which infect the fruit and vegetables, like potatoes and tomatoes.

The results of the antibacterial tests showed that among the secondary metabolites tested, only the alkaloids present a strong inhibiting activity on pectinolytic Erwinia species with a minimal concentration of inhibition of 1 to 10µg/ml. Theses alkaloids present too an antifongic effect on different kinds of fungal species: Mucor, Botritus, Fusarium, Penicillium, Trichoderma and Aspergillus.

Keywords: alkaloid, *nigella sativa*, *nigella damascena*, anti-bacterial activity. *Erwinia spp*., Antifongic activity, *Mucor*, *Botritus spp*, *Fusarium spp*, *Penicillium spp*, *Trichoderma spp* and *Aspergillus spp*

Effect of *rpoS* mutation on the survival response of *Erwinia amylovora* under oligotrophic conditions

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Erwinia amylovora, causal agent of fire blight and a quarantine organism in Europe, is a plant pathogenic gram-negative bacterium, affecting economically important rosaceous plants worldwide. Fire blight is one of the most difficult-to-control diseases of pome fruit trees, mainly due to the ability of the pathogen to respond to changes in the environment, thus facilitating its survival in different reservoirs and transmission by various routes. However, the information about stress responses of *E. amylovora* in natural environments outside the host is still scarce. One major limitation of non-host environments is nutrient scarcity, which frequently stimulates a starvation response in bacteria, allowing their subsistence in a culturable state. In these conditions bacterial cells can additionally undergo some kind of oxidative stress. These stresses have been involved in the induction of the viable-but-non-culturable (VBNC) state. Both the starvation response and the VBNC state have been described as *E. amylovora* survival strategies under oligotrophic conditions, and might reflect distinct genetic responses not yet studied in this pathogen. Gram-negative bacteria can respond to nutrient limitation and other environmental stresses by activating the expression of *rpoS*, encoding the alternative sigma factor RpoS. This protein is an important global regulator of genes involved in the cell protection against environmental challenges, such as nutrient limitation. Therefore, the objective of this work was to study the role of the sigma factor RpoS in the starvation and the VBNC responses of the fire blight pathogen in oligotrophic conditions. To this aim, a mutant in the *rpoS* gene was obtained from *E. amylovora* strain CFBP-1430 by marker-exchange. Additionally, a complemented mutant carrying a broad-host range plasmid with a functional copy of the *rpoS* gene was obtained. Thereafter, natural water microcosms were separately inoculated with the wild type strain, the *rpoS* mutant and the complemented strain at a final density of 10^7 cfu/ml. Bacterial survival was monitorized by determining total, viable and culturable cell counts along the time. Total and viable cell counts were recorded by flow cytometry, after cells staining with the Baclight viability kit (Invitrogen). Culturable cell counts where done on KB agar plates. Additional studies where carried out to characterize the *rpoS* mutant. The results showed that the *rpoS* mutant was able to grow in KB plates, but exhibited a more pronounced reduction in culturability than the wild type strain when exposed to oligotrophy in natural water. This can be related to the increased hydrogen peroxide sensitivity of the mutant compared to the wild type strain. These results indicate that *rpoS* gene helps *E. amylovora* to maintain culturability during starvation, showing the importance of this gene for the adaptation of fire blight pathogen to oligotrophy.

Keywords phytopathogenic bacterium*;* fire blight; oligotrophic water; starvation-survival response; viable but non culturable state; *rpoS*

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Effects of epigeic earthworms on the structure and activity of microbial communities during the first stages of decomposition

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Epigeic earthworms are known to play a critical role in the decomposition of organic matter, thus significantly accelerating decomposition rates and nutrient turnover. Vermicomposting is an example of an enhanced decomposition process, in which detritivore earthworms interact intensively with microorganisms and other fauna within the decomposer community, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties. The vermicomposting process includes two different phases regarding earthworm activity: (i) an active phase during which earthworms process the organic substrate, thereby modifying its physical state and microbial composition, and (ii) a maturation phase marked by the displacement of the earthworms towards fresher layers of undigested substrate, during which the microorganisms take over the decomposition of the earthworm-processed substrate. The length of the maturation phase is not fixed, and depends on the efficiency with which the active phase of the process takes place, which in turn is largely determined by the composition of the parent material and the earthworm species. The aim of the present study was therefore to evaluate the short-term effects of three different earthworm species (*Eisenia andrei*, *Eisenia fetida* and *Perionyx excavatus*) on the microbial community structure (phospholipid fatty acid profiles) and microbial activity (basal respiration and microbial growth rates) of three types of animal manure (cow, horse and rabbit manure) which differed in microbial composition.

For this, we carried out a mesocosm experiment with each combination of manure and earthworm species. A control treatment that consisted of each manure without earthworms was also included. Each treatment was replicated three times. All the mesocosms were stored in random positions in an incubation chamber at 20 ºC and 90% relative humidity. The high rate of consumption, digestion and assimilation of organic matter by these earthworm species resulted in the substrates being completely processed by the earthworms in one month. After this time, the earthworms were removed from the mesocosms and the processed material was collected for analysis. The same amount of sample was also collected from the control mesocosms.

Overall, bacterial and fungal populations assessed by PLFA biomarkers decreased in the three types of animal manure with earthworm presence, although in cow manure such a decrease was only detected in the presence of *E. andrei*. The earthworm activity also greatly decreased the bacterial growth rate, estimated by the incorporation of ^{3}H-leucine into proteins, after the active phase of vermicomposting. As occurred with microbial biomass, the presence of *E. andrei* greatly reduced the bacterial growth rate in mesocosms with cow and horse manure, while no such decrease was found for *E. fetida* and *P. excavatus*. Microbial activity in both manures followed the same pattern as the bacterial growth rate. These findings highlight the potential of *E. andrei* for biodegrading organic substrates. The species *E. andrei* and *E. fetida* are closely related, although *E. andrei* predominates in mixed cultures, especially when there is no substrate limitation, as occurred in this experiment, indicating that it is a more extreme r strategist than *E. fetida*, as shown by more rapid growth and reproduction.

Despite the consistent effects on bacterial growth, earthworm activity did not affect the fungal growth rate, irrespective of the type of manure. These contrasting effects on bacterial and fungal populations with earthworm activity are thus expected to have important implications on decomposition pathways, because there exist important differences between both microbial decomposers related to resources requirements and exploitation. In addition, there were no differences between earthworm-worked samples derived from the different types of manure after they have been processed by each earthworm species with regards to microbial community structure. These results point to epigeic earthworms acting as major shapers of microbial communities, as well as important drivers of organic matter decomposition and nutrient cycling, by modifying microbial composition and altering the levels of activity of microbial populations in the short-term, and in turn the rates of decomposition, as shown by the greater loss of carbon with earthworm presence.

Keywords microbe-earthworm interactions; PLFA profiles; microbial growth rates; decomposition rate

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Effects of PAHs and PAH-degrading *Mycobacterium gilvum* VM552 on production and motility pattern of fungal zoospores

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With the aim of developing new co-inoculation strategies, the rhizosphere fungus *Pythium aphanidermatum* was used as a model zoospore producer to test the influence of PAH bioremediation scenarios on the quantity and quality of zoospores. A mineral medium was optimized for the production of zoospores. Production and motility of zoospore was also determined in PAH-saturated media (phenanthrene, anthracene, pyrene, naphthalene and fluoranthene) and in the presence of the PAH-degrading bacterium *Mycobacterium gilvum* VM552. The results evidenced that an optimal medium for zoospore production was a mineral buffered solution (pH 6.5) containing 1 mM sodium phosphates, as well as other inorganic salts. This medium allowed the production of $5.33(\pm 0.53)\times 10^{4}$ zoospores mL^{-1}. Zoospore production decreased in PAH-saturated media, being phenanthrene as the most inhibitory chemical (90% decrease). Suspensions of *M. gilvum* VM552 cells showed an effect to zoospore production only at high cell densities (approximately 10^{8} cells mL^{-1}). The morphology and motility pattern of zoospore was unaffected in all experimental conditions. These results provide the first insights to understand the fate of fungal zoospores in PAH-polluted environments.

Keywords fungal zoospore, bioremediation, bioavailability, polycyclic aromatic hydrocarbon, *Mycobacterium gilvum*

Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on soil microbial diversity and ammonia-oxidizing bacteria after bovine effluent application in experimental microcosms

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The application of animal manure to soil can result in increased gaseous emissions such as NH_3, N_2O, CO_2 and CH_4 as well as nitrate leaching, contributing to climate warming and ground and surface water pollution.

Nitrification inhibitors are chemical compounds that delay microbial oxidation of ammonium to nitrate in soil for a certain period of time by depressing the activity of ammonia-oxidizing microorganisms. As a result, oxidation of ammonia to nitrate can be delayed, reducing N losses and increasing fertilizer use efficiency.

In this study, we assess the effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on microbial community-level physiological profiling (CLPP) and on the abundance of ammonia-oxidizing bacteria (AOB) using RT-qPCR in soil microcosms amended with bovine effluent. DMPP reduced the nitrification rate starting from the second week of incubation until at least the fourth week. DMPP treatments induced rapid changes in microbial heterotrophic metabolism showing significant differences after 24h of incubation, as well as in the size of ammonia-oxidizing bacterial communities, which was affected by DMPP thoughout the entire incubation period.

Keywords: Nitrification inhibitor, DMPP, bovine effluent, nitrogen use efficiency.

Enhancing survival and subsequent infectivity of conidia of entomopathogenic fungus using UV protectants

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Ultraviolet radiation (UV) can reduce the effectiveness of fungi used for biological control; Exposure to simulated solar radiation for a few hours can completely inactivate the conidia of the fungus. Here, the effect of exposure to an UV-B radiation dose of 30μw cm^{-2} on the conidial culturability and germination of selected strains of the entomopathogenic *Metarhizium anisopliae* and *Beauveria bassiana* incubated in the sabouraud dextrose agar (SDA) was investigated. The addition of TiO_2 and SiO_2 nanoparticles (NPs) (0.25 mL of 0.2% w/v aqueous solution), as well as the traditional UV protectants (0.25 mL) such as yeast extract, SM and some vegetable oils in culture medium was compared to see their performance of the culturability and germination of the spore suspensions (0.25 mL). The radiation time was ranged from 1 min to 2 h with the comparison of the control. The germination rate was measured at 24 h and 48 h. The results indicated that TiO_2 NPs exhibit the best performance, SiO_2 NPs the next, vegetable oil the third. After radiation for 24 h, the germination rate was increased from 10% to 60% with the addition of TiO_2 NPs. While that for 48 h, the germination rate was increased from 15% to 70%. The enhancing survival property decreased in the order: TiO_2 > SiO_2> Oil > NaCl > ZnO > benzophenone > sodium salicylate> methyl cinnamate.

Keywords UV-B radiation; UV protectants; Entomopathogenic fungus bioinsecticides; *Metarhizium anisopliae*; *Beauveria bassiana*; Morphology

Epiphytic survival and temporal dissemination of *Brenneria quercina* in a plot of *Quercus ilex* in Spain

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Brenneria quercina is a phytopathogenic bacterium responsible of bark cankers with exudates on the trunk and branches in different *Quercus* species, which may limit their development and can even cause death. In Spain, this pathogen has been identified in different regions, including the region of Valencia, and in some forests of great environmental value, being considered as one of the causes of oak decline syndrome. However, the biological cycle of *B. quercina* is still unknown, as well as its possible temporal dissemination and/or the damage of trees infected by this pathogen. To try to provide information on these aspects, we selected a plot of one hundred adult trees of *Q. ilex* in the province of Castellón (region of Valencia), in which *B. quercina* had been detected. In order to study the epiphytic survival of this pathogen in 2006 four samplings were done in five healthy and five affected randomly selected oaks. In these trees, the average leaf area per branch from randomly taken samples was also determined. To study the temporal dissemination of the pathogen in the plot and/or impaired growth of the symptomatic oaks with respect to the healthy ones, surveys and measurements of trunk perimeter of one hundred oaks, was conducted comparing the results with those of a first evaluation in 2000. The results have demonstrated, for the first time, the epiphytic presence of *B. quercina* on leaves and twigs of Mediterranean oaks in both the affected and the apparently healthy trees. However, the average leaf area per branch was significantly lower in affected than in healthy oaks. Similarly, it was observed a significantly lower growth in affected trees than in healthy oaks over 6 years. Regarding the temporal dissemination of the pathogen in the plot, the results have shown a significantly greater number of affected trees in 2006 than in 2000. In conclusion, the spread of *B. quercina* could result not only from infected tissues and/or exudates, but also through its epiphytic populations. Moreover, it has also been observed direct damages of trees as a result of infection by this pathogen and a significant increase in the number of diseased oaks in the plot analysed.

Keywords phytopathogenic bacterium*;* bacterial canker, oak decline; *Q. ilex;* forests; epiphytic populations;

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Expression of an exogenous ACC deaminase gene in chickpea mesorhizobia promotes plant growth under salinity

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Salinity is one of the most severe abiotic factors limiting crop yield and plant productivity. It is known that salinity induces an increase in ethylene levels in plant tissues that can cause plant death. The enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase degrades ACC, the immediate precursor of plant hormone ethylene, into ammonia and α-ketobutyrate, thereby lowering the levels of this hormone in a developing or stressed plant, and thus relieving the plant of the ethylene effects. It was proposed that ACC deaminase, present in soil bacteria, could enhance plant growth by reducing the levels of ethylene in plants, especially when plants are facing stressful conditions, such as salinity.

Our main goal was to study the symbiotic performance, in chickpea plants under salinity, of two *Mesorhizobium ciceri* strains transformed with an exogenous ACC deaminase gene.

The *M. ciceri* EE-7-ENMP and *M. ciceri* G-55-Guarda strains, previously characterized as salt-sensitive and tolerant, respectively, were transformed with the exogenous ACC deaminase gene from *Pseudomonas putida* UW4 by triparental conjugation using the plasmid pRKACC. A plant growth assay was conducted in a growth chamber using chickpea plants inoculated with the pRKACC transformed or the wild type strains, under control and salt-stress conditions. After eight weeks, the plants were harvested and several parameters were measured.

As expected, the chickpea plants were severely affected by salinity. Interestingly, both transformed strains showed an improvement of chickpea plants growth compared to the corresponding wild type strains, under salinity. However, salt-tolerant wild type strain promoted a slight increase on plant growth compared to the salt-sensitive one. Under salinity, all strains showed a reduced ability to nodulate chickpea, except the transformed salt-sensitive strain, that increased significantly the nodule number compared to control conditions.

These results show that salinity affects chickpea plants growth, nevertheless its inoculation with *Mesorhizobium* strains transformed with an exogenous ACC deaminase gene can improve plant tolerance to salt stress. The results also suggest that a salt sensitive *Mesorhizobium* strain can increase its symbiotic performance in a higher extent by expressing an exogenous ACC deaminase gene. To our knowledge, this is the first study on the symbiotic performance of mesorhizobia transformed with an exogenous ACC deaminase gene, in chickpea plants submitted to salinity. These results suggest that ACC deaminase can play an important role in plant-rhizobium interaction under this particular stressful condition, a finding relevant for applications in sustainable agriculture.

Keywords rhizobia; chickpea; ACC deaminase; salinity

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Function of a peptide pheromone from *Streptococcus bovis* in the goat rumen

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Backgrand

In ruminants, *Streptococcus bovis* often predominates when ruminants are fed a diet containing a large amount of readily fermentable carbohydrate, such as starch. Feeding high-carbohydrate diets generally leads to an increase in lactate production in the rumen, which causes a drop in ruminal pH. Since *S. bovis* is relatively acid-tolerant among ruminal bacteria, the proportion of *S. bovis* in ruminal microbiota often increases when ruminal pH is low. In addition, *S. bovis* produces higher percentages of lactate when culture pH is low, thus suggesting that *S. bovis* contributes to the progress of rumen acidosis. In addition, S. bovis may contribute to bloat, since this bacterium produces capsular polysaccharide. Therefore, it is desirable to suppress the overgrowth of *S. bovis*, or the overproduction of lactate by *S. bovis*. It has been proposed that peptide pheromone-signaling system is a bacterial intercellular communication mechanism for controlling gene expression in response to environmental stress or population density. Previously, we sequenced the genes of the two components of the peptide pheromone-signaling system, ComD and ComE, and its signal peptide ComC (AB284382). In order to control the growth of *S. bovis* via the ComC peptide pheromone signaling system, we examined the function of this system in the mixed microbes from the Japanese goat.

Resalts

When *S. bovis* was grown in the presence of mature ComC peptide, the growth rate and transformation frequency increased in the dose response manner. The synthetic full length peptide had no effect. Deletion of the ComC gene resulted in decreased growth rate and decreased transformation frequency. These results indicate that *S. bovis* mature ComC peptide might stimulate the growth and induce genetically competent cells, and post-transcriptional processing of ComC is necessary to change the active form of this pheromone. When synthetic mature ComC peptide was added to the cultures of mixed ruminal microbes from a goat, the number of *S. bovis* per total bacterial counts estimated from the 16SrRNA (cDNA) contents increased. This result shows that ComC functions in the cultures of mixed ruminal microbes, which suggests the possibility that ComC functions in the ruminal ecosystem. Addition of ComC resulted increase in butyrate production without lactate accumulation. The cDNA level of *Megasphaera elsdenii* was increased significantly by the addition of mature ComC peptide, although *Selenomonas ruminantium* and *Ruminococcus albus* were unaffected. The increase in butyrate production may reflect a conversion from lactate to butyrate by butyrate-producing bacteria such as *M. elsdenii*. Amylase activity in the mixed culture was increased by the addition of mature ComC peptide. CMCase activity was unaffected. However, tannase activity was slightly decreased, as well as xylanase activity. This result indicates that *S. bovis* ComC does not function as a signal peptide for *Streptococcus gallolyticus*. The mature ComC peptide was purified from the Japanese goat rumen, which were fed the high grain diet, and analyzed by MALDI-TOF mass. This result indicates that *S. bovis* produces and uses mature ComC peptide in the rumen. The level of *S. bovis comC*-mRNA in the rumen was increased when the diet was changed to the high grain diet from the basal diet. This result was consistent with the increase in *S. bovis* populations, which confirms the possibility that ComC functions in the ruminal ecosystem.

Acknowledgments
This study was supported in part by a Grant-in-Aid for Scientific Research (No. 23580377) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT).

Keywords peptide pheromone; rumen bacteria; ruminal acidosis; *Streptococcus bovis*; two-component signal transduction system

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Fungicidal Effect of Polymers Nu Film 17 and Nu Film P

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Due to the importance of having negative consequences, application of chemicals is reduced as much as possible, whereas biological control is becoming increasingly important. Fungicides are used for control of plant diseases caused by fungi. Addition of polymers during the application improves the efficiency of fungicides. Nu Film 17 and Nu Film P are emulsifiable concentrate formulations of a specific ß-pinene polymer, designed for use as a spray tank additive, to be applied in combination with insecticide and fungicide pest control programs. They are used in pest control programmes when the spray applications are made to crop foliage, during the normal growing and spraying season. Nu Film 17 and Nu Film P are film-forming polymers, which encapsulate the pesticides and biopesticides and provide protection from various weathering factors, including rainfall and wind erosion. They shield the pesticide and biopesticide spray residue from heat and ultra-violet radiation, which often may cause pesticide and biopesticide degradation. Pesticide volatilization is minimized by the protective film. Loss of pesticide deposit by the abrasive activity of leaves rubbing together is also prevented. Nu Film 17 and Nu Film P slowly releases the pesticide at a predictable rate, thus maintaining biological control over a longer than normal period of time. Because of this, it has been found that the use of Nu Film 17 and Nu Film P reduces the quantity of pesticides needed in spray applications. It improves initial depositing of the pesticide so that lower rates of pesticide can be used. It increases the normal life of most pesticides by 50-100 %, again reducing the quantity of pesticide used by increasing the interval between sprays. High costs of aircraft operation make the increase of the interval between sprays especially advantageous for aerially applied pesticides. Nu Film 17 is used at the rate of 1-1 l.5 L/ha, and Nu Film P is used at the rate of 0.3-1 L/ha.

The experiments were carried out in the nursery „Rogot“ on the oak seddlins Quercus robur L., aged 6 yeasrs. The paper tested the possibility of using all the positive properties of polymers in the course of experiment. Polymers Nu Film 17 and Nu Film P, that create leaf protective cover (film) and reduce infection by powdery mildew, were used in process of treating sample plots. The trial were set according to the instruction of methods PP 1/152 (2) AND pp 1/69 (2). The treatment plan was made set according to fully randomised block design.The experiment was conducted in four repetitions. The basic plot had in area of 25 m2 and consisted 8 trees (1x3m apart). Phytotoxicity was estimated by PP methods 1/135 (2), the intensity of infection according to Towsend-Hauberger, the efficiency by Abbott, the analysis of variance with Duncan test and PP/181 (2).

The data on efficacy of polymers Nu Film 17 and Nu Film P on powdery mildew infestations on the oak leaves are presents in Table 1. Based on the variance analysis of the randomized block design, it was determined that the difference between the mid repetitions was statistically significant at the probability of 95%, since $F_0 > F_{0.05}$. Moreover, a statistically significant difference was found between mid treatments at the probability of 99%, since $F_0 > F_{0.01}$. Statistically significant difference between mid treatments of variant with Sumpor and Sumpor with polymers were random. Difference between the mean values of AQ10 (0.3 g)+Nu Film 17 (1l) and AQ10 (0.3 g)+Nu Film 17 (1,5l) i AQ10 (0.7 g)+Nu Film 17 (0.3l) show that there is a statistically significant difference in the probability 95%. Difference between the mean values of AQ10 (0.5g) and AQ10 (0.5 g)+Nu Film 17 (1l), AQ10 (0.7g)+Nu Film 17 (1,5l), AQ10 (0.5 g)+Nu Film 17 (0.3l) i AQ10 (0.7 g)+Nu Film 17 (1l) there is also a significant difference in the probability of 95%. The same statistically significant difference in verovatnosci 95% exists for variant control + Nu Film P (0.3 g), as well as variants of AQ10 (0.7g) and AQ10 (0.3 g) + Nu Film P (0.3l), Control + Nu Film 17 (1l) and Control + Nu Film P (1l). There are statistically significant difference between mid treatments of other variances and all other treatments, at the probability of 99%. By means of a multiple comparison procedure (Duncan test, 1955), ten homogenous groups were identified with statistically significant differences at 99%, which match the previously explained groups studied in the variance analysis. Nu Film 17 and P are film-forming polymers, which encapsulate the pesticides Sufur SC and biopesticide AQ-10, and provide protection from various weathering factors, including rainfall and wind erosion. They increase the normal life of pesticides and biopesticides by 50-100 %. No adverse effects of sulphur SC, biofungicide AQ-10 and polymers Nu Film 17 and Nu Film P on the treated plants and other organisms were noted, either in the nursery or on the locality.

Keywords: polymers, fungicidal efficacy, diseases

Genista numidica and their endosymbionts diversity

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The nitrogen-fixing leguminous plants are key components of the natural succession in semi-arid Mediterranean ecosystems and constitute a fundamental source of N input to the ecosystem. The ability of legumes to establish a symbiosis with soil bacteria, traditionally referred to as rhizobia, allows the former to benefit from the fixed atmospheric nitrogen in their biological form" nodule" and grow more efficiently on nutrient-limited soils.

The majority of rhizobia from wild legumes are diverse. Include 13 genera such as *Rhizobium, Bradyrhizobium* and *Burkholderia,* distributed between *Alphaproteobacteria* and *Betaproteobacteria*.

The *Genisteae* (Adans) Benth legume tribe of *Fabaceae*, includes several Genre: *Retama, Lupinus, Genista,..* They have known for their great ecological significances in Mediterranean countries. The genus *Genista*, consisting of 87species among 23 grow in Algeria. *Genista numidica,* indigenous legume *Genista* specie of mediterranean coastal area, is a spontaneous shrub that has not been examined for its root nodule bacteria diversity. In the same context that our work takes aim: study of *G. numidica* endosymbiosis diversity.

The taxonomic diversity of bacteria from root nodules of *G. numidica* growing in coastal region of Algeria was investigated following phenotypic and genotypic characteristics.

The nodules harvested from *Genista numidica* root grown in Algerian soil show a great diversity. Some of them present a determinate form and other an indeterminate form. The total of 60 bacterial strains isolated from these nodules was characterized using phenotypic characteristics.

G. numidica endosymbiosis are Gram-negative; rod shaped microorganisms, mobile, usually containing granules of poly b-hydroxybutyrate.

In Yeast Extract Mannitol agar medium with bromothymol blue indicator (0.0025%, w/v) some *Genista numidica* microsymbionts were acid producers other synthesized alkaline products. And with addition of different carbon and nitrogen source, *G. numidica* isolates show diversity in their assimilation of this different source.

Also, it was found that bacteria isolated from nodules of *G. numidica* show an important diversity between rapid and slow growth, salinity and pH tolerance. The isolate grew at temperatures from 26° to 37°C with an optimum growth in 28°C. All of the isolates were able to grow at pH around 6 and 8 in buffered Yeast Mannitol Broth. However some strains grew at pH 9 and 10. Most of the tested strains grew in medium containing 100 and 200 mM NaCl, Some strains were able to grow at the presence of 400 and 500 mM NaCl. Their doubling growth time was a mixture of fast, intermediate and slow-growers respectively 2, 4, 6 and 10 hours.

Thus, the combination of stress factors such as pH and salinity as they are imposed in nature allows us to select interesting *Genista numidica* microsymbiosis to fertilise arid and saline soil of Mediterranean zone.

Therefore genotypic characterization by PCR- RAPD gene sequencing is an important step to complete the identification of the isolated strains.

Keys words: *G. numidica,* Rhizobia, diversity, Phenotypic, Genotypic, PCR- RAPD.

Identification of bacteria of the genus *Azospirillum* isolated from the rhizosphere of durum wheat (*Triticum durum*)

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Several studies have shown that the PGPR (Plant Growth Rhizobacteria)promote growth and have many beneficial effects, these rhizobacteria, including several genera *Azospirillum, Bacillus*, *Rhizobium* and *Psodomonas* may provide nitrogen as ammonium to plant, legumes and some grasses (Ivan R et al.,2006, Sonia E et *al*.,2007).These bacteria have many beneficial effects on host plant such as biological fixation of nitrogen, lute against pathogens reduction of diseases, growth hormones synthesis and other effects (B.E.Baca and *al*.,2007).That's why we decide to study the characterization of theses rhizobacteria specially the genus *Azospirillum*.

First of all, bacterial strains were isolated from the rhizosphere of durum wheat (*Triticum Durum*) grown in fields in several regions of eastern Algeria. These strains were identified as *Azospirillum* phenotypic characterization based on morphological characters such as the shape the physiology (temperature and pH), followed by determination of the effect of PGPR strains (production of IAA and ammonium, solubilization of phosphate). We noticed that the bacterial strains have an optimal growth in a pH of 6.8 and a wide temperature range of 28 to 37C°. These 10 strains of the genus *Azospirillum* use mannitol and fructose, except Azo4 and Azo7 witch cannot use sucrose for the first and lactose for the second. All strains have optimal growth at concentrations equal to 200mM, we selected four strains that have been very tolerant of NaCl even at very high concentrations (700mM).Consequently, rhizobacteria have the capacity to produce IAA at concentrations that differ from one strain to another; and can also produce ammonium. Therefore, the bacteria of genus *Azospirillum* were PGPR effects that will be used to improve the growth of plants such as wheat.

Keywords: *Azospirillum,* Characterization.

Identification of genes involved in toxicity of the Mexican bacteria *Serratia entomophila* Mor4.1 pathogenic against root damaging larvae of *Phyllophaga blanchardi*

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The soil bacteria *S. entomophila* strain Mor4.1 (*SeMor4.1*; *Enterobacteriaceae*) is pathogenic to larvae of several species of the *Phyllophaga* genus (Coleoptera:Scarabaeidae) inducing reduction in food consumption and mortality after oral ingestion. The larvae of *Phyllophaga* are responsible of severe damage to important crops world wide, including Gramineae, vegetables and ornamentals.

Experiments done by injecting either, the bacteria or the cell free culture broths, exhibit insecticidal activity against larvae of *Phyllophaga, Anomala* sp and larvae of the lepidopteran *Manduca sexta* (Nuñez-Valdez et al., 2008 Appl. Environ. Microbiol. 74:802-10). It is suggested that *Mor4.1* produces and secretes virulence factors with a wide spectrum of action, with toxic activities at the level of the insect gut and also at the level of the hemocoel. To identify the virulence factors, a fosmid *SeMor4.1*genome library was prepared in *Escherichia coli* and five insecticidal clones were selected by injection bio-assays.

The analysis of the DNA sequence of the clones named C8 and G8 is presented. Most ORFs showed high homologies to *Serratia proteamaculans* 568 genes. The GC content represents a mosaic-like structure, going from 51% to 68% and 43% to 64% respectively. ORFs coding for putative virulence factors as the hydroxamate-dependent iron uptake operon and the LPS biosynthesis core were identified. Analysis of sub-clones, Tn5 insertion and deletion mutants indicated that a putative ATP-dependent RNA helicase HrpB, a putative Penicillin-binding protein 1b, a dUTPase, a flavoprotein and a heptosyltransferase III are associated with insecticidal activity. The presence of virulence and tRNA genes and also the GC content suggest that C8 and G8 DNA might be part of pathogenicity islands on the genome of *SeMor4.1*. The characterization of the virulence factors of *SeMor4.1* will be the basis for future biotechnological application of pathogenic bacteria for the management of insect pests.

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Identification of *Rhizobium leguminosarum* bv. *viciae* genes expressed under control of symbiotic genes of pea (*Pisum sativum* L.)

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The development of legume-rhizobial symbiosis is determined by complex mechanisms of genetic integration of symbiotic partners. Identification of specialised genes of micro- and macrosymbionts will provide the basis to reveal the molecular mechanisms of plant-microbial interactions. In spite of intensive investigation of nitrogen fixing symbiosis, little is known about differentiation of nodule bacteria into symbiotic form, bacteroids, a key stage of symbiosis that permit further development and persistence of symbiotic compartments, e.g. symbiosomes, and symbiotic organs, the nodules. Investigation of the main regulatory bacterial genes, probably controlling genetic interactions with the host genes at the stage of bacteroid differentiation, will provide the basis for understanding mechanisms of genetic integration between symbionts at late stages of nitrogen-fixing nodule development. A series of such genes have been identified in the course of cooperation between EEZ-CSIC and ARRIAM. Using methods of differential gene expression analysis, functional genomics and molecular genetics of bacteria in symbiosis with well characterized pea mutants impaired for development of symbiotic organelles, the *Rhizobium leguminosarum* bv. *viceae* genes *pRL90308, RL3274, RL1870* and *RL1877* were characterized and proposed to be involved in regulation of symbiotic structures development and interaction with pea at the late stages of symbiosis. The detail procedure and results will be reviewed and discussed.

Along with intensive investigation of model legume symbiotic systems, it is important to study beneficial interactions of agriculturally important crops with corresponding microsymbionts, particularly the symbiotic system of pea (*Pisum sativum* L.) - *R. leguminosarum* bv. *viceae*, in order to apply this knowledge to modern sustainable agriculture.

Keywords legume-rhizobial symbiosis; *Rhizobium leguminosarum* bv. *viceae;* differential gene expression; molecular genetics; sustainable agriculture.

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In vivo versus *in vitro* mycorrhizal inocula: root colonization and plant growth stimulation potential

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The presence of the arbuscular mycorrhizal (AM) symbiosis in natural and agroecosystems has been well studied in the last decades and the role of the fungal partner at increasing crop yields and plant health has stimulated the search for mass production methods. The obligate biotrophic condition of arbuscular fungi requires to produce them only on a plant carrier, either *in vivo*, in symbiosis with a host plant, or *in vitro*, in a root organ culture (ROC). Both types of inoculum can be found under commercial trades including a variety of fungi, formulations and components, making it difficult to elucidate differences in performance among them.

This work presents a comparative evaluation of *in vivo* and *in vitro* AM inocula, obtained after cultivating the isolate of *Glomus intraradices* Shenck and Smith (BEG 72) both in a plant culture association, and in axenic culture on RiT-DNA transformed carrot roots. In order to evaluate the influence of the production method on root colonization and on plant growth performance, several mycorrhizal type propagules from soil inoculum and from *in vitro* plates were used as inoculum source in leek plantlets grown in 100 ml individual containers filled with sterilized sandy soil. Full inoculum, excised mycorrhizal root fragments, individual spores, three inoculum sieving fractions (125 to 250 µm; 50 to 125 µm; 20 to 50 µm) and mycelium were evaluated.

Leeks were grown in the greenhouse for eight weeks and watered regularly. At the end of the experiment (repeated twice concerning the evaluation of mycorrhizal root colonization) plants were cut and processed to determine fungal infection and plant growth parameters. Results showed that among the inoculation treatments, only mycelium and the 20 to 50 µm fraction were not infective, regardless of the production method. All the other inoculum sources produced *in vivo* achieved a higher mycorrhizal colonization in host plant roots than *in vitro* inoculum, irrespective of the source of propagules used, and could induce a significant stimulation on plant growth. When propagule sources of *in vitro* inoculum were compared, it was observed that the full inoculum treatment was much more effective than all the other treatments based on propagule types, a result that was not recorded for *in vivo* inocula.

Keywords: symbiosis, *Glomus intraradices*, AM inoculum production.

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Interaction of biological control agent *Serratia plymuthica* A30 with blackleg causing biovar 3 *Dickeya* spp. in vitro and in planta

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In Europe pectinolytic bacteria belonging to *Dickeya* spp. cause increasing losses in (seed) potato production. This is related to presence of a new, unclassified genetic clade of biovar 3 *Dickeya* spp. provisionally named *D. solani.* Effective strategies to control *Dickeya* spp. have not been developed yet. We have characterized a biological control agent *Serratia plymuthica* strain A30, an endophyte isolated from rotten potato tuber tissue and active against *D. solani*. This antagonism requires direct contact between the control agent and the pathogen and is most likely based on antibiosis. In a potato slice assay, strain A30 eliminated the pathogen and prevented potato tissue maceration by *D. solani* when inoculated in densities at least 100 times higher than the pathogen. To study the interaction between S. plymuthica A30 and *D. solani* in planta, fluorescent protein tagged strains (marked with GFP and DsRed) were exploited. In repeated greenhouse experiments, a tuber treatment with strain A30 protected potato plants against *D. solani* effectively, resulting in a decrease in the incidence of stem infection of, on average, 97%. Using confocal laser scanning microscopy, the antagonist could be traced in vascular and parenchymatic tissue of tubers, roots and stems at least till 28 days after planting. Results indicated that *S. plymuthica* A30 outcompeted *D. solani* in planta. We used random transposon mutagenesis and genome analysis to characterize potential genes of *S. plymuthica* A30 involved in biocontrol.

Interactions of MRSA ST398 and inhibitor producing *S. aureus* strains *in vitro* and *in vivo*

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In view of the increasing antimicrobial resistance amongst animal and human pathogens, bacterial antagonism may become an important alternative in the control of MRSA, such as the pig-adapted MRSA CC398. The aims of this study were to: (1) identify bacterial antagonists of MRSA CC398 *in vitro* using a well-diffusion assay and, (2) characterize the effectiveness of these antagonists in the control of MRSA CC398 *in vivo* using a gnotobiotic piglet model. Thirteen out of thirty tested bacterial strains were able to successfully antagonize MRSA CC398 isolates *in vitro*. Of these, *S. aureus* strains 502A (MSSA CC5) and J94 (MSSA CC45) were the most adherent strains to pig corneocytes (P<0.05) in a corneocyte binding assay, and these were selected for the gnotobiotic piglet study. Two-week old piglets in three sterile chambers were initially colonized with MRSA CC398 (isolate PIL69). Two days later, piglets in two chambers were inoculated with antagonist strains (chamber 1: 502A, *n*=5; chamber 2: J94, *n*=4), whilst the third chamber served as control (PIL69 only, *n*=2). Quantitative microbiology was performed periodically over a period of 23 days from nasal mucosae, skin behind ears, and from sacrum, using MRSA selective media (mannitol salt agar with 2 µg/ml oxacillin). Growth of the $\log_{10}$-transformed MRSA CC398 colonies forming unit per swab were compared between animals in the three chambers using a nonlinear regression model. Results indicated that MRSA rate of growth was not inhibited but instead promoted with the presence of antagonists (P < 0.01). Lack of correlation between *in vivo* and *in vitro* results may reflect *in vivo* effects of the host that allow sustained and advantageous colonisation of the CC398 strain in pig skin habitats. Further *in vivo* research is required to study host–microbe and microbe–microbe interactions.

Keywords: MRSA, gnotobiotic piglets, bacterial antagonism

Investigation of microorganisms and enzymes on the composting of tree pruning and sewage sludge

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The composting is an aerobic biological process controlled by micro-organism where the organic solid wastes are biodegradated in a product of nutritional value (humus). This stabilized product is applied in agriculture as organic fertilizer and soil conditioner. Scientific researches are encouraged to reuse the solid wastes due to its increased acumulation with the population growth and the technological development. The sewage sludge and the tree pruning are organic waste generated in large quantities in urban area. The aim of this study was to determine the microbiological content and enzyme activities during the composting of tree pruning and sewage sludge.

The laboratory composting was performed in polyethylene boxes with tree pruning and sewage sludge in the ratio of 1:1, in the presence of slaked lime 50% and an inoculum. This inoculum was obtained from the consortium of indigenous microorganisms of the sewage sludge by biostimulation in Erlenmeyer flasks of 500 mL, at 150 rpm, 25°C for 48 hours. An experimental design of randomized blocks was conducted under three treatments in triplicate. The composting was watery and plowed weekly.

In the field experiments, eight cells were built outdoors, directly in the soil (80 cm of diameter and 60 cm of height) in layers of tree pruning, sewage sludge and cattle manure in the presence or absence of lime. Four treatments in duplicate were performance. The composting was watery and plowed twice a week.

The samples were collected at the central of the cells before the plowing of the wastes during 60 days of composting. Each 10 g sample was suspended in 100 mL of sterile buffered water at pH 7.2 in Erlenmeyer flasks. The sample extractions were performed at 150 rpm for 10 min at 25°C. Microbiological determinations of total coliform, fecal coliform, standard count of bacteria, counts of filamentous fungi and yeasts, *Salmonellas* and helminth eggs were performed according to the Standard Methods for the Examination of Water and Wastewater. The samples were diluted with sterile buffered dilution water. Enzyme activities were determined by agar diffusion method with culture media in Petri dishes in the presence of the substrates: carboxymethyl cellulose (cellulases), gelatin (proteases), gallic acid (phenoloxidases) and tannic acid (tannases). The extracts were inoculated and incubated for 24 – 72 h at 30°C.

Total and fecal coliforms were determined (maximum of 10^9 bacteria/kg) during the composting of pruning trees and sewage sludge. Bacteria, yeasts and filamentous fungi were determined with maximum values of 10^9, 10^7 e 10^8 CFU/kg, respectively. The sanitization of sewage sludge occurred only in the presence of 50% w/w of lime at the composting in the laboratory due to the absence of total and fecal coliforms, *Salmonellas* and helminth eggs.

The activities of cellulases, proteases and phenoloxidases were determined throughout the composting of tree pruning and sewage sludge. The activities of tannases were determined after 15 days of the biodegradation process. The highest percentages of activities were determined between 15 to 40 days of composting. The presence of lime at 50% w/w inhibited all four enzymes investigated while the inoculum and the cattle manure induced the enzyme activities.

Pruning trees and sewage sludge are biodegraded by microorganisms in the composting process and hydrolytic and oxidative enzymes have maximum activities in the active phase of waste degradation.

Keywords: solid waste, tree pruning, sewage sludge, composting, enzyme activities.

Isolation and characterization of plant growth promoting rhizobacteria from rhizosphere soils and their effect on chickpea production in Indo-Gangetic plain of Eastern Uttar Pradesh, India

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ABSTRACT: Soils samples were collected from rhizosphere soils of different crops (chickpea, mungbean, redgram, wheat and rice) from 42 villages of different districts of Mirzapur, Varanasi, Azamgarh and Jaunpur of Eastern Uttar Pradesh, India at 2006. 50 bacterial isolates were isolated from rhizosphere soil. Out of 50, we selected only 28 effective isolates on the basis of biochemical and physiological traits generally related to biocontrol and plant growth promotion, such as: indole acetic acid (IAA), ammonia, HCN and siderophore production, as well as their capacity to solubilize phosphates, inhibit the growth of soil born phytopatogenic fungi *in vitro*. All effective isolates were positive for IAA and ammonia production. Twelve isolates were positive for strong phosphate solubilizer. Ten isolates were positive for HCN production and five for siderophores production. Eleven isolates were positive for growth inhibition of phytopatogenic fungi (*Fusarium oxysporum* f.sp. *ciceri* and *Rhizoctonia solani*). According to these traits, four isolates (*Pseudomonas aeruginosa* BHUPSB01, *Pseudomonas putida* BHUPSB04, *Bacillus megaterium* BHUPSB14, *Paenibacillus polymyxa* BHUPSB17, *Rhizobium leguminosarum* BHURC04 and *Azotobacter chroococcum* MTCC-446) were selected and evaluated individually and in combinations for plant growth promotion on chickpea plants under plant growth chamber as well as field. Treatment combination *R. leguminosarum*+ *A. chroococcum*+ *P. aeruginosa* was found most significant increase in shoot length and root length followed by *R. leguminosarum*+ *A. chroococcum*+ *P. putida* and *R. leguminosarum*+ *A. chroococcum*+ *B. polymyxa* than control and other combinations under control condition after 15 days of inoculation. Similarly *R. leguminosarum*+ *A. chroococcum*+ *P. aeruginosa* was showed maximum significant plant growth and gain yield of chickpea than control and other treatment combinations under two year field experiments (2007-08 and 2008-09). Field experiments were conducted at Agriculture Farm, Banaras Hindu University with three replication and 11 treatments. Hence, treatment combination *R. leguminosarum*+ *A. chroococcum*+ *P. aeruginosa* could be used as effective biofertilizer for chickpea production under Indo-Gangetic plain of Eastern Uttar Pradesh.

Key words: Rhizosphere soil; IAA; plant growth; chickpea.

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Isolation and study of endophytic bacteria from *Sphagnum fallax* and *S. magellanicum* mosses with high biocontrol activity and PGPR-properties

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The aim of this study was to isolate promising bacterial strains associated with *Sphagnum* mosses possessing beneficial for agricultural crops properties as perspective objects for agricultural biotechnology. About 50 samples of Sphagnum mosses were collected during expeditions in Alps (Austria) and Kolskiy peninsula (Leningrad region, Russia). About 250 strains of endophytic bacteria were isolated from green tissues of *Sphagnum* plants by using original methods. Antagonistic properties of isolated strains against a number of phytopathogenic fungi and bacteria have been studied. It was shown that more than 60% of all isolates demonstrated strong antifungal properties (*in vitro*).

The principal part of this work included study of colonization activity of isolated strains and their PGPR-properties. The colonization activity was studied using gnotobiotic systems with radish and wheat plants whereas localization of introduced bacteria on the roots of cultivated plants was demonstrated by methods FISH and CSLM. The analysis of 16S rRNA gene nucleotide sequences of strains with high biocontrol and colonization ability revealed their close homologues among the genera *Pseudomonas, Serratia, Flavobacterum, Burkholderia* and *Collimonas*. The positive influence of promising bacterial strains on the plants was demonstrated in greenhouse experiments.

Keywords Sphagnum mosses, endophytic bacteria, biocontrol, PGPR, gnotobiotic system.

Isolation of *Bacillus thuringiensis* strains with cytotoxic activity against MOLT-4, a leukemia cell line*

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Parasporal protein inclusions from *Bacillus thuringiensis* are widely studied as a biological agent in order to control pest. However, recently it has been attracted attention for other biological activities. One of the most remarkable is anti-cancer activity. The Parasporins are a new category of Cry proteins which are non-hemolytic neither insecticide but they preferentially kill cancer cells. The aim of this study was the detection of Parasporins in 100 *Bacillus thuringiensis* strains isolated from Mexican soil-samples and their evaluation of cytopathic effect (CPE) against leukemia cells MOLT-4. In order to identify putative Parasporin genes, we designed six pairs of primers to amplify the eighteen Parasporin genes in a multiplex system. Our screening revealed that two strains named IB-88 and IB-84, contain the genes to encode potential Parasporins; however the parasporal protein profile on SDS-PAGE not correspond with the previously reported. PCR products were cloned and the nucleotide sequence from IB-88 strain showed a high identity (98%) to Parasporins Class 2, while IB84 is very similar (97%) to Class 4. The CPE of protein extracts was evaluated in 96 well plates after 24h of exposure to MOLT-4 cells. The results showed that IB-84 has a CPE higher than IB-88 with a cell survival rate less than 5 %. The nucleotide sequences and *in vitro* assays results, suggest that both are two novel Parasporins with a high potential as therapeutic agent.

Keywords: *Bacillus thuringiensis*, Parasporin, MOLT-4.

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Ligninase activities and decolorization of Remazol Brilliant Blue and Methyl Orange by a ligninolytic fungus isolated from semi-arid region of North Mexico

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Introduction. Synthetic dyes are widely used, mainly by textile industries. It is estimated that 10% of the worldwide dye production is discharged into the environment through wastewater streams[1], and cause irreparable damages to the environment. Ligninolytic fungi are well known for their ability to degrade an extensive range of recalcitrant compounds due to their nonspecific enzymatic system composed principally by Laccase, Lignin peroxidase (LiP) and Manganese peroxidase (MnP)[2]. In this context, several ligninolytic fungal strains were isolated from semi-arid region of North Mexico and evaluated for laccase, LiP and MnP production and their potential in decolorization of Remazol Brilliand Blue (RBBR) and Methyl Orange (MO), an anthraquinone and azo dye respectively.

Methods. Fungal strains were isolated from semi-arid region of Coahuila, North Mexico using Kirk's medium with tannic acid as carbon source. Laccase, LiP and MnP activity and decolorization of RBBR and MO (0.01 and 0.025%; w/v) were determined in a basal medium under three different conditions, *viz*., 25°C, pH 7, 0.02% ammonium tartrate, 0.05% glucose, 100rpm; 35°C, pH 6, 0.01% ammonium tartrate, 0.05% glucose, 100rpm and 35°C, pH 6, 0.01% ammonium tartrate, 0.1% glucose, 100rpm. These growth conditions were selected based on the analysis of optimal conditions by Taguchi method and none of the medium contained tannic acid. Samples were analyzed for enzyme activity at every third day and decolorization efficiency was determined daily. Laccase activity was measured by the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) at 420nm. LiP activity was determined through oxidation of veratryl alcohol at 310nm. MnP was measured by the oxidation of phenol red at 610nm. One unit of activity was defined as 1µmol of product formed per minute under the assay conditions. Decolorization of RBBR and MO were monitored at 593nm and 464nm, respectively.

Results and discussion. Of the eight ligninolytic fungal strains isolated based on Bavendum reaction, one of the strains was selected for its high ligninolytic enzyme production. It was observed that the culture conditions and the dye concentration affected by the enzyme activity. Rectors with RBBR recorded the maximum activities at 25°C, pH 7, 0.02% ammonium tartrate, 0.05% glucose, 100rpm and were 6013.9, 1322.9, 2957U/l for Laccase, LiP and MnP, respectively. But MO elicited higher activity than RBBR and were 12750, 9435.5 and 5381.2U/l of Laccase, LiP and MnP, respectively under the same conditions. Although other two tested conditions were found to be optimum by Taguchi method for laccase, LiP and MnP production using tannic acid as carbon source, it was observed that the presence of dye completely inhibited the enzymatic activity. In the case of decolorization assay, RBBR recorded higher color removal than MO. The percentage of decolorization was 96% (0.01%) and 74% (0.025%) for RBBR, while MO recorded only 53% (0.01%) and 45% (0.025%). The higher degradation of RBBR than MO could be related the structural differences of the dyes tested. Although other conditions did not record enzyme activity, decolorization of dyes was observed. Analysis of the data showed that there is a positive correlation between the production of Laccase and the decolorization of RBBR and MO[3]. It was also observed that the presence of MnP increased the decolorization of both dyes.

Conclusion. Optimum conditions obtained by Taguchi method for the maximum enzyme activity did not show elicit similar response in the presence of dye. The fungal strain tested decolorized the anthraquinone dye, RBBR better than MO, an azo dye. A positive correlation between laccase and decolorization of dyes was observed. The efficiency of decolorization increased in the presence of MnP in addition to laccase.

Keywords decolorization, ligninolytic enzymes, dye, laccase, lignin peroxidase, manganese peroxidase

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Litter in Poultry houses, a threat to animal welfare?

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Microbiological risk and animal health are topics of great importance in poultry production. In the project CHIP "Chicken and Hen Infection Protection" the ammonia emission from litter materials in poultry houses, as well asthe microbial communities inhabiting the materials,has been in focus as factors affecting animal welfare. Ammonia is produced from fecal material which is mixed with the litter continuously through the production period. The produced ammonia will affect the health and wellness of the birds by affecting the acidity of the litter and by reducing the air quality in the stable. The type of litter will furthermore affect the microbial communities of the litter and hereby pose a possible pathogenic threat.Several different litter types, like sphagnum, straw and wood shavings, are used in Danish poultry production and these are often chosen exclusively from economic and practical perspectives.

In a pilot batch-experiment we found that the litter type will affect both the ammonia emission from the fecal matter, the pH levels of the litter, and the levels of potential pathogens/chosen microbial indicator organisms.In an additional laboratory study, where the air flow in stables were mimicked, the ammoniaemission from tree different dung-mixed litter types, sphagnum, straw and wood shavings were monitored along with levels of chosen microbial indicator organism.

Metatranscriptome of a Grassland Soil Microbial Community

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Environmental genomics, or metagenomics, has greatly advanced our understanding of the genetic diversity and potential of microbial communities. However, in situ activity and the response of microbial communities to environmental change can only be monitored through functional units such as mRNA transcripts and proteins. Owing to the advent of massively parallel sequencing technologies, metatranscriptomics has become one of the cutting-edge techniques in functional microbial ecology.

Until now, metatranscriptomics has mainly been applied to marine environments rather than to soils, because (i) extraction of mRNA from soil is very challenging, (ii) soil microbial communities are more diverse than marine assemblages and vary greatly in their composition between sites and, as a consequence, (iii) the database of reference genomes for functional annotation is poor as compared to marine microbial communities. We developed a pipeline for generating soil metatranscriptome data, which includes extraction and enrichment of mRNA of high purity and integrity, cDNA library construction for Roche 454 Titanium pyrosequencing, and bioinformatic data analysis; as exemplified for a typical grassland soil microbial community. In order to investigate the phylogenetic composition and functional activity, a total of 17,348 and 339,652 cDNA sequences were obtained from extracts of total RNA and enriched mRNA, respectively. Bacteria accounted for about 90% of all rRNA sequences with *Actinobacteria* and *Proteobacteria* constituting the dominant phyla. Archaea represented a minor but distinct population closely related to *Nitrososphaera viennensis*, a representative of the mesophilic crenarchaeal nitrifiers. Among the cDNA sequences obtained from enriched mRNA, 43,010 reads were classified as non-ribosomal RNA by BLAST search against SILVA rRNA database. These putative mRNA sequences were subjected to functional annotation and taxonomic binning using BLAST against NCBI non-redundant protein database. About 40% of putative mRNA sequences were novel using an E-value threshold of $< e^{-5}$. Most of the annotated mRNA transcripts were derived from bacteria (approx. 93%). *Actinobacteria* and *Proteobacteria* contributed the greatest percentage of transcripts, indicating that taxonomic binning of transcripts and phylogenetic analysis of rRNA provided consistent results. About 1.5% of transcript sequences were functionally annotated as stress response, such as e.g. oxidative and osmotic stress.

Keywords metatranscriptomics, microbial community, functional activity, next generation sequencing

Microbial diversity of root soil of some boron-tolerant plant taxa naturally distributing in boron-rich soils of Kirka/Turkey

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Boron is a relatively rare element in the Earth's crust. The worldwide commercial borate deposits are estimated at 10 million tonnes. Turkey and the United States are the world's largest producers of boron. Turkey has almost 72% of the world's total boron reserves. Boron does not appear on Earth in elemental form but is found combined in borax, boric acid and borates. Economically important sources of boron are rasorite (kernite) and tincal (borax ore). They are both found in the Mojave Desert of California, but the largest borax deposits are located at the Central and Western Turkey including the provinces of Kırka (Eskişehir), Kütahya, Bursa and Balıkesir.

In this preliminary study, four different boron-rich root soil samples belonging to four different tolerant plant taxa (*Gysophila sp., Alyssum sp., Glaucium sp*. and *Puccinella sp*.) were collected from Kırka (Eskisehir-Turkey). These samples were analysed both culture-dependent and culture-independent methods to chacterize microbial communities. Total DNA were extracted and 16S rRNA gene for Archaea and Bacteria were amplified by PCR. The prokaryotic diversity inhabiting these soil samples were examined by denaturing gradient gel electrophoresis (DGGE) technique. Totally ten bands obtained with Archaeal primers were sequenced. The closest matches (and percentage of similarity) for the sequences retrieved were determined by a BLAST search. DGGE showed the existence of *Crenarchaeote* and *Euryarchaeote* members in these boron rich soil samples. Culture-dependent studies showed that most of the isolates are Gram positive, rod shaped and can tolerate up to 100 mM boric acid in their culture medium.

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Microsatellite-primed PCR characterization of hydrocarbon-degrading *Aspergillus* sp and *Penicillium sp* isolated from cotton and flax seeds

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Aspergillus and *Penicillium* species, isolated from cotton and flax seeds, were evaluated for their ability to degrade crude oil. Isolates showed variation in their ability to degrade such materials with different concentrations. *Penicillium* isolates no.C2-C19 and *Aspergillus* isolates no F8-F23 were the best degraders of benzene. However, type and concentration of hydrocarbon influenced effectiveness. Microsatellite-primed PCR (MSP-PCR) analysis was applied for identification and characterization of the pre-selected isolates. MSP-PCR showed low variation among the tested isolates, indicating close relationship among them. Unweighted pair-group method with Arithmetic Averages (UPGMA) cluster analysis based on Pearson Similarity Coefficient showed that the isolates were grouped 41.4%-100%. There was a clear-cut genetic marker obtained to differentiate between low and high hydrocarbon-degrading fungi. Computer- assisted cluster analysis of MSP-PCR profiles has been shown to have a remarkable congruence with cluster analysis of hydrocarbon-degradation profiles. Results obtained in the present study indicated potential for use of *Aspergillus* and *Penicillium* species for utilization of the different hydrocarbons and bioremediation of oil in the environment.

Keywords: Hydrocarbon degradation, *Aspergillus, Penicillium* ,Microsatellite-primed PCR

Mining the bacterial biosynthetic pathways of Sierra Nevada soils

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Soil bacteria represent an important source of new biologically active compounds, but it has been estimated that 99% of this bacterial diversity is unculturable or difficult to culture. Gaining access to this diversity by means of the construction of metagenomic libraries provides an alternative source of new molecules with novel biological activities. The availability of Bacterial Artificial Chromosome (BAC) vectors allows the construction of High Molecular Weight (HMW) DNA metagenomic libraries in order to explore antimicrobial compounds produced by biosynthetic genes from soil microbial communities.

We describe the generation of metagenomic libraries of different soils from Sierra Nevada (Granada, Spain) to mine new secondary metabolic pathways of the bacteria present at different altitudes. We also describe the development of a shuttle BAC vector that allows the heterologous expression of metagenomic DNA in alternative hosts and facilitates the searching for novel biologically active compounds.

Keywords Metagenomic library; soil; bacterial artificial chromosome; secondary metabolic pathways.

Monitoring infection risk for air and soil borne fungal plant pathogens using Antibody and DNA techniques and mathematical models describing environmental parameters

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Accurate information about the presence of sufficient pathogen inoculum is required to predict plant disease occurrences in field settings. Traditionally, plant disease forecasting systems have relied upon environmental data singularly to predict disease occurrences in crops. Mathematical models describing the effect of temperature and wetness on pathogen infection have been developed for many types of plant pathogen. However, detecting and quantifying pathogen inoculum during a time period when environmental risk for a disease is high would enable protective disease control strategies to be implemented. Currently there are few methods that can detect levels of fungal inoculum in air samples rapidly and accurately. Using an innovative spore trapping system (Mircrotitre immunospore trap (MTIST)) allied to an immunological test (plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA)) we report on the potential to monitor airborne field inoculum of *Mycosphaerella brassicicola.* In conjunction with a mathematical model we are able to ascribe the likely onset of ringspot disease occurrence in crops of Brussels sprout and cauliflower. In addition, using an air sampler which collects 8 x 24hr air samples (Fig.1.) we have developed, using specific monoclonal antibodies incorporated in to immunochromatographic test strips, 'in-field' on-site rapid tests (Fig.2) which measure for a range of target airborne plant pathogens. Improved management of disease and, reduced applications with effectiveness of the fungicides applied, has been achieved utilizing information based on inoculum availability and an environmental disease risk forecast.

Fig. 1. Burkard 8-day cyclone multi-tube sampler

We also report on the use of quantitative PCR to detect crop pathogens in soil and, using immunomagnetic fishing, the development of assay systems to extract target pathogens from soil and carry out on-site tests using immunochromatographic test strips.

Fig. 2 A semi-quantitative competitive lateral flow device with *Albugo candida* zoosporangia numbers tested between 0 – 4800.

Keywords: monoclonal antibody, QPCR, disease forecast, lateral flow, air sampling

Moroccan Actinobacteria isolates as potential agents against *Ceratitis capitata*

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Pest control has been dominated by the use of synthetic chemical insecticides. However, the intensive application of such compounds has raised environmental and toxicological concerns, especially the development of resistance in target pest and non-target beneficial insect, and contamination of the environment, particularly water supplies.

These problems have lead growers to seek more environmentally safe and cost-effective pest control strategies. One of the most promising approaches is the biological control of insect pests using bioinsecticide based upon organisms such as the Gram-positive Actinobacteria. Previous study showed that Moroccan habitats especially rizhospheric soil of endemic plants are a rich source of Actinobacteria species producing bioactive secondary metabolite.

In the present work, Moroccan Actinobacteria isolates were selected and tested for their capacity to produce insecticidal compounds using a biological and chemical screening.

The biological screening was conducted for the selected Actinobecteria isolates from the brine shrimp bioassay, the fermentation broth of actinobecteria was subjected to a biological screening against the Mediterranean fruit fly larva, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae).

The chemical screening was performed for the most promising isolates to detect acetylcholinesterase (AChE) inhibitors. AChE is an enzyme that degrades the neurotransmitter acetylcholine. An inhibitor of AChE block the function of acetylcholinesterase and thus cause more acetylcholine to accumulate in the synaptic cleft. Excess acetylcholine causes neuromuscular paralysis throughout the entire body, leading to death by asphyxiation. The screening for AChE inhibitors was conducted according to the method of Zhongduo *et al.* (2009).

From our primary screening 14 isolates showed a positive response in anti-brine shrimp screening (higher than 60%) and six isolates showed strong anti-brine shrimp activity (higher than 80%). These last were further tested through bioassay with *C. capitatta* larvae. The bioassay result shows that four isolates have a larvicide activity, the isolates OS5, AS1, B89 and 98 were the most toxic with corrected larval mortality of respectively 16, 34, 42 and 98%. On the other hand, The inhibition of adult emergence from the larva stade for the isolates OS5, AS1, B89 and 98 was respectively 16, 42, 74 and 100% compared to control. The inhibition of adult emergence from the pupa stade for the isolates OS5, AS1, B89 and 98 was respectively 1.22, 15.02, 60 and 100% compared to control.

The chemical screening results indicate that the methanolic crude extract of our most promising isolate show white spots on TLC plate at the concentration of 100µg/ml except the isolate 37.

Key words: Insecticide, Actinobacteria, *Ceratitis capitata*, acetylcholinesterase inhibitors.

Nematicidal crystalliferous and Coleopteran- specific strains and putative novel *cry* genes in Iranian native *Bacillus thuringiensis* collection

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The characterization of nematode and coleopteran-effective strains and novel cry genes in the Iranian *Bacillus thuringiensis* (*Bt*) collection (70 isolates) is presented. Characterization of nematicidal strains was based on PCR analysis using 12 specific primers for *cry5, cry6, cry12, cry13, cry14* and *cry21* genes encoding proteins active against nematodes, crystal morphology as well as their nematicidal activity on two free-living nematodes (*Acrobeles complexus* and *Panagrolaimus rigidus*) and two important parasitic nematodes, root-knot nematode (*Meloidogyne incognita*) and sugar beet cyst nematode (*Heterodera schachtii*) at laboratory and green house levels. PCR results with primers for these genes showed that 22 isolates (31.5%) contain a minimum of one nematode-active cry gene. Strains containing the *cry6* gene were the most abundant and represented 22.8% of the isolates. *Bt* strains harboring *cry14* genes were also abundant (14.2%). The *cry21* and *cry5* genes were less abundant, found in 4.2% and 2.8% of the strains, respectively. In total, six different nematode-active *cry* gene profiles were detected in this collection. Twenty-two *Bt* isolates containing nematode-active cry genes were selected for preliminary bioassays on free-living nematodes. Based on these bioassays, five isolates were selected for detailed bioassays on parasitic nematodes in In vitro and greenhouse assays. Isolates AL11 and YD5 led to 80% and 69% reduction of *M. incognita* egg numbers on tomato root respectively in greenhouse assay. Also isolates GN15 and YD5 decreased number of cysts of *H. schachtii* per 1 g of soil by 65% and 57% in greenhouse assay respectively. So, based on our results new profiles of nemacidial *cry* genes and effective *Bt* strains against nematodes were reported which can be used in biocontrol programs for plant parasitic nematodes.

The characterization of the strains containing Coleopteran-specific was based on PCR analysis using 31 general and specific primers for *cry1B, cry1I, cry3A, cry3B, cry3C, cry7A, cry8A, cry8B, cry8C, cry14, cry18, cry26, cry28, cry34* and *cry35* genes, protein band patterns as well as their insecticidal activity on *Leptinotarsa decemlineata* (Colorado potato beetle) and *Xanthogaleruca luteola* Mull (elm leaf beetle). Larvae . Forty six isolates (65.7%) contained minimum one Coleopteran-active cry gene. Based on universal primers, strains containing *cry18* and *cry26* genes were the most abundant and represent 27.1% and 24% of the isolates, respectively, whereas *cry14, cry3, cry28, cry34, cry35, cry7* and *cry8* genes were less abundant, found in 14.2, 12.5, 10, 7, 7 and 5.6% of the strains, respectively. Based on specific primers, isolates containing *cry1I* were the most abundant (48.5%). Two strains YD5 and KH4 containing Coleopteran active cry genes showed higher activity (more than 90%) against *X. luteola* and *L. decemlineata* larvae than *Bt* subsp. morrisoni pathovar tenebrionis. The strain YD5 showed high toxicity against coleopteran (X. *luteola* and *L. decemlineata*) pests and plant parasitic nematodes, also previously we showed that this strain has high toxicity against lepidopteran pests (*Heliothis armigera* (Coton boll worm) and *Phthorimaea operculella* (potato tuber moth)), so, it can be used as a powerful biocontrol agent. Finally, economic media based on available agricultural wastes was optimized for mass production of the strain YD5. In addition, effects of different factors, including quantity of primary inoculation, aeration and pH on growth rate, and spore and crystal production were evaluated in fermentor conditions. Fermentation experiments showed that hydrolyzed starch, corn steep and sea salt were as good carbon, nitrogen and mineral sources for YD5. Two percent primary inoculation, pH 7 and 90% oxygen saturation were the best fermentor conditions for YD5 spore and crystal production (5.5 × 109 CFU/ml). Finally, formulation based on wettable powder was optimized for commercial production of the strain.

To find novel *cry* genes in the collection, about 110 universal and specific primers were synthesized. These primers allowed us to detect about 380 known *cry, cyt* and *vip* genes in the native *Bt* isolates. The native isolates showed high diversity in *cry* genes contains. Thirty isolates, when assayed for *cry1C, cry5, cry6, cry8b, cry9, cry10, cry11, cry18, cry24* and *cry35* genes, showed unexpected size bands. Cloning and sequencing of the amplicons allowed both the identification of known *cry* genes and the detection of 9 putative novel *cry1C* sequences.

Key words: *Bacillus thuringiensis*, *cry* genes, *Meloidogyne incognita*, *Heterodera schachtii*, Novel *cry* genes, *Leptinotarsa decemlineata, Xanthogaleruca luteola,* fermentation

Pathogenicity of the Streptomyces strains on potato by a factor other than thaxtomine

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A few of *Streptomyces* species are pathogenic on some plants such as potato. Main pathogenicity factors among this species on potato are thaxtomine, concanamycin and a compound named as FD-981. Potato scab disease is one of the most important diseases in potato growing area in Hamedan province. Potato tubers shown raised, netted, shallow and deep pitted lesion symptoms were collected from many potato fields and the *Streptomyces* strains were isolated. Based on the phenotypic features and induced symptoms the isolated *streptomyces* strains were not uniforms. They induced symptoms on the tested plants including potato, parsnip, horse radish, carrot and other tested plants. Most of the tested strains harbored a linear plasmid exemined by pulsed field gel electrophoresis and they had sequences related to insertion sequences, nec1 and thaxthomin biosynthetic genes. Raised and netted scab disease inducing strains produced thaxtomin determined by thin layer chromatography but not pitted lesion inducing strains. The last strains which did not produced thaxtomin also did not hybridized to thaxtomin biosynthesis gene probes. Deep pitted inducing representatives strains produced disease inducing toxins other than thaxtomin.

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Phenotypic and genotypic characterization of indigenous *Sinorhizobium meliloti* strains isolated from different regions in Croatia

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The studying of rhizobial biodiversity opens up the possibility to preserve and maybe to exploit some indigenous strains with hidden symbiotic or ecological potential, particularly under unfavourable conditions. In order to improve the beneficial effect of alfalfa inoculation it is important to characterize the indigenous strains and to obtain information about actual composition of rhizobial field population. The main aim of the present study was to characterize indigenous rhizobia associated with alfalfa (*Medicago sativa* L.) in different regions of Croatia. The soil samples for rhizobial isolation were collected from three different regions in Croatia (Istrian peninsula, north-west Croatia and central Croatia). Greenhouse pot experiment was established in order to obtain nodules for isolation procedure. Due to better description of field sites, main physical and chemical characteristics were determined in all soil samples. Over 250 isolates were obtained from root nodules of alfalfa. To study their diversity and characterize them in relation to environmental conditions of their soils of origin, a polyphasic approach was used. Stress tolerance assays revealed significant variations in pH tolerance while almost all isolates showed similar tolerance to elevated salt concentrations and growth temperatures. Among all isolates recovered from different regions, significant number of isolates showed tolerance to acidic pH values with dominance of strains originating from Istrian region where 38% of isolates tolerated pH 4.5. The actual composition and genetic diversity of natural field population was studied by two PCR fingerprinting methods. PCR-RFLP of 16S rDNA revealed most of isolates to be closely related to *S. meliloti*. Cluster analysis of RFLP patterns obtained with *RsaI*, showed that none of the isolate was identical with *S. medicae* type strain. However, several isolates produced slightly different RFLP pattern from *S. meliloti* type strain and other isolates. Dendrogram derived from AFLP profiles revealed considerable genetic diversity among *S. meliloti* isolates. Only a few strains were identical or nearly identical to each other. The strains originated from the same region were mostly grouped within the same cluster. These results were confirmed by further analysis. Analysis of molecular variance (AMOVA) allowed relating the genetic structure of the symbiotic population to various factors, including location, soil pH and the type of vegetation. Majority of the factors considered were significant as source of genetic variation, indicating that these populations were structured according to their geographical location and environmental conditions. The certain number of isolates, showing unspecific rhizobial characteristics, were sequenced. The results confirmed the presence of other bacterial species within the nodules. Therefore, further investigations are needed for the assessment of hidden diversity within the nodules. Better understanding of rhizobial ecology and selection of locally adapted and genetically defined strains as well as their targeted application regarding the environmental conditions of certain production area is one among many approaches to improve the nitrogen fixation efficiency and crop productivity.

Keywords nitrogen fixation; rhizobia; *Sinorhizobium meliloti*; indigenous strains; stress tolerance; genetic diversity

Phosphate Solubilization by Fungi Isolated from Alkaline Soils

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Biofertilizers have been identified as an alternative to chemical fertilizers to increase soil fertility and crop production in sustainable farming. In this study, a phosphate solubilizing fungus has been isolated from rhizosphere of *Sesamum indicum.* On the basis of phenotypic characteristics and ITS region sequencing, the fungus has been identified as *Aspergillus awamori* S36. The soluble phosphate production was 1.25 g l^{-1}. This isolate was further examined for *in vitro* phosphate solubilization using different substrates *viz*., dicalcium phosphate (DCP), tricalcium phosphate (TCP), ferric phosphate (FP) and Udaipur rock phosphate (URP) at varied temperature. Solubilization of DCP by the isolate was significantly higher ($p<0.05$) followed by TCP and URP at all temperatures but performed especially well in the range of 25-35°C. Phosphate solubilizing ability was measured in presence of various carbon and nitrogen sources. Fructose was found to be the best carbon source whereas, sodium nitrate was found to be the best nitrogen source followed by ammonium sulphate. The soluble P content was significantly higher when the initial pH for TCP solubilization was 8.0 although it showed good phosphate solubilization capacity even at pH 10.00. Optimum concentration for TCP solubilization was 7.5 g l^{-1}. Soluble phosphate concentration in the culture medium was directly proportional to the titratable acidity and inversely related to pH. *Aspergillus awamori* S36 appears to adapt well to the stress conditions and has shown the potential to solubilize inorganic phosphates under semi-arid conditions. Further, pot experiments showed that inoculation of *A. awamori* S36 significantly increased the growth and yield of mungbean crop compared to the control uninoculated soil. The P content was significantly increased in the plants. In conclusion, *A. awamori* S36 can be used as a strong phosphate solubilizer in agricultural environment.

Keywords Biofertilizers; *Aspergillus awamori;* phosphate solubilization; ITS region sequencing

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Role of soil enzymes produced by PGPR strain in barley growth and nutrient uptake parameters in the filed conditions

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Soil enzymes originate from animal, plant and microbial sources and the resulting biological activity includes the metabolic processes of all these organisms. Under favorable conditions microorganisms supply most of the enzyme activity in soil. N_2-fixing and P-solubilizing bacteria have great potential to enhance nutrient availability with increase the soil enzyme activity. The present study was conducted to investigate the effects of seed coating by N_2-fixing and P-solubilizing PGPR strains on soil enzyme activities (dehydrogenase, urease, alkaline phosphates, and acid phosphates) in relation to soil microbial population and barley (*Hordeum vulgare* L.) growth in comparison to control and optimum and half of N fertilizer doses application under field condition in 2008. The treatments included control (no inoculation and fertilizer), Nitrogen (40 kg N ha^{-1}), Nitrogen (80 kg N ha^{-1}), *Bacillus* OSU-142, (5) *Bacillus* M-3, *Azospirillum* sp.245, OSU-142 + M-13 + *Azospirillum* sp.245, *Bacillus megaterium RC07*, *Paenibacillus polymyxa RC05*, *Raoultella terrigena* and *Burkholderia* BA7, OSU142 AMP Res, M-3 Amp Res, sp.245 Amp Res, *P. polymyxa* 2/2, *B. megaterium* T17, and Mixed + 40 kg N ha^{-1}. All PGPR inoculations and fertilizer applications significantly increased enzyme activities tested and plant nutrient uptake of barley compared to the control. Statistical significant effects were determined between the soil microbial population and enzyme activities, responding sampling time during the plant growth period. The highest dehydrogenase activity was obtained from mixed inoculations with three strains (OSU-142 + M-13 + *Azospirillum* sp.245), and the highest urease activity was obtained form 80 kg N ha^{-}1 and 40 kg N ha^{-1}. On the other hand, *Bacillus* M-3, *Bacillus megaterium RC07*, M-3 Amp treatment had the highest alkaline and acid phosphates activity, which were in good relation to soil P and micro element availability in soil rhizosphere..

Key words: seed coating; soil enzyme, plant growth-promoting rhizobacteria; macro and micro element availability

Screening of actinomycetes isolates from argan forest able to enhance seed germination argan tree (*Argania spinosa* L. Skell)

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This study was conducted in an attempt to enhance germination of argan tree (*Argania spinosa* L. Skell) seeds. It is an endemic species of the western south Morocco and remains extremely difficult to transplant or establish on any meaningful scale outside Morocco. Actinomycetes were isolated from the upper 2 – 4 cm of the soil layer and the rhizosphere in a well-developed argan forest region near Essaouira city. In order to screen isolated actinomycetes able to improvethe argan tree seed germination, we investigated four characteristics of germinating seeds including germination index, germination percentage, germination rate and vigor index after two pre-treatments (warm and cold stratification). Most actinomycete isolates exerted a significant inhibition on germination compared to control.The percentage of actinomycetes that inhibited seed germination was significantly higher in isolates obtained from rhizosphere than in those obtained from the soil. However, three actinomycete isolates from soil and one from rhizosphere were selected for next experiments because they enhanced seed factors studied. Warm stratification was the most effective pre-treatment in our study.

Keywords: *Argania spinosa* . Actinomycetes .Germination . Stratification

Seasonal changes in soil fungal communities after biosolarization and its repeated use in pepper crops in Southeast Spain

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The removal of methyl bromide (MB) as a common soil disinfectant in 2005 in sweet pepper grown in greenhouses in Southeast Spain led to essay alternatives with the minimum environmental effect. A broad spectrum of non-chemical alternatives has been deeply studied for controlling the main pathogens of the crop, keeping acceptable yield levels and reducing soil fatigue effects. These effects that take place when soil disinfestation is not carried out between two consecutive pepper crops in the same soil are specific for sweet pepper and they seem to be related to non-pathogenic soil fungi accumulated at the end of cropping season. Among the different alternatives, the use of organic amendments is recommended by their great number of advantages for soil properties. In this work, biofumigation (manure amendments alone, B) and biosolarization (biofumigation combined with solarisation, BS) disinfectant effects were evaluated and compared with MB. Experiments were conducted at one greenhouse over a 3-year period. The impact of soil disinfestation was measured by soil analysis during the growing season to detect the density of non-pathogenic soil fungi depending on the disinfection treatment.

Isolated soil fungi belonged to the following genera: *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp., emphasizing the first genus for its abundant presence. The use of manure amendments with a plastic cover was able to increase the non-pathogenic soil fungal density respect to the other treatments studied: MB and control. Although the first year of carrying out, BS had a higher soil fungal density, its reiteration reduced it making it similar to MB. Moreover, the progressive reduction of organic amendments did not led to a lower efficacy than methyl bromide. In conclusion, the use of B or BS showed to have a similar effect than MB on non-pathogenic soil fungi in pepper crops.

Keywords sweet pepper; manure amendments; soil fungal populations

Selection of potential biocontrol agents on the example of rhizosphere isolates antagonistic towards *Pectobacterium* sp. and *Dickeya* sp.

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Pectinolytic plant pathogenic bacteria belonging to *Pectobacterium*and *Dickeya*genera are causative agents of bacterial plant diseases worldwide. They cause the important economic losses in the potato production in Europe. The elimination of the pathogens and prevention of the disease spreading is based on hygienic measurement and application of the certified pathogen-free propagation material.

The application of biological control agents (BCAs) together with physical and chemical treatments might be an alternative strategy for potato and ornamental plant protection.

This work aimed for selection of the bacteria originated from the rhizosphere of various vegetable crops in Poland for their antagonistic activities towards plant pathogenic bacteria: *Pectobacterium atrosepticum,P. carotovorum* subsp. *carotovorum* and*Dickeya* sp. (especially newly isolated in Europe and aggressive *Dickeya sp.* bv 3' strains).

Out of more than thousand isolates, 18 were chosen on the basis of their ability to inhibit growth of the tested pathogens in a plate assay, the production of strong iron chelators and biosurfactants, and/or inactivation of acyl-homoserine lactone (AHL) signal molecules important in quorum sensing (QS) mechanisms involved in production of pectinolytic enzymes by tested pathogens.

The isolates were gathered into two groups: Group I composed of 12 isolates inhibiting the pathogens' growth in a plate assay and group II comprising 6 isolates able to inactivate AHL. The ability of these isolates to inhibit potato tubers tissue maceration caused by *Dickeya* sp. and/or *Pectobacterium* sp. was tested in a tuber slices assay and in case of *Dickeya* sp. also on chicory leaves. In group I, most isolates wereable to attenuate maceration of tuber tissue caused by the pathogens belonging to *Pectobacterium* genus. These isolates were identified mostly as *Pseudomonas* sp. Despite the inability of the group I isolates to inhibit tissue maceration caused by *Dickeya* sp. strains on potato slices, they were highly effective against *Dickeya* sp. when tested on the chicory leaves.

TheAHL-inactivating isolates of group II, all classified as*Bacillus*spp.,were active against most of the pathogenic strains from both *Dickeya* and *Pectobacterium* sp.. It is especially interesting due to the fact thatQS mechanism does not play such an important role in the pathogenicity of *Dickeya* sp. as it is known for bacteria from *Pectobacterium* genus.

We propose that the selected bacterial strains may serve as potential biocontrol factors of pectinolytic plant pathogens.

Keywordsbiological control, soft rot pathogens, *Pseudomonas, Bacillus*, potato, quorum sensing, acyl-homoserine lactone

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Silencing of pds gene in *Atropa belladonna* using hetrologous VIGS

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Introduction, *Atropa belladonna* is a well known medicinal plant which is important for its alkaloids and therefore the genes involved in the pathway need be studied. In this study, we used Virus-induced gene silencing (VIGS) to silence phytoene desaturase (*pds*) as a marker gene in Atropa belladonna *in a heterologous mode*.

Method, Recombinant TRV vector (NtpTRV2) containing exogene from tobacco injected into Atropa seedlings *(3 weeks old)*. The results showed that the plants injected with NtpTRV2 photobleached after 3 weeks of infiltration. Spectrophotometric analysis showed that the amounts of chlorophylls, carotenoids and total protein in leaves of *Atropa belladonna* significantly reduced*.*

Result, The amounts of chlorophyll a, chlorophyll b , carotenoids and total proteins in NtpTRV2 treatment decreased 10.9, 7.4, 9.3, and11.27 folds than those of control plants, respectively. This was the first report of efficient silencing of a gene in Atropa belladonna *using* VIGS. Such an approach could be used as a powerful tool for silencing and characterizing the target genes involved in alkaloids metabolism in this plant.

Key Words: VIGS, Photobleaching, *Atropa belladonna,* phytoene desaturase, gene silencing.

Soil bacteria contributing to nitrogen cycle in Finnish Lapland forest limit under different vegetation

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In the terrestrial ecosystems in high latitude or alpine zone, factors controlling forest limit and tree growth are still unclear. Although nitrogen is considered as one of the most important limiting factors for primary production of trees in latitudes north from Arctic Circle, nitrogen cycle of forest limit has not been elucidated yet. In this study, nitrogen fixation of soil bacteria in northern high latitude forest limit in Finnish Lapland was investigated, using acetylene reduction assay in gellan gum soft gel medium (Hara et al., 2009).

The study sits, Tundra (site-T), birch forest heath (sites-B1, -B2, -B3) and dry pine forest (site-P), were located in Lapland near Kilpisjärvi Biological Station, northern Finland (68-69˚N, 20-22˚E). Humus with living roots(O-horizon), podzolicsoil (E-horizon) and mineral soil (A- and B-horizon) were respectively collected from each site in early September 2010, and acetylene reduction assay for each soil was performed in the medium previously applied for East Siberian larch forest soil (Hara et al., 2009). The soils of O-horizon and A-horizon from site-T showed high activities, whereas almost all other samples from sites-Bs and -P did not show any significant activities (Table).

Effects of incubation temperature and concentration of carbon source onnitrogen-fixation were examined. The soils from O-horizon and A-horizon at site-T showed the highest acetylene reduction at 15˚C and at 0.5% carbon source. Conversely, the soils showed relatively lower activity at high temperatures (20˚C or 25˚C) and at high concentrations of carbon source (1.0% or more), i.e. in conditions widely used in acetylene reduction assay for nitrogen-fixing soil bacteria. These trends were similar to those found from East Siberia (Hara et al., 2010a) and a planted larch forest established on volcanic sand in Hokkaido, northern part of Japan (Hara et al., 2010b). DNA was extracted from cultured medium used for acetylene reduction assay, and 16S rRNA gene targeted DGGE was performed. GC-clamped PCR amplicons of *Clostridium* spp. were frequently detected from the bacterial biome in site-T soil, which probably contributed the most to the high acetylene reduction of Tundra soil.

Table [a]Acetylene reduction of soil from each siteand vegetation

Site	site-T (Tundra)	site-B1 (Birch forest)	site-B2 (Birch forest)	site-B3 (Birch forest)	site-P (Pine forest)
O Layer	1.11 ± 0.06	0.54 ± 0.94	0	0	0
A Layer[b]	0.94 ± 0.03	0.23 ± 0.40	-	-	-
E Layer[b]	-	-	0	0	0
B Layer[b]	-	0	0.54 ± 0.91	0	0
Dominant tree		*Betulapubescensssp. czerpanovii*	*B. pubescensssp. czerpanovii*	*B. pubescensssp. czerpanovii*	*Pinus sylvestris*
Cover plants	*Betula nana Empetrumnigrum*	*B. nana, E. nigrum Vacciniumvitis-idaea*	*B. nana, E. nigrum V. vitis-idaea*	*V. vitis-idaea Pleuroziumschreberi*	*V. vitis-idaea P. schreberi*

[a]Three hundreds mg of soil samples were incubated in 10 mL of gellan gum soft gel medium, and acetylene reduction assay was performed as described in our previous study (Hara et al., 2009).Values represent the means and standard deviations for three replicates (nmol C_2H_4 h^{-1} $vial^{-1}$), except for site-B3 (no replication).
[b] A-, E-, and B- Horizons were not found vertically. -: not found.

Keywords; Nitrogen fixation; boreal forest; soil bacteria; gellan gum soft gel; Tree limit; acetylene reduction

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Starved bacteria retain their size but lose culturability - Lessons from a 5000 years old undisturbed A-horizon

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The vast majority of soil bacteria are unable to formvisible colonies on agar media. One hypothesis is that unculturable soil bacteria are dwarf cells that may either be small starved forms derived from larger species or represent inherently small species. We test the hypotheses that cells of extremely starved soil bacterial communities are smaller and less culturable than cells of bacterial communities from a richer soil, and that culturability is related to cell size by comparing an extremely starved community from a 5200-year-old A-horizon buried under a burial mound with a community from a modern agricultural A-horizon.

We serially altered cell suspensions through alters with successively smaller pore sizes (0.8 mm, 0.6 mm and 0.4 mm) and assessed total cell number and culturability, i.e. the ability to form colonies on two types of agar media, in each size fraction. Cell size distributions were assessed in unaltered suspensions. Average cell size was only moderately reduced in the starved community, where culturability was low for all size classes. In contrast, culturability was much higher in the modern community, where culturability decreased dramatically with decreasing cell sizes.

Keywords Bacteria, CFU, AODC, Cell diameter, Cell viability, Culturability, Starvation, Palaeosoil, Size

Survey of chickpea rhizobia in Portugal: genetic diversity and stress tolerance

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Rhizobia are soil bacteria able to establish nitrogen fixing symbiosis with legumes. The biological nitrogen fixation contributes to an ecological and sustainable agriculture, as it reduces the need for chemical N-fertilizers. The first studies on the chickpea-rhizobia symbiosis, showed that two rhizobia species were able to nodulate this legume, namely *Mesorhizobium ciceri* and *M. mediterraneum*. Recently, several isolates assigned to other *Mesorhizobium* species were described to induce effective nodules in chickpea plants. Our aims were to examine the biogeography of chickpea rhizobia species, to search for a predominant species and to identify the most efficient microsymbiont, considering Portugal as a case study. In addition, chickpea rhizobia ability to tolerate different stresses, namely high temperature, acidity and salinity, was investigated. In order to understand the molecular bases of stress tolerance in mesorhizobia, the transcript levels of major chaperon genes (*groELS, dnaKJ* and *clpB)* from tolerant and sensitive isolates were compared. A total of 110 isolates were obtained from continental Portugal and Madeira Island (1). The 16S rRNA gene phylogeny revealed that isolates are highly diverse, grouping with most *Mesorhizobium* type strains, in four main clusters (A-D). Interestingly, only 33% of the isolates grouped with *M. ciceri* (cluster B) or *M. mediterraneum* (cluster D), the formerly described specific chickpea microsymbionts. Most isolates belong to cluster A, showing higher sequence similarity with *M. huakuii* and *M. amorphae*. Cluster C comprises isolates close to *M. tianshanense*. An association between isolates origin province and species cluster was found and suggests biogeography patterns: most isolates from the North, Centre and South belong to clusters B, A and D, respectively. A correlation was found between isolates species cluster and origin soil pH, suggesting that pH is a key environmental factor, which influences species geographic distribution. Isolates showed a high diversity in their ability to grow under temperature stresses (heat shock at 48ºC; heat stress at 37ºC; cold stress at 15ºC) (2). Isolates from distinct species groups differed significantly in their ability to endure temperature stress. Isolates from the chickpea specific microsymbionts species groups (*M. ciceri* and *M. mediterraneum*) showed the highest tolerance to all tested conditions. An association was found between isolates origin province and temperature stress tolerance. Interestingly, analysis of the *groEL* and *dnaK* gene expression levels, after heat shock, showed a much higher induction in tolerant than in sensitive isolates. These results suggest that higher chaperon gene expression levels are related to higher tolerance to heat shock. However, further studies are required to clarify the role of GroESL and other chaperone machineries in differential stress tolerance of chickpea rhizobia.

To our knowledge, this is one of the few surveys on chickpea rhizobia and the first systematic assessment of indigenous rhizobia in Portugal. The obtained isolates collection, highly diverse in terms of species, symbiotic effectiveness and stress tolerance, provides an important source of rhizobia strains. Within this new collection of isolates, stress tolerant and symbiotically efficient mesorhizobia with potential agronomical importance have been identified.

Keywords rhizobia; chickpea; diversity; stress; chaperone

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The application of hydrocarbonoclastic fungi for the bioremediation of weathered crude oil

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Hydrocarbon waste, including crude oil residues in tank bottoms represents an ongoing and growing environmental problem throughout the world as they are particularly persistent within the environment and have the potential to induce a vast array of serious negative health effects for all biological entities. Recycling companies in Australia currently collect over 250 million litres of waste oil and an estimated 75-80% of this material is recycled into a useful product. The remaining 20% still requires treatment to facilitate safe disposal. In addition, the treatment and disposal of hydrocarbon waste remains a major environmental challenge in most developing countries in Africa and Asia which do not have adequate recycling facilities.

It is well established that soil contains a reservoir of microorganisms with the potential to metabolise a wide range of pollutants. In instances where the soil has been exposed to pollutants, the microbial consortia can react on two ways. The first is to develop mechanisms to prevent cell damage as the result of exposure to levels of toxic compounds that would otherwise cause serious injury. The second is to develop the ability to utilise that pollutant as a source of energy. Bioremediation offers a sustainable treatment that reduces the levels of a pollutant present in a soil, for example to sub-toxic concentrations or brings them in line with compliance criteria for safe use or for disposal. In so doing the risk following release to the environment is ameliorated. Exploiting microorganisms with the ability to degrade the pollutant, or convert it into biomass, provides the best opportunity for successful implementation of an environmentally friendly and economical bioremediation treatment.

To date the application of fungi to bioremediation has been limited; yet their filamentous growth and their ability to degrade an array of complex aromatic compounds (e.g. lignin) suggest that they have been under-utilised in this area. The aim of this study was to assess the potential of fungal isolates from previously contaminated environments to both survive in contaminated environments and degrade weathered crude oil. The isolation of fungi capable of such biochemical activity was achieved by using weathered crude oil as the sole carbon-source in isolation plates. From the isolates obtained, screening against a range of hydrocarbons identified a strain that exhibited high levels of hydrocarbonoclastic activity. This isolate was further tested as a candidate to bioaugment the breakdown of weathered crude oil by enhancing the hydrocarbon-degrading activity of a natural microbial population indigenous to previously contaminated soil. Additional optimisation of culture conditions led to the development of new media formulations which enhanced the hydrocarbon-degrading activity of the isolate.

Following successful laboratory scale trials with the fungal isolate, microcosm trials were undertaken to assess the potential of this combined bioaugmentation/biostimulation strategy for the remediation of a soil contaminated with weathered crude oil From an initial concentration of 110,000 mg kg^{-1}, after 70 d the concentration of total petroleum hydrocarbons had reduced to 43,333 mg kg^{-1} in the mesocosm containing the fungal isolate, compared to a final concentration of 86,000 mg kg^{-1} in the uninoculated control mesocosm. Profiling of the microbial community using denaturing gradient gel electrophoresis confirmed the presence of the isolate during the bioremediation process and showed that the strategy did not adversely affect the soil microbial community. The result highlights the potential of hydrocarbonoclastic fungi for use in bioremediation.

Keywords bioremeiation of oil oil, bioaugmentation, biostimulation, hydrocarbonoclastic fungi, weathered crude oil.

The Effect of Agricultural and Industrial Developments on the Quality of Water at UMhlathuze River (Northern Coast of Kwa-Zulu Natal, RSA)

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UMhlathuze River is the main recipient of domestic, sewage, industrial and agricultural waste from local industrial and agricultural practices. Scarcity of water resources and the contamination of UMhlathuze River by agricultural and industrial developments make communities around UMhlathuze area susceptible to potential outbreaks of water-borne diseases as well as the risk of ingesting carcinogenic substances. In determination of the effect of human developments on the UMhlathuze River, four different sites representing different human activities were used for sampling along the river. Temperature and pH were monitored in situ and were found to vary between 19-21°C and 6.2 - 7.8 respectively. COD was found to be higher in areas affected by both industrial and agricultural activities. Spectroquadrant Pharo 300 (Merck) was used for chemical analysis of water. Agricultural waste recipient area had high concentration of phosphate, ammonia and nitrate. Parts of the river receiving effluent from treated wastewater had high sulphide, nitrate and ammonia concentrations. Industrial areas had high concentration of heavy metals (e.g. Aluminium). Microorganisms found in all sites included *Escherichia coli* and species of *Salmonella, Shigella, Citrobacter, Serratia* and *Enterobacter,* although their quantities differed from agricultural to industrial sites. Agricultural and industrial development activities practiced around UMhlathuze River were found to have a huge contribution to the continued deterioration of the quality of water at UMhlathuze River.

Keywords: UMhlathuze River, water pollution, water quality, public health, agricultural and industrial developments

The effect of powder of Mint, Ginger and Cinnamon on *in vitro* degradation of alfalfa hay by sheep rumen microorganism

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The aim of this study was to investigate of effect of powder of mint, ginger and cinnamon on *in vitro* degradation and gas production parameters of alfalfa hay by sheep rumen microorganism. Rumen fluid was supplied from two fistulated Arabi sheep were fed a 40:60 concentrate: forage (250 g concentrate, 550 g lucerne hay and 200 g wheat straw) in prior to the morning meal, and was added to the anaerobic mineral buffer solution (1:2 v/v). The experimental samples were a mixture of lucerne hay without and with 5 mg powder of Mint, Ginger and Cinnamon (4 replicates per each). Gas production were assessed by incubating approximately 300 mg experimental sample (1.0 mm screen, triplicate) with 30 ml of rumen buffer mixture in 100 ml glass syringes based on Menke and Steingass procedure. Gas production (ml) were recorded at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h. Cumulative gas production data were fitted to the exponential equation $Y=b\ (1-e^{-ct})$. Data of *in vitro* gas production, and OMD were subjected to analysis as a completely randomized design using the General Linear Model (GLM). Data analysis showed that the highest gas production from fermentable fraction of alfalfa by sheep rumen microorganism (57.95 ml) was for 5 mg mint powder ($P < 0.05$), that is followed by ginger and cinnamon (42.5 and 39.6 ml, respectively) ($P >0.05$). The highest gas production after 24 and 72 hours incubation was for alfalfa treated with 5 mg mint powder (31.5 and 45 ml, respectively) ($P >0.05$). Therefore, the results suggest that with 5 mg mint powder has had better effect on degradation and gas production parameters alfalfa by sheep rumen microorganism compared to 5 mg powder of ginger and cinnamon.

Keywords alfalfa hay; mint; ginger; cinnamon; gas production

The fibrolytic activity of rumen bacteria on *in vitro* disappearance of sugarcane pith processed with high pressure steam in Holstein steer

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This experiment was conducted to investigate rumen bacteria activity in degradation untreated sugarcane pith and or treated with high temperature steam (210 ºC, 3 min, 19 bar, 70% moisture), by using *in vitro* disappearance of dry matter (DM) and neutral detergent fiber (NDF) in anaerobic bacteria pure culture. Rumen fluid was collected from two fistulated Holstein steers and centrifuged (1000 rpm, 10 min), then supernatant was used to grow bacteria in medium containing fungicide solution (benomyle: 500 ppm/l medium and metalaxyle: 10 mg/l medium) under anaerobic conditions at 39 ºC for 24 h. These isolates were used (1:9), as a source of inoculum for culturing bacteria in a serum bottle containing 45 ml of culture medium and 1g of experimental sample (untreated and steam treated sugarcane pith) under anaerobic conditions (using three times subculture) for 24, 48, 72, and 96 h. Samples of sugarcane pith used as the substrate of culture media were collected from each bottle after washing twice with distilled water, and then disappearance of DM and NDF were measured. The result showed disappearance of DM after 96 h incubation by rumen bacteria will be 68.42 and 76.12 g/100 g for untreated sugarcane pith and treated with steam, respectively ($P<0.05$). Steam treatment caused to increase disappearance NDF of sugarcane pith (405.0 *vs* 369.3 mg/g) ($P<0.05$). Therefore it appears that the growth and activity of rumen bacteria of Holstein steer on sugarcane pith and its degradation are influenced by steam processing.

Keywords sugarcane pith; rumen bacteria; degradation; high temperature steam

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The use of anaerobic digested slurry as an organic amendment: a potential risk or a chance for the soil?

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The overproduction of animal manures- over 1500 million tonnes per year are produced in the EU-27 - has led to inappropriate disposal practices such as their indiscriminate and inappropriately-timed application to agricultural fields. These practices can cause several environmental problems, including an excessive input of potentially harmful trace metals, inorganic salts and pathogens; increased nutrient loss, mainly nitrogen and phosphorus, from soils through leaching, erosion and runoff; and the emission of hydrogen sulphide, ammonia and other toxic gases. However, if handled properly, manure can be used as a valuable resource for renewable energy production, as well as a source of nutrients for agriculture, as it provides high contents of macro- and micronutrients for crop growth and represents a low-cost alternative to mineral fertilizers.

The health and environmental risks associated with the management of animal manures could be significantly reduced by stabilizing them before their use or disposal. Composting and vermicomposting are two of the best-known processes for the biological stabilization of solid organic wastes and they have been widely used for processing raw manures. Vermicomposting involves the bio-oxidation and stabilization of organic material but, in contrast to composting, it depends on the joint action of earthworms and microorganisms and does not involve a thermophilic stage. However, the on-farm production of renewable energy from animal manures has increased interest over the last few years in anaerobic digestion as a methodological alternative for the treatment of such organic substrates. This process involves the degradation of the organic matter in the absence of oxygen producing biogas, a renewable energy source; and a biologically stable and partially hygienic product that can be applied as an organic amendment to the agricultural soils. However, the effects of land spreading the digested slurry instead of the untreated manure still need to be defined in order to broaden our knowledge about the benefits and risks of using this co-product as an organic amendment because pathogens, mainly those with a spore forming capacity such as *Clostridium* species, might be spread together with the digested slurry in soils. The aim of the present study was therefore to evaluate, at a microcosm level, whether and to what extent the use of the anaerobically digested slurry modifies the soil chemical and microbiological properties compared to the undigested cattle manure and two well-recognized biomasses, i.e. compost and vermicompost.

For this, all the amendments (cattle manure, anaerobically digested slurry, compost and vermicompost) were mixed with soil heaps by turning at the same N rate, considering the soil bulk density of 1 g cm^{-3} and a plough of 20 cm. A control treatment that consisted of soil without the addition of any amendment was also included. The experiment was set up in columns, which were filled with 2 kg soil each (fresh weight) resulting in a total of 60 experimental units (5 amendment levels x 4 incubation times x 3 replicates). After an equilibration period of four days at 4 °C, 15 samples were collected to analyze (incubation time zero months). The remaining columns were arranged in a completely randomized design and maintained in a room with a constant temperature (22 °C) and were destructively sampled after 15, 30 and 60 days.

Overall, the nitrification rate along with the nitrate content were found to be higher in all the treatments over time, although such increase was more pronounced after 60 days of incubation. With respect to the pathogens, we found that *Clostridium perfringens* was more abundant in compost than in the other amendments; whereas *Escherichia coli* and faecal coliforms CFUs appeared to be higher in vermicompost, cattle manure and anaerobic slurry. Upon application to soil of the different amendments, *E. coli* CFUs were detected in cattle manure-amended soil at the start of the experiment (zero time) and after fifteen days incubation. However, no *E. coli* CFUs were found in control soil and soil amended with compost, vermicompost or anaerobic digested slurry, irrespective of the incubation time. At the start of the experiment, the addition of the different amendments into soil induced higher faecal coliforms CFUs relative to the control; and, after thirty days incubation faecal coliforms were still detected in cattle manure and slurry-amended soils, although the abundance of this pathogen was lower compared to zero time. *Clostridium perfringens* CFUs were found in all the treatments, including the control soil, for all the sampling times probably due to the fact that *Clostridium* species have a spore forming capacity and in turn, can persist in soil over time.

Keywords *Escherichia coli***;** Faecal coliforms; *Clostridium perfringens*; microbial activity; organic amendments

The Use of the Plants for the Removal of Toxic Metals form Contaminated Soil in Agricultural Land around Aluminum Industrial Complex

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Human environment is a complete set of physical and natural environment and it also includes the interactions between human and environment. Due to increasing population and increasing organic and inorganic contaminants, it is essential to provide a reliable, low cost, and relatively quick method for removing contamination without any undesirable side effects for the environment. Phytoremediation is an appropriate way in this regard. This study aimed to determine the heavy metal accumulation capacity around the Iralko aluminum factory, located in Markazi (Iran) using plant Hultemia persica. This study employs a "factorial experiment" with completely randomized design under greenhouse conditions. It conducted four treatment plants, 3Repeat and 4 treatments of heavy metals (zinc, nickel and chromium) in control, which were 2.5,5 and 10 times more than average concentration of elements in soil to evaluate the Phytoremediation methods and compare the estimated absorption in refining metals-contaminated soils. In this study the average concentration of Zink in soil is 107 mg/kg and the bioavailability is 0.4mg/kg which is more than other two metals. After analysis of plant tissues, results shows that the Zink has the highest concentration with 38.74% in leaves and then 36.04% in the stem of the plant. Considering the morphology terms and resistance to water shortages this plant is more efficient in absorption of zinc than absorption of Cr and Ni from the soil around the Iralco factory. On the condition that they not used as livestock forage in inappropriate circumstances of pastures

Key words: phytoremediation, Iralko، Hultemia persica

Ultra pure, autochthonous mycorrhiza-based industrial inoculants enhance crop quality and production while increasing microbial biodiversity and soil stability

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Mycorrhizal symbiosis is a well known, widespread benefitial interaction between a restricted group of soil fungi (the glomalean fungi) and roots of most of the economically interesting plants. Many scientific reports have confirmed that the correct functioning of this symbiosis confers a better nutritional and health status to plants, increasing crop yield and quality of fruits. However, extensive application of mycorrhizas in agriculture and environment restoration has been up to now hindered by the lack of adequate inoculum. Indeed, ex-vitro raised mycorrhizal inoculum often contain a small quantity of low quality fungal propagules, usually contaminated by other non-desired microorganisms. Moreover, most of the actually available mycorrhizal inocula contain non-autochthonous mycorrhizal fungi, therefore less adapted to soil conditions than indigenous populations, and which could disturb soil stability and microbial population biodiversity and dynamics.

After more than 20 years of scientific research at the Spanish Reasearch Council, the young SME MYCOVITRO S.L. exploits in exclusivity a patented procedure allowing to design and produce in vitro-raised mycorrrhizal inoculants, containing autochthonous fungi. These innovative products, commercialized under the generic name GLOMYGEL® have been tested under agronomic conditions for 4 years now, rendering important benefits in crops, which would be presented. This is a clear example of benefits obtained by applying biotechnological tools involving microorganisms to agriculture and environment.

	TOMATO		EGGPLANT	
Campaign	2009/2010	2010/2011	2009/2010	2010/2011
Total surface (Ha)	0,6	0,6	0,4	0,4
No. of plants	3,500	3,500	2,200	2,200
Variety	Torry	Torry	Cristal	Cristal
Production (Kg)	**40,000**	**60,000**	**29,400**	**32,000**
N fertilizer cost	29€	--	29€	--
P fertilizer cost	100€	--	90€	--
GLOMYGEL® cost	--	40€	--	20€
Total cost	**129€**	**40€**	**119€**	**20€**

The application of GLOMYGEL in tomato and eggplant industrial production resulted in an increased crop production, while reducing input cost.

Keywords ultrapure inoculants, mycorrhizas, in vitro production, autochthonous fungi, GLOMYGEL®.

Utilization of *Selenomonas ruminantium* for the prevention of rumen acidosis and suppression of methanogenesis in the rumen, and its genomic sequence and transcriptional analysis

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Backgrand

Beef and dairy cattle are usually fed high-concentrate diets, but feeding such diets sometimes causes excessive lactate accumulation in the rumen, which is reffered to as lactic rumen acidosis. Lactate produced in the rumen is secondarily fermented by lactate-utilizing bacteria, mainly *Megasphaera elsdenii*. However, when the rate of lactate production exceeds the rate of lactate consumption, lactate accumulates in the rumen. Therefore, it is desirable to augment lactate consumption for the prevention of lactate accumulation. *Selenomonas ruminantium* is also reported to utilize lactate in the rumen. In addition, some strains of this bacterium metabolize lactate to mainly propionate and succinate. This can bring about reduction in methanogenesis, because propionate is formed by accepting electrons. It is paticularly important to reduce methanogenesis because ruminal methanogenesis represents a loss of feed energy for the host animal, and methane adds to the greenhouse effect. Aiming at enhancing lactate utilization by *S. ruminantium*, the novel strain with high lactate consumption rate was isolated and examined the effect of the addition of this strain into the *in vitro* cultures of mixed ruminal microbes. Subsequently, the genome sequence was analyzed to design the Microarray probe.

Resalts

Approximately 100 colonies of lactate consumers were isolated from rumen samples from goats, sheep, or steers by using a growth medium containing lactate as an energy source. *S. ruminantium* with the ability to utilize lactate was present at levels of 15 ~ 40 % of the total lactate-using bacteria in these animals. The percentages of *S. ruminantium* tended to be slightly higher when concentrate diets were fed. The cell number of *S. ruminantium* that reduced nitrate and nitrite was 80 ~90 % of the total number of *S. ruminantium*, suggesting that most strains of *S. ruminantium* with the ability to utilize lactate are able to reduce nitrate and nitrite. The highest strain to utilize lactate was selected from the total isolated *S. ruminantium*. The introduction of *S. ruminantium* into the *in vitro* cultures of mixed ruminal microbes stimulates lactate utilization. When *M. elsdenii* was added at the same level, stimulation of lactate utilization was similar when *S. ruminantium* was added. Addition of *M. elsdenii* increased methane production, which suggested that H_2 production increased due to increased cell number of *M. elsdenii*. However, the addition of *S. ruminantium* greatly decreased methane production. The proportion of propionate was also increased by addition of *S. ruminantium*. Therefore, in order to decrease methanogenesis and to prevent lactate accumulation simultaneously, it is desirable to increase lactate utilization by *S. ruminantium*. A single shotgun pyrosequencing run of *S. ruminantium* genomic DNA using a Genome Sequencer FLX system (454 Life Sciences) resulted in 210,000 high-quality reads that were assembled using Newbler soft ware into approximately 100 contigs >500 bp long. The sequence was annotated using Annotation Engine (J. Craig Venter Institute) and manually curated using Manatee (http://manatee.sourceforge.net/). The draft genome comprises approximately 2,000,000 bases including approximately 2,000 predicted CDSs. A functional classification of CDS was categorized into 23. Microarray probes were designed based on the *S. ruminantium* genome. Comparison of the levels of mRNA from *S. ruminantium* cells grown lactate with those from cells grown glucose by Microarray analysis showed that several transcriptions were changed. The transcription level of FAD-FMN-containg dehydrogenase, which seems to be responsible to the conversion of lactate to pyruvate, was much higher in lactate grown cells. The levels of electron transfer flavoprotein and ferredoxin were also higher in lactate grown cells. However, the transcription levels of sugar transporters were decreased in lactate grown cells.

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Keywords genome sequence; methane production; rumen bacteria; ruminal acidosis; *Selenomonas ruminantium*

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Viral and bacterial infections associated with camel (*Camelus dromedarius*) calf diarrhea in North Province, Saudi Arabia

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Diarrhea and deaths in new-born camel calves, was noticed by veterinary investigators and pastoralist in Saudi Arabia to be very high. Hence, it is thought to be necessary to investigate this problem from the virological and bacteriological point of view. The role of pathogenic bacteria and viruses in six different towns of North Province (Al-Assafia, Arar, Domat Aljandal, Hail, Skaka and Khoa), in Saudi Arabia was studied. Survey was conducted in diarrheic camel calves aged 12 months or younger. In our study calf diarrhea was reported in184 out of 2308 camel examined clinically during one year, the prevalence of diarrhea was found to be 8.0 % in calves ranging from one month to one year. The result of analysis showed no significant difference in morbidity rate between the different area of study as well as in the two seasons studied. In the present study group A rotavirus and *Brucella abortus* were detected in 14.7% and 8.98% respectively using ELISA technique. On the other hand characterization and isolation of other pathogenic bacteria from fecal samples was adopted using different culture methods. E. coli was isolated from diarrheic calf camel (58.2%) 99/170 samples during dry and wet season. *Salmonella* spp, and *Enterococcus* spp were detected in 12 % and 8.8 % of the specimens, respectively. In this study toxin producing *E. coli* strains (Entero toxogenic *E. coli*) were isolated from 7 % from diarrheic camel, which indicates the strong correlation between the camel calf diarrhea and detection of enterotoxogenic E. coli. This study represented the first report for the detection of group A rotavirus and *Brucella abortus* antigen and antibodies in calf camels in Saudi Arabia. It is recommended that the disease should be controlled by vaccination in calf camels.

Keywords Diarrhea; group A rotavirus; brucellosis; *E. coli*; camel calves

Environmental, Marine, Aquatic Microbiology. Geomicrobiology

A microcosm study on the die-off response of the indicator bacteria, *Enterococcus faecium* and *Enterococcus faecalis*

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The transmission of waterborne disease is a matter of concern in public health. While it is impossible to measure every pathogen in waters, a surrogate that is easily measurable and can accurately indicate the health risk is necessary. Among numerous indicator bacteria, *Enterococci* is recommended by the US Environmental Protection Association (USEPA), World Health Organisation (WHO) and the European Union (EU) for marine beach water quality management for its persistency in saline waters and strong correlation with waterborne diseases. Despite being widely utilized across North America and Europe, the use of *Entercococci* is still in embryonic stage in Hong Kong. The reason is that little is known about the die-off response of the local species compared to their temperate cousin especially in the environmental condition in subtropical waters.

In this study, a microcosm experiment is set up to investigate the die-off response of local strains of *Enterococci* to the range of environmental conditions in Hong Kong. Four strains of *Enterococcus faecium* and *Enterococcus faecalis* were selected from survivors in effluent samples from three representative sewage treatment plants in Hong Kong (Sha Tin, San Wai and Stonecutters Island) before and after disinfection. The die-off rates (k) of *Enterococci* were determined from the rate of change of *Enterococci* concentration (following Chick's Law) under different combinations of light intensity, salinity, nutrient (BOD) and temperature conditions. The relative influence and interaction of the four factors are studied with 3D response surface analysis.

From the response surface analysis, it is observed that 1) Light intensity is the primary factor governing the elimination of *Enterococci*; 2) While *Enterococci* has high tolerant to salinity at low light intensity (almost the same die-off rate as in fresh water), its tolerance reduces significantly with the increase in light intensity; 3) Increase in nutrient concentration has positive effect for the survival of *Enterococci* and thus reduces its die-off rate; 4) Depending on situation, both positive and negative effect may come from temperature on the survival and die-off of *Enterococci*. Based on the results, a die-off equation is constructed for *Enteroccoci*, which will be useful for future water quality management.

Keywords water quality; bacteria indicator; *Enterococci*; $(GTG)_5$-PCR; die-off rate; response surface analysis

A preliminary study on nymph of *Ameletus inopinatus* (Insecta: Ephemeroptera) gut microbiology

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The major function commonly attributed to the microorganisms in the guts of such animals is the depolymerization and fermentative breakdown of the cellulosic or lignocellulosic component of their diet, which leads to degradation products Mayflies are hemimetabolous insects well known for their short-lived (ephemeral) adult phase, which usually lasts from two hours to three days. Life forms of mayfly larvae are diverse, but they fall into four broad categories: burrowing, flattened, swimming, and creeping. Immature specimens of *Ameletus inopinatus* are aquatic and can be found in the calmer reaches of small rivers or streams between stones and boulders. *Ameletus inopinatus* occurs in water without organic enrichment. In this preliminary study *A. inopinatus* samples were collected from Yarımca village (Bozdağ-Eskişehir-Turkey). Molecular, culture independent rRNA-based studies have been performed to characterize the archaeal and bacterial communities in their gut. Total microbial DNA extracted from the gut and 16S rRNA genes for Archaea and Bacteria domains were amplified by PCR. Amplified products were separated in denaturing gradient gel electrophoresis. Totally six bands were obtained with Bacteria spesific primers. The prokaryotic diversity inhabiting *Ameletus* gut was examined by also fluorescence in situ hybridization (FISH) and DAPI staining. It is concluded that community was dominated by Bacteria domain.

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Activity of extracellular enzymes in waters of eutrophic lake

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Variations in hydrolytic activity of six extracellular enzymes in subsurface waters in eutrophic Lake Chełmżyńskie were measured. The ranking of potential activity rates of the assayed enzymes was: lipase > aminopeptidase > phospatase > α-D-glucosidase > chitinase > β-D-glucosidase. It was found that the selected extracellular enzymatic activities were all characterized by a distinct seasonal variability and depended on the location of the research site (ANOVA, p-value < 0.0001). Significant differences in enzyme activity between different parts of the studied lake were demonstrated, with higher values of lipase and phospatase in spring and in autumn in the part of the lake near the town Chełmża, and lower values of these enzymes in spring and autumn in the zone of the lake far from the town. In summer, activity of phosphatase and aminopeptidase were significantly higher in the part of the lake far from the town than near the town. Enzyme chitinase showed higher activity in the zone of the lake far from the town both in summer and in autumn.

Keywords eutrophic lake; extracellular enzymes; planktonic bacteria

Acute Toxicity of Surfactants to Aquatic Organisms

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The massive use of surfactants in detergents and cosmetic formulations and their subsequent disposal in aquatic systems require surfactants to be as environmentally friendly as possible. This implies the need for low toxicity and biodegradable surfactants.

Many types of bioassays are available to establish the toxicity levels of compounds for aquatic organisms, but many of these tests are also time-consuming and not routinely applicable. Moreover, the use of higher organisms as test species may also be ethically undesirable. Although several bioassays using microorganisms have been described, most of the bacterial screening tests have been based on measurements of luminiscence, because they are rapid, reproducible, simple to use, cause no ethical problems, and are cost-effective. One of these methods is the wellknown Microtox® (Farré et al., 2001), using the luminiscent bacterium *Vibrio fischeri*. These assays provide a rapid response and, while not flawless, serve to compare different contaminants. In addition to providing assays with liophilized bacteria, these assays present high degrees of reproducibility, as demonstrated with interlaboratory tests.

In order to select efficient surfactants with a lower impact on the aquatic environment, another test organisms have been used: the fresh crustacea *Daphnia magna*, the microalgae *Selenastrum capricornutum* (freshwater algae) and the microalgae *Phaeodactylum tricornutum* (sea water algae).

The anionic surfactants assayed are commercial ether carboxylic derivative surfactants and the commercial dodecylbenzensulfonic acid. The non-ionic surfactants are fatty-alcohol ethoxylates, amine oxide based surfactants and nonylphenol polyethoxylate.

The use of these aquatic organisms allows to classify the surfactants according to the European Union Directive nº 67/548/ECC.

The results indicates that the toxicity values depend on the test microorganism. As a general rule, for all surfactants studied, *V. fischeri* is the most sensitive microorganism, compared with *D. magna* and microalgae, which is the least sensitive. As different test microorganisms have different sensitivity to the toxics, it is necessary to establish the most appropriate microorganism to classify the surfactant as very toxic, toxic, harmful or safe, in order to establish the maximum permissible concentrations in aquatic ecosystems.

Keywords *D. magna*; Microalgae; surfactants; toxicity; *V. fischeri*

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European Economic Community (EEC). Council Directive of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (67/548/EEC)

Arbuscular mycorrhizal fungi in heavy metal contaminated soils

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Environmental and health problems caused by excessive accumulation of metals in soils are leading to the search for new technologies for soil remediation. Phytoremediation, a sustainable and inexpensive technology based on the removal of pollutants from the environment by plants, is one of the most promising technologies. However, metal phytoextraction can be influenced by soil microorganisms living in intimate association with plant roots. Among soil microorganisms, arbuscular mycorrhizal (MA) fungi are the only ones providing a direct link between soil and roots, and are, therefore, of great importance in heavy metal availability and toxicity to plants.

AM fungi, belonging to the phylum Glomeromycota, are soil microorganisms that establish mutualistic symbioses with the majority of higher plants. They colonize the root cortex biotrophically and develop an external mycelium which overgrows the soil surrounding plant roots. AM fungi are present in almost all habitats and climates, including disturbed soils and those contaminated by heavy metals. The extensive extraradical hyphal network produced by these fungi allows the plant to access a great volume of soil, leading to the enhancement of plant nutrient uptake. Besides promoting plant growth, AM fungi can enhance plant tolerance to environmental stresses, including heavy metal toxicity.

The significance of AM fungi in soil remediation has been widely recognized. Buffering heavy metal-stress by AM fungi had been attributed at least partially, to selective immobilization of the metal within those root tissues containing fungal structures or to the high metal sorption capacity of the extraradical mycelium. Despite the significant role of AM fungi in plant interactions with soil metals and the ubiquity of AM fungi, only recently some progress has been made towards understanding the cellular and molecular mechanisms used by AM fungi to control heavy metal homeostasis and to avoid their toxicity.

The aim of the present work was to study the mechanisms evolved by AM fungi to survive in heavy metal contaminated soils. Our data show that both avoidance and compartmentalization strategies are used by these microorganisms to avoid uncontrolled accumulation of heavy metals in the cytosol and to grow in contaminated environments. Avoidance mechanisms mainly include binding of the metal to the fungal wall and immobilization in the soil by fungal exudates. Through the activity of specific metal transporters, the excess of metal in the cytoplasm is translocated to subcellular compartments, mainly vacuoles, where it would cause less damage. At the level of the fungal colony, AM fungi have also evolved compartmentalization strategies based on the accumulation of the metal into specific fungal structures, such as extraradical spores and intraradical vesicles. In addition to the avoidance and compartmentalization strategies, AM fungi have also evolved mechanisms to combat the oxidative stress produced by heavy metals or to repair the induced oxidative damage. The potential of AM fungi for enhancing phytoremediation of heavy metal contaminated soils will be also discussed.

Acknowledgments: This work was funded by the Spanish Ministry of Science and Innovation (Project AGL2009-08868)

Keywords arbuscular mycorrhizal fungi, bioremediation, Glomeromycota, heavy metals

Arsenic Resistance in an Extremophilic Yeast: a Cytological and Molecular Approach

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Metal resistant microorganisms are often associated with acidic environments as low pH promotes heavy metal solubilization. A new and unique acidophilic yeast identified as*Cryptococcus* sp. was isolated from two distinct acidic environments: acid mine drainage in Portugal (with high heavy metal content) and a volcanic river in Argentina (low metal content). This new species is the only acidophilic yeast known to date and both analyzed strains – one from each environment – showed high resistancelevels to several heavy metals, despite their different ecological background. Assessment of the resistance mechanism(s) in these *Cryptococcus* sp. strains is of most importance, as it may elucidate some functional aspects of the acidophilic eukaryotes.

Arsenic is a heavy metal toxic for most organisms in very low concentrations, and tolerance for this metal varies widely amongst microorganisms. Growth experiments in different arsenate (As(V)) concentrations allowed determination of minimum inhibitory concentration(MIC) for both tested strains.The observed MIC values show that these yeast strains tolerate up to seven times more As(V) than the model yeast *Saccharomyces cerevisiae*.

Known mechanisms for arsenate resistance in microorganisms include thiol-mediated detoxificationand efflux pumps. Thiol peptides are described as being involved in numerous cell stress-related responses, including oxidative stress. The possibility of involvement of a thiol-mediated detoxification mechanism was assessed in both strains by analyzing thiol accumulation in cells grown with and without arsenate. To do so, these differentially growncells were incubated with a thiol-specific fluorescent probe (5-chloro-methyl fluorescein diacetate– CMFDA) and observed under fluorescence microscopy. Differential cell staining would unveil the involvement of thiol molecules in arsenate resistance and would suggest cytoplasmic or vacuolar accumulation of thiol-metal complexes. However, no significant differential stainingwas observed, suggesting that these strains do not accumulate arsenate inside the cell, or that metal accumulation is not the main arsenate resistance mechanismresulting in thiol-metal complexes bellow detection limit.

Metal efflux by plasmatic membrane pumpsis also described as a generalized metal resistance mechanism, in particular for arsenic.Homologous genes responsible for arsenic efflux (*ars*genes) were found in several microorganisms, including bacteria, archaea and yeasts (namely *Cryptococcus neoformans*). In this metal resistance mechanism, the energy-dependent efflux is mediated by the *arsB* gene product (efflux pump)thatmay be associated with an ATPase (*arsA*)that allows a more efficient efflux and, therefore, a higher resistance level. Since PCR detection of *arsA* and *arsB* with designed primers based on *C. neoformans* sequences was negative, a dot-blot hybridization strategy was applied, using PCR DIG-labeled probes from *C. neoformans* and genomic DNA from both selected strains.Positive results were observed pointing to the presence of homologues of *arsA* and *arsB* genes in *Cryptococcus* sp.. Isolation, sequencing and transcriptional analysis by real-time PCR of *ars*-like genes must be performed to further assess their role in the arsenic resistance of*Cryptococcus* sp..

Keywords Extremophilic yeasts; Arsenic resistance mechanisms; Fluorescence microscopy

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Assessment of Waterborne Pathogenic Bacteria in Domestic Greywater Systems

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Recycling greywater (GW) represents the largest potential source of water savings in domestic residences. The aim of this project was to detect, identify, and monitor bacterial pathogens in treated (not disinfected) GW effluent and the irrigated soil. GW treated by Recirculating Vertical Flow Constructed Wetlands (RVFCW) was collected six times over the course of a year from three households as well as soil samples from their respective irrigated yards. We then used microbial culture dependent and independent methods to evaluate the pathogen load in each sample. The GW samples and irrigated soil were tested for *Campylobacter jejuni, Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi, Shigella* spp., *Staphylococcus aureus* and *Vibrio Cholerae*. Our results indicate that the culture independent methods are more effective and reproducible in the detection and enumeration of bacteria, moreover, the bacteria found in GW matched the ones found in the irrigated soils. However, GW may not be the sole contributor to the presence of the soil pathogenic bacteria as control freshwater irrigated soil contained the same strains, albeit at different concentrations.

Keywords Greywater; pathogens, irrigation, soil, bacteria

Bacteriophages in Agriculture: Aerial Control of a Plant Pathogen in the Orchard

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Agricultural applications of bacteriophages for the control of plant diseases date back to the early part of the twentieth century. We have initiated a program with the goal of developing a bacteriophage-based biological control agent for fire blight. The infection and destruction of commercial apple and pear cultivars by *Erwinia amylovora* has become a global problem. Apple and pear trees are most susceptible to the fire blight pathogen during the open bloom period. The pathogen multiplies on the pistils and free water washes the bacteria into the hypanthia. Under optimal weather conditions ingress of the pathogen into inner tree surfaces occurs and results in the death of the blossom, shoot, branch and tree. Disease control programs focus on decreasing the bacterial populations in the flower. The search continues for a biological agent with high efficacy.

The initial project goal was to isolate, purify and identify a diverse collection of *Erwinia* spp. phages. Soil samples from diseased orchards and aerial portions of infected trees served as sources of the bacteriophages. To obtain a heterogeneous phage collection the initial enrichment process used a mixture of six bacterial isolates. The resultant AAFC Vineland Phage Collection contains approximately 50 isolates. Restriction fragment length polymorphisms (RFLPs) and transmission electron microscopy were used to characterise the isolates. The collection contains six distinct RFLP groups of tailed phages belonging to *Myo*-, *Sipho*- and *Podoviridae*. The phages display a broad host range when tested against a collection of *E. amylovora* wild type isolates from North America and Europe.

The major hurdles in aerial applications of phages were ultra violet light and dry orchard conditions that were deleterious to the bacteriophages. The solution to the problem was the use of an orchard epiphyte, *Pantoea agglomerans*, which would serve as the phage carrier. Our phages also showed a very broad host range among 235 wild type isolates of *P. agglomerans* and many should be useful carriers.

A forced pear flower bioassay was developed to screen for the biological control activity of the 50 phage isolates. The carrier served a dual role acting as “feeder” for the phages and as a biological control agent that directly controlled the pathogen. Bioassay data provided critical concentration and timing data that were transferred to the field trials and were used to optimise these parameters. Field studies showed that the control afforded by phage-carrier treatments was not statistically different from that afforded by streptomycin, which is the most effective treatment for the prevention of blossom blight. The population dynamics of the phage, carrier, and pathogen were monitored by real-time PCR over the course of selected treatments. In treatments exhibiting a significantly reduced incidence of fire blight, the average blossom population of *E. amylovora* had been reduced to pre-experiment epiphytic levels. An average phage population greater than 1×10^5 PFU/blossom at the time of pathogen arrival was required to significantly reduce the chance of *E. amylovora* infection.

We are currently looking at mechanisms by which bacterial resistance to the phages may develop in order to develop strategies to circumvent this potential problem.

Keywords biopesticide, *Erwinia amylovora*, *Pantoea agglomerans*

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Behavior of Saccharomyces cerevisiae UE-ME$_3$ in presence of diuron at beginning of exponential phase

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The diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, is an herbicide used on autumn-winter crops, due to its ability to block the chloroplast electron chain at level of photosystem II. Although most of the living beings that contact with diuron are heterotrophic, this shows high toxicity for them, since it can also block the respiratory chain, behaving as ROS inductor. This herbicide is classified as POPs, because the chemical and biological degradation at ground level is very low. The main objective of this study was to evaluate the effect of this phenylurea in *Saccharomyces cerevisiae*, at beginning of exponential phase, a early stage of cell growth.

S. cerevisiae UE-ME$_3$ grown in YEPD medium until the exponential phase were inoculated in the absence and presence of 5, 25, 50, 75 µM diuron and allowed to grow for 200 min. Samples of each culture after lysis by sonication were used to obtain the supernatant and the pellet post-12000 *g* that were used for CAT A enzyme activity determination by spectrophotometry, glutathione, MDA and ROS content by fluorescence and GR, GPX, G6PD, CAT T enzymatic activities determination by spectrophotometry [1, 2, 3, 4, 5, 6, 7, 8, 9].

The results show that cells exposed to 5 µM diuron present a significant increase in MDA, GSH and GSSG content. Also it was detected a significant decrease of cytoplasmic redox status estimates by GSH/GSSG ratio in cells grown in the presence of 50 µM diuron. With respect to the glutathione reductase were not detected significant changes in any of the chosen situations for this study. However, it was observed a significantly decrease of G6PD and GPx enzyme activities, in cells exposed to 50 and 75 µM diuron, whose lack of NADPH availability, probably block the glutathione cycle. In addition CAT T activity presents also a significant increase in cells grown in 50 µM diuron, an answer that seems compensates by the drop of GPx activity. These facts suggest an active process of cell death in *S. cerevisiae* grown in the presence of 50 µM diuron. There was also observed a significant and dependent increase of cytoplasmic ROS, MDA level and CAT A activity. This profile response points us a main role of peroxisomal lipid oxidation, in cells grown in presence of 50 and 75 µM diuron that can trigger cell death by eventual energy constraint.

Keywords: diuron; *Saccharomyces cerevisiae*; antioxidant power

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Biocontrol of bacterial pathogen by cyanobacteria in *Arabidopsis*: Are cytokinins involved?

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Cyanobacteria constitute an extremely large group of organisms comprise most of the world's biomass. Beside their widely use in biofertilization of crop plants, some attention has also been devoted to their role in plant immunity. However, biocontrol by cyanobacteria has been attributed to the ability of these organisms to produce antibacterial and antifungal compounds. Here, we found that cyanobacteria promote resistance of *Arabidopsis* against hemi biotrophic pathogen, *Pseudomonas syringae* pv tomato DC3000, by modulating plant's cytokinins. Exogenous application of cytokinins mimicked the activity of cyanobacteria. Several folds increase in the accumulation of camalexins was recorded *Arabidopsis* tissues supplied with cyanobacterial or exogenous cytokinins. Enhanced levels of camalexins and activation of *Pr1* in inoculated leaves was the key to restrict the growth of the pathogen as shown by GFP assay. Introduction of auxins (IAA and 2,4-D) to the system nullified the effect of cytokinins as well as cyanobacteria. It may be concluded that *Chroococcidiopsis* sp. Ck4 controls the growth of pathogen in *Arabidopsis* by increasing cytokinins concentration associated with enhanced levels of camalexins and *Pr1*, which can be reversed with exogenous auxins.

Key words: Camalexins, Cytokinins, *Chroococcidiopsis*, IAA and *Pr1*

Biodegradation of Acetaminophen by Microbial Consortium of Domestic Sewage Sludge

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The present study aimed at assessing the potential of microorganisms originating from sewage sludge to degrade Acetaminophen, a pharmaceutical compound known by general name Paracetamol. Paracetamol is considered as a pollutant with potential ecotoxic effects. The biodegradation studies were conducted in batch mode using both cell suspension and activated sludge biomass as inoculums. Initial paracetamol concentration studied was 10 mg/L. At the end of the incubation period of 168 hours, 90% removal of paracetamol was observed in test set with cell suspension as inoculums and 73% removal of paracetamol was observed in test set with activated sludge biomass as inoculums. Present study indicates that activated sludge utilizes paracetamol as sole source of carbon.

Keywords*:* Paracetamol; biodegradation; activated sludge; carbon source

Bioemulsifier produced by marine hydrocarbonoclastic bacteria (MHB) modulate the expression of *Aeromonas salmonicida* virulence factors and induce the immune response in *Oncorhynchus mykiss*

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Aeromonas salmonicida is a fish pathogen that can cause hemorrhagic septicemia, furunculosis, fin rot and soft tissue rot in salmonids and non-salmonid fish, producing important economic loss to the aquaculture factory. The virulence observed on *A. salmonicida* is due to the expression of quorum sensing (QS) and not QS related virulence factors. Between QS related virulence factor, there is the expression of a serine protease enzyme, witch activates toxins like aerolysine and glycerophospholipid cholesterol acyltransferase (GCAT). Among QS non-related virulence factors, there is the expression of lipase, lateral flagella and nuclease. Since these virulence factors have an active role on *A. salmionida* infection, the gene modulation of their expression is a potential target for infection prophylaxis and treatment. In this context, this work studied the relationship of this pathogen with autochthonous microorganisms of marine ecosystems, determining the existence of microbial competition mediated by the modulation of virulent behavior. The autochthonous microorganisms are marine hydrocarbonoclastic bacteria (MHB) isolated from inter-tidal ponds of Valparaíso shore. The MHB were selected by their ability to produce biosurfactants (BS) during growth on sulphur heterocyclic hydrocarbons as the only carbon source. A total of 37 strains of MHB were isolated, characterized and identified by physiological, metabolic, biochemical and molecular assays. Surface-active properties of BS were evaluated by surface tension and emulsifier activity. Microbe-microbe and microbe-fish interactions were evaluated using Quorum Sensing biosensors, cells fight-challenges against fish pathogens and by the immune response of *Oncorhynchus mykiss*. The effects against the pathogen *Aeromonas salmonicida* were evaluated by qRT-PCR of virulence transcripts obtained from *A. salmonicida,* exposed to different concentrations of BS or viable cells of the selected MHB. The immune response of *Oncorhynchus mykiss* was evaluated by TNF-α and IL-1β. The obtained results show that two strains of MHB produce an extra cellular BS with emulsifier and biological features, able to affect the expression of virulence factors in *A. salmonicida* and to induce the immune response in trout (*Oncorhynchus mykiss)*. The selected strains of MHB were identified as member of *Cobetia* genus, and are featured by the ability to produce a surface-active extract that reduces the surface tension of water from 76,0dynes/cm to 57,0 dynes/cm and present an Emulsification Index (E24) of a 70%. The bacterial cells of MHB and/or their extract are able to modulate the expression of aerolysine (*aer*), GCAT (*gcat)*, nuclease (*nuc*), lateral flagella (*laf*), serine protease (*ser*) and lipase (*lip*) genes in *A. salmonicida*. The expression of *gcat* and *laf* was ten times lower that in unexposed *A. salmonicida*. *aer* and *ser* expression levels were six and three times lower, respectively, while *nuc* and *lip* expression showed a decrease near to 80 %. The immune parameters were IL-1β and TNF-α, and there were evaluated through indirect ELISA assay. In *O. mykiss* exposed to BS produced by MHB, the expression of IL-1β was a hundred percent higher than unexposed fish. Project F-D07I1061 funded by Fondef Conicyt, Government of Chile.

Keywords hydrocarbonoclastic; biosurfactants; fish-pathogen

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Bioleaching of metals (Cu, Fe and Ag) from chalcopyrite ore by *Acidiphile* group of bacteria

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Bioleaching is an economical method for the recovery of metals from low-grade ores that requires low investment and operation costs. Furthermore, it is generally more environmentally friendly than many physico-chemical metal extraction processes. The main goal of this work was to determine and optimize the bioleaching efficiency for seleted metals (Cu, Fe and Ag) from chalcopyrite ore by chemolithotrophic bacteria, namly, *Acidothiobacillus ferroxidans* (DSMZ 271)*, Leptospirillum ferroxidans* (DSMZ 882) and *Acidothiobacillus thiooxidans* (DSMZ 272). The copper ore (chalcopyrite) samples were obtained from a mining industry. The effect of iron substrate in chalcopyrite on the bioleaching of Cu and Ag was also investigated. Abiotic leaching of the three metals using organic acids (citric and oxalic acids) and mineral acids (sulfuric acid, nitric acid and hydrochloric acid) was studied at the concentrations of 10, 50, 100 and 200 mg/L, and compared with biotic leaching. Results showed that Cu and Fe leaching by *A. ferrooxidans, A thiooxidans* and *L. ferroxidans* were 85%, 38%, 98% and 5%, 10%, 50% , respectively. The leaching efficiency of these three isolates for Ag was 5%, 40%, and 6%, respectively. Bioleaching studies showed that *L. ferroxidans* had the higher leaching efficiency for Cu and Fe in the chalcopyrite ore whereas for silver the leaching efficiency by *A. thiooxidans* was higher. The higher extraction of Cu and Fe by *L. ferrooxidans* was due to the formation of Fe(III) in the solution phase which facilitated the dissolution of Cu from the chalcopyrite under acidic conditions. The maximum microbial leaching efficiency (98%) was obtained in iron-containing medium, 20-40 mesh ore sizes, at pH 2.2. Overall, bioleaching resulted in higher metal extractions compared to abiotic controls. *L. ferroxidans* was found to be most suitable for the efficient extraction of Cu anf Fe from the chalocopyrite ore. As for Ag, work is currently in progress for identifying the most suitable bacterial isolate. Mechanistic insights into the bioleaching process will be discussed.

Keywords Bioleaching; Chalcopyrite; Chemolithotrophic bacteria; Copper; Iron; Silver

Biotechnological potential of bacteria isolated from native fruits of the Cerrado in Minas Gerais- Brazil

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The Brazilian has been constantly anthropogenic, mainly due to agricultural expansion affecting the macro-and microbiota of this biome. In this biome there is still little known microbial biodiversity. The aim of this work was to select and cellulolytic bacteria antagonistic to toxigenic fungi present on the surface of native fruits of the Cerrado as *Psychotria hoffmannseggiana*. One hundred and sixty isolates were characterized and tested for their ability and enzymatic activity antagonistic to *Aspergillus carbonarius*. Fifteen percent of the isolates showed cellulolytic activity and 37% were able to inhibit the growth of *A. carbonarius*. Thus, it was possible to verify the biotechnological potential of bacterial isolates naturally present in fruits native Cerrado.

Keywords: bacteria, fruits of the Cerrado, cellulolytic activity, antagonic activity

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Biotoxicity assessment of heavy metals in soils by solid-phase applications of microbial biosensors

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The environmental problems caused by industrial and agricultural pollution have increased the demand for the development of toxicity assessment methods. Although traditional analytical techniques can identify and quantify specific pollutants, it fails to distinguish between contaminants that are available to biological systems and contaminants that exist in the environment in unavailable forms. Biological assays are able to complement them by showing the bioavailability and ecotoxical effects of compounds.

In recent years, reporter-gene constructed bacteria (microbial biosensor) for specific pollutants have been successfully applied for heavy metals. The stress induced by toxic compounds can cause disruption to the metabolism of organisms. So once changes occur, it is essential to quantify this impact. In this study, the bioluminescent biosensors produce light which correlates with the dose present in presence of target analyte (Cu and Zn). Understanding the speciation of heavy metals and its relationship with biological responses is an important factor for the soil assessment.

Bioavailability is the major factor controlling the toxicity of heavy metals in soil. Until now, our assessment of bioavailability has been restricted to measuring the aqueous soluble fraction of samples and the biosensor responses. This fails to consider the complex environment of the soil solid phase which is likely to host most of the labile and bioavailable pollutants and be the more dynamic in both space and time to perturbations.

The deployment to soil will be likely to be coupled with an approach such as “rhizon sampler” where the solution will be extracted from the soil and then assimilated onto the solid phase extraction (SPE) surface. In this study, a range of novel solid phase devices were compared to assess the reproducibility of given assays and their relationship with aqueous phase assays.

Solid phase devices were prone to considerable variability while physical syringes enabled sample equilibration and higher consistency. The performance of the biosensor was not solely determined by the extraction procedure used but was influenced by the presence of a suitable osmotic buffer and the solubility of the heavy metals. This approach was complemented by the application of inducible biosensors for metals (Cu and Zn).

In general, the toxicity and bioavailability of heavy metals in solid phase was lower than in aqueous phase demonstrating the need to measure both phases and develop a relationship between them and the likely mobility and partitioning of the target analytes.

Future research will consider the bioavailability of hydrocarbons and in addition to assessing its related toxicity. This will be further inferred to a reliable method of the potential for biodegradation and bioremediation.

Keywords biosensors; biotoxicity; heavy metals; solid-phase applications

Characterization of the 90-kb plasmid of *Rhodococcus erythropolis* CCM2595

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The strain *Rhodococcus erythropolis* CCM2595 is an efficient degrader of phenol, hydroxybenzoate, p-chlorophenol, aniline and other aromatic compounds. A large circular plasmid (90,224 bp), designated pRECF1, was discovered in this strain by sequencing its complete genomic DNA. 94 putative open reading frames (ORFs) were identified on pRECF1 DNA and functions were assigned to 45 ORFs. Sequence analysis revealed various levels of similarities of products of 74 pRECF1 ORFs to those encoded by other plasmids of rhodococci (e.g. pREC1 and pREL1 from *R. erythropolis* PR4, pBD2 from *R. erythropolis* BD2 and pKNR from *R. opacus* B4). However, the organization of the pRECF1 genes is unique. This documents that the *Rhodococcus* plasmids possess mosaic structures, which evolved by horizontal transfer of genes.

The pRECF1 *rep* gene codes for a replication protein showing highest similarity to the Rep proteins of large circular plasmids pREC1 and pSOX of rhodococci that are related to the Rep proteins of mycobacterial plasmids classified into pLR7 family. The products of *parA* and *parB* genes are most probably involved in plasmid segregation and stable maintenance. The presence of genes coding for homologs of a relaxase and the MobC and TraG-like proteins of conjugative plasmids from the strains of genus *Arthrobacter* suggests that pRECF1 is a conjugative plasmid. The 26-kb region of pRECF1, containing 31 ORFs, is homologous with the regions of large linear *R. erythropolis* plasmids pREL1 and pBD2. This region contains the genes involved in resistance to heavy metals and arsenic and in biogenesis of cytochrome *c* as well as the genes coding for transporters of still unknown functions. The arsenic resistance operon consists of three *arsC* genes coding for arsenate reductases, *arsA* and *arsB* genes encoding arsenite-translocating ATPase and arsenite export protein, *arsO* coding for putative FAD-dependent oxidoreductase, *trxB* encoding thioredoxin reductase and two regulatory genes, *arsR* and *arsD*, coding for putative repressors.

In contrast to other plasmids of rhodococci, no genes involved in catabolism of organic compounds were found on pRECF1. The high capacity of the strain *R. erythropolis* CCM2595 to degrade various organic pollutants is thus based exclusively on chromosomal genes.

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Keywords *Rhodococcus erythropolis*; large circular plasmid

Characterization of the *Legionella* and the non-*Legionella* bacterial community composition in a biological treatment plant

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Legionella is the etiological agent of Legionaires' disease (LD) and the non-pneumonic legionellosis Pontiac fever. An infection results from inhalation of aerosols containing the bacteria. In 2005 and 2008 three outbreak of LD were reported in the Fredrikstad/Sarpsborg community, Norway (3), and the two aeration ponds of the biological treatment plant at Borregaard Ind. Ltd. were identified as the main amplifiers and primary disseminators of the outbreak *Legionella pneumophilia* strains ST15 and ST462 (4). Biological treatment plants are used to degrade organic substances in wastewater from wood refinement processes, and *L. pneumophilia* serogroup 1 (SG1) has been detected in corresponding aeration ponds at concentration levels up to10^{10} CFU/L (4). *L. pneumophila* was identified up to distances of 200 m downwind from the ponds at the biological treatment plant. The highest concentration level of viable legionellae was identified directly above the aeration ponds (1). Air sampling studies at the plant indicated the presence of respirable aerosol particles of a medium size of 3.5 µm estimated to contain about 147 *Legionella* cells per aggregate. Additionally, 44 taxonomic different bacterial genera were measured above the aeration ponds indicating that the aerosol might contain a mixture of bacteria including *Legionella* (2). As a consequence of the outbreaks and the high concentration of legionellae, the aeration ponds were shut down in September 2008, in which this process lasted for four months (September to December 2008). From November 19, 2008, a biocide was added the system. The aim of the present work was to analyze the *Legionella* and the non-*Legionella* bacterial communities in the aeration ponds prior to and during the shut down process.

Frequent analysis using plate counting on GVPC agar showed a decrease of total legionellae cells and *L. pneumophila* (SG1 and SG2-14) from 10^{10} to 10^{6} CFU/L during September to December 2008. Quantitative real-time PCR of *L. pneumophila* (mip) showed a similar trend. The *Legionella* spp. community was characterized in samples from September 4-16 2008, using nucleotide sequencing of the *rnpB* gene of *Legionella*. Species detected included *L. pneumophilia*, *Legionella oakridgensis* and *Legionella londiniensis*. The non-*Legionella* bacterial community was investigated by using 16S rRNA sequencing and by microbiological growth methods. Genetic fingerprinting (DGGE) was used to monitor potential changes in the bacterial community during the shut down process, and then samples from September 4 and 25, November 3 and December 1 were chosen as selected dates for extensive analysis. A clone library of the amplified 16S rDNA fragments was developed and a total of 400 clones were analyzed. In general, the filamentous bacteria belonging to the phylum *Chloroflexi* were present in the clone library from September mainly. These bacteria are supposed to be important in wastewater treatment, however, only a few species have been cultured. At November 3 the *Proteobacteria* was dominating the aeration ponds (clone library). *Proteobacteria* is a metabolic diverse group important in several degradation processes in nature including nitrogen metabolism. A total of 63 *Pseudomonas* isolates were detected by growth analysis. *Pseudomonas* species are important in the nitrogen cycling in the environment; however, potential opportunistic pathogens occur. A decrease in *Pseudomonas* spp. similar to the decrease of *L. pneumophilia* was observed using real-time PCR.. This work showed that during the shut down process the concentration level of *Legionella* spp. decreased followed by a decrease of *Pseudomonas* spp. and a change in the composition of the bacterial community in the aeration ponds.

This work contributes in enhancing our knowledge about the complex bacterial community present in biological treatment plants which might have an impact on the bacterial composition of the aerosols emitted from such plants and on the proliferation of *L. pneumophilia* .The overall aim is to reduce the dissemination and transmission of pathogenic *Legionella* species from aerosolizing devices to reduce the exposure risk to *Legionella* containing aerosols.

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Community-Driven Anaerobic Chromate Reduction by Yellowstone National Park Hot Springs Microorganisms

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Industrial practices including electroplating, leather tanning, pigment manufacture, corrosion inhibition, and fungicide production generate large quantities of chromium-laden wastewater that must be treated before discharge. Hexavalent chromium, or Cr(VI), is acutely toxic, mutagenic, teratogenic, carcinogenic, and is highly mobile in the environment, mainly due to its soluble nature. Cr(VI) can be reduced to non-water soluble trivalent chromium, becoming 100-fold less toxic than Cr(VI). Cr(VI) reduction has been described in many microorganisms from the domains Eubacteria and Archaebacteria, however, no methanogenic Archaebacteria have demonstrated Cr(VI) reduction. Interestingly, thermodynamics predicts that methanogens will preferentially utilize Cr(VI) as a terminal electron acceptor over CO_2, thereby contributing to community Cr(VI) reduction. Soil, water, and biomat samples from Yellowstone National Park were used to construct anaerobic microcosms under methanogenic and Cr(VI) reducing conditions, with H_2 as the electron donor. Solfatara Creek and Nymph Creek-1 samples exhibited rapid chemical reduction, masking any biological chromium reduction. After a 4 month acclimation period samples from five remaining samples were re-amended as necessary with ~1mM Cr(VI). These sites included a second Nymph Creek sample, and samples collected from Octopus Spring, Queen's Laundry, a thermal feature near White Dome, and Narrow Gauge terrace. The most successful sample, Narrow Gauge, was able to reduce 1448 uM Cr(VI) in 14 days and 1230 uM Cr(VI) during the second 14 day period under Cr(VI)-reducing conditions. Similarly, Nymph Creek-2 reduced 986 uM and 1031 uM Cr(VI) in the same periods. Inhibition of sulfate-reducing bacteria (SRBs) with molybdate resulted in decreased Cr(VI) reduction by all samples. Narrow Gauge, Nymph Creek-2, Octopus Spring, a feature near White Dome, and Queen's Laundry reduced a total of 1393 uM, 1880 uM, 327 uM, 569 uM and 132 uM Cr(VI), respectively. In two samples, Nymph Creek-2 and Narrow Gauge, inhibition of SRBs did not impact Cr(VI) reduction as greatly as inhibition of methanogens by BESA. In these samples 1385 uM and 2086 uM Cr(VI) were reduced by Nymph Creek-2 and Narrow Gauge, respectively. It is often reported that SRBs reduce most of the available electron acceptors within communities, yet these results suggest methanogens may play a role in community driven anaerobic Cr(VI) reduction. While CH_4 has not yet been measured, it is expected that CH_4 will only be produced when Cr(VI) is absent.

Keywords hexavalent chromium, metal reduction; hot springs

Comparative Evaluation of Two Bioreactors for Bioleaching of Cu, Fe and Ag from chalcopyrite by *Leptospirillum ferrooxidans*

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Bioleaching is an emerging, environmentally-benign technology with significant potential to add value to the mining industries. The main objective of the present study was to compare the performance of a continuous stirred tank reactor (CSTR) and an air-uplift reactor (ALR) with mesophilic iron oxidizing bacteria, *Leptospirillum ferrooxidans* (DSMZ 882), at different oxygen supply rates (100 L/h and 200 L/h) to acheive the maximum extraction of Cu, Fe and Ag from chalcopyrite ore. The bioleaching experiments were conducted under controlled operating conditions (pH 2.0, pulp density 2% and temperature 35^oC) for both the bioreactors. The leach solutions were periodically collected and analyzed for metal ion concentrations i.e., Cu, Fe and Ag using inductively-coupled plasma mass spectrometry (ICP-MS). The bacterial population was measured in the solution (free cells) using UV-Visible spectrophotometer and Epi-fluorescence microscope. The attachment behaviour of the isolate onto the chalcopyrite ore was examined based on scanning electron microscope (SEM) images. Results obtained pertaining to the recovery of the three metals in the bioreactors and the bioleaching effeciency for these metals from chalcopyrite ore will be discussed. Mechanisms involved in the bioleaching process will be elucidated.

Keywords: Bioleaching; Chalcopyrite; Air-uplift bioreactor; Continous stirred tank reactor

Comparison of Extended-spectrum-ß-lactamase (ESBL) carrying *Escherichia coli* from sewage sludge and human urinary tract infection

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Objectives: Extended-spectrum-ß-lactamase (ESBL) carrying enterobacteriacae are a increasing problem in hospital and community. Beside their human reservoir, ESBL carrying enterobacteriacae can also be found in the environment.

Different groups of ß-lactamase genes are the genetic basis of the ESBL mediated resistance.

In this study, ESBL carrying Escherichia coli from sewage sludge and human urinary tract infection were investigated for their resistance pattern and the occurence of six different ESBL gene groups.

Methods: A sample of 50 ESBL E.coli from sewage sludge collected from five different sewage treatment plants in the area of Styria between January and September 2009 and a sample of 50 ESBL E.coli strains from urinary tract infections, isolated at the Medical University Graz (Austria) in the same time period, were analysed.

Strains were screened for 5 ESBL gene groups (CTX-M, TEM, SHV, VEB, GES) by PCR, and the amplified genes were subsequently sequenced.

Further, strains were tested for resistance to 15 different antibiotics (except cephalosporins).

Results: With 37 isolates (74%) of ESBL E.coli from urinary tract infection and 22 isolates (44%) ESBL E.coli from sewage the CTX-M-15 gene was the most common ESBL gene. CTX-M-1 was identified in 11 (22%) strains in ESBL E.coli from urinary tract infection and 20 (40%) strains ESBL E.coli from sewage.

CTX-M-3 (3 strains) and SHV-15 (one strain) were also present in E.coli from sewage.

5 isolates had no positive PCR product for any of the tested ESBL gene groups.

53% of all strains were carrying, in addition to CTX-M, the non ESBL ß-lactamase TEM-1.

ESBL E.coli from urinary tract infection showed a higher resistance rate to most tested antibiotics as well as to combinations of antibiotics. For example, 32% of the ESBL E.coli from urinary tract infection and 14% of the ESBL E.coli from sewage were resistant to Gentamycin, 14%/0% to Amikacin, 82%/44% to Ciprofloxacin and 40%/8% to Ampicillin/ Clavulanic acid. ESBL E.coli from both sources showed similar resistance rate to tetracycline (56%/60%) and carbapenems (0%/2%).

Conclusions: CTX-M genes were the dominant ESBL group in the analyzed samples. All ESBL gene variants present in human urinary tract infection were also present in sewage sludge. In contrast, the resistance pattern to non cephalosporins differed between strains from the different sources.

Keywords Sewage sludge, E.coli, ESBL

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Complete sequence of the genome and two novel plasmids of *Methylocystis* sp. strain SC2 and analysis of genes involved in methane-oxidation

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Methanotrophic bacteria consume the greenhouse gas methane, thereby mitigating global warming. Our laboratory has recently shown that *Methylocystis* sp. strain SC2 possesses two isozymes of particulate methane monooxygenase (pMMO). The two pMMO isozymes are differentially expressed in response to changes in methane concentration. To investigate this phenomenon further, we currently perform a genomic and transcriptomic analysis of strain SC2. The genome of strain SC2 was completely sequenced and assembled (3.77 Mb). One major finding of the genome analysis is that strain SC2 harbours two novel plasmids of sizes 143 Kb and 225 Kb. The plasmids were completely sequenced and certain features of them lead us to speculate that they are specific to methanotrophs. The most interesting feature is the presence of a singly located copy of *pmoC* in the 143-kb plasmid. Strain SC2, in addition, possesses five *pmoC* copies in its genome, of which three copies are part of the *pmoCAB* gene clusters (*pmoC1a,b* and *pmoC2*) encoding the pMMO isozymes and two others are singly located copies. The translated amino acid sequence of the plasmid-borne *pmoC* showed greatest homology (85%) to the PmoC1a,b. All three singly located *pmoC* copies, regardless of whether located on the genome or plasmid, were transcribed during growth of strain SC2. Single copies of *pmoC* have been repeatedly found in type I and type II methanotrophs. Conclusive results on the functional role of the genome-encoded, isolated *pmoC* copies are not yet available. However, elucidation of the functional role of a plasmid-borne *pmoC* appears to be the more intriguing task.

Keywords: methane-oxidation, *Methylocystis* strain SC2, plasmid, *pmoC*

Conjugative transference of antibacterial resistance from bacteria belonging to microbiota associated to the culture of Chilean scallop larvae

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The larval culture of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819) is one of the most important commercial industries of Chilean mariculture. However, intensive conditions of larval culture have resulted in increasing problems of bacterial diseases, prompting the necessity of an intensive use of antimicrobials for treatment and prevention. This fact increases the possibility to select antibacterial-resistant isolates in the scallop culture environments and the risk to transfer this resistance to scallop and human pathogens by conjugation.

The aim of this work was to evaluate the *in vitro* transference of resistance to florfenicol (FFC), tetracycline (TET) and other antibacterials by conjugation from two strains of *Pseudomonas monteilli* (OX239, OX262), isolated from the scallop hatcheries, located in the Coquimbo Region in the North of Chile, to two strains of the fish and shellfish pathogen *Vibrio splendidus* (CK13, CK16), recovered from epizootic events occurred in Chilean hatcheries, and also to the laboratory strain *Escherichia coli* K-12. Experiments of resistance transference were carried out by mating assays in Tryptone Broth (TB). One mL of an overnight culture of donor strain was mixed with 9 mL of recipient strain in a 250 mL of Erlenmeyer flask containing 100 mL of TB and incubated overnight at 25ºC. Then, 100 µL aliquots were spread on Tryptone agar plates added with FFC (32 µg/mL) or TET (16 µg/mL) plus nalidixic acid (100 µg/mL) as selective antibacterials. Plates were incubated at 25ºC for 24 h and transconjugants were assayed for their antibacterial resistance profiles by disc diffusion test. As is shown in the table, the multidrug resistant strains of *P. monteilli* were able to transfer by conjugation the resistance to various antibacterial agents to *E. coli* K-12, and more interestingly, to the pathogenic strains of *V. splendidus*. The frequencies of transfer of FFC and TET ranged between $2.3x10^{-7}$ to $1x10^{-5}$, and $5.8x10^{-8}$ to $4.3x10^{-7}$ per recipient cell, respectively.

	Inhibition zone (mm)								
Mating pairs	**TET**	**CHL**	**FFC**	**SXT**	**FLU**	**CTX**	**AMP**	**TMP**	**NAL**
Donor OX239	0	0	0	0	9	19	0	0	21
Recipient E. coli K-12	25	27	25	33	20	31	21	5	0
transcongujant 1	0	7	0	0	7	20	0	0	0
Donor OX239	0	0	0	0	9	19	0	0	21
Recipient CK16	27	30	30	39	25	33	24	33	0
transcongujant 8	0	0	0	0	0	35	22	29	0
transcongujant 11	0	0	0	0	0	20	0	0	0
Donor OX239	0	0	0	0	9	19	0	0	21
Recipient CK13	30	27	29	22	25	25	14	20	0
transcongujant 25	0	0	0	0	16	21	0	0	0
Donor OX262	16	0	0	17	14	15	0	0	29
Recipient CK16	27	30	30	39	25	33	24	33	0
transcongujant 10	17	0	0	0	14	14	0	0	0

TET: tetracycline; CHL: chloramphenicol; FF: florfenicol; SXT: sulfamethoxazole/trimethoprim CTX: cefotaxime; AMP: ampicillim; TMP: trimethoprim; NAL: nalidixic acid

These results warn about the risk that this transfer of resistance may naturally occur in environments with high use of antibiotics, as it is happening in intensive aquaculture.

This work was supported by the grant FONDECYT 1090793 from CONICYT-CHILE

Keywords *Argopecten purpuratus;* Chile; antibacterial resistance; aquaculture

Correlation between spore-forming bacteria and physicochemical parameters in water samples from irrigated rice ecosystems of Southern Brazil

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In most of the rice field regions the water applied in irrigation systems comes from rivers and springs that permeate urbanized areas. This fact adds an item to the factors that contribute to diversity of components available in the water used for irrigation in rice areas, because anywhere with human presence there is a concomitant residual waters release. In aquatic ecosystems, where it is possible to include the irrigated rice fields, the microbial communities are described as potential degradation agents of anthropogenic molecules, as they are organisms with high adaptation versatility, which makes possible their persistence in these agroecosystems. Thus, the distribution patters are often discussed in the context of environmental factors such as temperature, pH, nutrients availability and carbon sources. These ecological factors influence microbial activities and develop an important role determining the spatial-temporal dynamic of microorganisms in natural environments. This study aimed to estimate the diversity of cultivable spore-forming bacteria in water samples from two rice areas in Rio Grande do Sul, Brazil, and relate them to the water physicochemical characteristics between the irrigated rice phases, during one agricultural year. The samples were taken at the Experimental Station from the Rio Grande do Sul Rice Institute, Cachoeirinha (29º55'30"S and 50º58'21"W) and at the Arroio Duro Dam perimeter in Camaquã (30º51'04"S and 51º48'44"W), in representative water sources from irrigation and draining channels, in the 2007/08 agricultural cycle. The 100mL water samples were filtered in 0.22µm porosity nitrocellulose membranes and stored at -20°C. After rehydration in saline solution, the bacterial growth was performed in Tryptone Soy Agar and Nutrient Agar at 30°C in the presence of free oxygen for approximately 24 hours. The physicochemical parameters evaluated in the water samples were: electric conductivity, pH, nitrogen, phosphorus, potassium and calcium. Bacteria growth in solid medium were characterized according to morphocytochemical parameters (Gram differential staining and phase contrast microscopy analysis), and were identified by sequencing after total DNA extraction and PCR amplification of the 16S ribosomal gene applying the initiators 27F (5'AGAGTTTGATCCTGGCTCAG3') and MH1 (5'**C**CTTGTTACGACTTCACCC3'). Results revealed 152 colonies isolated, with 13 catalogued taxa, being 84.2% identified at the species level and 15.8% at the genus level. From the total, stand out as the most representative taxa, in decreasing order of frequency: *Bacillus thuringiensis* (38.1%); *Bacillus* spp. (15.1%); *Lysinibacillus sphaericus* (13.1%); *Bacillus megaterium* (11.8%) and *Bacillus pumilus* (11.2%). The Canonical Correspondence analysis showed that the two first axes indicate the existence of short gradient and therefore bigger species dispersion. However, the concentration of the points along the center indicates that the environmental variables explained little about the variation found. Nevertheless, it is possible to infer that the electric conductivity and the nutrients nitrogen and potassium were the principal physicochemical factors that influenced the quantity of spore-forming bacteria communities. The similarity index regarding these bacterial species frequency was 84.2% between the irrigation channels in Cachoeirinha and Camaquã, and corresponded to 69.7% in the draining channel in Camaquã. This difference is mostly due to the lack of *Bacillus pumilus* in draining water samples from Camaquã areas.

Keywords Water; Irrigated Rice; Spore-forming bacteria; Taxonomy; 16SRNA

Degradation kinetics of the herbicide Propanil in a biofilm reactor

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The widely used herbicide (N-(3,4-diclhorophenyl) propanamide); (3,4-DCPA) or Propanil is inhibitory of photo-system II of plants. It is listed by the USEPA as slightly toxic; however, the partially degraded form of 3.4- dichloroaniline (3,4-DCA) is more toxic than the original molecule. Both compounds affect aquatic organisms and, in several countries, they have been found in superficial and ground waters. Thus, the development of processes for the 3,4-DCPA removal is of concern.

This work proposes the use of a microbial consortium, isolated from agricultural soils; to degrade both compounds in a biofilm reactor. The consortium was selected in an aerated column, packed with fragments of a porous volcanic stone (tezontle) and total volume of 900 mL. The column's liquid volume was 400 mL. Initially, the column was operated as a repeated batch-reactor. Once colonized the porous support by the microbial consortium, the reactor was operated in continuous regime. Air and a minimal salts medium, containing the herbicide (50 mg L^{-1}), were concurrently supplied through the packed column at various volumetric loading rates of propanil ($B_{V,DCPA}$ [mg L^{-1} h^{-1}]). For evaluation of the volumetric removal rates ($R_{V,DCPA}$ [mg L^{-1} h^{-1}]), and removal efficiencies ($\eta_{V,DCPA}$) = $R_{V,DCPA}$/ $B_{V,DCPA}$) of Propanil, several analytical methods were used. Spectrophotometric method (λ_{MAX} = 242 nm), liquid chromatography (HPLC), chemical oxygen demand (COD), and total organic carbon (TOC).

The $B_{V,DCPA}$ values were varied from 1.9 to 10 mg L^{-1} h^{-1}. The results obtained with this bioprocess show that the microbial community is able to use the herbicide as a carbon and nitrogen source. In any case, accumulation of the toxic and recalcitrant compound 3,4-DCA or any other aromatic byproduct was detected. The removal efficiencies, spectrophotometrically measured, varied from 98.5 to 100%. When $\eta_{V,DCPA}$ was determined by HPLC, TOC and COD methods, the values were 100, 99 and 97% respectively.

Keywords Propanil; 3,4-dichloroaniline, biodegradation, biofilm reactor

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Degradation of a mixture of two azo dyes and their intermediates by a bacterial consortium immobilized in a support of porous volcanic stone

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Azo dyes are generally regarded as xenobiotic compounds very recalcitrant to degradation, causing severe pollution of water bodies near textile industries. By azo-reduction of these compounds, aromatic amines are generated. In some cases, these byproducts could be more toxic and recalcitrant to degradation than the original azo dye.

It is common to find mixtures of dyes in the effluents from the textile industry. For this reason, the main objective of this study was to evaluate the ability of a selected microbial community to degrade a mixture of two widely used azo dyes, Acid Orange 7 (AO7) and Acid Red 88 (AR88), and its degradation products, the 4-aminobenzene-sulfonic acid (4ABS), 1-amino-2-naphthol (1A2N) and 4-aminonaphthalene-sulfonic acid (4ANS).

The biodegradation process was carried out in a continuous culture biofilm reactor, using a microbial community immobilized in a support of porous volcanic stone, fed with AR88 and AOA7 (50 mg L^{-1}, each) as the sole sources of carbon energy, nitrogen and sulfur. In the microbial consortium, five cultivable bacteria were isolated and identified; *Pigmentiphaga kullae*, *Labrys neptuniae*, *Kocuria* sp., *Bacillus* sp., and *Curvibacter gracilis*.

The laboratory-scale packed-bed reactor was operated in continuous steady-state regime, with volumetric loading rates of azo dyes ($B_{V,\ AOR}$) in the range of 4-85 mg L^{-1} h^{-1}. All runs were made at room temperature, maintaining an aeration rate of 1.5 L min^{-1}. Both azo dyes and their azo-reduction byproducts were determined by HPLC. In parallel, the removal of contaminants was also determined by chemical oxygen demand COD.

Within the $B_{V,AOR}$ values probed, the two azo dyes and their byproducts were efficiently removed. The volumetric removal rates of AO7 ($R_{V,AO7}$) were always proportional to the volumetric loading of the azo dyes supplied, with removal efficiencies close to 100 %, while the AR88 removal efficiency was 97%. For the 4-ABS, the removal efficiency was about 98 %. In any case, and perhaps by the instability of 1A2N, this byproduct was detected in the reactor's effluent. In the case of 4ANS, a slight accumulation was observed (5.3 mg L^{-1}); so its removal efficiency was around 90 %. This fact could explain why the overall removal efficiency of contaminants, measured as COD; was about 94.5 %.

With these results, it can be concluded that the bacterial community was able to degrade a mixture of two azo dyes commonly found in the effluents of the textile industry; and that the accumulation of aromatic amines did not affect the decolorization rate of these azo dyes.

Keywords: azo dyes, aromatic amines, Acid Red 88, Acid Orange 7, biofilm reactor

Degradation of sulfamethoxazole by pure strains isolated from an acclimated membrane bioreactor

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Antibiotics such as sulfamethoxazole are found at relatively high concentration in surface waters worldwide. Their presence, due to the possibility of spreading resistances is of great concern. Sulfamethoxazole has been shown to be especially difficult to remove in biological wastewater treatment plants, and results can be highly variable. Currently, only energy-intensive processes such as ozonation and methods based on it are able to completely transform sulfamethoxazole. Nevertheless, possible by-products of these treatments have not yet been tested for negative impacts on the environment. Until now, only two studies reported the biodegradation of sulfamethoxazole by pure strains. However, the apparent biodegradation rates remained below 30% in both cases. This evidences the need to isolate and identify bacterial strains able to biodegrade sulfamethoxazole to higher extent.

Several strains able to grow on sulfamethoxazole as a carbon and energy source could be isolated from a membrane bioreactor acclimated to sulfamethoxazole. Homogeneous suspensions of single isolates, and a consortium were tested for degradation of ^{14}C- sulfamethoxazole. Mineralization was detected by trapping the formed $^{14}CO_2$ in NaOH. Within few days, a significant amount of the total applied radioactivity could be recovered in the CO_2 traps, indicating a fast mineralization. To our knowledge, this is the first report evidencing mineralization of sulfamethoxazole in axenic bacterial cultures. Currently, metabolites of sulfamethoxazole generated by the strains are investigated.

A deeper understanding of sulfamethoxazole degradation by bacteria may contribute to the optimization of biological wastewater treatment plants for the efficient removal of pharmaceuticals.

Keywords sulfamethoxazole, antibiotic, biodegradation

Detection of biosynthetic gene sequences and antimicrobial activities from *Micrococcaceae* (Actinomycetales) isolated from marine sponges

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Marine biological diversity is thought to exceed that of the terrestrial environment. Marine ecosystems harbor indigenous and novel marine actinomycetes that are widely distributed among nearly all habitats. The discovery of new groups of marine bacteria from unexplored or underexploited habitats is believed to offer sources of potentially novel bioactive compounds.

Sponge-specific microbial communities have been reported to produce therapeutically important molecules. Sponges (*Porifera*) are among the oldest metazoan and are considered as microbial fermenters and as filter feeders. They are capable of lodging high bacterial population densities. These multicellular animals constitute an extremely rich reservoir for the isolation of a large diversity of actinobacteria, many of them representing still today a wide untapped source of secondary metabolites. This diversity of new chemical structures, with antibacterial, antifungal, antiviral and antitumor activities, is hypothesized to be a significant reservoir of new therapeutic agents.

We surveyed 44 strains isolated from marine sponges collected from Florida Keys (USA). Phylogenetic characterization of the isolates based on 16S rRNA gene sequencing clearly assigned the strains to two actinomycete genera: *Kocuria* and *Micrococcus*.

The biosynthetic potential of these strains was analyzed by PCR screening for genes involved in the production of bioactive secondary metabolites: polyketide synthase (PKS-I and PKS-II) and nonribosomal peptide synthetase (NRPS). The majority of the strains yielded an amplified metabolic gene sequences as PCR product for at least one of these biosynthetic clusters.

The isolates were assayed for the production of biological activities using microwell fermentations in 12 different media (528 extracts). The extracts were tested in zone of inhibition assays against clinically relevant strains: Gram-positive (*Bacillus subtilis* and methicillin-resistant *Staphylococcus aureus*), Gram-negative (*Acinetobacter baumanii* and *Pseudomonas aeruginosa*) and yeast (*Candida albicans).* Bioactivities against MRSA were observed in 3 of the 528 extracts corresponding to three different strains. The production of secondary metabolites was detected only in one fermentation media. This antibacterial activity was reproduced in new fermentations and the active component is currently being purified and identified.

This survey demonstrated the presence of biosynthetic genes (PKS and NRPS) with potential to produce secondary metabolites in members of the family *Micrococcaceae*. Therefore, this family of actinomycetes represents a source for the discovery of new medically relevant small molecules. Because of their relative abundance in marine environments, intrafamilar diversity, and ease of growth, these bacteria can serve as model organisms for improving the process of natural product discovery using tools for predicting their genetic capacity for biosynthesis and their responses to media manipulations designed to express their chemical diversity.

Keywords: sponge, marine actinomycete**,** secondary metabolite, biosynthetic gene.

Detection of keratinophilic fungi in sandy coastal plains of Northeast Brazil

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Fungi are widely distributed in the environment and are highly producers of several enzymes such as cellulases, chitinases, proteinases and keratinases. At the moment, there are no studies relating the keratinophilic fungi in coastal environments. In this way, this study aimed to endorse two sampling methodologies to obtain keratinophilic fungi in the environment. Thus, samples were collected from sandy sediments, situated in open shrubland and forest periodically flooded. We used the method TOKAVA for fungi isolation. Another experiment (*in situ*) was performed for keratinophilic fungi, which cast polyethylene bags containing dried chicken feathers were deposited under the leaf litter for 30 days. For isolation of fungi used the technique of serial dilutions and were transferred to Petri dishes containing agar with chloramphenicol supplemented with crushed feathers. We found 31 morphotypes of filamentous fungi in different plant formations of the coastal sandy dune, using the method of TOKAVA, whereas in the experiment *in situ* were isolated 25 morphotypes. The results show a greater morphological diversity of fungi selected by the method of TOKAVA, suggesting that these organisms, although resistant to environmental stress provides the sandbank, grow better under controlled conditions. According to the results obtained, TOKAVA methodology used for the detection of keratinophilic fungi were more efficient than the method *in situ*, indicating a greater possibility of studies of the ecological diversity of fungi producing keratinase

Keywords: keratinase, fungi, sandy dune

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Determination of Amount of Oxytetracycline (OTC) Residues in Muscle of Common carp By HPLC Method

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The aim of this study was to determine the amount of OTC residues in muscle of comon carp in multiple dose by HPLC Method .For this purpose, 100 fishes were divided into ten experimental groups, and each groups was divided into three repetitions.

Regarding to the weight range of fishes (40-80 g) and also the injectable OTC dosage rate of 25-50 mg/kg, 0.02 ml (or 25 mg/kg) of injectable solution of OTC was intramuscular injected and the next day, tissues were sampled from 5 fishes and the other fishes were injected by 0.02 ml of OTC solution.

This process was continued for 10 days.

At the end of sampling process, measurement of OTC residues was carried out by HPLC after solid phase extraction (SPE).

The results indicated that the lowest concentration of drug in muscle belonged to the first day was 1.32µg/g and the highest concentration belonged to the 6th day was 9.4µg/g. Then the OTC concentration decreased to 4.4µg/g in the 10th day.

Regarding the high concentrations of OTC in muscle after consecutive injection for 10 days, a long time is needed for OTC to be eliminated from the tissue and reach to Maximum Residue Limit (MRL) in muscle (0.2 µg/g).

Keywords Common carp, HPLC, Oxytetracycline,Residues

Determination of Anaerobic and Anoxic Biodegradation Capacity of Sulfamethoxazole and the Effects on Mixed Microbial Culture

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Antibiotics which are known as xenobiotic compound accumulate in the ecosystem day to day s a result of the increase in the antibotic consumption in the worldwide. Approximately 90% of the utilized antibiotics are excreted by urine and feaces, etiher as active substances or metabolites. After fate of a few part of these compounds by different processes in urban wastewater treatment plants, they are directly discarged to the receiving water bodies. Despite they are accepted as micro pollutants, they have an important threat against the ecosystem, especially for aquatic and terrestrial organisms. Antibiotics also primarily affect the microbial community in the sewage system. Even in the high concentrations of antibiotics, which are observed in the direct discharge of hospital and/or pharmaceutical industry wastewaters to sewage system, the inhibition effects of these compounds on the microbial community are observed. It cause serious problems in the organic matter degradation; therefore the inhibition effects of antibiotics on microbial population pose a great risk. Also antibiotics especially cause the increase of antibiotic resistance pathogens and these organisms firstly threat public health, then plants and animals.

This study involved setting-up batch biodegradation test to investigate biodegradation characteristics of sulfamethoxazole (SMX), which is a common antibiotic to treat urinary tract infections, under anoxic and anaerobic conditions. The biodegradation test bottles were set up under nitrate reducing, sulfate reducing and methanogenic conditions. Experimental test was carried out for 120 days. Gas production in the test bottles was monitored daily during this period. Wet chemical analysis in terms of dissolved organic carbon, electron acceptor and SMX concentration measurements and also quantification of microbial groups by Q-PCR were carried for four different sampling times.

Biodegradation ratios were estimated with three different approaches. a) Theoretical CO_2 and biogas production, which were calculated according to assumption of complete biodegradation of SMX in the system, were compared to experimental CO_2 and biogas production within the batch tests. b) Evaluation of DOC removal. c) SMX concentration measurements for solid and liquid phases. The effects of SMX on different microbial groups in terms of *Bacteria*, *Archaea*, sulfate reducing bacteria and methanogens were determined by DNA based microbiological analysis.

The results indicated that ultimate biodegradation of SMX cannot be achieved under three electron accepting conditions. Under nitrate reducing conditions, 38% of SMX was mineralized to CO_2 and 2% of the parent compound was converted to its soluble microbial products (SP) and/or transformation products (TP). Under sulfate reducing conditions, 32% of the SMX was mineralized to CO_2 and 8% of it was probably converted to TP of the SMX or SP. Under the methanogenic conditions, 23% of the SMX was converted to CO_2 and methane via methanogenesis whereas; 18% of SMX remained in the liquid phase as undetected compound. SMX concentration analysis within the sludge samples indicated that 29% of the SMX was sorbed to sludge in all electron accepting conditions. Overall SMX removal rate was calculated as 69%. However ultimate biodegradation rate was changed between 23%-38%. Microbial groups were not adversely affected by SMX significantly. Under methanogenic conditions, the results showed that there was a strong correlation between antibiotic concentration and the amount of bacteria and methanogens. This correlation was a strong proof of the utilization of SMX and showed that the bacterial and archaeal community continue to work together while this compound was used as sole carbon source. Also a high correlation was found between TOC concentration and sulfate reducers under sulfate reducing conditions.

Keywords: sulfamethoxazole; anoxic; anaerobic; biodegradation test; Q-PCR; OECD 311

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Differential response to isoproturon by two strains of *Saccharomyces cerevisiae*

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The isoproturon (IPU), 3-(4-isopropylphenyl)-1,1-dimethylurea, represented by the structure:

is a phenylurea widely used as herbicide which blocks photosynthesis, inhibiting chloroplasts electron chain at level of photosystem II. Therefore, the presence of isoproturon in the living cells can generate ROS and consequently oxidative stress. Because it exhibits low water solubility and chemical/biological degradation, accumulates in soils as waste and therefore persists in biological systems for long periods, being listed by European Union among 33 special substances that threaten the earth surface. In addition, the literature indicates that exposure to this phenylurea can change human blood parameters, and trigger cancer. So it is urgent to determine their mechanisms of toxicity in eukaryotes. The aim of this study was to compare the response of two strains of *S. cerevisiae*, wild-type UE-ME_3 deposited in the collection of enology laboratory of University of Évora, Portugal and IGC-4072, deposited in the Portuguese Yeast Culture Collection of New University of Lisbon, Portugal, that has been used as biological model in toxicity approaches because show high survival capacity to pesticides.

S. cerevisiae UE-ME_3 and IGC-4072, at mid-exponential phase were inoculated in mineral medium (MB) or MB with 100 µM isoproturon and incubated in a water bath with orbital shake at 28 ºC during 72 h. Samples from each treatment were used to obtain growth curves and to prepare post-12000 *g* supernatant, used for determination of protein [1] and antioxidant capacity by the 2,2-diphenyl-1-picryl-hidrazil (DPPH) method [2], and glutathione [3] contents, as well as glutathione reductase (GR) [4], glutathione peroxidase (GPx) [5], glucose-6-phosphate dehydrogenase (G6PD) [6] and catalase T (CAT T) [7] enzymes activities.

S. cerevisiae UE-ME_3 grown in MB medium in the absence and presence of IPU show a similar growth profile and a stabilization of the biomass, non-protein thiols content, cell viability (cfu) and cell redox status estimated by GSH/GSSG ratio. This response seems depend on GR and G6PD activities increase, which probably activates the glutathione cycle, signs of adaptive response to phenylurea. However, *S. cerevisiae* IGC-4072 strain showed signs of cell death in the presence of IPU, exhibiting a more slowly growth profile, a decrease of dry weight and cell viability, as well as a significant increase of free radicals scavenger (DPPH) in presence of phenylurea, without significant changes of the GSH/GSSG ratio. As the value of the GSH/GSSG ratio is significant lower in IGC-4072 cells and GR activity undergoes a marked decrease in the presence of the IPU it is suspected that the worst IPU adaptive capacity displayed by this strain depends on a poor antioxidant power mediated by the glutathione.

Keywords: *S. cerevisiae*; isoproturon; antioxidant capacity.

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Effect of *Bacillus subtilis* and inulin, single or combined, on intestinal microbiota of *Sparus aurata* L.

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The use of probiotics and prebiotics has been regarded during last years as an alternative viable therapy in fish culture, appearing as a promising biological control strategy and becoming as an integral part of the aquaculture practices for improving growth and disease resistance. This strategy offers innumerable advantages to overcome the limitations and side effects of antibiotics and other drugs and also leads to high production. Probiotics and prebiotics could also offer alternative strategies to maintain a microbiologically healthy environment.

The present study evaluated the effect of dietary *Bacillus subtilis* and/or inulin on the intestinal microbiota of Sparus aurata L. by 16S rRNA gene analysis using polymerase chain reaction-denaturing gradient gel electrophoresis. Fish were feed with one of these diets: 1) non-supplemented commercial pelleted diet (control group); 2) diet supplemented with 10 g kg^{-1} inulin (I); 3) diet supplemented with 10^7 cfu g^{-1} *B. subtilis* (B); 4) diet supplemented with 10^7 cfu g-1 *B. subtilis* and 10 g kg^{-1} inulin (BI). Six specimens from each group were sampled at two and four weeks. The whole intestines including gut contents were collected and stored at -80 ºC until analysis. Samples were used to determine the diversity of the intestinal microbiota.

Results showed high similarity of the profiles obtained in the microbiota of fish fed with the diets I and BI, with a similarity coefficient of nearly 75%. This similarity was higher than that observed for fish fed the control diet and diet supplemented only with *Bacillus* with a similarity near to 60%. The Shannon index (H) was 2.13±0.13 and Specific richness (R) 8.75±1.29 for the control treatment, whilst for fish fed with B diet values of H=1.96±0.8 and R=8±0.6, for diet I were H= 1.41±0.67 and R=6.6±2.96 and for diet BI were H=1.62 ±0.16 and R=6 ± 1.09. The Rr values obtained for fish fed with diet C and I were less than 30 while for the other two diets were more than 30. The first case is associated with low numbers and diversity of species. Habitats with low Rr values have been associated with low habitable environments.

The results obtained have demonstrated that administration of I and BI to gilthead seabream results in a different intestinal microbiota being that more similar that between the diets C and B.

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Keywords inulin, *Bacillus subtilis*, intestinal microbiota, *Sparus aurata*.

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Effect of DNA polymerases on DGGE patterns

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The denaturing gradient gel electrophoresis (PCR-DGGE) and other PCR-based fingerprinting methods (TGGE, SSCP, T-RFLP, ARISA) has been widely used to determine the structure of microbial communities. The assay is a multi-step procedure containing the extraction of total DNA, PCR amplification with specific primers, the separation of amplicons based on sequence differences and the image analysis of the patterns. The DNA preparation method and the amplification circumstances strongly determine the outcome of PCR-DGGE analysis. DNA polymerase itself may affect the obtained patterns due to differences in processivity, inhibitor resistance and accuracy. Based on this consideration, the performance of two highly processive and accurate DNA polymerases (*KOD Hot Start* and *Phusion*) was compared to the generally utilized *Taq* polymerase in PCR-DGGE analysis. The DGGE pattern of the different polymerases were highly similar, however some predominant bands produced by *KOD Hot Start* were missing of the patterns of *Taq* and *Phusion*.

Sensitivity of the polymerases was tested against the potent PCR inhibitor humic acid. Our data show, that *Phusion* could tolerate 0,3 μg/ml humic acid, while *Taq* was inhibited by less than 0,3 μg/ml humic acid. In contrast, *KOD Hot Start* polymerase was able to perform positive results in the presence of 5 μg/ml humic acid.

Based on the above results we conclude that the choice of the polymerase enzyme indeed can influence the generated bacterial community profiles.

Effect of initial pH on bio-hydrogen production from enzymatic hydrolysate of acid-pretreated sugarcane bagasse by elephant dung

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This research investigated the effect of initial pH on hydrogen production by elephant dung from enzymatic hydrolysate of acid-pretreated sugarcane bagasse (SCB). In order to obtain the hydrolysate, the acid-pretreated sugarcane bagasse was hydrolyzed with 20 U/g commercial cellulase derived from *Trichoderma reesei*. The resulting hydrolysate had the glucose concentration of 3.19 g/L which was subsequently used as the substrate to produce hydrogen by elephant dung in batch fermentation at various initial pH of 3-8 with an increment of 0.5. Elephant dung was heat-treated in boiling water for 2 h before used as the seed inoculums in order to inhibit methanogenic activity. The experiments were conducted in 10 mL serum bottles with a working volume of 7 mL. Culture of the hydrolysate at the initial pH 7 provided a maximum hydrogen production (HP) and hydrogen yield (HY) of 410.97 mL and 0.93 mol H_2/mol total sugars consumed, respectively. At the optimum initial pH 7, the treatment without Endo-nutrient addition showed a higher HP and HY than the treatment with Endo nutrient addition 2.0 (823.68 mL) and 2.27 (2.12 mol H_2/mol total sugars consumed) folds, respectively. Main soluble product was acetate and butyrate suggesting the hydrogen fermentation from the enzymatic hydrolysate of acid-pretreated SCB is the acetate-butyrate type.

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Effect of oxidizing agents on metabolic path way of fermentative hydrogen production by *clostridium acetobutylicum*

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The excessive use of fossil fuels is one of the primary causes of global warming and acid rain, which affects the earth's climate, weather condition, vegetation and aquatic ecosystems. Considering the energy security and the global environment, there is a pressing need to develop non-polluting and renewable energy source. Hydrogen is a clean energy source, producing water as its only by-product when it burns. Microbial H_2 production, one of the biotechnology applications, is from renewable raw materials such as organic wastes. Hydrogen can be obtained from dark fermentation by hydrogen-producing bacteria. Increasing of hydrogenyield through themetabolicpathways is obtained byreoxidationof residual NADH to NAD^+according to the following reaction:

$NADH + H^+ \rightarrow NAD^+ + H_2$

Therefore, the yield of hydrogen will be improved if metabolic fluxes can be adjusted and/or redirected to increase the amount of NAD^+ available in cells.In this study the influence of oxidizing agents on the metabolic path way of biohydrogen production by *clostridium acetobutylicum*was examined.

Keywords: biohydrogen, Regulation of metabolic pathways, oxidizing agents, *clostridium acetobutylicum*

Effect of the hydrocarbons contamination on the microbiological resilience of soils

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Different bacterial species that inhabit the soil environment can form a robust community and provide to the soil the capacity of perform a range of ecological functions when stressed by climatic fluxes and anthropogenic inputs. However, how these communities respond to changes in the environment and what makes them stable in the presence of potential stressors have not always been clear. There are different opinions about whether hydrocarbons pollution reduces the microbial diversity, and therefore produces less resilience of soil microbial communities.

To investigate the incidence of previous history of hydrocarbon contamination from the soils on the capacity of response of soil microbial communities', two places near petrochemical industrial area were sampled: petrochemical industry site S1 (THC 232.37 mg-1 dry soil) and petrochemical industry site S2 (THC 414.77 mg-1 dry soil). Also, a third place, outside the contaminated area was studied and considered as control, P. Shannon-Weaver diversity index (*H*), determined from DGGE profiles, showed similar levels of diversity among the bacterial communities of the different sampled soils.

Three different stresses were imposed to the sampled soils, in microcosms systems. To evaluate the response to Cd contamination two different concentration of $CdSO_4$ were applied 140 and 400mg Cd kg-1 dry soil. The saline stress was provoked by the addition of 2.18 meq NaCl /100gdry soil and 7.79 meq NaCl/100 g dry soils. The acid stress was provoked by adjustment of the soil pH to 4.5 or 5.5 with dilute HCl (1mM). Following imposition of the treatments, all soil microcosms were incubated at 26±2 ºC and 70% WHC for two weeks. This time period was chosen to allow sufficient equilibration of the microbial communities after imposition of each stress treatment and for decomposition of organic C released from microorganisms killed by the treatments. The activity of different enzymes, heterotrophic bacteria count (R2-agar) and the physiological diversity estimated by Biolog EcoPlate (average well colour development AWCD), were determined before and after the stresses were applied.

Different response patterns were observed by principal components analysis (PCA), in relation with the previous pollution history from the soils. Three enzymatic activities (dehydrogenase, phosphatase and urease) and the AWCD were able to highlight the different behavior among the three microcosms after the stresses. Only saline stress provoked a negative impact on the dehydrogenase activity in P microcosms, reducing their resilience. The Cd contamination and the acid stress reduced the dehydrogenase activity in S1 microcosms. The most contaminated microcosms, S2, showed the lowest enzymatic activities and surprisingly, the acid stress stimulated its dehydrogenase activity and AWCD values. In addition, no change was observed in heterotrophic bacteria counts of P microcosms after the stresses were applied. A significantly decrease in heterotrophic bacteria counts was observed in S2 microcosms by effect of acid stress (pH 4 and pH5). Only the acid stress at pH 4 provoked a decrease in heterotrophic bacteria population in S1 microcosms.

The different history of hydrocarbon contamination of soils was not evidenced into the bacterial community diversity; however different resilience patterns were demonstrated.

Keywords: hydrocarbons; resilience; soil microbial community.

Effects of coal ash application on the microbial population of a tropical soil

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Abstract - Coal combustion in thermal power plants produces coal ash (CA), a solid residue composed of the noncombustible matter plus a small amount of carbon remaining from incomplete combustion, comprising fly and bottom ash (FA and BA). FA contains the finest particles from coal mineral matter, mostly silt- and clay-sized glassy spheres; BA, quite different, is a coarse, granular, incombustible byproduct, collected from the bottom of furnaces, with grain sizes spanning from fine sand to fine gravel. Byproduct characteristics depend on the type of furnace and the coal used.

Conventionally, CA is disposed of in landfills and in ponds, allowing for diverse xenobiotic compounds to enter the environment. Waste deposits from coal combustion present leaching of potentially toxic elements, such as heavy metals, as well as a lack of essential nutrients in soil and a disruption of its structure. As a cementitious or pozzolanic material (one that hardens when mixed with water, or with water but only after activation with an alkaline substance, respectively), some fly ashes may be useful for cement replacement in concrete and other building applications, as shown by several studies. Likewise, CA has been evaluated for use as soil conditioner for agricultural purposes, as they may help bypass productivity constraints in agricultural soils; their efficacy, though, still remains highly variable. On-going studies show that CA addition improves soil physical and mechanical characteristics for the application as base road pavements.

Ecosystems impacted by xenobiotics generally present altered biodiversity and proprieties; this study evaluated the effects of FA and BA 5% w/w addition on the microbiota of a tropical soil. Microcosms and batch assays monitored soil parameters for seven weeks. Results show that ashes did not significantly alter soil pH and available carbon, unchanged in time; despite a drop in microbial degrading activities observed with FA application (fig.1), both ashes caused a significant increase in microbial biomass (fig.2), while community metabolic structure shifted only slightly. Therefore, as no significant changes happened in microbial activity, a 5% CA application seems appropriate in this soil for engineering purposes; biomass enhancement has to be further evaluated, though, to better assess CA usefulness for agricultural uses.

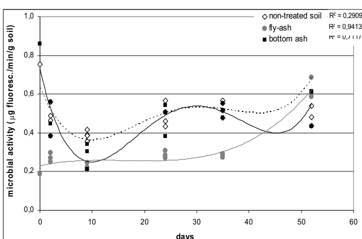

Figure 1 – Microbial activity during the assay (μg hydrolysed FDA x min^{-1} x g^{-1} soil)

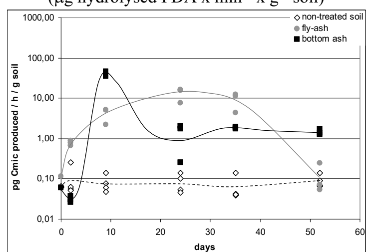

Figure 2- Production of microbial carbon (pg Cmic x h^{-1} x g^{-1} soil)

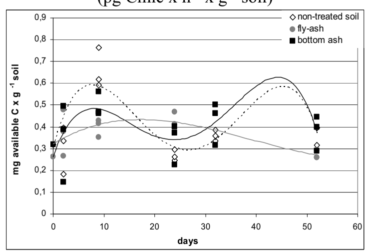

Figure 3 – Available carbon in soils throughout the experiment (mg Cav x min^{-1} x g^{-1} soil)

Keywords coal ash; soil microbiota

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Effects of different quorum sensing molecules on attachment and biofilm formation of acidophilic, moderately thermophilic microorganisms

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Bioleaching is a process where metal sulfides are dissolved by bacterial and archaeal oxidation-driven-processes. Leaching microorganisms attach to mineral surfaces, enhancing metal sulfide dissolution. Natural leaching processes result in one hand in a type of water pollution called acid mine drainage (AMD). On the other hand, bioleaching processes are used in biomining technologies for recovering metals such as copper, gold, nickel or zinc.

In several microorganisms biofilm formation is partially regulated by cell-to-cell communication systems known as Quorum Sensing (QS). In order to optimize microbial leaching efficiency, the role of QS in this context has to be understood. Therefore the interactions between moderately thermophilic bacteria in bioleaching and biofilm formation on pyrite were studied.

Several QS molecules such as homoserine lactones (AHLs) or furanones were tested in cultures of *Acidithiobacillus, Leptospirillum* and *Acidimicrobium* species. Cell attachment to pyrite and leaching behavior were quantified with and without addition of different QS molecules. Also biofilm formation was visualized using the floating filter technique in combination with Epifluorescence Microscopy (EFM) with the nucleic acid stain DAPI and fluorescently labeled lectins (which bind polysaccharides). *L. ferriphilum* responded to different AHLs. Depending on the type of AHL, attachment and leaching rates either increased or decreased. Moreover, growth of *Acidithiobacillus, Leptospirillum* and *Acidimicrobium* was inhibited by use of furanones. Thus, new strategies may be developed to control bioleaching processes.

Keywords bioleaching, quorum sensing, attachment

Effects of excreted OTC in manure on biogas digesters in terms of microbial populations and biogas production

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Most of the veterinary antibiotics metabolized poorly in livestock animal. Oxytetracycline (OTC) is a common antibiotic in treating cattle diseases due to its low cost and limited side effects. Excretion of OTC in livestock manure can be problematic for biogas digesters since digestion of manure from OTC medicated animals can decrease biogas production by inhibiting microbial populations. In this study, commercial OTC solution was applied once by intramuscular injection to a 450 kg dairy cow and manure was collected for next 20 days. OTC detection analysis by High Performance Liquid Chromatography (HPLC) showed that excretion of injected OTC begins after 24 hours with concentration of 10.38 mg/kg and present in manure in decreasing concentrations for 12 days. Excretion of OTC was mainly on first five days and after 12 days a total 10% of injected OTC was recovered from manure samples. In order to observe effect of OTC on microbial populations and biogas production, manure slurries collected on days 1,2,3,5,10,15,20 were digested in 120 ml serum bottles with 5% Total solid (TS) concentration for 30 days. During digestion biogas composition and production were observed along with changes in microbial populations and OTC concentrations. 155±8 L/kgTVS biogas yield were observed in control serum bottles. Inhibitions on cumulative biogas productions were 46%, 58%, 57%, 51% and 21% for the manures collected on days 1, 2, 3, 5 and 10, respectively. Although there was a huge decrease in biogas production, biogas composition remained unaffected (58±5% methane). HPLC analysis of manure slurries also showed decrease in detectable OTC concentration. Starting concentrations of OTC were decreased to nearly its half concentration in 20 days (Table 1.).

Table 1. OTC concentration in manure and serum bottle digesters

Manure collection day	**OTC concentration of fresh manure (mg/kg)**	**OTC concentration of manure slurries at digestion day (mg/l)**	
		0th	**20th**
1	10.38	3.34	1.56
2	4.54	1.4	0.85
3	4.13	1.39	0.4
5	3.71	1.01	0.3
10	0.45	0.15	N.D.*
15	N.D.	N.D.*	N.D.*
20	N.D.	N.D.*	N.D.*

*N.D.: Not detected

Microbial community structures of serum bottle digesters were identified by 16S rDNA clone library formation. Change of community structures were also observed by 16S rDNA targeting Denaturing Gradient Gel electrophoresis (DGGE) and Real Time PCR (QPCR). Clone library of microbial communities indicate bacterial communities were composed of mainly by *Firmicutes* group (82%) and Proteobacteria (15%). Archaeal communities were represented by *Methanosarcina mazei* (52%) and *Methanobacterium* spp. (46%). Statistical analyses of clone libraries with environmental parameters were made by Canonical Correspondence Analysis (CCA). CCA of bacterial clone library show negative correlation between bacterial groups and OTC concentrations. CCA of archaeal clone library proved to be more explanatory. *Methanobacterium* and *Methanosarcina* species were in negative correlation with OTC concentration whereas biogas production and digestion time were in positive correlation with *Methanosarcina* species. Q-PCR analyses are in still progress and planned to be completed shortly. After completing, results will be incorporated into this manuscript.

Keywords: oxytetracycline, 16S rRNA, biogas, anaerobic digestion, Canonical Correspondence Analysis (CCA)

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Environmental anthropogenic impact on Polychaetes of Adriatic Sea of the Salento peninsula (Italy)

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We studied Polychaete marine community distribution (specimens and species) along the coast of the Villanova (Brindisi, Italy). We sampled two sites up to a depth of 50-150 cm below the sea level. The first sampling point was into the port of Villanova, whereas the second sampling point was close the outflow of a stream-channel named "Lama d'Antelmi". The surface water of the channel was monitored seasonally during 2010 together with coastal groundwater in some monitoring wells. Sludge and substratum below the sea was sampled for Polychaetes and other biota, during March, May and July 2010. Polychaetes have been recovered and classified by the Department of Zoology of Bari University. At the Villanova port we have sampled 21 species and 127 specimens of Syllidae and Orbiniidae, with maximum count (66) in July. At the second sampling point we have found only 15 specimens of Sabellidae during May 2010.

Sampling results of water quality and Polichaete species have been treated for a statistical analysis in order to find the relationship between anthropogenic activities and Polychaete count distribution. We compared Villanova sampling results to the data collected at Oasis of Torre Guaceto, i.e. a protect uncontaminated site located 10 km from Villanova. We have found sensitive parameters, such as nitrates and others chemical water constituents, which may explain the reduction of Syllidae and Polychaetes with respect to the Torre Guaceto area, as a consequence of human activities (municipal treatment plant effluents, fertilizers-livestock untreated wastewaters, tourism impacts, etc.). The ecological reading of marine ecosystem has been carried out by using AZTI Marine Biotic Index-New (http://ambi.azti.es). The present study suggests measures and strategic activities, which must be applied at Villanova in order to re-address the marine ecosystem to the natural and uncontaminated status. The proposed measures have been evaluated by using STELLA software (www.iseesystems.com). The best ecological status can be achieved by diverting channel outflow in the groundwater after wastewater treatments. The artificial recharge can be used as a dynamic barrier also to stop the sea water intrusion, by increasing groundwater volume for farmer supplies.

Keywords: Polychaetes, anthropogenic coastal impact, ecological ecosystem quality.

Environmental surveillance of human parechovirus and enterovirus in sewage using molecular methods

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Human parechoviruses (HPeV) and human enteroviruses (HEV) are small RNA viruses belonging to the family of the *Picornaviridae*. Infections in humans can be asymptomatic or cause mild symptoms like diarrhoea and flu-like disease. Occasionally more severe diseases like meningitis, sepsis and paralysis occur. To monitor the circulation of HPeV and HEV it is important to detect viruses from both the asymptomatic and symptomatic infected individuals.

Sewage samples were taken in the bible-belt in the Netherlands where many people that have not been vaccinated against poliomyelitis on religious grounds live closely together. Sewage samples were taken on 15 different locations, primarily near schools, from September 2010 until March 2011, resulting in 3 sampling sets. RNA extraction was done on 5 ml of the sewage sample with a magnetic bead extraction kit (bioMerieux), and viral RNA was detected in these sewage samples with a real time RT-PCR method, targeting the 5'UTR of either the parechovirus or the enterovirus genome. Per sample, the real-time PCR was performed in triplicate and the RNA samples were tested undiluted, 10 times and 100 times diluted, to increase the detection probability by reducing the concentration of inhibitory substances influencing the PCR. When the viruses were detected in the sewage sample they where further identified by a molecular typing method: a (semi)nested-PCR using primers situated within the VP3-VP1 or the VP1 region of the genome of HPeV and HEV, respectively. Subsequently, the PCR-products were cloned in a vector and approximately 5-10 clones per sample were sequenced to determine which virus types were present in the sample.

Sewage samples were taken in September/October and November 2010 and March 2011. In respectively 47%, 53%, and 20% of the 15 samples HPeV RNA was detected. Based on sequencing analysis three different HPeV types were found, with HPeV1 (9x) being the most prevalent. Furthermore, HPeV3 (2x) and HPeV6 (1x) were detected. Typing failed in 6 samples in which HPeV was detected, because no VP3/VP1 PCR product was obtained. HEV RNA was detected in 87%, 100%, and 67% of the 15 samples. In total 17 different HEV types were found, of which echovirus 30 and coxsackievirus A16 were the most prevalent types detected at 7 different locations. For both HPeV and HEV the detection RT-PCR (5'UTR) is known to be more sensitive than the typing method based on the (VP3-) VP1 region. From 13-40% of the 5'UTR positive samples it was not possible to obtain a (semi)nested (VP3-) VP1 PCR product, and therefore the viruses in these samples could not be typed. Method optimisation is needed to obtain more sequence data on the (VP3-) VP1 region of these viruses.

Concluding: Because three different types of HPeV were detected in sewage as well as 17 different HEV types, it can be concluded that environmental surveillance in sewage samples using molecular methods is suitable to monitor the circulation of specific human pathogenic picornaviruses in the population. Because no information on virus infectivity is obtained with these molecular detection methods, quantification of public health risks through environmental exposure is complicated.

Keywords Environmental surveillance, human parechovirus, human enterovirus, sewage

Evaluation of bacterial diversity of Some Hot water springs in the Limpopo Province of South Africa

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The bacterial diversity of six hot water springs; Eiland, Mphephu, Sagole, Siloam, Souting, and Tshipise hot water springs in Limpopo, South Africa was determined by pyrosequencing of two 16S rRNA hyper variable regions; V1-3 and V4-7. Very diverse bacterial genera representing all the different major bacterial phyla were detected in the hot water springs studied. Analysis of the community DNA revealed that the phyla Proteobacteria, Cyanobacteria, Bacteriodetes, Planctomycetes, Firmicutes, Deinococcus-Thermus, Fusobacteria, Acidobacteria, Chloroflexi and Verrucomicrobia dominated and could be detected at varying abundances in the different hot springs. The bacterial diversity detectable and classifiable varied for the variable regions used. Analysis of the community DNA revealed that the most abundant phyla, as detectable by the V1-3 or V4-7 region respectively were as follows: Proteobacteria 100 & 92.64%, Actinobacteria 0.00% & 0.42%, Bacteriodetes 0.00% & 0.28%, and Cyanobacteria 0.00% & 0.14%, at Eiland; Bacteriodetes 45.66% & 54.65%, Proteobacteria 41.09% & 34.54%, and Firmicutes 0.36% & 0.28%, for Mphephu; Proteobacteria 32.89% & 32.37%, Cyanobacteria 6.21% & 23.93%, Bacteriodetes 3.35% & 3.27%, Fusobacteria 4.49% & 5.79%, Firmicutes 1.72 % & 1.64%, Planctomycetes 1.34% & 0.63%, Chloroflexi 0.48% & 1.51%, Acidobacteria 0.76% & 0.63%, Verrucomicrobia 0.38% & 1.01 and Deinococcus-Thermus 0.57% & 0.63% at Sagole; Proteobacteria 54.43% & 48.06%, Cyanobacteria 0.57% & 5.46%, Bacteriodetes 6.93% & 13.03%, Firmicutes 1.25 % & 1.58%, Planctomycetes 8.30% & 10.74%, Chloroflexi 0.91% & 0.88%, Acidobacteria 0.23% & 0.50%, Verrucomicrobia 1.14% & 2.46 and Deinococcus-Thermus 0.45% & 0.70% at Siloam; Proteobacteria 63.23 & 80.30%, Cyanobacteria 2.47% & 3.03%, Bacteriodetes 2.69% & 4.55 %, and Planctomycetes 0.00% & 0.76%, at Souting; Proteobacteria 26.37 & 56.67%, Cyanobacteria 19.73% & 20%, Bacteriodetes 2.54% & 6.67%, Deinococcus-Thermus 0.78% & 0.00%, Firmicutes 0.78% & 0.83%, Fusobacteria 0.00% & 0.83%, Chlorobi 0.59% & 0.00% and Planctomycetes 0.20% & 0.00%, at Tshipise; The number of genera detectable was: 15 (V1-3) and 9 (V4-7) Eiland; 15 (V1-3) and 9 (V4-7) at Tshipise, 29 (V1-3) and 26 (V4-7) at Mphephu; and 31(V1-3) and 33 (V4-7) at Sagole, 25 (V1-3) and 14 (V4-7) Souting. The associated physicochemical properties of the hot springs were determined and are discussed in relationship to the determined microbial community.

Keywords: Thermophilic, Hot springs, bacterial diversity, 454 pyrosequencing, South Africa

Evaluation of biogas production potential of *Spirulina Platensis*

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Today carbon emissions (especially CO_2) to the atmosphere are a big problem for the world in terms of global warming. Some species of algea are very attractive for removol of CO_2 from the combustion exhaust gases because of their capability of using atmospheric carbondioxide as carbon source. Algal biomass can be converted to energy (methane) via the combined algal-bacterial process. In this study, the algae are produced in photobioreactors from ligt and carbon in the first step. In the second step, the algal biomass is used as a substrate for anaerobic digester for the production of methane by anaerobic bacteria.

In this study, biomass of the blue-green algae '*spirulina platensis*' was converted to methane by using complately mixed anaerobic digesters. The technical feasibility of utilization semi-microscopic blue green algae '*spirulina platensis*' as a source of renewable energy was investigated. The study conducted in two parts.

In the first part; the anaerobic digestion (AD) of *spirulina platensis* was evaluated in batch systems (one – phase anaerobic digestion system) which consist of an anaerobic batch reactor. The contents were mixed inorder to obtain a homogenized content, provide good contact with the microorganisms and prevent settling. The study was conducted under 3 runs and 6 different experimental conditions by using two batch reactors (2.5 and 3.0 l working volume). For six different starting concentration on dry weight basis; 0.6, 1.0, 1.5, 2.0, 3.0 and 5.0 % volatile solids (VS) concentration, 93 %, 92 %, 92.5 %, 91 %, 90.5 %, 89 % conversion of volatile solids was obtained (VS reductions) respectively. The organic fraction (VS) was 50%. The corresponding biogas productions (ml) per g of organic load (VS) were 260, 280, 290, 285, 240, and 210 respectively during the 35 days of retention time operational period under mesophilic conditions (35-38 ° C). The average methane content was between 65-68%. Higher proportion of biogas (70%) was produced between days 15- 27.

In the second part of the study, co-digestion of *spirulina platensis* and sewage sludge was investigated in a two-phase anaerobic digestion system. The system consisted of two anaerobic semi-continuous (one day feeding) reactors; the first one was acidogenic reactor (2 liter working volume) and the second one was methanogenic reactor (5 liter working volume). The temperature in the reactors was maintained at 35-37°C. Therefore, the two-phase anaerobic digestion system was tested under two sets.

During first set the anaerobic digestion of only sewage sludge was investigated in the semi-continuous system. The system was operated with a total hydraulic retention time of 14 days (4 days in acidogenic reactor and 10 days in methanogenic reactor). The constant loading rate was 1.5 % of total solids which contained 1% of volatile solids (68% of TS) by feeding 500 g of raw sewage sludge daily to the acidogenic reactor. The system achieved about 60% volatile solids reductions at the end of methanogenic phase. The major part of the VS reduction was achieved in the methanogenic reactor (75%). After achieving steady state, 2620 ml biogas/day was produced in two reactors system where, 2400 ml of the biogas was produced from the methane-digester and the rest, 220 ml, was from the acid-digester. Therefore, for 5-g VS daily feeding, 525 ml biogas/(g-VS.day) was obtained. The biogas from the acid-digester contained 60% CO_2, 38% methane and 2% other gases whereas, 72% methane, 27 %CO_2 and 1% others produced from the methane-digester. As expected, the methane digester generated 94-96 % (v/v) of the system methane production.

During second run the system was fed with a mixture of waste activated sludge (66.6%) and *spirulina platensis* (33.3%) in VS basis. The system was operated for 14 days HRT. The reactor temperatures were kept under mesophilic conditions. The constant loading rate of mixed substrate (200-g *spirulina platensis* and 300-g sewage sludge) contained 1.50% TS which 0.91% VS with a 500-g daily feeding raw mixture to acidogenic reactor. The pH values changed between 5.5-5.8 in the acid reactor and between 7.0-7.2 in the methane digester during the operation. During this operation 62.5% VS reductions was achieved. As it was in the first run the major part of this reduction was achieved in the methanogenic reactor (78%) and the rest (22%) in the acidogenic reactor. Total 2880 ml biogas was produced per day from two reactors. 2650 ml of this biogas was produced in the methane-digester and the rest (230 ml) was in the acid-digester. For 4,5 day of HRT, 640 ml biogas/(g-VS.day) was achieved. Therefore, the co-digestion with *spirulina platensis* improved biogas production. The biogas produced from the acid digester consists of 65% CO_2, 32% methane and 3% other gases compared to 75% methane, 24 % CO_2 and 1% others in the methane reactor. The methane-reactor generated 94-97 % (v/v) of the system methane production.

Key Words: biogas production, co-digestion

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Expression profiling of marine microbial communities with 454 pyrosequencing

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The project "**M**icrobial **I**nteractions in **MA**rine **S**ystems" (MIMAS) (www.mimas-projekt.de) aims at investigating the seasonal changes in the microbial communities at Helgoland Roads, a long term ecological research site in the North Sea, Germany. The application of "Meta-Omics" approaches like Metatranscriptomics and Metaproteomics will shed light into the functions of the active fraction of genes, while Metagenomics will address the genetic potential of the bacterial community as a whole. Furthermore the correlation with oceanographic and environmental data will give new insights in the ecological role of marine bacterial communities and their response to environmental changes.

Metatranscriptome analysis of marine microbial communities has been performed via Roche 454 pyrosequencing. The obtained sequence data shows promising findings. Several high abundant transcripts could be assigned to proteins with known function. For example, transcripts encoding for proteins involved in housekeeping processes, transport and photosynthesis have been detected. Interestingly, a high amount of transcripts encoding for cell wall-associated hydrolases were expressed as a response to the algae bloom. These transcripts may play an important role in bacterial interaction with algae during bloom situations.

Taxonomic analysis of the Metagenome and tag sequencing of the 16S ribosomal RNA genes revealed high abundances of *Bacteriodetes,* as well as *Alpha*- and *Gammaproteobacteria* during the spring bloom. Metatranscriptomics supports these findings with particular high messenger RNA activities for the three phyla. Detailed gene expression and pathway analysis in KEGG gave first insights into the metabolic distribution. Differences in the abundances of transcripts involved in several metabolic pathways such as energy and carbohydrate metabolism could be detected as a response to the algae bloom. Moreover, genes encoding for tonB-dependent transport were primarily assigned to *Bacteroidetes* while *Alphaproteobacteria* feature ABC transporters. These finding are supported by the Metagenome analysis.

To complement and extend the studies of the bacterial community pure culture experiments are planned. The marine model organisms *Rhodopirellula baltica* SH1^{T} will be studied under defined conditions to elucidate further information on the activity and function of the so far 'unknown proteins'.

Our results demonstrates that the integrated application of Meta-Omics methods provides access to shifts in microbial community composition and gene expression patterns that are significantly correlated with seasonal environmental changes.

Keywords: Metatranscriptomic, Metagenome, marine microbial communities, 454 pyrosequencing

Field study on the behaviour of heavy metals in contaminated soil by stimulating the indigenous sulfate-reducing bacteria

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Stabilization is a well known technique for immobilization of heavy metals in geological media such as soil, sediment and groundwater. Chemical stabilization techniques have shown high effectiveness in lowering mobility of heavy metals; however, sometimes they are too expensive to be operated. The technique using microbial precipitation of heavy metals as sulfide by sulfate-reducing bacteria draws significant attention especially in remediation of groundwater. However, the research and practical application of the technique to the soil contaminated with heavy metals were rare. This study examined the potential of stimulating indigenous sulfate-reducing bacteria to stabilize heavy metals in contaminated soil in the vicinity of abandoned gold mine.

The studied soil was mainly contaminated with As which was due to mineral mining and processing. The results of laboratory experiments, in which glucose and sulfate were amended to the soil to stimulate indigenous bacteria, indicated that indigenous bacteria can control the behavior of Fe and SO_4^{2-}. Aqueous concentrations of Ni, Zn, Pb, Cr, and As decreased in microbial experimental set over time when compared with abiotic controls.

Glucose and sulfate were also amended to field test cells (2m × 2m × 0.5m). After 117-day incubation of indigenous bacteria, total concentration of As in the soil, which was determined after aqua-regia digestion, appeared to be similar between before and after amendment process. However, partial extraction of As using 1 N HCl revealed decrease in As concentrations with 60%, 60% and 75% at depth of 0.2, 0.5 and 0.8 m, respectively, after stimulation of indigenous bacteria. Conceivably, stimulation of indigenous bacteria in contaminated soil might lead to effective stabilization and long-term stability of As and heavy metals in soil.

Keywords Microbial stabilization; Heavy metals; Arsenic; Soil; Sulfate-reducing bacteria

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Fly density and environmental factors in street vendor foods and its contamination with *Escherichia coli*

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Research to measure the fly density and environmental factors in street vendors foods was done along the Margonda Street, Depok City, West Java Province, Indonesia used crossectional design.

A total of 100 street vendors were selected randomly, from which different types of foods were sampled for fly density and *Escherichia coli*. Fly grill was used to measure the fly density, otherwise the analysis of *E. coli* using the Most Probable Number (NPM) method. Also, environmental factors of street vendors and personal hygiene of food hanlers were observed. Analizing of the data used Chi Square Test and Logistic Regression.

It was found that the fly density was a quite high (more than 6) with the average in each location were 9.38 in Point-1, 8,53 in Point-2, and 10.33 in Point-3. Forty one (41) percent of samples contaminated by *E. coli* and 14% contaminated by Salmonella. From the environmental factors of street vendors and personal hygiene of food hanlers , the variables which were in logistic regression equation were the existence of trash bin, food utensils which washed with unflowed water, food utensils which washed with soap, and the condition of food serving.

The dominant factor in contaminating E. Coli was the existence of trash bin (OR= 0,15: 0,04-0.60). There was a association between the level of fly density and *E. coli* contamination in vendor’s street food.

Keywords *Escherichia coli*; fly density; food; Salmonella; street vendor

Genetic diversity of *E. coli* that persisted in the sediment of a subtropical intertidal mudflat

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Escherichia coli is used as an indicator of fecal pollution in standard water monitoring practices. The general assumptions are that the feces of warm-blooded animals is the only source of *E. coli* in water, and that *E. coli* population decays as the impacts of pollution subside. However, many recent studies have reported genetically distinct *E. coli*populations that appeared to be able to persist in the environment autochthonously. These so-called naturalized *E. coli* can seriously impair the reliability of pollution monitoring. Most of the previous studies on naturalized *E. coli* have focused on freshwater beach sand and riparian soils. Knowledge about the diversity and naturalization of *E. coli* in marine environments has been scant. In the subtropical environment of Hong Kong, we have conducted a year-round (2009/2010) monitoring of *E. coli* abundance and diversity in the sediment and seawater of an intertidal mudflat that receives low levels of fecal pollution from septic tanks and feral cows. A total of 3,000 *E. coli* isolates have been obtained from the sediment, seawater and fecal sources of the mudflat. The isolates were assigned to phylogenetic groups using a multiplex PCR approach, and analyzed for genotypic diversity using REP-PCR DNA fingerprinting.The isolates of the sediment had the highest genotypic diversity, followed by those of the seawater, and finally those of the fecal sources. Cluster analysis of the DNA fingerprint patterns separated the isolates according to their sources and phylogroup assignment. Isolates belonging to phylogroups B2 and D were of low abundance (< 10%) in the fecal sources. However, 40 % of the isolates obtained from the environmental matrices belonged to phylogroups B2 and D; most of themwere genotypically distinct and persistent in the sediment. This finding is in contrast to what had been reported for the freshwater environments where the persistent genotypes always belonged to phylogroup B1. Using multiloci sequence typing, we have reconstructed the evolutionary relationship among a number of isolates of the fecal sources and the environmental matriceswith*Salmonella* as the outgroup.The results indicated that the sequence types that persisted in the environmental matrices were descents of those in the fecal sources. The significance of our findings in the understanding of the naturalization of *E. coli* in marine sediment will be discussed in the presentation.

Keywords: *E. coli*, marine sediment, naturalization, REP-PCR DNA fingerprinting, multiloci sequence typing

Growth of fungal strains isolated from Livingston Island on phenolic compounds - biodegradation potential

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Sixteen strains of filamentous fungi were isolated from soil samples collected from Livingston Island, Antarctica. The isolates taxonomic identifications were performed based on morpho-dimensional parameters following the most suitable identification keys for the different genera. The affiliation of the investigated strains was established to the particular genera. The obtained fungal isolates were members mostly to the genera *Penicillium*, *Aspergillus* and *Cladosporium*.

All strains were studied for their ability to adapt to aromatics containing media. Most of the investigated strains demonstrated good tolerance to the presence of 0.5 g/l phenol in the culture medium. More than that the investigations showed that strains were able to grow in a culture medium containing phenol in concentrations varying form 0.1 to 0.7 g/l as a single source of carbon and energy.

The experiments carried out with hydroxyl-, methyl- and nitro- phenol derivatives revealed the capability of some of the strains to grow and utilize several of these aromatic compounds. The strains *Aspergillus* sp. AL1, *Aspergillus sp.* AL8, *Aspergillus* AL9, *Aspergillus* sp. AL15, *Penicillium sp.* AL5 and *Penicillium* AL11 were able to grow and utilize as a sole carbon sources 0.3 g/l of each examined aromatic compound. There were not found strains able to utilize any of tested nitrophenols. The representatives of *Cladosporium* as well as strain *Lecanicillium* sp. AL12 did not show any capability to degrade phenol derivatives.

Keywords fungi; phenol; phenol derivatives

High-Affinity Methane Oxidation of Upland Soil Cluster alpha

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Methane-oxidizing bacteria are able to utilize methane as their major source of carbon and energy for growth. Although cultured methanotrophic strains can't grow at low methane concentration (<50-100 ppmv), evidence of biological methane oxidation in upland soil has indicated the presence of high-affinity methanotrophs capable of consuming atmospheric methane (1.75 ppmv). The key enzyme in the methane oxidation pathway is the particulate methane monooxygenase (pMMO), which catalyzes the oxidation of methane to methanol.

PCR analyses of upland soil samples targeting conserved regions of *pmoCAB* have detected clusters of *pmoA* sequences that could not be attributed to any cultured methanotroph. One of these clusters was named the "upland soil cluster alpha (USCα)". As these USCα-*pmo* genes are consistently recovered in upland soil with measurable methane uptake, they are believed to be responsible for the atmospheric methane oxidation, thereby holding a key role in global methane cycle. Metagenomic studies revealed that these *pmoA* fragments are part of a complete *pmoCAB* operon affiliated to *pmoCAB* of alphaproteobacterial type II methanotrophs. Therefore, it is probable that USCα-*pmoCAB* encodes an active pMMO enzyme. Based on this assumption, it should be possible to express the USCα-pMMO in a cultured *Alphaproteobacteria* methanotroph, namely *Methylocystis* strain SC2. This particular methanotrophic strain has two pMMO isozymes (pMMO1, pMMO2) that have significantly different affinities to methane. By utilizing pMMO2, strain SC2 can survive under very low methane concentration. The expression of pMMO1, the pMMO with low affinity, is repressed under low methane concentrations (<600 ppmv), while pMMO2 is constitutively expressed. Due to the high-affinity pMMO2, strain SC2 is capable of sustaining its function at methane concentration below 100 ppmv. Our aim is to replace *pmoCAB2* (expressing pMMO2) with USCα-*pmoCAB* and to examine its methane oxidation activity in strain SC2.

We have designed a 5-Kb fragment containing the entire USCα-*pmoCAB* along with its native promoter, plus regions from both ends of *pmoCAB2* operon of strain SC2. The construct was synthesized and cloned into the Copy Control^{TM} pCC1 vector. For selection of recombinants in strain SC2, galactokinase and kanamycin resistance genes were inserted into this construct and electroporated into *Methylocystis* strain SC2. The transformants after first recombination have been selected on kanamycin-containing plates. Presence of pMMO1 allows screening of the putative mutants under high methane concentration. After second recombination, the transformants will be sensitive to kanamycin. After successful gene replacement, the transformants with USCα-*pmoCAB* will be identified by PCR with USCα-*pmoCAB*- and SC2-*pmoCAB2*-specific primers. Function of the USCα-*pmoCAB* encoded system can be characterized by culturing the SC2 mutant strain under low methane concentration. By observing physiology of the mutants, it will be possible to reveal the involvement of USCα-*pmoCAB* in high-affinity methane oxidation.

Keywords *Methylocystis* strain SC2; upland soil cluster alpha; *pmoCAB*; pMMO; gene replacement

Identification and characterization of the microbiota from rainbow trout (*Oncorhynchus mykiss*) and its aquatic environment as potential probiotics for a sustainable aquaculture

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Background

Aquaculture has the potential to contribute to the increasing demand for food of aquatic origin; however, the production intensification, the disease control and the environmental deterioration prevention means a significant challenge to this important economic sector. In this respect, the use of Lactic Acid Bacteria (LAB) of aquatic origin as probiotics constitutes an alternative strategy to the antibiotic treatment for disease control in the aquaculture farming.

Objectives

The objectives of this study were (i) the isolation and taxonomic characterization of LAB and total microbiota from rainbow trout (*Oncorhynchus mykiss)* in 5 different growth stages (larvae and fry [F1, F2, F3 and F4]) and its aquatic environment (feedstuffs, vegetation and tank waters), and (ii) the evaluation of the antimicrobial activity of LAB strains against the main Gram-negative and Gram-positive fish pathogen s.

Methods

The microbiota from rainbow trout (larvae: 5 days after hatching [DAH], F1: 30 DAH, F2: 90 DAH, F3: 240 DAH, and F4: 330 DAH), feedstuffs, vegetation and tank waters was isolated on MRS (for LAB microbiota) and TSA (for total microbiota) media. Ten different colonies from each origin and media were randomly selected and taxonomically identified by DNA sequencing of *16S rRNA* and/or superoxide dismutase (*sodA*) genes amplified by PCR. Antimicrobial activity of 1,052 representatives from all the aquatic origins was determined by a stab-on-agar test against the most relevant fish pathogens (*i.e.*, *Streptococcus iniae*, *Lactococcus garvieae*, *Yersinia ruckeri*, *Vibrio campbellii* and *Aeromonas salmonicida)*.

Conclusions

LAB and total microbiota from rainbow trout varied considerable depending on the fish growth stages, but Gram-negative bacteria such as *Aeromonas* spp., *Acinetobacter* spp. and/or *Citrobacter* spp. were the dominant microorganisms detected in all the stages. Interestingly, LAB population in total microbiota (principally *L. garvieae*) progressively increased along fish development. The most frequent LAB species were *L. lactis* and, to a lesser extent *L. garvieae*, *Pediococcus acidilactici*, *Weissella soli*, *Enterococcus* spp. and *Carnobacterium* spp. A total of 72.7% of the LAB showed antimicrobial activity against, at least, one of the fish pathogens tested, revealing that the rainbow trout and its aquatic environment are an interesting and appropriate source for the isolation of LAB with a potential application as probiotics for a sustainable aquaculture.

Keywords: Aquaculture, rainbow trout, probiotics, Lactic Acid Bacteria.

Identification of Thermophilic Cyanobacteria Isolates Obtained From Afyonkarahisar City-TURKEY

MERAL YILMAZ; GIZEM ARIK, MERIH KIVANC

Afyonkarahisar is a rich city from the point of view of geothermal energy potential. There are many hotsprings in city borders. In these hot springs water temperatures ranges from 50–90ºC. In this study, it was identified the cultivable cyanobacterial isolates obtained from 5 different hot spring locations based on 16S rRNA gen analysis. For this purpose water samples and mats collected. A total of 165 different isolates of cyanobacteria were obtained from culture medium BG11 and $BG11_0$. *Mbo*I and *MspI* restriction enzymes were used for the amplified ribosomal DNA restriction analysis (ARDRA) of 165 isolates. 8 different restriction profiles were obtained. One-two representative samples were selected from each profile for the partial 16S rDNA sequences analyses. BLAST results of 16S rRNA gene sequences in the GenBank (NCBI). based on the revealed that *Oscillatoria sp., Plectonema sp.*, *Thermococcus sp., Geitlerinema sp*., *Synechococcus sp.* genera may be cultivated from Afyonkarahisar hotsprings.

Improved growth performance of the mangrove *Avicennia marina* seedlings using a 1-aminocyclopropane-1-carboxylic acid deaminase-producing isolate of *Pseudoalteromonas maricaloris*

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Out of 62 bacterial isolates obtained from the mangrove *Avicennia marina* rhizosphere that grows along the Abu Dhabi coast, United Arab Emirates, an isolate of *Pseudoalteromonas maricaloris* (Wild type strain) (WT) produced relatively high levels of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase *in vitro*. Application of this WT strain under greenhouse conditions to *A. marina* seedlings significantly (P<0.05), reduced endogenous levels of ACC in the roots and shoots, and significantly (P<0.05) increased the levels of *in planta* endogenous plant growth regulators (PGRs) including indole-3-acetic acid (IAA), indole-3-pyruvic acid (IPYA), putrescine (Put), spermidine (Spd) and spermine (Spm) in roots and shoots compared with control mangrove seedlings. WT application has also significantly (P<0.05) increased photosynthetic pigment contents, photosynthetic carbon assimilation, plant water use efficiency and promoted mangrove seedlings growth characteristics including increased dry weight and length of roots and shoots, total leaf area and the number of the side branches compared with control mangrove seedlings. In comparison, an ACC deaminase non-producing mutant strain (NPM) failed to reduce endogenous levels of ACC in the roots and shoots and also failed to increase endogenous PGRs and photosynthetic pigments and did not promote seedling growth. Both WT and NPM strains were incapable of producing *in vitro* detectable levels of IAA, IPYA, Gibberellic acid (GA_3), zeatin (Z), Put, Spd and Spm in the culture filtrates. This study demonstrated for the first time the ability of ACC deaminase-producing bacteria to promote mangrove growth under greenhouse conditions. *P. maricaloris* has potential as biological inoculants to promote the growth of mangrove seedlings in afforestation programs in nutrient impoverished sediments in hyper-saline coastal areas in the UAE.

Keywords biological inoculants, ethylene, plant growth-promoting bacteria, plant growth regulators, rhizosphere, UAE

Inactivation of microorganisms by sunlight. The effect of solid particles

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Various diseases such as cholera, gastroenteritis, and different hepatitis, can be transmitted by contaminated water.Many microorganisms including bacteria, viruses, and protozoa are usually present in aquatic environments; some of them, pathogens, may be harmful for human health. They reach the water through feces, organic and inorganic particles, wastewater from industries, and other wastes produced by infected people. Numerous methods are used for treatment and disinfection of contaminated water to preserve people health and improve life quality. Disinfection by sunlight is a well known process that affects the survival of microorganisms in aquatic environments. However, this inactivation may not lead to a total removal of microorganisms due to different factors. Two main seasons: Wet Season (December-March) and Dry Season (April-November) can be differentiated in the Province of Salta, located in the northwest of Argentina. Rainfall during summer (Wet Season) increases the flow and the turbulence of rivers, resuspending small particles like clays that enhance the turbidity of the water. Turbidity refers to cloudiness of water and has no health effects itself but may indicate the presence of disease-causing organisms (EPA). These particles can interfere with sunlight disinfection providing a medium for microbial growth, increasing their persistence in the water column and becoming a potential health hazard because of the subsequent human ingestion.

The aim of the present study was to evaluate the effect of solid particles on the inactivation of microorganisms when exposed to natural sunlight.

Sediments from two aquatic environments (Campo Alegre Reservoir and Vaqueros River) were collected, dried and classified in different sizes in the laboratory. Water from the Wierna River was used to prepare two matrixes: one with a high concentration (5000 mg/l) of solid particles, diameter < 44 μm, (maximum turbidity in NTU) and another with no sediments (no turbidity in NTU). Each matrix was spiked with 10^6 UFC/ml of two bacteria: *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212). Cellulose dialysis tubing (M.W. 12,400 SIGMA) was cut into 10 and 20 cm-length pieces, which tied at the ends, were meant to contain the microcosms to simulate the aquatic environment. These “bags” where filled with both matrix and deposited into two glass containers (50×30×40 cm) containing also water from the Wierna River. One of the containers was exposed to the sunlight while the other was not; the latest was covered with black bags. Water from the glass containers was renewed every three days. Solar radiation was measured at every hour. During one month, samples were removed in duplicates from each container and taken to the laboratory for microbiological analysis. *Escherichia coli* (Method 1103.1-EPA, 2002) and *Enterococcus* (Method 1106.1-EPA, 2002), were determined by the Membrane Filtration Method using a three branch stainless steel manifold system (Sartorius, USA). Decay rates were calculated using a first order decay model ($C_t=C_0*e^{-kt}$).

Results indicate that microorganisms, with and without particles, decay faster when they are exposed to sunlight compared to those who were shielded from it. Also in both cases, with and without sunlight, microorganisms survived longer in the presence of solid particles than in water without them. Finally, it was observed that *Escherichia coli* was more persistent than *Enterococcus faecalis* in the presence of solid particles, maybe because *Escherichia coli* has a better adherence to particles in freshwater than *Enterococcus faecalis*, which seems have more affinity with seawater.

Keywords: sunlight inactivation, disinfection, water quality, solid particles, microorganisms

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Indoor mould ecology resembles ecological strategies in natural environments

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The ecological requirements of mould species, together with aspects on succession and species turnover, can be used to understand the establishment, development and even age of mould growth in damp buildings. This is important when potential health and building damages caused by moulds have to be assessed by legal instances, as these cases often end in litigation.

Growth requirements of moulds under laboratory conditions cannot be extrapolated to those in the building environment. In buildings, as in natural environments, moulds encounter fluctuations in temperature, humidity and nutrient conditions, competition against other microbes, and even animal predation.

Our results are based on 15950mould samplescollected in Norwegian buildings during the years 2001-2006. Both the building material (growth substrate) and type of construction (ecological niche) are knownfor each mould type.A total of 28 mould genera, 17 building materials, and 13 construction types have been cross-analyzed.

The results show specific growth preferences for many common indoor genera that resemble ecological strategies in natural environments. *Chrysosporium*, *Cladosporium*, *Stachybotrys*, *Trichoderma*, and *Ulocladium* can be used as indicators of specific water casualties indoors. Species turnover in particular constructions can often give a clue on the type of water damage (condensation, leakage, infiltration) and even the age of mould damage.

Influence of temperature on *Saccharomyces cerevisiae* UE-ME_3 response to titanium dioxide nanoparticles

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Titanium dioxide is a polymorphic material which can be found in nature in three mineral phases: rutile, anatase and brookite, the most unstable and of less interest. The form of NP-rutile TiO_2 (<100 nm) is described as one of the most toxic compound. While living organisms have been exposed with nanoparticles from millions of years ago and may be adapted to low levels of these materials, the increase of industrial capacity of synthesis, manipulation and massive use in electronic, energy and catalysis processes has increase the environmental levels of nanomaterials in several regions of the planet. The nanotoxicology is an emerging field for research, since fixed mass, density and surface reactivity are features of nanoparticles that contribute for the generation of ROS. The main intention of this work was to determine the influence of temperature and titanium dioxide nanoparticles on the growth of *S. cerevisiae* UE-ME_3, a wine wild-type strain of Alentejo, Portugal.

Cells growing at mid exponential phase in liquid YEPD medium with 2 % (w/v) glucose, at 28 or 40 ºC, were exposed during 200 min to 0.1 or 1.0 µg/mL of titanium dioxide nanoparticles (NP-TiO_2), prepared by sonication, at same temperature conditions. Samples of each treatment were used to obtain the post-12000 *g* supernatant for proteins, glutathione, ROS, MDA contents as well as GR, GPx, CAT A and LOX activities determinations [1, 2, 3, 4, 5, 6, 7, 8, 9].

The results show that the temperature influence differently the response of *S. cerevisiae* UE-ME_3 to titanium nanoparticles, since cells grown at 28 °C show dry weight, protein and glutathione contents higher than values determined in yeast cells grown at 40 ºC. In addition, it was observed a significant increase of glutathione content in cells exposed to nanoparticles at 28 ºC, response only observed in cells grown in the presence of 0.1 µg/mL of NP-TiO_2 at 40 ºC. However the GSH /GSSG ratio is greater in yeast cells grown at 40 °C, response which can be interpret by a sharp decrease of glutathione disulfide content, apparently justified by a significant decrease of GPx activity, more evident effect in cells exposed to NP-TiO_2. Furthermore, *S. cerevisiae* grown in presence of 1µg/mL NP-TiO_2 at 28 ºC reveal a lower value of GPx activity, as well as, higher values of ROS contents and LOX activity than control, which can explain the elevated MDA contents in these cells. In other hand the values of CAT A, LOX enzyme activities and ROS are significantly highest in cells grown at 28 °C. However, there was a significant decrease in CAT A and LOX activities, as well as, an increase of ROS in cells grown in the presence of 0.1µg/mL NP-TiO_2 at 28 °C. These results suggest that NP-TiO_2 at 28 °C induces oxidative stress and cell death. Although the biomass markers suggest a decrease of cell survival in cultures at 40 ºC, this response probably result from surface interaction of nanoparticles on cell membranes and denaturating effects induced by temperature.

Keywords: yeast, titanium dioxide nanoparticles, oxidative stress

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Inhibition of bacterial pyrite leaching by surfactants

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Bioleaching is the dissolution of metal sulfides like pyrite or chalcopyrite by bacterial oxidation processes. Beside the desired leaching effect in heap or tank leaching for winning valuable metals such as copper or gold, unwanted bioleaching causes acid mine drainage (AMD)/acid rock drainage (ARD) as a natural process. Wherever metal sulfides are exposed to the environment, e.g. in coal mines, bioleaching can cause pollution and acidification of surface- and groundwater. Little is known about inhibition methods or substances against unwanted bacterial leaching. Addition of surfactants to active leaching cultures decreases bacterial cell number and leaching. No bacterial growth was indicated in ironII-ion- or pyrite- containing media including surfactants. By the use of a LIVE/DEAD-kit (® BacLight™) we were able to show that mainly all bacteria were inactive.

To investigate the inhibition effect of surfactants *in situ*, a percolator system was used filled with pyrite containing material from a brown coal area where unwanted bacterial leaching occurs.

Each percolator was filled with 3kg soil, and 5L water was cycled. Surfactants were added after bacterial leaching was established. Bacterial leaching was determined by ironII-ion concentration and bacterial metabolic activity. Metabolic activity was measured by calorimetric measurements. Additionally, growth capability of ironII- and sulfur oxidizing bacteria were tested without surfactants.

The addition of surfactant inhibited leaching within one week and was stable till the end of the experiment after eight weeks. Microorganisms grow in fresh growth media was negligible.

Based on these findings applications for inhibition of bacterial leaching in brown coal mining areas can be established.

Keywords: bioleaching, inhibition, AMD/ARD, surfactants

Isolation and Characterization of Thermophilic Bacteria from Beach Hot Spring in Kagoshima, Japan

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A total of 48 thermophilic bacterial strains were isolated from a beach hot spring in Ibusuki, Kagoshima prefecture, Japan. The isolates were cultured at temperatures between 50 to 75°C on the medium containing 1.5% NaCl. 31 strains hydrolyzed starch, while 4 strains hydrolyzed casein. On the basis of 16S rRNA gene sequence analysis, 34 strains were identified as *Rhodothermus marinus*, 8 as *Thermus thermophilus*, 3 as *Albidovulum inexpectatum*, and 1 as *Thermoactinomyces vulgaris*. Two strains demonstrated a distant phylogenetic relationship (less than 95% sequence similarity) with published species, suggesting that they might be novel species. A culture-independent molecular analysis is now being conducted for the characterization of natural microbial communities in the environment.

Keywords Thermophilic bacteria; Beach hot spring

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Isolation and molecular characterization of an extreme halophilic, multi-drug resistant denitrifying bacterium *Halomonas sp* (sm-sr10) from Sundarban, India

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Recently, when studying on the microorganisms surviving in the water of halophytic mangrove forests of Sundarban, India, an extremely halophilic bacterium was isolated and characterized. Designated as sm-sr10, this pleomorphic, Gram + bacterium grow up to 4M NaCl added medium containing tryptophan, beef and yeast extract. It is identified as *Halomonas Sp* at biochemical and 16S rDNA level (GenBank Acc. no **HM446042**). The scanning electron microscopic picture of 24 hr culture reveals its size (Len-0.97 µM and Diam-0.67 µM at 20 kV) with a rough surface. This alkaliphilic, non-capsulated and non-endospore forming bacterium showed negative results for amylase, gelatinase and positive for catalase. It could utilize D-glucose, D-fructose, sucrose, maltose, lactose and mannitol. The sm-sr10 efficiently reduced nitrate and nitrite. It showed resistance to streptomycin, kanamycin, neomycin and methiciline and poses a large plasmid (~50 kb). The 16S rDNA sequence reveals that sm-sr10 belongs to genus *Halomonas* and family *Halomonadaceae*. The phylogenetic tree, constructed by MEGA4 and nucleotide homology data suggest its similarity with *Halomonas shengliensis* SL014B-85 and *Halomonas sp*. MOLA 69 (99%); with *Halomonas sp*. Ad-1 and *Halomonas sp*. C-12 (98%) and with *Halomonas sp*. whb34 (97%). Present results and further studies have implications for the understanding of saline adaptations, stress and high degree of drug resistance of sm-sr10 in partially polluted Sundarban regions and there is scope to utilize this microorganism for biotechnological purpose.

Isolation of a bacterial community able to remove aerobically a mixture of 4-chlorophenol and 2,4,6-trichlorophenol as the sole carbon source, in a two stage packed bed reactor

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To carry out the biodegradation of a mixture of 4-chlorophenol (4-CP) and 2,4,6-trichlorophenol (TCP) as the sole source of carbon and energy, the ability of a microbial community isolated from a river exposed to industrial pollutants in the state of Tlaxcala, Mexico, was studied.

In the isolated microbial community, six cultivable bacteria were found, but only three of them were predominant, *Stenotrophomonas* sp., *Agrobacterium* sp. and *Burkholderia cepacia*.

The ability of this microbial community to remove aerobically chlorophenols was evaluated using a 1.2 L two stage reactor packed with a porous volcanic stone (tezontle). The biodegradation kinetics of the chlorophenols mixture was studied in the biofilm reactor continuously operated in steady state, at different volumetric loading rates of the mixed substrates ($B_{V,CL}$).

While the concentration of chlorophenols mixture was maintained constant (25 mg L^{-1} of each one), de $B_{V,CL}$ values were varied from 0.36 to 12.6 mg L^{-1} h^{-1}. The TCP was removed more efficiently than 4-CP by the microbial community. After analysis by HPLC of the outflowing liquid, it was determined that TCP removal efficiencies were 100% at volumetric loading rates lower than 8 mg L^{-1} h^{-1}. In this operational conditions, the maximum removal efficiency of the chlorophenols mixture was 96%, while the average removal efficiencies of the chemical oxygen demand (COD) and total organic carbon (TOC) were 77% and 87% respectively.

Keywords chlorophenols; packed bed reactor; biodegradation

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Making Biological Brick as Building Material by Bio-calcification

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In this study, an indigenous bacterium was isolated from soil, identified as *Staphylococcus pasteurii* HF 2011by 16s rRNA sequencing method and registered in EMBL with Accession Number of FR839669. This facultative aerobic microorganism was employed as a urease positive bacterium for calcite precipitation of sandy soils. The 40mm height and 70 ×70 mm^2 cross section area PVC cubic sample was positioned and packed with sandy soil (from Sistan desert) to a dry density of 1.53 g/cm^3. Each end of the cubic was fitted with scotch for preventing disturbance of soil particle through the injection of bacterial suspension and reactant solutions. Fluid reservoirs containing the injected fluids were placed at the top of the cubic. Bacterial cell growth and urease activity were determined by spectrophotometer and conductivity method, respectively. After 30 days, the mechanical behaviors of the packed soil were determined from the interpretation of the results of XRD and unconfined compressive strength tests. The compressive strength of the brick was obtained as 8 MPa, which is appropriate for building construction material.

Keywords : biological Brick, Building material, *Staphylococcus pasteurii,* biocementation

Marine antifouling coating for the sustainable and environmentally friendly farming of *Argopecten purpuratus*: Natural products modulators of microbial Quorum Sensing as additives

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In general, the scallop production is a harvest activity, but due to the world-wide problem of fishery the global trends is to restrict this activity. In this context, Chile early established as production strategy areas for farm and specific aquaculture techniques. As result, the production by aquaculture of the North Chilean Scallop (*Argopecten purpuratus*) has an important economic significance. Actually, the 95 % of chilean scallop productions by aquaculture is exported to European countries. Nevertheless, to consolidate the development and growth already reached, Chile is facing important challenges in order to make aquaculture a sustainable activity, environmental friendly and sure for the human health. On this context, the strategies for the control of problems associated to management techniques are mayor complications. The main problem associated to North Chilean Scallop is the biofouling that affect the submerged cultivation installations. The goal of the present work is to develop alternatives based on the chemical diversity present on natural products from different origins. Specifically is presented a coats formulated with natural extracts for the treatment of submerged installations. Natural extracts were selected in basis to his ability for affect the biofilm development, (of *Cobetia marina* and *Listonella anguillarum*), and modulate the microbial cell communication using Quorum Sensing biosensors. Likewise, the toxicity of selected natural extracts was evaluated *in vivo* by growth and mortalities of young scallops. Natural extracts were obtained from *Canelo* (Drimys winteri) and biofouling present un farm areas. The chemical characterization of natural extracts selected was by Gas chromatography–mass spectrometry (GC-MS) and NMR spectroscopy. Finally physical and chemical properties of selected extracts were evaluated in order to his incorporation in a coating formula able to resist marine environment. Results obtained indicate that characterized organics and hydro-alcoholic fractions obtained from Canelo and biofouling respectively modulate the Quorum Sensing response, avoid the development of *L. anguillarum* biofilms and can be used as additives for marine coatings. The coatings formulated are for marine environments and is non-toxic for North Chilean Scallop. Project funded by Corfo-Innova, Government of Chile.

Keywords hydrocarbonoclastic; antifouling; coatings; Chilean scallops

Microbial assessment and antibiotic susceptibility of pathogenic bacteria of Domat Al-Jandal Lake, Saudi Arabia

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Lake Domat Al-Jandal is a product of irrigation project in Domat Al-Jandal that started in 1987 and lies four km to the north of Domat Al-Jandal province and about 33 km of Skaka, AL Jouf Province, Saudi Arabia. The lake is formed in a low basin of water surrounded by high hills from all its sides, over an area of 1.1 million square meters, shapes are not regular, surrounded by 8 km, and depth varies from 4 to 17 meters. Water is sweet, but after passing over saline area it becomes salty, collected in tanks then pumped from current lake area, which is able to carry about 30 million square meters of water. A study on microbial assessment and antibiotic resistance of water and sediment in Domat Al-Jandal Lake was carried out. Samples included water and sediments from the Western, Eastern, North and South Basins of the lake. The analytical results obtained indicated that there are considerable variations among the examined samples with respect to their microbial composition. Microbiological analyses indicate that the only issue of true concern is the total coliform, which was too numerous to count and, therefore, warrants more attention. Total coliforms, thermotolerant coliforms, *E. coli*, *Enterococcus* spp., *Salmonella* sp., *Staphylococcus* spp. and *Pseudomonas aeruginosa* were detected in samples. Antibiotics susceptibility testing was selected, pathogenic bacterial isolates revealed resistance against most applied antibiotics. The levels of resistance of bacterial to various antibiotics differed considerably.

Keywords Microbial assessment; antibiotic susceptibility; Domat Al-Jandal Lake; Saudi Arabia.

Microbial community in a biofilter treatingodours from a valorization center for municipal solid waste treatment (MSW)

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Municipal solid waste treatment (MSW) stations with energetic valorization make use of the biogas produced by the digestion of organic matter to generate energy. However, complex odours associated with the MSW activities, like hydrogen sulfide (H_2S), organic reduced sulfur compounds (e.g.,CH_3SH), and volatile organic compounds (VOCs) have to be controlled. The current work aims to manage these compounds using biological air treatment systems, such as biofilters. A biofilter filledwith a mixture of wood-chips and compost was implemented at a MSW. Inocula from lab scale reactors treating organic compounds was used during the start up. The microbial communities present in this matrix, in the inoculum and in matrix after an acclimatization period was investigated. Colony forming units (CFU/g) ranged from 10^8 (matrix), 10^6 (inoculum) to 10^8 (after acclimatization) CFU/g. Microbial communities differed at each stage. After 16S rRNA analysis, 26 different isolates (26% being *Actinobacteria*) were identified from the initial matrix, 11 from the inoculum (46% being *Firmicutes*) and 29 from the matrix after the acclimatization period (38% being *γ-Proteobacteria*).Twenty seven of the recovered isolates were able to oxidize sulphur compounds in solid medium (Sulphur Oxidizing Medium), in continuous successive transfers. Of those, 37% belong to the *γ-Proteobacteria* phylum. Their capacity of growingin liquid medium is under evaluation.

Microbial Populations of Sungurlu Salterns in Turkey

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Sungurlu is the largest district of Çorum Province in the Black Sea Region of Turkey, located at 72 km south-west of the city of Çorum. Sungurlu salterns are located 740 m above sea level. These salterns consist of little ponds which are fed by hypersaline spring water rich in sodium and chloride. The microbiota inhabiting these salterns was examined by fluorescence in situ hybridization (FISH), 16S rRNA gene Denaturing Gradient Gel Electrophoresis (DGGE) analysis, and cultivation techniques. Isolation was carried by using 23% Modified Growth Medium. Both extremely halophilic Bacteria and Archaea were isolated. The microbiota inhabiting the ponds was dominated by *Archaea.* Archaeal and Bacterial populations also detected with universal FISH probes. Isolates were analyzed and selected according to their Amplified Ribosomal DNA Restriction Enzyme (ARDRA) profiles. Most of the sequenced DGGE bands were related to *Halorubrum* (for the Archaea community) and *Halomonas* (for Bacteria). Archaeal and Bacterial populations also detected with universal FISH probes.

Keywords: Sungurlu Salterns, DGGE, FISH

Acı Göl/Türkiye'nin Mikrobiyal Populasyonları

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Acı Göl tektonik orjinli sığ ve tuzlu athalassik bir göldür. Çardak ve Dazkırı arasında Afyon ve Denizli illerinin sınırında (37°49'N–29°48'E) 836 m rakımda Türkiye'de yer almaktadır. Tuzluluğu ‰80 ila ‰200 arasında değişen tuzlu bir göldür. Sığ bir göl olduğundan tuzluluğunda sık değişimler olur. Bu göldeki bakteriyal çeşitlilik Denatüre edici gradient jel elektroforezi (DGGE), floresan in situ hibridizasyon (FISH) ve kültüre alma teknikleri ile araştırılmıştır. %23 Modifiye büyüme ortamı ile izaolasyon yapılmıştır. Hem Arkeal hem de Bacteria domaini izole edilmiştir. Dizi analizi yapılmış bantların çoğu *Haloarcula, Haloterrigena*, *Halorubrum* (Arkeal kommünite için) ve *Halomonas*, *Halovibrio*(Bacteria için) ile benzer bulunmuştur. Evrensel FISH probları ile hem Arkeal hem de Bacteria populasyonları tespit edilmiştir.

Anahtar Kelimeler: Acı Göl, DGGE, FISH

Microbial Risk Assessment for Food and Water Safety: challenges to developing countries

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New challenges in the safety of food and water supply require new strategies for evaluating and managing food and water risks. Microbial risk assessment (MRA) is a process that evaluates the likelihood that adverse human health effects will occur following exposure to a pathogenic microorganism or to the medium in which the microorganism occurs. This evaluation determines how changes in pathogens, food preparation, water supply, distribution and consumption, can adversely affect human health. Information obtained may then be used to inform decisions about appropriate management of the food and water supply systems. Although microbial risk assessment has been used to estimate disease burden from water and food supplies in developed countries, the method has not been adequately evaluated in developing countries due to dearth of relevant data. Microbial risk assessment offers many benefits when used in the appropriate circumstances because it has the potential to improve the understanding of key issues, enables an objective evaluation of risk management options, and provides a scientific justification for actions. In the application of MRA, the gap between developing countries and some industrialized countries is quite extensive. However, many developing countries have realized and recognized the need to, at least, understand and move toward using MRA. The appropriateness of the need to address the gap and the challenges facing the developing countries are hereby discussed.

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Microbiological control of the floor in open air children playgrounds

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Playgrounds are part of township kindergartens, used by children, adults and animals that share the environment, such as: several species of birds, dogs, cats and rats. In order to identify, quantify and characterize the microbial flora in the soil of six (6) playgrounds located in Lisbon, samples were collected bimonthly for a period of nine (9) consecutive months. The six (6) randomly selected playgrounds vary in geographical location, number of users, social status of the target population, surroundings and cleaning conditions. Associated to these variables it is also included the local weather variation between December 2010 and July 2011.

The six (6) samples consist of 5mm pebblestone, which have been collected near the surface of playground toys and trees.

For the analysis, it was used 25g of each pebblestone suspended in 225ml of BPW. After shaking, the suspension was filtered in different amounts, of 500µl and 10ml, in order to obtain a quantitative relation. Agar culture media was selected accordingly to the microorganism to detect: fecal and total coliforms (MacConkey, bioMérieux), fecal Enterococci (Slanetez and Bartley Medium, Oxoid) *Staphylococcus* spp. (Baird-Parker bioMérieux), Fungi (Rose Bengal Chloramphenicol, Oxoid), *Candida albicans* (Brilliance Candide, Oxoid) and *Salmonella* spp (Brilliance Salmonella, Oxoid). After isolation of microorganisms, detected in the cultures, biochemical tests (API, bioMérieux) were conducted, in order to identify the genus and species. One of the objectives of this work is to characterize the antimicrobial resistance of the detected bacterial organisms. To achieve this purpose, the isolated bacteria were scattered in culture media with ampicillin. In isolates that grow in this medium it was studied the antimicrobial susceptibility for a panel of different antibiotic classes.

It was possible to say that climatic changes, normal in every season and included in this study, had a major impact on microbial flora: when there is an increase of temperature, there is a significant decrease in the number of bacteria, while with fungi such phenomenon is not registered.

Among the six playgrounds, differences may be noted in the total amount of microorganisms. Low rates are directly related to the cleaning conditions, social status of visitors and animal control. In playgrounds where the number of visitors is lower but there is no guard and all animals have free access to the facilities the number of microorganisms incresed.

However it should be noted that in five (5) of the six (6) playgrounds similar quantities and species of ampicillin resistant microorganisms were found, such as *Escherichia coli, Vibrio parahaemolyticus, Enterobacter faecalis, Enterobacter cloacae*.

The remaining playground that showed the lowest levels of ampicillin resistent microorganisms is located in a large public garden, where there is an extensive vegetation, that is supervised by township employees and it has a very good clean status.

Our results demonstrate that a microbial control of children playgrouns is importat as many of the microorganisms found are part of commensal flora that could be potencial pathogens to humans.

Keywords playgrounds, microorganisms, antibiotic resistance

Molecular basis of electron transfer mechanisms supporting extracellular respiration in *Shewanella oneidensis*

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Bacterial extracellular respiration has become of great interest to the science and engineering community due to the potential use of these organisms in bioremediation of contaminated environments with heavy metals and in biotechnological applications for energy production in microbial fuel cells. Fulfillment of this promise requires a thorough understanding of the cellular physiology and molecular details of the process of reduction of insoluble terminal electron acceptors. The dissimilatory metal reducing bacteria *Shewanella oneidensis* MR-1 (SOMR1) has been chosen as a model organism due to its metabolic versatility, the availability of a sequenced and annotated genome, genetic tractability and ease of laboratory manipulation under a relatively wide range of conditions. The electron transfer pathway of SOMR1 to inorganic minerals and metals is sustained by several c-type multiheme cytochromes that shuttle electrons from cytoplasm and inner membrane oxidizing enzymes toward the outside of the cell during anaerobic respiration. Extensive genetic and biochemical data suggest that the pathway to move electrons from the intracellular quinol pool to extracellular metal oxides involves series of intermolecular electron transfer events in the cytochromes CymA, STC, MtrA, OmcA and MtrC. However the molecular mechanisms of electron transfer between these proteins and the specific interactions between them and metal oxides remains to be elucidated. This knowledge can only be acquired with detailed thermodynamic and kinetic information of each protein capable of discriminating the role of the various hemes to obtain a clear view of the electron transfer mechanisms performed by SOMR1.

STC is one of the most abundant multiheme cytochrome found in the periplasm of SOMR1. This small cytochrome of 12 kDa molecular mass and containing four c-type hemes was shown to be involved in iron reduction. The determination of the 3-dimensional structure of STC led to the proposal that this protein may work as a nonspecific electron harvester, but latter it was proposed that hemes I-III would feed electrons to heme IV. Only with the full thermodynamic characterisation, specific roles for the hemes were proposed, with heme I serving as the electron entry gate and heme IV as electron exit. Stopped-flow experiments allowed the discrimination of the kinetic properties of the individual hemes of STC. These results established a functional specificity of each redox centre, which is at the basis of a directional electron flow within this cytochrome. Isothermical titration calorimetry and transient-kinetic studies of specific mutants of STC allowed the identification of specific aminoacid residues in the vicinity of heme I and heme IV that are involved in iron binding and reactivity.

Keywords *Shewanella oneidensis* MR-1; multiheme cytochromes; electron transfer; iron oxides

Nanoiron cytotoxicity toward the cyanobacterium *Anabaena planktonica* and the green alga *Chlamydomonas* sp.

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Nanoiron particles have often been proposed and tested for environmental applications. There is no doubt about nanoiron strength for remediation of various aromatic compounds, polychlorinated biphenyls and other pollutants. On the other hand, it is important to carefully consider environmental aspects of nanoiron toxicity toward soil and water microbial communities. Although several studies have been published on the nanoiron toxicity toward bacteria and invertebrates, effects on cyanobacteria and algae has not been investigated yet.

We studied the effects of zerovalent nanoiron on common freshwater species *Anabaena planktonica* and *Chlamydomonas* sp. from non-axenic batch cultures. We used two types of nanoiron: suspension of unmodified nanoiron and suspension of nanoiron coated with sodium polyacrylate.

The effect of nanoiron was first determined spectrophotometrically using Cytotox 96® Assay. The cytotoxicity was calculated from the amount of lactate dehydrogenase (LDH) enzyme released from cells after nanoiron action. Final concentrations of nanoiron in the samples ranged from 100 to 2500 mg/l. The highest cytotoxicity (~ 60%) of unmodified nanoiron for *A. planktonica* was detected after 2-h incubation at concentration of 750 mg/l. Lower toxicity of other nanoiron concentrations was caused either by rapid oxidation of nanoiron at lower concentrations (100-500 mg/l) or formation of less reactive aggregates at higher concentrations (> 1000 mg/l). The cytotoxicity of the coated nanoiron could not be measured using Cytotox 96® Assay, because the samples were artificially colored by sodium polyacrylate.

Moreover, we determined viability of the culture *Chlamydomonas* sp. after nanoiron treatment using cell analyzer CASY® TT. We presumed that cells with impaired cell walls and outer membranes were not viable and thus we were able to calculate the proportion of dead and viable cells in the sample. Highest impact on *Chlamydomonas* showed sodium polyacrylate-coated nanoiron at concentration of 500 mg/l. Cell viability decreased after 4 hours to ~ 27% of total cell number, while unmodified nanoiron at the same concentration had much lower impact (~ 65% of viable cells). This matches well with results from Cytotox 96® Assay, where the cytotoxicity of unmodified nanoiron (500 mg/l) toward *Chlamydomonas* sp. was 30%.

Finally, we employed fluorescence microscopy to verify direct impact of nanoiron on single cells of *Chlamydomonas* sp. We used a kit for detection of reactive oxygen species (Total ROS detection kit®). Reactive oxygen species were, in our case, generated by zerovalent nanoiron via the Fenton reaction. Almost all cells treated with 500 mg/l of sodium polyacrylate-coated nanoiron were fluorescently labeled. This test was not possible to perform with *A. planktonica* due to high interference between autofluorescence of cyanobacterial phycobilins and fluorescence of the fluorescent dye.

To conclude, nanoiron showed cytotoxicity toward both *A. planktonica* and *Chlamydomonas* sp. Nanoiron was able to disrupt cell walls and membranes and intracellularly generate reactive oxygen species. The sodium polyacrylate-coated nanoiron was more harmful, which was caused by prolonged time of its reactivity and better dispersibility.

Keywords zerovalent nanoiron; cytotoxicity; *Anabaena planktonica*; *Chlamydomonas* sp.

Nanopods: A New bacterial structure for deployment of outer membrane vesicles

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Bacterial outer membrane vesicles (OMV) project metabolic function into the environment *via* the proteins and other molecules they contain. Production of OMV is widespread in proteobacteria, but OMV have been extensively studied only in organisms that inhabit fully hydrated environments (i.e., pathogens). But, many, arguably most, bacterial habitats are only partially hydrated. For example, in soil, water is characteristically distributed as films, which are on average thinner than are typical OMV (*ca.* ≤ 10 nm water film *vs.* 20 to > 200 nm OMV). Thus, for soil bacteria, the free release of OMV would be of limited effectiveness for expansion of their sphere of metabolic influence, as OMV diffusion would be highly constrained. We have identified in the phenanthrene-degrading soil bacterium *Delftia* sp. Cs1-4 a new bacterial surface structure, termed a "nanopod", that is a conduit for projecting OMV significant distances (*e.g*., ≥ 6 µm) from the cell. A nanopod is an undulating, tubular element in which chains of OMV are encased within a sheath of a surface layer protein, termed Nanopod protein A (NpdA). Nanopods produced by phenanthrene-grown *Delftia* sp. Cs1-4 contain a variety of enzymes including gamma-glutamyltransferase (GGT), for which activity in nanopods was verified. Specific metabolic functions for nanopods are not yet known. However, a connection with metabolism of glutathione conjugates of phenanthrene is hypothesized since nanopod formation was induced by growth on phenanthrene, and such metabolites have been tentatively identified by mass spectrometry. Also, GGT is best known for its function in glutathione metabolism. Orthologs of NpdA were identified in three other genera of the *Comamonadaceae* family that have diverse lifestyles, including free-living soil bacteria (*Delftia acidovorans* SPH1, *D. acidovorans* ATCC 15688, *Acidovorax delafieldii*), plant pathogens (*Acidovorax avenae s*ubsp. *avenae* ATCC 19860, *A. avenae* subsp. *citrulli* AAC00-1) and an earthworm symbiont (*Verminephrobacter eiseniae* EF01-2). Nanopods are new bacterial organelles, and establish a new paradigm in the mechanisms by which bacteria interact with their environment. Specifically, they create a pathway through which cells can effectively deploy OMV, and the biological activity that these transmit, in a diffusion-independent manner. Nanopods would thus allow bacteria to expand their metabolic sphere of influence in a manner previously unknown. It will be interesting to determine how, or if, bacteria tailor nanopods with functionality suited to support diverse lifestyles.

Oxidation of pyrite by the action of microorganisms

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As part of the mechanisms that carry out the oxidation of pyrite are those who are dependent on the action of the soil microorganism. In order to estimate the importance of these processes have been chosen soil samples from mining areas and have attempted to determine the existence of some kind of microbial population present in them from their leachates. It has sought the number of total bacteria and the possible existence of others that may come from any source of organic pollution. Of the eight soil types analyzed microbial growth was apparent in six of them, but only in the means for total heterotrophic bacteria counts, while two of them this estimate was not feasible. Our results are preliminary and have isolated the bacteria that are viable land pending a molecular characterization that allows us to explain in greater detail the oxidation processes that take place in these soils.

Keywords: Pyrite, leachates, acid-soil.

Passage through *Tetrahymena tropicalis* enhances the resistance to stress and the infectivity of *Legionella pneumophila* Lens

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L. pneumophila is a Gram-negative bacterium prevalent in fresh water, which accidentally infects humans and responsible of the disease called legionellosis. In natural environments, *L. pneumophila* replicates inside protozoan hosts, primarily amoebae. Growth of this bacterium in amoeba leads to an increase of *L. pneumophila* survival and infectivity. Ciliates of the genus *Tetrahymena* are ubiquitous in surface freshwater and capable of supporting the multiplication of *L. pneumophila*. However, the stationary-Phase Forms (SPFs) of *L. pneumophila* differentiate into Mature Intracellular Forms (MIFs) without apparent replication in the species *T. tropicalis*.

Our results presented here show that MIFs released from *T. tropicalis* are more resistant to gentamicin, survive better in a nutrient-poor environment, and are more infectious to the human pneumocyte cells than SPFs. These results strongly suggest a potential role of ciliates in increasing the risk of legionellosis.

Keywords: *Legionella* survival, Mature Intracellular Forms, *Tetrahymena* pellets

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Presence of virulence traits and antibiotic resistance among enterococci isolated from Eurasian otter (*Lutra lutra* Linnaeus, 1758) in Portugal

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Enterococci are ubiquitous microorganisms found as part of the normal intestinal microbiota of many animals, among which the free-ranging Eurasian otter (*Lutra lutra* Linnaeus, 1758). For the present investigation twenty-nine enterococci were isolated from fecal samples of Eurasian otters free-living in reservoirs and associated river stretches in Alentejo region, South Portugal.

After identification by molecular methods the isolates were allocated to the species *Enterococcus faecalis* (n=19)*, E. faecium* (n=9) and *E. durans* (n=1). Molecular typification by PCR-fingerprinting demonstrated the high diversity of the enterococci included in this collection. Regarding the screening for virulence factors, three strains produced cytolysin and six were gelatinase-positive. The genes *ace* and *acm* were detected in five enterococci each, *ebpABC* in seventeen isolates, *gelE* in fourteen and *cylA* in three strains. A multiresistant phenotype was observed for all the enterococci under analysis with all isolates being resistant to cephalexin, cephotaxim, nalidixic acid, clindamycin, streptomycin and sulphamethoxazole/trimethoprim. The antibiotic resistance genes *tet(M)* and *pbp5* were detected in seventeen isolates each, whereas *vanB* and *vanD* were identified in thirteen and five enterococci, respectively. The majority of the *van*-harboring isolates belong to the species *E. faecium*. The *aac(6')-Ie-aph (2")* gene, encoding for gentamicin resistance, was observed in all gentamicin-resistant otter isolates analyzed.

Since all otter-enterococci present a multiresistant profile and several harbor virulence and/or antibiotic resistance genes, the role of free-living Eurasian otters in dissemination of virulent/resistant strains among other animals sharing the same ecological niche cannot be disregarded, as well as the health risk they may represent for humans directly interacting with them or their habitat.

Prevalence of sulphonamide resistance genes in sediments of the aquaculture environment

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Concerning to the human and animal public health, there is an emergence of antibiotics resistance appearance in the environment, due to use of antibiotics particularly the prophylactic and therapeutic use of antibiotics in aquaculture. The works presented here investigates the prevalence of three different sulfonamide-resistance genes, sul1, sul2 and sul3, in sediments of two aquaculture farms and their pristine areas (200m surrounding from the farms) in the Baltic Sea. Both of the aquaculture farms had stopped using antibiotics for 6 years prior to the first sampling. In addition, HPLC measurements of sulfonamides concentration in sediments also will be conducted to address the absence of selection pressure in the farms environment.

We qualitatively monitored two of the sulfonamide-resistance genes, sul1 and sul2, presence in the both aquaculture farms by using standard polymerase chain reaction (PCR)-based. Quantitatively, qPCR-based will be performed to determine sul1 and sul2 resistance genes in the sediment samples which had been collected during summer for four years. Interestingly, our results showed parallel pattern with a recent published results of the persistence of tetracycline resistance genes in the same sampling aquaculture farms.

Keywords; sulfonamide resistance genes, aquaculture.

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Quantifying microbial degradation of trace pollutants with Isothermal Titration Calorimetry (ITC)

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Pollutants belonging to the group of hydrophobic organic compounds (HOC) are of high interest due to the ubiquity, persistence, and potential health effects. Their environmental fate is often determined by biological (microbial) degradation. The degradation often occurs in the aqueous phase. HOC are only soluble in aqueous solutions in trace concentrations and tend to interact with bioreactor materials and sampling devices. Furthermore, HOC are frequently volatile, so that conventionally derived degradation parameters are often biased. We report on the development and validation of a novel calorimetric approach that serves to gain real time information on the kinetics and the physiology of HOC bioconversion in aqueous systems while overcoming weaknesses of conventional biodegradation experiments.

For testing the method, different soil bacteria were exposed to pulsed titrations of different HOC and the thermal responses were monitored. The strain/HOC combinations were selected as examples for complete [1] and partial biodegradation and complete degradation with storage product formation, respectively. Heat production rates were interpreted thermodynamically and biodegradation kinetic parameter (e.g. in terms of Michaelis-Menten kinetics) were derived. Comparison with conventional methods shows the suitability to extract kinetic degradation parameters of organic trace pollutants from simple isothermal titration calorimetry (ITC) experiments, while thermodynamic interpretation provided further information about the metabolic fate of HOC compounds [2].

The practical advantages of ITC in comparison to conventional methods are beside of delivering this additional information:

- Systematic failures are minimized because the measurement and reaction are done in the same vessel.
- The reliability of the derived kinetic parameters is improved due to multiple automatic injections and the on-line monitoring of the biodegradation process.
- The handling is convenient because sampling is not required anymore and the manual efforts are strongly reduced.
- In case of side product formation the influence of this product on biodegradation kinetics can be studied in one experiment due to multiple automatic injections.
- The recently emerging multichannel calorimeter makes the method potentially suited for high throughput measurements.

Keywords Hydrophobic organic compound; Biodegradation; Isothermal Titration Calorimetry

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Sand Dunes Fixation as a Novel Application of Bio-calcification by *Bacillus pasteurii*

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This work is an investigation of the new method for fixation of sand dunes through the use of microbial carbonate precipitation by *Bacillus pasteurii* .The analysis of the results was performed by using the interpretation of results obtained from X-Ray diffraction, Scanning Electron Microscopy and physical test.

Keyword: Bacillus pasteurii, Sand Dunes Fixation, Microbial carbonate precipitation

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Seasonal variability changes in microbial population, diversity and physico-chemical quality of beach waters in Durban, Kwazulu-Natal province of South Africa

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Although water is generally considered a recyclable resource, proper management and protection is required to prevent over-exploitation and pollution due to industrial growth, urbanization and anthropogenic problem. In this study, the seasonal variability of microbial distributions and physico-chemical quality of six beaches in Durban, KwaZulu-Natal Province of South Africa was investigated using standard methods. PCR-DGGE method was employed to evaluate seasonal changes in the bacterial community. Temperature, pH and turbidity profiles varied significantly ($p < 0.05$) from 13 to 27°C; 6.37 to 8.30 and 0.57 to 2.37 NTU respectively, across the seasons. Higher BOD_5 values ranging from 1 to 9.05 mg/L were recorded during spring and summer, while COD and conductivity values ranged from 43 to 149 mg/L and 4730 to 5190 mS/m respectively. Total coliform (TC) and faecal coliform (FC) counts varied significantly ($p < 0.05$) and ranged from 1.468×10^2 - 6.40×10^2 cfu/100ml and 0.61×10^2 - 3.37×10^2 cfu/100ml respectively. *Vibrio cholerae* (VC), Salmonella (SAL) and Shigella (SHIG) counts ranged from 1.364×10^2 - 4.44×10^2 cfu/100ml, 0.6×10^2 - 3.075×10^2 cfu/100ml, and 0.844×10^2 - 3.775×10^2 cfu/100ml, respectively. Significant correlations were observed between VC and turbidity ($r = 0.545$), VC and BOD_5 ($r = 0.459$), SAL and temperature ($r = 0.545$), SHIG and temperature ($r = 0.600$) and SHIG and BOD_5 ($r = 0.492$). pH displayed negative correlations with TC ($r = - 0.558$), VC ($r = - 0.542$), SAL ($r = - 0.538$) and SHIG ($r = - 0.609$). The DGGE profiles of the bacterial communities revealed a total of 127 different bands over the seasonal periods, showing a wide bacterial diversity over the four seasons. The observed seasonal fluctuations of the microbial and physico-chemical environmental variables among the investigated beach waters indicate the dynamic nature of these aquatic resources. Appropriate quality monitoring of these waters is needed for proper protection of public health and vulnerable water resources.

Keywords: Beach waters; DGGE; faecal coliforms; public health; recreational water quality

Study of dissemination and removal of multidrug resistant *Salmonella* in two sewage treatment plants from Comunitat Valenciana (Spain)

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The aim of the present study was to gather more information about the levels of faecal indicator coliform bacteria in different stages of sewage treatment plants (STP) and their relationship with the occurrence of *Salmonella* spp. Moreover, it investigates the resistance of *Salmonella* spp. Isolated strains to different commonly used clinical antibiotics. This is a Public health issue, as in our geographical area regenerated water is used for agricultural irrigation.

Water samples were collected from two different sewage treatment plants with tertiary treatment located in Comunitat Valenciana (Spain). These included a total of 45 water samples comprising 21 from STP1, at the entry (untreated sewage), at secondary treatment effluent and at tertiary treatment effluent (UV desinfection), and 24 from STP2, including one more point of sampling after sand treatment filtration process, just after secondary treatment.

Presumptive faecal coliform concentrations were measured by using standard methods based on membrane filtration: a total of 100 ml of a water sample obtained after tertiary treatment was filtered, each membrane was deposited in plates containing mFC agar and incubated at 44 ºC. *Salmonella* was determined according to ISO 6579:2002 analysis. Isolates with a typical biochemical profile were tested for antibiotic resistance by disk diffusion test according to the NCCLS guidelines. Susceptibility to amikacin, ampicillin, amoxicillin/clavulanate, sulfamethoxazole-trimethoprim, ceftriaxone, ciprofloxacin, chloramphenicol, carbenicillin, gentamicin, nalidixic acid, tetracycline and cephalothin was evaluated.

Faecal coliform indicators were present in the 7 analyzed water samples after tertiary treatment from STP1; four of them yielding levels up to 1000 cfu/100 ml. Similar results were obtained from the STP2 water samples;

Among the 45 water samples tested, more than 50% of samples (24) were positive for *Salmonella*, 13 from STP1, but only two after tertiary treatment. Eleven water samples from STP2 were positive for *Salmonella*.

A total of 76 strains of *Salmonella* spp. were isolated and tested for antibiotic resistance. 53 of them (69.7%) were susceptible to at least one antimicrobial agent. Multiple resistances (three or more antibiotics) were observed in 11 of the isolates (8 %). Tetracycline resistance was the most common, and nine antibiotic resistance patterns were verified in total. Nalidixic acid resistant strains (9.2%) were isolated from SPT1 and all of them showed a ciprofloxacin reduced susceptibility.

Results show the microbial presence of faecal indicators and *Salmonella* spp. at each sampling point, including their presence in the regenerated water, in both STPs, which indicate a risk for human health and environment.

Keywords Wastewater; *Salmonella*; sewage; treatment plant; tertiary treatment; enteric pathogens; faecal indicators

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Study of Indicators Coliform Accumulation in *Amphibalanus amphitrite* (Cirripedia) in the Littoral area of Gnaveh, Persian Gulf,Iran

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Background: Benthic animals are useful for the monitoring of environmental quality due to their habitat and lifestyle.Therefore, among the barnacles to date, only Amphibalanus amphitrite has been proposed as a potential biomonitor for sewage-derived nutrients in coastal marine ecosystems.

Methods: Sampling(Samples of water and barnacles *Amphibalanus amphitrite)* was designed to test the pollution gradient in littoral areas of Gnaveh, Persian Gulf,Iran, in April and May 2010.The bacterial analyses were performed according to the standard methods for seawater and shellfish examination, using the Most Probable Number (MPN) technique for the total (TC) and thermotolerant coliforms (TTC) test.

Results: The results showed that Comparatively with the water samples, the highest coliform values came from the barnacles, with TC values ranging from $< 3.0 \times 10^3$ to $\geq 2.4 \times 10^6$ MPN.g^{-1}, and TTC ranging from $\geq 2.4 \times 10^3$ to 2.9×10^5 MPN.g^{-1}..Barnacles accumulate the TC *Ewingella americana*, and the TTC *Escherichia coli, Enterobacter aerogenes*.

Discussion: The coliform count of this study showed that Genaveh Port,Persian Gulf,Iran,Station was the most contaminated(Because sewage) site, and this fact is cited in the literature.

Conclusion: The results provided an indication of the level of organic contamination at the sampling locations and that this species could be a good organic pollution bioindicator.

Keywords: *Barnacle*, bioindicator, organic pollution,Persian Gulf

Susceptibility to antibiotics of marine bacteria

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In recent years there has been a growing increase in the number of marine bacteria associated with both external and internal microflora of various species of farmed fish. These bacteria have been described as commensals or asymptomatic and origin of contamination appears to correspond both terrestrial and certain microorganisms that have begun to study prebiotic or probiotic agents administered in the feed. We were chosen at random, a group of these bacteria and have faced some of the antibiotics that can be used in aquaculture, finding that some of them are resistant to maximum dose of antibiotic established by the FAO and related to the maximum residue levels (MRLs) allowed.

Keywords: Marine, microflora, comensals.

The Effect of Two Different Growth Media on the Cytotoxicity of Actinomycetes and Fungi Associated with New Zealand Marine Invertebrates

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A total of 104 isolates (13 actinomycetes and 91 fungi) obtained were cultivated on liquid and solid media. The actinomycetes were grown on Starch Casein Broth (SCB) and Starch Casein Agar (SCA) and the fungi on Peptone Yeast Glucose Broth (PYGB) and Peptone Yeast Glucose Agar (PYGA). The resultant cultures, yielded 208 ethyl acetate extracts. Of the 208 extracts assayed, 118 (57%) were potentially active. Of the 118 extracts that were bioactive in a P388 quick screen assay, but only 41 (35%) inhibited the growth of P388 cells at <12,500 ng/mL. Actinomycetes showed significant preferences for the solid medium compared to liquid medium for cytotoxicity. Although no significant preference was seen for the fungi, some differences were observed in chemical profiles of the fungal extracts obtained from liquid medium in comparison to solid medium.

Keywords: Marine-derived actinomycetes, marine-derived fungi, cytotoxicity

The Mechanisms behind Stability of Soil Microbial Community toward Stressors

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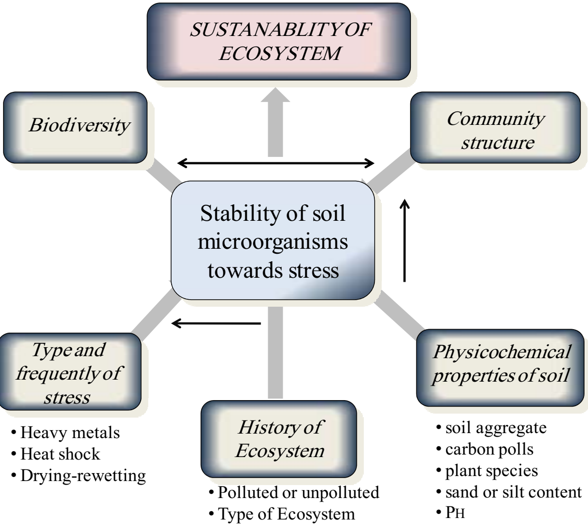
In previous years the response of soil microorganism to perturbation is quite interest to researchers looking for biologically appropriate variables regarding with new methods and tools especially molecular approach such as high-density 16S microarray (Phylochip) and functional gene array (Geochip) to founding reasonable answer for old question: who is there and what are they doing? Now it is time to use available data to express ecosystem function in term of microbial stability (resistance and resilience) to stress. One is clear that only a few resilience studies addressing soil ecosystem and especially paid their attention toward underground organisms (such as bacteria and fungi). So it is become difficult to investigate worldwide methods for determining stability of microorganism and their relationship to ecosystem process. Even though numerous studies have described the impact of variations in surrounding soil condition on microbial community structure (heavy metals), we know a little about factors influencing their stability.



-Factors effect on microbial stability towards stressor

Following literature, we found several important factors that play essential roles on stability of microorganism towards stressors or disturbances. In this paper we briefly summarize these factors expressing by heavy metals pollution as a stress conditions.

Keywords resilience; resistance; soil microbial community; Environmental stressors

Time-evolution of growing Gram-negative bacteria and biological oxygen consumption in marine microcosms polluted with gasoline

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In this paper we present the results concerning total number of cells capable of growth and multiplication in marine microcosms supplemented with gasoline and gasoline-enriched marine populations. The experimental variants are five types of marine microcosms: Black Sea natural sample- control (M3); control supplemented with gasoline (1% v/w) (M2), control supplemented with gasoline (1% v/w) and nutrients (ammonium nitrate 0.005% w/w) (M1), control supplemented with gasoline (1% v/w) and selected population (1 mL) (M4) and control supplemented with gasoline (1% v/w), nutrients (ammonium nitrate 0.005% w/w) and selected population – 1 mL (M5). Measurements were performed using the software Image J (to measure cell length) and Cell C (to count the cells). In experimental microcosms containing filtered seawater (Millipore 0.45 µm) supplemented with nutrients, gasoline and gasoline-enriched marine populations as shown above, direct viable count method was used to count living cells capable of growth and division, as shown in table 1.

Table 1. Cell size distribution during the direct viable count in microcosms 4 (sea water, gasoline and selected population)

incubation time (hours)	Cell size distribution%			
M4	<1µm	1-2µm	2-5µm	>5µm
0	75.3	24.6	0	0
2	46.4	36.6	16.6	0
4	46	32	20	2
6	32.6	24	40.6	2.6
8	18.6	21.3	52.6	7.3
10	10	18.6	62	9.3
24	6	14	67.3	12.6

The control microcosm (M3) there was a small fraction of elongated bacteria (6.7%), while in M4 supplemented only with gasoline and gasoline-enriched marine populations, the percentage of elongated cells during the experiment was as high as 79.9% [79.9= 67.3+12.6= (75.3-6)+(24.6-14)].

The direct viable count is further discussed in correlation with biological oxygen consumption in the attempt to take into account the intensity of this consumption together with the number of active growing microorganisms. The correlation between the percentage of cells capable of growth and multiplication, and oxygen consumption at the end of the experiment is positive (r = 0,880; y = 4,701x+312,5). Gas- chromatographic measurements suggests that toluene was consumed mainly in M4 and M5 as compared to M2 and M3 (control). The time evolution of cell densities are due to gasoline consumption and, probably, to lytic bacteriophages and/or predatory bacteria. Our results argue that gasoline can sustain the growth of endogenous microbiota as well as of previously gasoline-enriched marine populations, thus sustaining the further research on the use of gasoline-enriched marine populations for bioremediation of polluted sites (bio augmentation). These results are discussed with respect to other microbial parameters (time evolution of total cell count, permeabilized cells and capsulated cells).

Keywords microcosms; gasoline; biological oxygen consumption- BOD_5; toluene

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Toxicity assessment of novel environmentally friendly consumer products

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Release and dispersal of hazardous chemicals from consumer products e.g. plastic products, are of concern due to the continuous increase in production and global consumption of chemicals and articles. These materials are composed of polymers that determine its desired properties, but also contain certain additives (e.g. antioxidants, stabilisers, plasticisers, flame retardants, and catalysts) that enable processing or give specific properties. The large size of polymers limit transport across biological membranes and they are not very reactive, so they are inert and are not hazardous from a toxicity point of view. However, low molecular mass polymers, additives, and unpolymerised residual monomers in polymeric materials may be weakly or not bound at all to the polymeric macromolecules and could be easily released from processed materials. Some of these compounds are known to be hazardous to human health and the environment (e.g. formaldehyde, acrylonitrile, toluene diisocyanate, benzene, phthalates).

In this work we investigated if various new polymeric materials designed for applications such as food packaging, agriculture and automotive parts manufacture will emit hazardous chemical substances to water in concentrations causing acute toxic effects. These materials included green composites and polyurethane foams whose common feature was to contain wood or by-products from papermaking industry as a total or partial replacement of petrochemical components traditionally used for its production.

To determine ecotoxicity of newly developed materials, the one stage batch leaching method was used according to EN 12457-4 for soluble compounds extraction. The leachates were tested for acute toxicity to *Vibrio fischeri* (ISO 11348-3).

Althougth there were significant differences in ecotoxic levels among samples depending of its composition, none of the analysed samples had values of TU (Toxic Units) higher than 2, considered as the limit for differentiating between toxic and non-toxic products. Consequently, non direct ecotoxic effect can be expected from these materials and they can be considered safe for manufacture of consumer products.

This work is supported by European project from Seventh Framework Program FORBIOPLAST No.KBBE-212239

Keywords ecotoxicity; polyurethane; composites; *Vibrio fischeri*

Treatment of Landfill Leachate by Biological Aerated Filter (BAF)

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The leachate treatment was investigated by a submerged BAF system with 5 cm diameter. The filter column was filled with 30 cm natural porous volcanic rock (puzzalone) with 21 cm clearance above the filter media. The media in BAF systems was stationary and aeration was provided from the bottom of the filter. The total volume of the BAF system was 0.65 l. The leachate used in this study was obtained from an aged landfill where domestic solid wastes have been disposed since 1978. It was characterized by very low BOD_5/COD ratio (<0.1) and very high ammonia-nitrogen concentrations (>3000 mg/l).

The BAF system was fed with various dilutions of leachate giving 210-650 mg COD/l and 75-95 mg NH_3-N. The system was tested under different organic loading rates (4.7-1.3 kg/m^3/d) and hydraulic retention times (HRT) (0.8-5 h). The BAF system was operated for about five months (including acclimation period). The system was backwashed regularly in order to prevent clogging of the filter due to biofilm growth. The filter was backwashed every 48 hours for 20 min period with top water. The performance of the BAF system was tested under 4 sets.

High ammonia concentrations and low BOD/COD ratios of the leachate inhibited nitrification in the Set 1 (Table 1). The average COD and NH_3-N removals were 26 % and 30 % respectively. Therefore, the leachate was supplemented by an external carbon source to increase biodegradable fraction of the feed and in order to stimulate denitrification. In order to achieve better nitrification (ammonia removal) and to provide the carbon, nitrogen and phosphate ratios needed for the bacterial growth, besides glucose the feed was supplemented by a phosphate source (KH_2PO_4) in the Set 2 and Set 3.

The system was also fed by leachate mixed with low strength domestic wastewater in the Set 4. However, low COD (< 40 %) and NH_3-N (16.5 %) removals were observed in Set 4. Better performance of the system was achieved when an external P source and glucose were added to the leachate. NH_3-N removal of more than 85 % was achieved by the BAF system. However, maximum COD removal achieved during the operation was 63 %. The COD removal decreased with increasing organic loading rate.

The results showed that it is possible to achieve simultaneous carbon and ammonia removal in the biological aerated filter system.

Keywords Biological Aerated Filter, landfill leachate

Using the residue of spirit production and bio-ethanol for protein production by yeasts

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Abstract The residue (vinasse) formed during the distillation of bio-ethanol and cachaça, a traditional rum-type spirit produced from sugar-cane in Brazil, is highly harmful if discharged into the environment due to high values of COD and BOD. One possibility for minimizing the impact of vinasse in soils and waters is to use the residue in the production of microbial biomass for use as an animal feed supplement that will provide high levels on nitrogen (> 9 % d.m.) and low content of nucleic (≤10 % d.m.) This paper reports the production and quality of biomass produced from fermentation of *Saccharomyces cerevisiae* and *Candida parapsilosis* in culture media under twelve different culture conditions and the respective effects of each variable (glucose, yeast extract, peptone, potassium phosphate, vinasse, pH and temperature). Of the *S. cerevisiae* isolates tested, two (VR1 and PE2) originating from fuel alcohol-producing plants were identified as offering the best potential for the industrial production of single cell protein from vinasse due to highest biomass productivity. Our results showed a potential viable and economic use of vinasse.

Keywords: Bio-ethanol waste,· Cachaça distillation waste,· Microbial biomass,· *Saccharomyces cerevisiae*·, *Candida parapsilosis*

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Variation of metal content in 15 bacteria grown under macronutrient-limiting conditions: Ecological implications

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We compared the metal content (mg Kg^{-1} of dry weight for Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, V, Zn) in fifteen non-human pathogen bacterial strains (6 Gram – and 9 Gram +) under 4 different macronutrient condition treatments (C-limited, C:P=5; P-limited, C:P=1,000; N-limited, C:N=100; and Iron-limited, C:Fe=133,000). The experiments were run in triplicate for each treatment with their corresponding sterile control (medium without bacteria) in acid-washed and sterilized polycarbonate flasks. Bacteria were grown in semi-continuous culture at 30ºC at a rotation speed of 225 rpm and a dilution rate of f= 0.0004 d^{-1} (~ μ=0). After 3 days at steady-state, the bacterial cultures were centrifuged at 9,000 rpm for 10 min. at 4°C, washed thrice with 0.22 μm filtered NaCl 0.85%, and dried for 48 h at 60°C to obtain a bacterial pellet. Metal content of dried pellets was determined by extraction of metals using nitric acid prior to analysis using a Thermo X Series quadrupole ICP-MS (Keck Foundation laboratory for Environmental Biogeochemistry, ASU).

Results indicated that, independent of the macronutrient limitation treatment, the highest metal contents in the bacteria were always for Iron (Fe) and Zinc (Zn), and the lowest values were for Vanadium (V), Cadmium (Cd), and Chromium (Cr). Large differences (>1 or 2 magnitudes for Fe, Cr and Manganese (Mn)) were found in the metal content among the species within a given macronutrient limitation treatment. When comparing the content of a particular metal across the nutrient limitation treatments, significant differences ($p<0.05$) were found for several species, as well as between Gram + *versus* Gram – species, suggesting plasticity in the metal composition of many of the 15 bacterial species.

The content of iron in the Gram + bacteria *Bacillus megaterium, B. subtilis* and *Enterococcus faecium* was significantly higher than in the rest of the studied bacteria; in addition, the iron content was much lower in the N-limitation treatment in these three species. The implications of these results are considered in the context of literature evidence that expression of virulence factors and slime production by some bacteria may be regulated by iron [1,2]. Our results are compared with those previously reported for other microbial groups (*i.e.* phytoplankton) and their potential ecological consequences for the environment are discussed.

Keywords *Bacillus* spp.; bacterial virulence; iron content; *Enterococcus faecium;* homeostasis; nutrient limitation; plasticity

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Food Microbiology

A novel fibrinolytic enzymes-producing species of *Virgibacillus* isolated from fish sauce fermentation

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Fish sauce fermentation is typically prepared by mixing 1 part of anchovy with 3 parts of salt. Fermentation process takes about 12-18 months by the action of fish endogenous enzymes and varieties of halophilic bacteria. Some of these bacteria could a potential cell factory of useful enzymes, particularly proteinases. Recently, bacterial proteinases with fibrinolytic activity have been shown to prevent and reduce cardiovascular diseases by hydrolyzing the fibrin clots. The objectives of this study were to identify a fibrinolytic enzyme-producing bacterium isolated from fish sauce mash and to biochemcially characterize the enzyme. A moderately halophilic bacterium SK1-3-7 exhibited high fibrinolytic activity. This strain was Gram-positive rod, non-motile, and produced endospores. It grew well under anaerobic or aerobic conditions. Optimum growth conditions of the isolate were pH 7.0, 30-45°C and 3-15% NaCl, respectively. The strain contained meso-diaminopimelic acid in cell-wall peptidoglycan, and its DNA G+C content was 37.37 mol%. From 16s rDNA sequence analysis, the strain showed 82% similarity to *Virgibacillus halodenitrificans* DSM 10037. The DNA-DNA relatedness between strain SK1-3-7 and *Virgibacillus halodenitrificans* JCM 12304 resulted from DNA-DNA hybridization was 62.80%. These results indicated that the strain SK1-3-7 appeared to be a novel species of *Virgibacillus.*

Fibrinolytic enzymes from *Virgibacillus* sp.SK1-3-7 were partially purified using hydrophobic and ion-exchange chromatography. The enzymes with molecular mass of 20 and 36 kDa showed fibrinolytic activity on a fibrin zymogram. The enzymes were activated by 0.15 M NaCl and 20 mM $CaCl_2$. In addition, the residual fibrinolytic activity of 61% was found at 4 M NaCl, suggesting that the enzymes remained relatively high catalytic activity at extremely high ionic strength conditions. The enzyme was stable between pH 4-10 and below 60°C. *Virgibacillus* sp.SK1-3-7 enzymes hydrolyzed fibrin to a greater extent than did plasmin. In addition, the enzymes were resistant to pepsin and trypsin digestion. The enzymes were completely inhibited by phenylmethanesulfonyl fluoride (PMSF) and preferably hydrolyzed Suc-Ala-Ala-Pro-Phe-pNA, suggesting that the enzyme is a subtilisin-like serine proteinase, similar to nattokinase from *Bacillus natto*. The fibrinolytic enzymes from *Virgibacillus* sp.SK1-3-7 could be utilized as a part of nutraceutical products that reduce the risk of cardiovascular diseases.

Keywords *Virgibacillus*, fibrinolytic enzyme, subtilisin-like proteinase, fish sauce fermentation

A single strand conformation polymorphism — PCR method for analysing cheese fungal communities

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Fungi are important sensory factors of cheeses as they contribute to the sensory richness and diversity of these products. The composition, development and activity of the microbial community which is made of strains inoculated by the producer or originating from the environment, is the least controllable feature in cheese production. Techniques using a species pattern database have been applied to the study of cheese microbial communities. However, while databases have been proposed for the most prominent bacteria of dairy products, or the most common yeasts found in cheese, no studies included filamentous fungi in their databases. Develoment of tools for rapid identification of filamentous fungi are therefore needed since different species play an important role in the development of the organoleptic properties of cheeses whereas other may be at the origin of defects.

The aim of this study was to elect a method that is reproducible enough to allow the development of a species pattern database with a good power of discrimination between the most prominent fungi, including yeasts and filamentous fungi, in cheese. In the present study, we evaluated and optimized a protocol based on the analysis by SSCP-PCR of conformers obtained from amplicons derived from the ITS1 region of the nuclear ribosomal DNA (rDNA) to discriminate fungal species commonly found in cheeses.

Our study led to the development of a database comprising the SSCP electrophoretic mobilities obtained for 50 fungal species including 30 species of yeasts and 20 mold species commonly encountered in cheeses. A specific signature was obtained for 46 of 50 species included in our database. In addition, by using this SSCP-protocol, the composition of the fungal communities of 42 commercial cheeses have been determined. We showed that the image of the fungal communities obtained by using the SSCP-PCR method was in accordance with the ones revealed by using an ITS-cloning approach. Our sensitive and reproducible method constitutes a potent tool for rapid monitoring of fungal communities of cheese by manufacturers.

Keywords PCR-SSCP; fungal communities; cheeses

A Study of the Effect of Different Conditions on the Growth of Yeast Isolated from Green Table Olives

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Yeast are present throughout the spontaneous fermentation of table olives and they can have a great influence over the final product; by this reason, yeast could be used as starter cultures in order to improve the quality of table olives. The aim of the present work was to study the effect of the growth conditions over the behaviour of yeast isolated from table olives. In this study, 17 yeast strains obtained from table olives, and belonging to various species of the *Candida* and *Saccharomyces* genera, were characterized by their behaviour in different culture conditions. Moreover, interactions between yeast and lactic acid bacteria were analyzed, and a study of the flocculation ability was performed. Significative differences were found when yeast were grown in culture media with salt concentrations between 7 and 12%, and pH conditions between 3.5 and 7. Most of yeast analyzed could be useful as a starter culture to enhance the control of table olive fermentation and to obtain a high quality final product.

Keywords green table olives; yeast; starter culture; fermentation

Activity of food preservatives against *Bacillus cereus*

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Chemical compounds added to foods for inhibition of microorganism growth may be called “food preservatives” or more precisely, “food antimicrobials”. Food antimicrobials extend the shelf life of products by inhibiting the spoilage microorganisms or improve food safety by inhibiting pathogens especially that form toxins in foods. *Bacillus cereus* is spore-forming enteric pathogen that is normally present in soil, dust, and water. This pathogen may cause food-poisoning symptoms such as diarrhea and vomiting and for this reason it attracts more and more attention.

The aim of the work was to determine the sensitivity of *B. cereus* to some natural and synthetic chemical agents and evaluate the minimum inhibitory concentrations (MIC) that inhibit the growth of bacteria in agar medium.

Methods.
A traditional microbiological method (Mannitol-Egg Yolk-Polymyxin Agar) was used for isolation of *B. cereus* from the foodstuffs. 100 strains of *B. cereus* isolated from food products were confirmed as *B. cereus* on the chromogenic *BACARA* medium and used in the further investigations. Activity of food antimicrobials against *B. cereus* was determined by agar diffusion and dilution methods using the suspensions which density corresponded to approximately $1.5 \cdot 10^8$ cells/ml. To determine the activity of food antimicrobials on *B. cereus* the chemicals of the following concentrations were used: potassium sorbate ($C_6H_7O_2K$), 0.5 – 200 mg/ml; sodium benzoate ($C_7H_5O_2Na$), 10 – 200 mg/ml; sorbic acid ($C_6H_8O_2$) and benzoic acid ($C_7H_6O_2$), 1 – 50 mg/ml; sodium nitrite ($NaNO_2$), 1 – 150 mg/ml; sodium nitrate ($NaNO_3$), 1 – 300 mg/ml; potassium nitrate (KNO_3), 1 – 300 mg/ml and sodium metabisulphite ($Na_2S_2O_5$), 0.5 – 10 mg/ml. The lowest concentration of preservative solution showing a clear zone of inhibition was considered as the minimum inhibitory concentration (MIC).

Results.
All actively growing *B. cereus* cultures were resistant to 1.0 mg/ml of benzoic and sorbic acid, 0.5 mg/ml of $Na_2S_2O_5$, 10 mg/ml of sodium benzoate and to all investigated concentrations of KNO_3. Lowest concentrations of benzoic and sorbic acid that inhibited the growth of 78 % and 18 % of cultures in agar medium were respectively 10 mg/ml and 25 mg/ml. Other natural food antimicrobial – $NaNO_3$ – was less active against *B. cereus* as benzoic and sorbic acids: the concentrations that inhibited the growth of *B. cereus* were higher: 150 – 300 mg/ml. The MIC values for this antimicrobial were 150 mg/ml, 200 mg/ml and 300 mg/ml respectively for 39 %, 26 % and 29 % of cultures. All tested *B. cereus* cultures were susceptible to ≥100 mg/ml of $NaNO_2$, but MIC values (determined by agar dilution method) depended from the strain: 25 mg/ml, 50 mg/ml and 100 mg/ml respectively to 10 %, 59 % and 31 % of cultures. $Na_2S_2O_5$ was the most active synthetic food antimicrobial against *B. cereus*. The growth of *B. cereus* was inhibited by very low concentrations of this chemical (1 – 5 mg/ml) with the widest inhibition zones (among all tested food antimicrobials) ranging between 11.5 mm to 34 mm. The MICs 1.0 mg/ml, 2 mg/ml and 5 mg/ml of $Na_2S_2O_5$ were determined for 11 %, 26 % and 63 % of cultures. Other synthetic food antimicrobials – sodium benzoate and potassium sorbate – were not so active against *B. cereus* as $Na_2S_2O_5$. Their activity was higher at higher concentrations. All *B. cereus* were susceptible to ≥200 mg/ml of sodium benzoate and ≥100 mg/ml potassium sorbate. The MIC values 25 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml and 200 mg/ml of sodium benzoate were determined respectively to 2 %, 6 %, 17 %, 47 % and 28 % of *B. cereus* cultures. The MIC values 25 mg/ml, 50 mg/ml and 100 mg/ml of potassium sorbate were determined respectively for 6 %, 19 % and 75 % of *B. cereus* cultures.

Conclusions.
All *B. cereus* were susceptible to ≥ 50 mg/ml of benzoic acid and sorbic acid, ≥ 300 mg/ml of sodium nitrate, ≥ 100 mg/ml of sodium nitrite and potassium sorbate, ≥ 200 mg/ml of sodium benzoate and ≥ 5 mg/ml of sodium metabisulphite, i.e. to concentrations higher than recommended by the Lithuanian hygienic norm HN 53:2008. Benzoic and sorbic acids were more active against *B. cereus* than the other natural preservatives. Sodium metabisulphite was more active synthetic preservative than the sodium benzoate or potassium sorbate.

Keywords *Bacillus cereus*; food antimicrobials; activity, minimum inhibitory concentrations

Aerobic and microaerophilic growth kinetics of *Saccharomyces cerevisiae* in batch cultures

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Saccharomyces cerevisiae is widely used in various food industries as well as other biotechnological applications. In an asynchronously growing *S. cerevisiae* population, individual cells differ in their phase within the cell division cycle, their genealogical age, and their size because of clonal variability among other factors. The optimization of its growth in different conditions is of great interest in the field of biosystems engineering.

Among factors affecting *S. cerevisiae* growth, oxygen concentration is of major importance. Although oxygen concentration in laboratory experiments can be regarded as homogeneous, large scale fermentation processes usually undergo oxygen gradients in voluminous containers that can affect the fermentation evolution and yield. The purpose of this work is to evaluate the consequences or effects of two different initial oxygen concentrations on the *S. cerevisiae* growth in batch cultures.

Experiments conducted can be designated as aerobic or microaerophilic. The medium used in aerobic conditions contained: 10 g L^{-1} glucose, 5 g L^{-1} yeast extract, and 3 g L^{-1} casein peptone and pH was initially adjusted to 3.5 with ortophosphoric acid. To ensure microaerophilic conditions the medium was supplemented with 0.5 g L^{-1} sodium thioglycolate and 0.001 g L^{-1} resazurine. Both media were autoclaved for 15 min at 121ºC. The inocula, previously grown aerobically or microaerophilically, were inoculated in 1000 mL flasks with 600 mL of the fresh medium and incubated at 27ºC, using magnetic stirring (150 r.p.m) for approximately 40 hours. The initial oxygen concentrations in the media were 8.4 and 6.9 mg O_2 L^{-1} for aerobic and microaerophilic growth conditions, respectively. Tests were performed in quadruplicate. Samples were taken out to be analysed every 90 minutes.

Cultivable cell counts were obtained by plate counting on agar malt extract. Petri dishes were incubated at 27ºC 48-72h. Cell viability was determined by staining with methylene blue. Cell suspensions were loaded into the Neubauer counting chamber and observed under a microscope (x400). Viable yeasts looked colourless while dead cells were blue stained. Budding yeasts were determined with the same preparation. The evolution of oxygen was recorded by using a DT222A oxygen sensor.

The growth kinetics of *S. cerevisiae* in both initial oxygen concentrations studied are similar, as well as the durations of the growth phases (lag, log and stationary phase). Although in aerobic conditions, viable population and the number of budding cells are slightly higher than the cells growing in microaerophilic conditions, the average number of buds per cell in both conditions is only one. At the end of the aerobic trial the proportion of dead cells is similar to living cells, whereas in the microaerophilic trial the proportion of dead cells is always lower than that of living cells.

Because digital image acquisition was also carried out during the trials, we assume that the processing and analyses of these images in the near future will bring further and helpful information about these first results.

Keywords *Saccharomyces cerevisiae*; yeast growth; aerobic conditions; microaerophilic conditions

Alternative method for airborne contamination control in food industry

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To minimize food contamination and to extend shelf life, microbial contamination control in the air surrounding vulnerable production processes plays an increasingly important part of hygienic food manufacturing. Microbiological monitoring methods in pharmaceutical and medical industries are well established, and can be adapted by food industry.

Bertin Technologies (France) has developed a system dedicated to the contamination monitoring of airborne bio-particles. The goal is to propose a sampling method compatible with Rapid Microbiological Methods (RMM) in order to get reliable and specific data and reduce the current time-to-results which is critical for sensitive products production.

With the cyclonic technology, airborne particles are separated from the air and collected into a sterile liquid media which is compatible with immunoassay, PCR assay, cytometry, standard culture methods...

The qualification test according to ISO 14698 has been successfully conducted by HPA (Health Protection Agency, Porton Down, UK), and the physical and biological efficiencies of Coriolis® have been proved to be equivalent or better than the traditional sampling method (impaction).

With the Coriolis® technology, numerous studies have been conducted for the sampling of pathogenic bacteria, fungi or non-cultivable microorganisms. Applications using Coriolis® associated with RMM to detect airborne microorganisms in hospital environment, cleanrooms or composting facilities could be presented. Another study carried out by Canadian researchers to detect airborne virulent bacteriophage of dairy starter culture in a cheese factory will also be presented.

Coriolis® has been adopted by major pharmaceutical manufacturers, and is suitable for contamination control in food industry.

Keywords airborne, air sampling, air monitoring, rapid microbiological methods, bacteria, fungi, viruses

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An evaluation of the interference of age on the protective effect of probiotics in sheep

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Shiga toxin-producing *Escherichia coli* (STEC) are the main cause of several human illnesses, such as hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpurea. Domestic ruminants, especially sheep and cattle, are the main reservoirs of STEC and may transmit this pathogen to the public consumer. Unlike STEC, probiotics are "live organisms that, when administered in adequate amounts, confer a health benefit on the host", decreasing the infection and its dissemination. However, these benefits are conferred only after the colonization of probiotic strains in the gut of the animal, which may be impaired by normal microbiota. The aims of this study were to determine whether the inoculation of sheep with probiotic strains decreases the colonization of pathogenic bacteria and to determine whether the age of sheep interferes with this protective effect. Sheep that received oral inoculums at a concentration of 2×10^9 cells per mL of viable STEC bacteria, which are carriers of *Stx1*, *Stx2* and *eae* genes, were compared with other sheep that did not receive inoculums. When probiotic bacteria were inoculated together with the pathogenic bacteria, the number of pathogenic bacteria in the population was similar to the control. The protective effect of probiotic strains was largest in groups with younger animals than with older animals. These findings suggest that the use of probiotic strains in sheep may decrease the intestinal colonization by pathogenic strains as well as the fact that the age of the sheep may interfere in the protective effect of probiotics against colonization by STEC.

Key words: Probiotic, protective effect, *Escherichia coli*, sheep

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Antibacterial effects of *Prosopis juliflora* occurring in Iran

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Many plants of genus *Prosopis* (Leguminosae) are known to be of medicinal value.

In this study antibacterial activity of *Prosopis juliflora* was tested in -vitro against six gram positive and negative bacteria by paper disk diffusion and well methods.

In this purpose the methanolic extract at different concentrations (0.05, 0.1, 0.2, 0.3, 0.4 mg/ml) against Staphylococus aureus, Micrococcus loteus, Bacillus cereus ,Shigella sonnei, Pseudomonas aeruginosa and Escherichia coli were tested.

Comparison were made with Amoxicillin and Methicillin.Results showed that *Pseudomonas aureginosa* had the maximum resistant and *Micrococcus luteus* had the less resistant against this extract.

Further work needs to be done in this extract including fractionation to isolate active constituents and subsequent pharmacological evaluation.

Keywords antibacterial activity,*Prosopis juliflora*, medicinal plant,extract,Iran

Antibiotic resistant enterococci isolated from Portuguese traditional fermented meat products

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Enterococci can be found in a variety of environments, such as water and soil, constitute a significant part of the gastrointestinal tract of different animals and are usually isolated from different types of fermented foods, like dairy and meat products. Nowadays enterococci are considered emerging pathogens, due to an increasing number of antibiotic resistances and production of virulence traits. Although enterococcal food-borne infections have not been described so far, this combination of factors raises special awareness regarding the food safety of products harboring enterococci.

The present investigation studied several Portuguese traditional fermented meat products namely, Catalão, Chouriço Preto, Linguiça, Paio and Salsichão, for the presence of *Enterococcus* spp. 159 enterococci were presumptively identified at genus level by growth in selective media, at different temperatures (10-45ºC), pH values (9.6) and NaCl concentrations (6.5%) and assessment of Gram staining, catalase and oxidase activities. Subsequently, the diversity of the bacterial collection was assessed by PCR-fingerprinting with primers OPC-19 and $(GTG)_5$ and calculation of Simpson’s diversity index (D=0.927). Analysis of the dendrogram obtained from the PCR-fingerprinting patterns led to the selection of 78 meat-enterococci, representative isolates of all enterococcal groups, for further studies.

Then, the selected enterococci were identified by PCR amplification, which allocated the isolates to the species *Enterococcus faecalis* (n=45), *E. faecium* (n=13) and *E. durans* (n=9) while 13 isolates remained unidentified. The *Enterococcus* spp. were subsequently evaluated for their susceptibility to fourteen antibiotics, representing different drug-classes. The resistance phenotypes obtained were as follows: 1% (1/78) for ampicillin, 1% (1/78) for amoxycillin/clavulanate, 100% (78/78) for cefalexin, 74% (58/78) for cephotaxim, 3% (2/78) for chloramphenicol, 13% (10/78) for erythromycin, 69% (54/78) for gentamicin-10, 0% (0/78) for gentamicin-120, 100% (78/78) for nalidixic acid, 36% (28/78) for penicillin G, 100% (78/78) for streptomycin, 100% (78/78) for sulphamethoxazole/trimethoprim, 60% (47/78) for tetracycline and 19% (15/78) for vancomycin. The high levels of resistance observed amongst the meat-enterococci pose apprehension and gain special relevance if we consider the detection of Vancomycin-Resistant-Enterococci (VRE), once this antimicrobial agent is considered the last resort for treatment of life threatening enterococcal infections.

Overall, since the enterococci under analysis seem to be associated with a high level of antibiotic resistance, it is of major importance to further assess for their potential pathogenicity, in order to reliably establish the potential hazard of consuming such products, particularly for high risk population groups.

Keywords: Fermented meat products, *Enterococcus*, antibiotic resistance.

Antifungal activity and biocompatibility of chitosan hydrochloride against *Aspergillus* species

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Chitosan, a cationic amino polysaccharide, essentially β-1,4 D-glucosamine (GlcNAc) linked to N-acetyl-D-glucosamine residues, is naturally present in the cell wall of certain fungi, and can also be obtained by chitin deacetylation. Chitosan is a weak base and is insoluble in water and organic solvent; however water-soluble chitosan can be obtained through a chemical modification. In food technology chitosan is important due to its several functional properties and can be used as antimicrobial agent. The aim of this study was to investigate, in vitro, the antifungal activity and the biocompatibility of chitosan hydrochloride, against three pathogenic *Aspergillus* species. Chitosan from crabs (Sigma®) with 65% of deacetylation and viscosimetric molar weigh of 7.59×10^5 g/mol was modified for chitosan hydrochloride, soluble in pure water. The effectiveness of chitosan hydrochloride in inhibiting the growth of Aspergillus *ochraceus* (ATTC 22947), *Aspergillus fumigatus* (ATTC 40640) and *Aspergillus parasitic us* (ATTC 15517) was tested. Chitosan hydrochloride solutions at concentrations ranging from 10.0 to 0.002 mg/mL were prepared in distillated water (v/v), pH adjusted to 7.0. The antifungal activity was assessed by determining the minimum inhibitory and fungicide concentration using broth dilution method in Sabouraud medium. Chitosan hydrochloride was replaced with sterile distilled water in the positive control. The chorioallantoic membrane of chick embryo (CAM) was used to evaluate biocompatibility and toxicity of chitosan hydrochloride. The parameters vasoconstriction, hemorrhage and coagulation to evaluate the potential for irritation according to the method of HET-CAM, signs of inflammation, edema or neovascularization were observed. Microbial growth was observed in the positive control, and the viability of the *Aspergillus* strains was confirmed by growth in Sabouraud agar without chitosan hydrochloride. Chitosan hydrochloride showed identical minimum inhibitory concentration (CIM) and minimum fungicide concentration (CFM) for all *Aspergillus* tested. The exact mechanism of the antimicrobial action of chitosan hydrochloride is still unknown, but different mechanisms have been proposed, in reference to its chemical and structural properties. Signs of vasoconstriction, hemorrhage, coagulation, inflammation, edema or neovascularization were not observed by application of Chitosan hydrochloride. The results demonstrate biocompatibility and antifungal potential by chitosan hydrochloride against the *Aspergillus* species tested.

Key-Words: Polymer, Antimicrobial activity, Hydrochloride

Antimicrobial activity of lactic acid bacteria against some pathogenic bacteria

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In order to improve the health of population, searching for natural preservatives suitable for food production is performed. Lactic acid bacteria (LAB) are widely used for preservation of different food products because they produce antimicrobial compounds (organic acids, diacetyl, hydrogen peroxide, phenolic compounds and bacteriocins or bactericidal proteins) that can inhibit the growth of pathogenic and spoilage bacteria in foods. KTU Food Institute has an unique collection of LAB with investigated morphological, physiological, physical-chemical, biochemical and sensory properties. The antimicrobial activity of the some chosen strains from the collection on the growth of pathogenic bacteria such us *Listeria monocytogenes* and *Bacillus cereus* was investigated in this study.

The aim of the work was to determine the antimicrobial activity of some LAB strains from the KTU Food Institute collection against *Listeria monocytogenes* and *Bacillus cereus* and to select the best strains for the production of fermented products.

Methods.
The following 36 strains from the collection were tested for their antimicrobial activity*: Lactobacillus acidophilus* (3 strains), *Lactobacillus casei* (4 strains), *Lactobacillus helveticus* (4 strains), *Lactobacillus plantarum* (1 strain), *Streptococcus thermophilus* (4 strains), *Lactococcus lactis* subsp. *lactis* (4 strains), *Lactococcus lactis* subsp. *cremoris* (5 strains), *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (4 strains), *Lactobacillus delbrueckii* subsp. *bulgaricus* (5 strains), *Pediococcus damnosus* (1 strain), *Lactobacillus sakei* subsp. *sakei* (1 strain). The pathogenic test bacteria were *Listeria monocytogenes* ATCC 19111 and *Bacillus cereus* ATCC 11778.

The agar well diffusion method was used for detection of antimicrobial activity. *L. monocytogenes* and *B. cereus* were cultured in Brain heart infusion broth respectively at 30 ºC and 37 ºC for 24 h. One milliliter of the suspension (approx. density 5×10^5 cfu/ml) was inoculated into 20 ml of plate count agar maintained at 45 ºC and the resultant mixture was poured into the Petri dishes. After solidification of the agar, four wells (diameter 8 mm) were made. LAB strains for testing were cultured in 10 ml of MRS broth and incubated at 30 ºC or 37 ºC (according the optimal temperature of the strain) for 48 h. Cells were removed by centrifuging at 6000*g* for 15 min. The supernatant fluid was filtered through 0.2 mm pore size filter and 50 µl of it was added into each well. The plates were examined for evaluating of inhibition zones after incubation at 30 ºC or 37 ºC for 24 h.

Results.
The antimicrobial activity of the cell-free filtrates of each of the 36 strains from the collection was evaluated. Twenty-nine strains were shown to produce inhibition zones against pathogenic test bacteria *L. monocytogenes* and *B. cereus.* The diameters of the inhibition zones varied between 2.5 mm to 22.0 mm. Strains *L.acidophilus* 336 and *P. damnosus* were the most active against *L monocytogenes* and *B. cereus* and inhibition zones were ranging between 16.7 to 21.5 mm*.* All strains of *L. helveticum* and *L. acidophilus,* one strain of *L. plantarum*, one strain of *L.casei* resulted in the higher diameter of inhibition zone than the other LAB strains on the *L. monocytogenes*. The diameters of the inhibition zones were from 17.2 mm to 21.0 mm. *B. cereus* were the most sensitive to all *L. delbrueckii* subsp. *bulgaricus* strains and inhibition zones were from 16.0 to 22.0 mm. One strain of *Lactobacillus sakei* subsp. *sakei* resulted in antibacterial activity in neutralized (pH 6.5) culture supernatants, in all the other cultures the antibacterial activity was not determined.

The investigated LAB strains showed the higher inhibitory activity against *L. monocytogenes* than *B.cereus*. The *Lactobacillus* genus strains were more active than *Lactococcus* and *Streptococcus* genus strains. The all strains of *S. thermophilus*, one strain of *L. lactis* subsp. *cremoris,* one strain of *L. lactis* subsp. *lactis* biovar. *diacetylactis* and one strain *L. lactis* subsp. *lactis* did not show any inhibitory activity against tested pathogenic bacteria.

Conclusions.
All *Lactobacillus* genus strains inhibited test strain *L. monocytogenes* ATCC 19111 and *B. cereus* ATCC 11778. *Lactobacillus* genus strains were more active against pathogenic bacteria than *Lactococcus* and *Streptococcus* genus strains. This study of LAB collection will help to select the best strains with multi-functional properties for improving the microbiological safety of traditional food products and may increase their shelf life.

Keywords Lactic acid bacteria; antimicrobial activity; pathogenic bacteria

Antimicrobial Mechanism of Ib-AMP1 Against Foodborne Pathogens

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Antimicrobial peptides (AMPs) are believed as promising alternatives for sanitization agents, natural food preservatives, and antibiotics: since bacterial resistance is a serious problem. Ib-AMP1 is a plant derived AMP, purified from seeds of *Impatiens balsamina.* Studies suggested it is active against fungi, Gram (+) and Gram (-) bacteria at micro-molar level and is temperature and pH stable. We believe that Ib-AMP1 is a promising AMP; however, the mode of action of Ib-AMP1 is not yet known. To facilitate the application of Ib-AMP1: the objective of the present study is to determine the comprehensive mode of action of Ib-AMP1 against foodborne pathogens and food spoilage bacteria. Results suggest Ib-AMP1 inactivates *Salmonella enterica* serovar Newport, *Escherichia coli* O157:H7, *Bacillus cereus* and *Staphylococcus aureus* and *Pseudomonas aeruginosa* from 50µg/mL to 200µg/mL. Fluorescent staining cell membrane permeability assay data indicated that 31% and 56% of cells became permeable after treating with Ib-AMP1 at MIC (50µg/mL) and 2xMIC (100µg/mL) level, respectively. One minute after treating cells with Ib-AMP1 at 2x MIC and MIC levels almost 100% and 20%, respectively, of ATP leaked from the cells. Results suggest Ib-AMP1 targets the cell membrane forming large pores rapidly, leading to loss of membrane integrity. Future studies will focus on the activity of Ib-AMP1 under a range of pH and temperature conditions. Studies will also be conducted to determine the activity of Ib-AMP1 in various food matrices including fresh-cut produce, ground meats, and dairy products.

Key Words: plant antimicrobials; foodborne pathogens, food preservatives

Antimicrobial properties of plant extracts

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A variety of different food preservatives and food processing methods are used to protect food ingredients and food products from microbial spoilage. However, negative consumers attitude towards synthetic food additives resulted in an increasing interest in search for natural substitutes, particularly from plants isolated ingredients. Regarding isolation methods, carbon dioxide extraction is considered as a 'green' process used to extract phytochemicals. Liquid CO_2 extraction has the advantage that it is carried out at low temperature, which is preferable for heat sensitive components. The objective of this study was to evaluate the antimicrobial effects of CO_2 extracts isolated from thyme (*Thymus vulgaris)*, celery roots (*Apium graveolens*) and oregano (*Origanum vulgare*) on spoilage and some pathogenic bacteria. In addition the effects of the extracts as potential natural preservatives in minced meat were tested.

Methods.
Dried plants were extracted with liquid carbon dioxide in the pilot plant scale equipment. Extractions were performed at ambient temperature and 60 bar pressure by using different operation cycle programs. The antimicrobial properties of plant extracts were evaluated by the agar well diffusion method. For determination of total count of bacteria in meat 99.5 ± 0.1g of minced meat with 1.0, 0.5 or 0.1g ± 0.001g CO_2 extract was well mixed in a sterile vessels. Total count of bacteria was estimated periodically (after 0, 2, 24 and 48 hrs). For this purpose the method of injection into plates was used (EN ISO 4833: 2003 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30°C).

Results.
Agar well diffusion method has shown that thyme CO_2 extracts had inhibitory effects on the growth of the majority tested microorganisms. However, the strongest antimicrobial effect was observed for thyme extract containing larger amount of essential oil (5.1 %) comparing with other CO_2 extracts of thyme. As a result, this extract was chosen for further experiments to determine its effect on microorganisms in minced meat. The results showed that it inhibited the activity of microorganisms immediately after adding extracts to minced meat and after 2 hours of storage. The samples with extracts had less colony-forming units by 0.6 and 0.5 lg in a milliliter than control sample.

Initial tests have shown that celery root CO_2 extract had inhibitory effect on the growth of the majority test bacteria. However, on the contrary to thyme, celery root extract did not influence total count of bacteria after adding to meat. After 48 hours in samples with 0.1 % celery root extract in minced meat the number of cfu/ml was 1.17 times less than in control sample. The main compounds in the essential oil of celery root CO_2 extract were limonene (21.9 %) and carvone (17.7%). It is known that these compounds exhibit antimicrobial effect against microorganisms.

The lowest 0.1 % dose of oregano CO_2 extract had no influence on the total count of bacteria. After 2 hours, smaller amounts of viable microorganisms were found in the samples with 0.5 % and 1.0 % additives of oregano extracts than in the control sample. However, after 48 hours total count of microorganisms in samples with additives was similar to that of control sample.

Assessment of the influence of CO_2 extracts on the sensory quality of minced meat products revealed that in many cases concentrations of used plant extracts were too high (0.4 and 0.6%). However, 0.2 % extract additives were acceptable from the sensory point of view.

Conclusions.
Thyme, celery roots and oregano CO_2 extracts were the most effective in reducing the total number of microorganisms in minced meat; addition of 0.1 % celery root extracts after 48 h reduced the number of viable cells by 1.17 times. Sensory acceptable amount (0.2 %) of thyme and oregano CO_2 extracts was too small to exhibit more remarkable antimicrobial effect.

Keywords CO_2 extracts, antibacterial activity, natural additive

Apple juice reverse the oxidative effect of vanadium pentoxide in *Saccharomyces cerevisiae*

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The *Malus domestica*, Borkh, tients for over 2500 years, was domesticated and expanded in Europe by the Greeks and Romans. Due to their high adaptability to different climates and soils, the apple orchards were quickly installed throughout the world, from countries with relatively cool to subtropical climates. In Portugal, Romans have carried out their introduction and later by religious influence led to the spread of different varieties. The region of Beira Alta, Portugal, with its diversity of microclimates, of harsh winters and hot summers with high brightness, distinguished himself early as a conducive area to apple growing, being denominated IPG (Protect geographical region) for the Golden Delicious variety. In the literature phenolic compounds of apples are described as potential inhibitors of oxidative processes, involving reactive oxygen species (ROS), implicated in chronic disorders such as cancer and cardiovascular disease. The main objective of this study was to evaluate the role of Golden Delicious apple juice in cell proliferation of yeast *S. cerevisiae* UE-ME$_3$, and stress molecular markers, in the presence of a well-know oxidant, vanadium pentoxide.

S. cerevisiae inoculated in liquid YEPD medium, in the absence or presence of 2.0 mM vanadium pentoxide or in the presence of 2.0 mM vanadium pentoxide and 5% apple juice, were left to grow during 72 h, at 28 ºC. Samples of each culture were harvested for determination of cfu, preparation of cell lysates, by sonication in 10 mM phosphate buffer pH 7.0, to obtain post-12000 *g* supernatant, used for determination of proteins, antioxidant power (DPPH) by spectrophotometry, the glutathione and ROS contents, by fluorescence and ALP and GR enzyme activities by spectrophotometry [1, 2, 3, 4, 5, 6].

The results show that 2.0 mM vanadium pentoxide (V_2O_5) behaved as inducer of cell death, decreasing cell viability (cfu) and ALP enzyme activity, with a significant increase of intracellular ROS and GR activity. The glutathione-mediated antioxidant system showed an increase in GSH content and a decrease in GSSG content, which reflects into a increase of the GSH/GSSG ratio. Despite also occur a rise of citosolic free radical scavenger in cells growing in the presence of vanadium, this response was not adequate to the preservation of cell viability. The Golden Delicious apple juice increased cell viability and ALP activity as well as decreased ROS in *S.cerevisiae* grown in presence of V_2O_5, which seems a protector response. As the ability to free radical scavenger remained higher in the presence of apple juice and the GSH/GSSG ratio decreased, we presume that there protective effect depends on phenolic compounds (261.3 ± 23.4 g/mL) present in apple juice which amends the response mediated by glutathione.

Keywords *Malus domestica*; *Saccharomyces cerevisiae*; antioxidants

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Application of an active zein film containing partially purified lysozyme and Na_2EDTA to improve the storability of cold-stored ground beef patties

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Due to increased health concerns and environmental problems caused by synthetic plastics, edible and environmentally friendly biodegradable packaging materials from natural sources could be a potential alternative solution. Instead of adding high concentrations of antimicrobial compounds directly into whole food mass, incorporating antimicrobials into films and coating of food surface enables use of less amounts of antimicrobials to maintan safety at the critical food surface. In this study, the antimicrobial activity of zein films incorporated with 700 µg cm^{-2} partially purified lysozyme and 300 µg cm^{-2} disodium ethylenediaminetetraacetic acid (Na_2EDTA) was evaluated against *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* by the classical zone inhibition test. The developed films were also tested on ground beef patties during refrigerated storage (at 4 °C). At the zone inhibiton tests, the films inhibited *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes* effectively, but the most resistant bacteria against films was *Salmonella* Typhimurium. The number of mesophilic aerobic bacteria in ground beef patties coated with antimicrobial zein films containing lysozyme and EDTA was 1.83 and 0.61 decimal less than those of controls at the end of 5 and 7 days, respectively. Active packaging with zein films containing lysozyme and Na_2EDTA also slowed down the oxidative changes in patties effectively. The coating of patties with the antimicrobial films caused insignificantly small changes in lightness values (L*), but it decreased the redness indices (a*/b*) of patties significantly during 7 days of storage. This study clearly pointed the advantages of using lysozyme and Na_2EDTA in active packaging of patties with zein films.

Keywords: active packaging, ground beef patties, lysozyme, zein films

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Application of antimicrobials incorporated into polysacharides edible films on vacuum packed cooked loin

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To lengthen the shelf life and ensure the safety and quality of ready-to-eat products, active antimicrobial technologies appear to be a good alternative. In recent years, packaging research has focused more on the use of edible films and coatings since they offer the possibility to incorporate the antimicrobial compound into the edible coating and to be applied by dipping or spraying onto the food. The most commonly used antimicrobial compounds are both synthetic and natural in origin. Currently there is renewed consumer interest in natural products that are free of synthetic additives. This has forced food industry to investigate new natural additives that satisfy consumer demands. The natural antimicrobial compounds can be spices, essential oils, lactic acid bacteria (LAB) and /or their metabolites, etc. Spices are rich in phenolic compounds, such as flavonoids and phenolic acids, which exhibit a wide range of biological effects, including antioxidant and antimicrobial properties. Some studies have demonstrated that LAB are capable of controlling microbial growth in food products and more specifically in refrigerated, anaerobically packaged, sliced and cooked meat products. Taking into account that further research on the antimicrobial activity of the natural compounds is required to evaluate its usefulness in the shelf life extension of packaged foods such as meat and meat products, the objective of this study was to evaluate the effectiveness of composite polysacharides edible films against the common spoilage flora in cooked pork loin.

Cooked pork loins were manufactured, cooled in a chilling room at 2ºC and sliced at 1 cm thickness. The steaks were placed in trays (100 g per tray) and randomly divided into four groups. The first group (control) was vacuum packed and the second group (batch P) was sprayed on the cooked pork loin surface with polysacharide film (DOMCA S.A., Granada, Spain) and vacuum packed. Both groups were packaged without antimicrobial addition. The third (batch RP) and fourth (RPP) groups were sprayed on the cooked pork loin surface with rosemary extract (Amexol; Amerex, Madrid, Spain), 5g pure extract diluted in 1l polysacharide film (DOMCA S.A., Granada, Spain) and with rosemary extract plus *Pediococcus acidilactici* (Biamex; Amerex, Madrid, Spain), 100g extract diluted in 1l polysacharide film (DOMCA S.A., Granada, Spain), respectively. Then, both groups were vacuum packed. Finally, all samples were stored under illumination at 2ºC.

At selected times: 0, 7, 14 and 21 days of storage, two packs of each batch (control, batch P, batch RP and batch RPP) were opened and microbial analyses were carried out. The samples were analysed for: psychrotrophic bacteria, anaerobic bacteria, *Enterobacteriaceae*, pseudomonads, lactic acid bacteria and yeasts and moulds. The entire experimental procedure was done twice.

In general, the microbial counts studied increased steadily during storage. Psychrotrophic and anaerobic bacteria counts reached values of 7 log cfu/g at 21 days. Regarding batches, there were no differences in log values of microbial counts among control and the others, which indicate that no antimicrobial activities were observed through storage. An explication for the observed inactivity of antimicrobials added may because the amount used was insufficient for inhibition. Several researchers have reported the antibacterial effect of many natural antimicrobials, however, these studies were carried *in vitro* or *in vivo* using higher percentages than those in our study. It is necessary taking into account that the different amounts of antimicrobials used in our work were selected by means of sensorial analyses carried out previously.

As conclusion, the use of antimicrobial polysacharides edible films did not produce a significant antimicrobial effect, for that reason further improvements are necessary to develop a more successful application of edible coatings enriched with natural antimicrobials on cooked pork loin.

Keywords: cooked pork loin; antimicrobials; edible films.

Application of rapid methods for detection of aflatoxin and ochratoxin producers in peanut

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Aflatoxins are carcinogenic, mutagenic, and hepatotoxic compounds and ochratoxin A is a possible carcinogen to human. Malaysia is a tropical country favoring the growth of aflatoxin and ochratoxin producer strains. To reduce the health risk from consumption of aflatoxin and ochratoxin contaminated food, rapid, accurate and effective detection methods are highly needed. This research has been conducted to optimize a reverse phase high performance liquid chromatography fluorescence detection method for determination aflatoxins and ochratoxin A, and to isolate potential aflatoxin and ochratoxin producer strains from raw peanuts marketed in Malaysia and detect them using polymerase chain reaction. Effect of HPLC conditions namely mobile phase composition, flow rate and temperature on peak area of four target aflatoxins (B_1 , B_2, G_1 and G_2) from the spiked peanut was investigated using response surface methodology (RSM). The highest quantification value for target aflatoxins was obtained under the following HPLC conditions: mobile phase composition of ACN/H_2O/MeOH: 8/54/38, temperature of 24 ºC and flow rate of 0.4 mL/min. The LOD for aflatoxins were found to be 0.03, 0.01, 0.09 and 0.06 ng/mL for aflatoxin B_1, B_2, G_1 and G_2, respectively. Recovery of the method for aflatoxin B_1, B_2, G_1 and G_2, was 91.57, 89.37, 89.42 and 76.14 % respectively. A significant nonlinear response surface model was fitted to evaluate the effect of HPLC parameters (e.g. pH, temperature and flow rate) on the quantification level of ochratoxin A . The highest quantification value for ochratoxin A was obtained with the following HPLC conditions: mobile phase consisting of 5 mM sodium acetate (pH 2.36)/ACN/MeOH (40:30:30); excitation of 333 nm; emission of 467 nm; flow rate of 0.4 mL/min; and temperature of 24°C. The recovery and LOD values of ochratoxin A under the recommended optimal conditions were 95.4% and 0.05 ng/g, respectively. Sixty raw peanut samples were used for fungal isolation. Based on the results of morphological studies *Aspergillus* species were isolated. Totally 23 *Aspergillus* (black and green aspergilli) isolates were obtained from samples. Aflatoxin and ochratoxin production of the isolates in culture media has been analyzed by the optimized HPLC methods described. In order to detect aflatoxigenic and ochratoxigenic isolates, specific primers targeting the genes responsible in aflatoxin and ochratoxin A production were used. Three primer sets were used for detection of aflatoxigenic and three pairs for ochratoxigenic isolates. Results of PCR amplification of isolates were in agreement with HPLC chromatogram of aflatoxin B_1 and ochratoxin A production of isolates. Totally 14 aflatoxigenic, 6 ochratoxigenic and 3 non-aflatoxigenic/non-ochratoxigenic *Aspergillus* were isolated from raw peanuts marketed in Malaysia.

Keywords: *Aspergillus*, HPLC, PCR, aflatoxigenic, ochratoxigenic

Application of *recA* gene to identify *Lactobacillus* Species in Lighvan Cheese

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Starter and non starter lactic acid bacteria (NSLAB) are responsible for enhancing unique flavor properties in traditional dairy products such as Lighvan cheese through biochemical mechanisms. In addition to saving domestic genetic resources, industrial production of semi-traditional dairy products is one of the major objectives of non starter microflora identification in indigenous dairy products. Previous studies performed in this laboratory based on the conventional methods showed that lactobacilli were the main microbial group colonizing in this type of cheese after 4 months ripening. In this study eight batches of Lighvan cheese with 4 months ripening period were purchased from the local shop in the Lighvan village, Tabriz province, Iran. For microbial detection the sorbitol agar was used to detect non starter facultatively heterofermentative lactobacilli. The results of chemical evaluation of cheese including moisture, NaCl content, acidity and pH value showed that Lighvan belongs to the group of semi hard cheeses. At the microbial level the group of *Lactobacillus plantarum* was identified but it was impossible to distinguish the particular species of this group present in this cheese. It was decided to sort the problem out using *rec*A gene sequence. The amplification products obtained on agarose gel created three distinct bands with 318 bp size for *Lactobacillus plantarum*, 218 bp size for *Lactobacillus pentosus* and 107 bp size for *Lactobacillus paraplantarum*. Results obtained using *rec*A gene revealed that 86% of Lactobacilli isolates classified as *Lb. pentosus* and 14% as *Lb. plantarum*.

Key words: Lighvan cheese, Non-starter culture Lactic acid bacteria, Heterofermentative, *recA* gene

Aspergillus Section *Nigri* in soils, grape and must and Ochratoxin A in wines in Brazilian Northeast

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This study was carried out to evaluate the incidence of potentially ochratoxigenic fungi *Aspergillus* Section *Nigri* in grapes, grape vines and soils of the Brazilian Northeast, as well as to investigate the contamination with OTA in wines and grape juices in the region. Was analyzed a total of five samples of wine grapes and juice. The must of each grape variety of wine were obtained by crushing the grapes, and soil were collected per plot for each grape variety. The isolation of fungi from the berries and seeds was performed by Direct Plating on DRBC medium. For samples of must and soil was used the technique of surface serial dilutions in DRBC e DG18. The species of *Aspergillus* Section *Nigr*i were identified by morphological characteristics. All isolates were tested for potential production of OTA by Agar Plug Method. The quantification of OTA from wines and grape juices was performed by the method of High Performance Liquid Chromatography (HPLC) with fluorescence detection. Were identified the following species: *A. carbonarius*, *A. niger*, *A. niger* Aggregate, *A. tubingensis* and *A. foetidus*. OTA-producing species were *A. niger, A. carbonarius* and *A. foetidus*. Thus, one can consider that the main source of these species in the vineyards studied is the soil. The species of *Aspergillus* Section *Nigri* are naturally present in grape seeds, grape and wine region of the soil studied. However, in most samples (80%) of wine and juice analysis was not detected the presence of OTA, which is probably related to climatic conditions, management and processing of grapes for the elaboration of final products

Assessment of Microbial profile and some physicochemical effects during fermentation and production of aerial yam (*Dioscorea bulbifera*) flour

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Aerial yam *(Dioscorea bulbifera*) is among the nine varieties of yam cultivated in Nigeria but less consumed by the people as it is popularly regarded as food for low income dwellers. Hence, the yam is on the verge of extinction. Although it is only consumed after boiling, many are not aware of the nutritious nature of the yam. Diversification of the use of this yam species may be possible through processing it into flour and fermenting the yam before the flour production since it is rich in carbohydrate. To achieve these, the yam was subjected to fermentation process for 5days and the flour produced thereafter. The microbial, pH and titratable acidity (%TA) of the fermenting mesh was investigated. The proximate composition of the flour produced after fermentation was also assessed. The flour produced without undergoing fermentation served as control. The total viable counts (TVCs) increased from 1.64 $x10^8$cfu/ml by day 3 of fermentation and decreased to 7.62 $x10^2$cfu/ml at the end of fermentation. Similar trend was observed for the fungal counts during the fermentation. .While the pH decreased from 5.54 to 3.51 by day 5, the TA increased from 0.38 to 0.89 at the end of fermentation .The bacterial genera involved in the fermentation were *Lactobacillus, Staphylococcus, Escherichia coli , Bacillus* and *Pseudomonas* while the fungal genera *were Penicillium* and *Aspergillus.* The proximate composition of the fermented flour differed significantly ($p<0.05$) from the unfermented. However, fermentation process increased the protein content of the flour from 6.78% (control) to 9.26% with little decrease in carbohydrate content. The fermented aerial yam flour therefore can provide support for food industries as well as diversifying the use of this product in production of instant foods.

Key words: Aerial yam, fermentation, flour, microbial, proximate composition.

Bacterial stress induced by Nanosecond Pulsed Electric Fields (nsPEF): potential applications for food industry and environment

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Introduction

Pulsed Electric Fields (PEF) is a promising non-thermal technology for liquid treatment with antimicrobial activity at ambient temperature. Recent technological advances in pulsed power technology have stimulated interest in high-intensity nanosecond pulses in biological applications, including microbial decontamination of liquids [1], [2]. The added potential advantage of nanosecond pulses over the more usually employed microsecond pulses is that thermal effects can be expected to be reduced still further during the treatment of liquids. However, previous studies have demonstrated the great PEF resistance of Gram-negative bacteria probably due to the presence of the outer membrane. Therefore the objective of the study was to assess the effects (lethal and sublethal) of nsPEF on a Gram-negative bacterium : *E. Coli*.

Methods

To this purpose, 0,2 ml of the microbial suspensions (*E. Coli*, at the final concentration of 10^6B/mL) was placed into the treatment chamber with a sterile syringe and then nsPEF-treated. This study used exponential waveform pulses (30 000) at electrical field strengths ranging from 0.4 MV/m to 1.6 MV/m and pulse repetition rate of 500 Hz. In this investigation, the temperature during treatment never exceeded 20°C inside the treatment chamber. The occurrence of lethal or sublethal injury in the outer membrane was measured using the both culture media : Tryptone Soya Agar (TSA) and a selective recovery media containing sodium chloride (2%) (TSA 2%).

Results and conclusion

In these experimental conditions, no significant lethal effects of ns PEF on *E. Coli* can be observed since the bacterial concentrations remain approximately similar before and after the treatment. However, in nsPEF treated samples, a little difference between the TSA and TSA+NaCl 2% counting can be reported. This decrease of the total number of bacteria on selective media after nsPEF could display sublethal effects. The occurrence of potential sublethal effects, *ie* the loss of membrane integrity (reversible or not) should be confirmed with the use of fluorescent dyes (such as propidium iodide or sytox green) combined with fluorescence microscopy or flow cytometry.

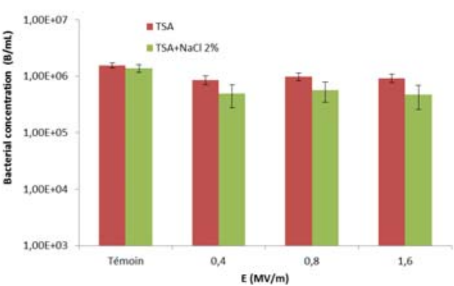

Figure 1 : Concentrations of E. Coli plated on TSA and TSA added with 2% of NaCl before and after nsPEF treatment (30 000 pulses) at electrical field strengths ranging from 0.4 MV/m to 1.6 MV/m

Significance and impact of the study

A better knowledge about the mechanism of microbial inactivation by ns PEF will aid the establishment of successful combined preservation treatments. Experimental verification of this mechanism would be interesting as it would open the door to a number of important applications for food industry and environment including the possibility of combining this technology with other nonthermal technologies such as bacteriocin.

Keywords : E. coli; Nanosecond pulsed electric fields; Lethal and Sublethal effects, bacterial stress

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Bacterial Superficial Contamination of Bovine and Ovine Carcasses Slaughtered at El-Harrach Abattoir (Algiers)

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The microbial load on sheep/beef carcasses was investigated in El-Harrach abattoir in Algiers. A total of 210 carcasses were studied using wet/dry swab technique. The samples were processed for total viable count (TVC), total coliforms (TC), fecal coliforms (FC), and *Salmonella* spp.

The recorded average charges were relatively high, especially for the bovine species. On 90 ovine and 70 bovine carcasses being the subject of *Salmonella* research, one ovine carcass (1,11%), and 7 bovine carcasses (10%) were contaminated. 13 strains of *Salmonella* were detected, the dominating serotype was S. Anatum (76, 92 %), followed by serotypes of subspecies *arizonae* (15, 38 %), and S. Abortus ovis (7, 69 %). The area of the breast and the posterior face of the foreleg were the most contaminated zones for the entire studied flora. *Salmonella* strains distribution on bovine carcasses showed a contamination rate of 58, 33% for the foreleg, of 33,33% for the breast, and 8,33% for the rearleg. The findings of this study reflect the bad conditions of carcass slaughtering and handling, and hygiene insufficiencies in El-Harrach abattoir, thus constituting a real danger to the public health.

Keywords: Abattoir, carcasses, ovine, bovine, bacteriological quality, *Salmonella*.

Bacteriophages in edible coatings for efficient biocontrol of *Listeria monocytogenes* in surface treatments

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Listeria monocytogenes is an opportunistic human pathogen, widely distributed in the environment and transmitted to humans via contaminated foods. The organism is well adapted to very different environmental conditions; it tolerates high levels of salt content, can grow at pH values below 6, with low oxygen, and is able to grow during cold storage.

Food-borne *Listeria monocytogenes* is a serious threat to human health, and new strategies to combat this opportunistic pathogen in foods are needed. On the other hand, currently there is renewed interest in consuming natural synthetic additive-free products. This has forced food industry to investigate new natural additives that satisfy consumer demands.

Bacteriophages are natural enemies of bacteria and are suitable candidates for the biocontrol of these pathogens. They are extremely specific regarding their bacterial hosts and generally do not cross taxonomic boundaries. With respect to the application of phages to foods, their inherent specificity results in the elimination of the target organisms only without compromising the viability of other autochthonic bacteria.

In recent years, the use of edible films and coatings for surface treatments in food has been extended. These offer the possibility to incorporate the antimicrobial compound into the edible coating and to be applied by dipping or spraying onto the food. Based on this, the aim of this study was to evaluate the efficacy of edible coating incorporating DMC-GB phage for control of *L. monocytogenes* strains in different foods known to frequently carry the pathogen. DMC-GB is a natural bacteriophage of Listeria that has been isolated and characterized by DMC Research Center SL in cooperation with microbiology research group of Institute of Water Research (University of Granada) led by Concepción Calvo, Ph.D.

Food samples (cooked ham, smoked salmon, sliced cheese and fresh fish) were surface-inoculated with a 10 strain cocktail of *Listeria monocytogenes* to a final concentration of 3 - 4 log CFU/cm^2 and then either sprayed with edible coating with or without DMC-GB phage at different concentrations. The edible coating used was a polysaccharide film developed by DOMCA S.A. (Granada, Spain).

Samples from each treatment were withdrawn in duplicate at selected times to determine viable counts of bacteria. *L. monocytogenes* counts were converted into log CFU per cm^2, and statistical analyses were performed using the SPSS PC 15.0 software (SPSS, Chicago, Ill. USA). Analysis of variance (ANOVA) was used for determining significant differences between controls and within phage treatments.

In all food tested, phage could reduce bacterial counts by up to 4-5 log units. The application of more phage particles was more effective than lower doses.

In conclusion, our data demonstrate that edible coating incorporating DMC-GB phage can be very effective for specific biocontrol of *Listeria monocytogenes* in contamination sensitive foods.

Behaviour of *Listeria monocytogenes* 437/07 serovar 1/2b on minced beef stored under aerobic conditions at 8 ± 1 ºC with presence of combined essential oils

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The chemical composition of the EOs of *Pistacia lentiscus* (*P. lentiscus*), *Myrtus communis* (M. communis), *Lavandula angustifolia* (*L. angustifolia*), were analyzed using a Gas Chromatography/Mass Spectrometry technique. The main components of EOs obtained were respectively β-Myrcene (15.18 %) + 1.8-cineole (15.02 %); 1.8-cineole (46.98 %) + cis-geraniol (25.18 %) and 1.8-cineole (37.8 %) + β-caryophyllene (20.9 %) for *P. lentiscus*, *M. communis* and *L. angustifolia*. EOs were screened for their ability to inhibit the growth of *L. monocytogenes* 437/07 serovar 1/2b using the standard agar-disk diffusion assay. Results obtained from disk-diffusion method, followed by measurements of Minimal Inhibition Concentration (MIC), indicated that *L. angustifolia* is most active (Φ=43.13 mm), with the lowest MIC value against *L. monocytogenes* (8 µl/ml). EOs were tested in minced beef stored in aerobic conditions at 8 ± 1 ºC, experimentally inoculated with foodborne pathogen at level of 3×10^6 cfu/g. A *L. monocytogenes* counts in treated samples were 0.91-2.23 $\log_{10}$ cfu/g less ($p < 0.05$) compared to controls at different intervals during storage. The number of *L. monocytogenes* in unsupplemented (control) meat from the first day to reach one week later 8.16 $\log_{10}$ cfu/g. Results showed that the combined EOs had remarkable antibacterial properties, higher than that individually application of EOs in meat. Sensory evaluation revealed that the aroma of minced beef treated with EOs was acceptable by panelists at the levels used. The results of the bioassays, together with the chemical profile of the EOs, support the possibility of using all EOs as potent natural preservatives to contribute in the reduction of experimentally inoculated *L. monocytogenes* in minced beef.

Keywords: Essential oils, *P. lentiscus*, *M. communis*, *L. angustifolia*, GC/MS, *L. monocytogenes*, antimicrobial activity, Disk diffusion, MICs, minced meat.

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Behaviour of *Salmonella* spp inoculated on four Iberian meat products as influenced by storage temperature

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Salmonella is an important pathogen that causes major problems of morbidity and mortality around the world. The pathogen is generally associated with contaminated foods like sprouts, almonds, eggs, meat and meat products. The incidence of food-borne illness caused by Salmonella has remained unchanged during 2005–2009, and Salmonella is consistently the most common bacterial pathogen in laboratory confirmed food-borne illness cases. Some reports have identified cured (fermented and dried) ready-to-eat (RTE) meat products as important vehicles for Salmonella infections. The manufacturing process of these products may reduce levels of Salmonella but not necessarily eliminate it from the final product. Most of the international microbiological food standards including the current European microbiological safety criteria require absence (in 25 g) of *Salmonella* spp. in food products intended to be eaten raw, as it is the case of dry cured products. Thus, this investigation aimed to determine the survival of Salmonella inoculated in ready-to-eat meat iberian meat products.

Different RTE dry cured iberian meat products (dry cured ham, dry cured loin and two dry cured sausages "salchichón" and "chorizo") were purchased at a local supermarket. Two packs of each meat product (control samples) were evaluated for detection of *Salmonella* spp, pH and a_w. The remaining packs were inoculated with a *Salmonella* (ATCC 14028) culture to obtain counts that reached 10^2 cfu/g in the products. Then, inoculated meat products were vacuum-packed and stored at 4, 8 and 12ºC for up to 365 days. Microbiological analyses (detection of *Salmonella* spp.) were performed on two packs taken from each meat product at selected times (0, 90, 180 and 365 days). The experiment involved two complete replications each using a different batch of each dry cured iberian meat products.

The results obtained in the control samples showed absence of *Salmonella* spp. in 25g in all samples analysed. In general, the pH and a_w of these samples were relatively low (dry cured ham -pH 5.93, a_w 0.920, dry cured loin -pH 5.99, a_w 0.885, "salchichón" -pH 5.03, a_w 0.828 and "chorizo" -pH 4.99, a_w 0.813).

Regarding inoculated samples, the "salchichón" samples stored at 4ºC were the only ones which showed presence of *Salmonella* spp. at 365 days. Overall, the results indicated that this pathogen was more sensitive to low temperatures when the pH of meat product was close to 6. However, at acidic pH, the damage was enhanced as temperature increased. On the other hand, differences were found among products with similar pH. Salmonella was inactivated more quickly in dry cured loin than in dry cured ham. The higher fat content of dry-cured ham in comparison with dry cured loin probably exerted a lower inactivation of this microorganism. Regarding fermented sausages, the survival of Salmonella was higher in "salchichón" than in "chorizo", probably due to the inclusion in the formulation of "chorizo" of antimicrobial ingredients, such as paprika, what did not allow the growth of this pathogen.

In conclusion, under conditions of this study, absence of Salmonella was not guaranteed neither by the physic-chemical characteristics of the dry cured meat products nor by the storage conditions used.

Keywords: *Salmonella* spp.; dry cured meat products.

Binding ability of lactobacillaceae

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The binding of probiotic bacteria allows durable health beneficial effects such as exclusion of pathogens, lasting production of beneficial bacterial molecules and immunomodulation. It is, along with survival in the gastrointestinal tract, one of the main characters that often contributes to the probiotic properties of bacteria. Since last decade, increasing amount of data dealing with the molecular origin of adhesion has permitted to enhance our comprehension of the binding mechanisms. Consequently, we investigated the presence of 12 genes involved in this mechanism in a collection of 152 lactic acid bacteria (LAB) isolated from African traditional pearl millet fermented slurries. Our main objective was to establish an approach that combined molecular screening and *ex vivo* methods to evaluate the probiotic potential of the microbiota of a traditional cereal-based fermented food.

As expected, all the screened house-keeping genes (*gapA*, *EF-Tu*, *groEL* and *enoA*) also involved in binding mechanisms were found in all strains. Concerning other genes involved in adhesion mechanisms, they were found in the majority of the bacterial collection since the genes *fpbA*, *srtA*, *Mub*, *cnb*, *MapA* were detected in 86 to 100% of the strains. The gene *msa*, encoding for the mannose specific adhesin was found only in 8% of the strains. The genes *slpA* and *cbsA* designed on species not present in our bacterial collection gave no amplification. On the whole, the distribution of the binding related genes is not species-specific but distributed equally among the isolates belonging to the six species of the collection. We then selected 33 isolates differing by their genetic equipment related to the binding mechanism for *in vitro* tests using both, mucus secreting (HT-29 MTX) and non mucus secreting cells lines (HT-29). Statistical analysis revealed that the gene *cnb* coding for the collagen binding protein were linked to the binding to HT-29 MTX cells while no discriminant gene where found for the binding to HT-29 cells. Furthermore, the binding ability was highly dependant on the strains and the cells lines models. These differences into binding phenotypes were probably due to the mucus layer produced by the HT-29 MTX cells lines. Finally, we showed that the microbiota of the model traditional fermented food we investigated was composed of LAB that had higher binding properties than well known probiotic LAB.

Keywords lactic acid bacteria, binding, mucus, genetic screening

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Biocide resistance in bacterial isolates from vegetable foods

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Disinfection of food contact surfaces and good hygienic practices are key step for prevention of transmission of human pathogens through the food chain. Biocides are commonly used for sanitation in the food industry. Bacteria challenged with biocides may acquire or develop biocide resistance mechanisms. Failure of biocide treatments due to microbial resistance is a threat when food spoilage or human pathogenic bacteria are involved in the resistance. In the present study, the incidence of biocide resistance in the natural microbiota of vegetable foods was studied.

Raw vegetable foods were purchased at local supermarkets. Samples were gently mixed with buffered peptone water to recover surface microbiota, and the cell suspensions obtained were incubated at 30ºC for 4 h for revivification. Then, cell suspensions were inoculated in tryptic soy broth supplemented with different biocides. After enrichment, samples were spread on selective media for presumptive Enterobacteria, Enterococci, Pseudomonads, *L. monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*. Bacterial colonies isolated from selective media were tested for growth inhibition in liquid broths containing a range of biocide concentrations.

The biocide preparation P3-oxonia was very active against all bacterial groups in a concentration range of 0.0025 to 0.025%. Benzalconium chloride was less active on Enterobacteria and Pseudomonads (0.025%) than the rest of bacteria (0.0025%). Cetrimide, hexadecylpyridinium chloride and poly-hexamethylen-guanine were effective in a concentration range of 0.025 to 0.25%. Triclosan and hexachlorophene were the biocides less active in growth inhibition.

Keywords: biocides; vegetable foods; food microbiota.

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Biodiversity of filamentous fungi in coffee beans cultivated in organic and conventional system

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The fruits of organic and conventional coffee are subject the contamination of several species of fungi and they can be related to the bad quality of the drink and the mycotoxins production. This study had as objective identifies the biodiversity of isolated filamentous fungi in the coffee beans produced in organic and conventional farms of a same place. Two hundred and twelve isolated belonging to eleven different genders were identified of the fifteen analyzed samples. The main found gender was *Aspergillus,* being isolated fungi of the Sections *Circumdati, Nigri, Flavi* and *Versicolores*. The samples that obtained the largest index of contamination were the ones that didn't pass for the disinfection process with hypochlorite of sodium to 1%. The samples of coffee beans of organic cultivation presented the largest wealth index and diversity inside of a same place with very close climatic conditions. A larger diversity of fungi in this system is fundamental to maintain the ecological balance and to supply tools to combat diseases and curses.

Characterisation of the spoilage microbiota in raw salmon steaks (*Salmo salar*) stored under vacuum or modified atmosphere packaging combining conventional methods and PCR-TTGE

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Salmon is an important product from aquaculture: 1 400 000 T were produced in 2008 for a value of more than 7 milliards of US dollars, atlantic salmon (*Salmo salar*) being the more reared species. Atlantic salmon farming account for 90% of farmed salmon market and more than 50% of the global salmon market (FAO, 2008). Chilled fish had important added value comparing with frozen fish, moreover, sea fish are more and more raw eaten (sushi, tartare…). Granting the consumers request, these products are found on cold shelves under modified atmosphere packaging (MAP) enriched with CO2. This type of packaging usually modifies the microbial flora, inhibiting mainly the aerobic Gram negative bacteria, and thus increases the shelf life of these seafood products making distribution easier. Microbiota composition at the spoilage time is important to determine which species are likely to be considered as specific spoilage microorganisms in further studies and to develop adapted control methods. In this work, a combination of culture-dependent and -independent methods, PCR-TTGE, was used to analyse 3 raw salmon batches stored at chilled temperature (4°C followed by 8°C after a cold chain break) in modified atmosphere packaging (MAP) (50% CO_2 / 50% N_2) or vacuum, in order to characterise the spoilage related microbiota of raw salmon. Sensory evaluation; microbiological enumeration and chemical analysis were performed after 3, 7 and 10 days of storage. At the onset of spoilage, 65 bacterial isolates were picked from the plates. Thus, 13 different genus or species were identified by phenotypic and molecular tests: *Serratia* sp, *Photobacterium phosphoreum*, *Yersinia aleksiciae*, *Hafnia alvei*, *Buttiauxella gaviniae*, *Pseudomonas sp*, *Carnobacterium maltaromaticum*, *Carnobacterium divergens*, *Lactococcus piscium*, *Lactobacillus fuchuensis*, *Vagococcus sp*, *Leuconostoc gasicomitatum* and *Brochothrix thermosphacta.*. The PCR-TTGE profiles and the bands identification allowed to visualise for all the batches a shift of the dominant populations during the storage probably due to the temperature change and the packaging. At the beginning of the storage, *Pseudomonas* sp dominated the raw salmon microbiota whereas in the following days (7 and 10), *P. phosphoreum* and *L. piscium* were identified as the main bacterial groups. This study enhances the knowledge of MAP and vacuum packed raw salmon spoilage microbiota.

Keywords : seafood, culture-independent method (PCR-TTGE), sensory analysis, *Photobacterium phosphoreum, Lactococcus piscium*

Characterization of an intracellular β-glucosidase activity from *Oenococcus oeni* ST81 isolated from wine

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In a previous work (Mesas *et al*., 2011) we isolated from a 12 %Vol red wine from Ribeira Sacra (Spain) a strain of *Oenococcus oeni* called ST81. Such strain was characterized as a high level producer of β-glucosidase activity, able to undergo malolactic fermentation and non producer of biogenic amines. Because the interest of β-glucosidase activity in winemaking due to its ability to liberate volatile aglycones as a source of aroma and flavour compounds (D'Incecco *et al*., 2004; Swiegers *et al*., 2005), the aim of this study was the characterization of the β-glucosidase activity of *O. oeni* ST81.

Cells from 400 ml of an MRST (MRS + tomato juice 50 ml l^{-1}) culture grown up to early stationary phase (absorbance at 600 nm ~ 1.5-2) were pelleted by centrifugation (9000 g), washed twice with 20 ml of NaCl 154 mmol l^{-1} and suspended in 15 ml of disruption buffer (phosphate-citrate buffer 125 mmol l^{-1}, pH 5). The cells were chilled and maintained in an ice bath during disruption in a Branson 450 sonifier (5 pulses of 30 sec, with 60 sec intervals using the micro tip at 60 W) as per Alegre *et al*. (2004). Cell debris was pelleted by centrifugation (12500 g) and the supernatant used as crude extract. The activity of β-glucosidase was determined according to the procedure described by Barbagallo *et al*. (2004) as follows. Appropriate crude extract or cell suspension (between 5 and 10 µl) was mixed with disruption buffer containing 5 mmol l^{-1} of p-nitrophenyl β-D glucopyranoside up to 500 µl. Unless otherwise indicated, the reaction was made at 30ºC for 30 min and then stopped by adding 1 ml of Na_2CO_3 1 mol l^{-1}. Samples were centrifuged (12500 g) and the supernatants determined at 400 nm. One unit (U) of enzyme activity was defined as the amount needed to hydrolyse 1 µmol min^{-1} of substrate. Protein concentrations were determined as per Bradford (1976) using bovine serum albumin for the standard curves. The results of β-glucosidase activity obtained comparing cell free extract, cell debris and whole cells suggest that β-glucosidase of *O. oeni* ST81 is an intracellular activity. Optimum temperature and pH were 40 ºC and 5 respectively. The presence of ethanol 4 to 16 %Vol in the reaction mixture increases up to a 140% the activity of the β-glucosidase of *O. oeni* ST81. Glucose 200 mmol l^{-1} inhibits 40% of the activity of β-glucosidase while fructose has no effect at similar concentration. While Co inhibits the activity of β-glucosidase and Mn increases it, other ion metals have little or no effect. The results obtained for β-glucosidase of *O. oeni* ST81 seems to be closely related with those of *Lactobacillus brevis* (Michlmayr *et al*., 2010) and *Leuconostoc mesenteroides* (Gueguen, *et al*., 1997), two lactic acid bacteria that also exhibit intracellular β-glucosidase.

Keywords β-glucosidase; *Oenococcus oeni*; intracellular activity

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Characterization of enterohaemorrhagic *Escherichia coli* O157:H7

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A total of 10 *Escherichia coli O157:H7* isolates from vegetables and two type strains were investigated and subtyped by using random amplified polymorphic DNA (RAPD), Antimicrobial susceptibility and lipopolysaccharide (LPS) profile patterns. Genomic DNA patterns generated by RAPD are highly specific for different strains of an organism and have significant value in epidemiologic investigations of *Escherichia coli O157:H7*. Ten food isolates of *Escherichia coli O157:H7* and two type strains of *Escherichia coli O157:H7*, one primer (RAPD 2, 970-11) were found to give excellent differentiation between Egyptian isolates and type strains by RAPD-PCR. Using only the presence or absence of variable bands. All of *Escherichia coli O157:H7* isolates were easily distinguished from the type strains of *Escherichia coli O157:H7*. RAPD using 970-11 primer is potentially useful typing tool for *E. coli* isolates of serotype O157:H7 and possible other *Escherichia coli O157:H7* type strains. The antimicrobial susceptibility of the different recovered isolates were tested against nine of antibiotics. All the isolates were resistant to bacitracin and penicillin G. The analysis of the lipopolysaccharide (LPS) patterns revealed that all the isolates have the same LPS pattern.

Characterization of the total phenolic content, antioxidant and antimicrobial properties of acorn extracts (from Quercus ilex and Quercus suber)

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Presently, due to the knowledge that our diet can actively influence our health, functional foods have attracted special attention, in particular their phenolic and antioxidant components. Several compounds obtained from plants, that have demonstrated antimicrobial and antimutagenic activity, are being actively characterized for use in functional foodstuffs.

Acorn is a fruit from several trees that belong to the *Fagaceae* family. In Portugal the predominant species of this family are *Quercus fagin* (Oak), *Quercus ilex* (Holm Oak) and *Quercus suber* (Cork Oak). The acorn has been used around the world for several years and for many different purposes. Racchout is a traditional Turkish drink prepared with acorns similar to hot chocolate. In Europe it has been used to prepare Eichel Kaffe (acorn coffee), a beverage with astringent and anti-diarrhea properties.

Our study aimed to analyse the antimicrobial properties of shell and cotyledon extracts of acorns from *Quercus suber* and *Quercus ilex*, taking into account the impact of the diferrent thermal processing of the samples on the total antioxidant capacity (TAC) and total phenolic content (TPC). Both cotyledon and shell were studied fresh and roasted and, in addition, a coffee-like beverage, produced from the cotyledon. The extracts were tested against several contaminant and potentialy pathogenic microorganisms: *Bacillus cereus* ATCC 11778, *Salmonella spp* ATCC 3076, *Escherichia coli* NCTC 9001, *Pseudomonas fluorescens,* a food isolate, Methicillin Sensitive *Staphylococcus aureus* NCTC 8532 (MSSA), Methicillin Resistant S. *aureus* ATCC 29213 (MRSA),*Listeria innocua* NCTC 10528, *Candida albicans,* a clinical isolate and *Yarrowia lipolytica,* a food isolate.

Generally the *Quercus ilex* and *Quercus suber* extracts had the same amount of TAC and TPC when considering the cotyledon extracts. When considering the shells, *Quercus* suber had higher values of TAC and TPC. Of all of the extracts tested, none were effective against *C.albicans* or any of the Gram negative microorganisms. The coffee-like extracts didn't exhibit any antimicrobial activity and the shell extracts had the most effectiveness. The active extracts were further characterized by the determination of the MBC and death curves.

Keywords *Quercus suber, Quercus ilex, Antimicrobial Activity, Functional Foods*

Comparison Between Antibacterial Effects of *Prosopis juliflora* and *Zingiber officinale Rose* occurring in Iran

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In Iran, as it is known, the medicinal plants have been used since very old times in traditional medication and wide diversity of the medicinal plant spieces, so conducting such study may be of high importance.

In this study antibacterial activity of *Prosopis juliflora* and *Zingiber officinale* Rose(Ginger) were tested in -vitro against some gram positive and negative bacteria by paper disk diffusion and well methods.

In this purpose the methanolic and ethanolic extracts at different concentrations (0.05, 0.1, 0.2, 0.3, 0.4 mg/ml) against *Staphylococus aureus*, *Staphylococus.epidermidis* **,***Micrococcus loteus*, *Bacillus cereus* ,*Shigella sonnei*, *Pseudomonas aeruginosa* ,*Escherichia coli*, and *K.pneumonia* were tested. MIC and MBC were determined by macrodilution.

According to the results from this study there are hopes that methanolic and ethanolic extracts of *Prosopis and Zingiber* may be used for treatment of some resistant types of bacteria such as *Pseudomonas areuginosa* and *Escherchia coli* .also it can be used as preserving material for food stuff in food industries.

Keywords Antibacterial activity;*Prosopis juliflora*;*Zingiber officinale Rose ;*medicinal plant;Iran

Correlation between moisture content and water activity of different varieties of maize and wheat grains from Spain

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Fungi are ubiquitous contaminants of cereals which can produce toxic metabolites in some environmental conditions. Their infection levels are mainly determined by the prevailing of these environmental conditions, where water availability and temperature are major determinants of cereal spoilage. The moisture content (mc) is the total water in a product, including the molecularly bound water, and is expressed in percent of its weight. But it is the free water that is available to microorganisms for growth, and its measuring value is called water activity (aw), the aw-value. On the other hand, standard methods for determining mc in grains are tedious and time-consuming whereas water activity instruments are simple to operate.

In this study aw-values and mc were determined in a total of 269 cereal grain samples:
144 samples of different varieties of maize including dried grains and undried harvested grains and 125 samples of different varieties of wheat. The samples were kindly provided by the Field Station IRTA-Mas Badia Foundation and the GENVCE Group ("Grupo para la Evaluación de Nuevas Variedades de Cultivos Extensivos en España") and they were from different areas of Spain. Water activity of samples was measured with a Novasina LabMaster AW. Moisture content was measured by drying according to the EC standard method of testing for moisture content.

Maize and wheat grains had aw-values in the range from 0.246 to 0.839 with a mean value of 0.572 and from 0.419 to 0.541 with a mean value of 0.489, respectively. Moisture contents of maize and wheat grains from the different samples ranged from 4.3% to 16.76% with a mean value of 10.77% and from 10.5% to 12.66% with a mean value of 11.63%, respectively. A significant positive correlation was observed between mc and aw-values of both cereals.

Keywords cereals; maize; moisture content; water activity; wheat.

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Destabilization and off-flavors generated by *Pseudomonas* proteases during or after UHT processing

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P. fragi, P. lundensis and members of the P. fluorescens group are known to compromise UHT-milk, due to the production of heat-stable proteases in the raw milk. While pseudomonads are readily inactivated by the heating conditions applied by the dairy industry, their proteases remain active in the heat treated UHT products. The presence of *Pseudomonas* proteases may cause instability problems and spoilage in UHT milk upon extended storage.

To determine the correlation between off-flavor development and protein degradation in UHT milk for the different Pseudomonas groups, 575L raw milk was pasteurized to reduce the bacterial load. Subsequently, 7 (60L) containers were aseptically filled with the pasteurized milk and inoculated with 6 different *Pseudomonas* strains. The 6 inoculated milks and the blanc milk were further stored at 6.5°C for 3,4 and 5 days to mimic normal and extreme pre-processing conditions. After the storage period the milk was heated under UHT conditions (140°C, 5s). Milk was aseptically filled in 0.5L HDPE bottles and stored at 37°C to accelerate proteolysis and off flavor development. On regularly moments samples were taken for sensorial analysis and proteolysis determination.

The results illustrate that high *Pseudomonas* count milk is impossible to process under the applied UHT conditions and that the correlation between off-flavor development and proteolysis is different for each investigated *Pseudomonas* species. Thus, not all *Pseudomonas* proteases are equally capable in generating off-flavors. On the other hand, it can be concluded that *Pseudomonas fragi* contains the severest spoilage risk when it comes to generating off-flavors.

Keywords *Pseudomonas*, off-flavors, proteolysis

Detection and prevalence of Entamoaeba gingivalis in children attending the dentistry school in UANL Mexico

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Objective: The aim of this study was to know how the presence of Entamoeba Gingivalis is associated with the prevalence of dental caries and the rate of oral hygiene in children from 2 to 12 years old attending the Pediatric Dentistry Graduate School in the School of Dentistry, University of Nuevo Leon. Methods: Two groups were defined according to age: Group 1: from 2 to 5 years old and Group 2: from 6 to 12 years old, neither of them had received periodontal dental treatment. Outpatients were carried out a random sampling. 61 samples of dental bacterial plaque in sterility conditions were taken from children. A 1 – mm cylindrical sterile wooden device was used in the sample taking. Samples were put into six tubes with TYI and PEHPS added with antibiotics and antifungal. They were incubated at 37°C for 5 days and observed with an inverted microscope. It was considered positive when observing amoebas suspended in the culture medium stuck to some of the 61 sample tube walls. Result: Entamoeba Gingivalis (2.6%) and Trichomonas Tenax (5.2%) were found in children attending the Pediatric Dentistry Graduate School in the School of Dentistry, with P<.05. Conclusion: The presence of Entamoeba Gingivalis and Trichomonas Tenax depend on patients' gender and oral hygiene conditions, regardless age. The association of gender with the presence of parasites cannot be established since the prevalence found was too low. CEO and CPO rates were very high in all ages, a fact which proves highly poor sanitary conditions.

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Detection of *horA, horC* and ORF5 genes in *Pediococcus* sp. of the brewing process by PCR

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Beer has a good microbiological stability because it has unfavorable conditions for microbial growth, specially the antimicrobial activity of iso-α-acids from hop. Despite these unfavorable characteristics for many microorganisms, species of lactic acid bacteria (LAB), most importantly *Lactobacillus* and *Pediococcus,* can cause beer spoilage. The resistance to hop compounds is given, partly, by the product of genes *horA, horC* and ORF5. The objective of the present work was to evaluate the capacity of *Pediococcus* sp, strains M, A, G1 and G5 for growing in increasing concentrations of iso-α-acids and to detect the genes that give the resistance to hop compounds. The bacterial growth showed that all strains could adapt to increasing concentrations of iso-α-acids to a maximum concentration of 150 µg/ml, which is 10 times more than the concentration of an average beer. All three genes were tested for PCR amplification using plasmid DNA as template. The analysis of the PCR products showed the presence of *horA* in all strains, meanwhile all strains were negative for *horC* and ORF5. This results show that *horA* is a useful marker for the detection of beer spoilage *Pediococcus* sp.

Key words: PCR, *Pediococcus,* beer-spoilage, *horA.*

Determination of the presence of *Listeria monocytogenes* in modified-*atmosphere-packaged* vegetables by the UNE-EN ISO 11290-1:1997 and Multiplex PCR procedures

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Listeria monocytogenes is a foodborne pathogen of great interest due to its unique epidemiological features, its ubiquity and the severity of the diseases it causes. Because of its wide distribution, this organism has many opportunities to contaminate food in different steps of production, this being the most common route by which humans acquire infection. Being tolerant to hostile environmental conditions, the bacteria can reach and stay long in a variety of fresh and processed foods, as well as on the surfaces of the equipment. Given the increase in the consumption of raw and pre-cooked in our society, not surprisingly, it is considered one of the main threats to food safety.

The purpose of this work was to study the presence of *Listeria monocytogenes* in modified-***atmosphere-packaged*** (MAP) vegetables available to consumers in Valencia by cultural and by a multiplex PCR technique.

Matherials and Methods: We studied 70 samples of modified-***atmosphere-packaged*** (MAP) vegetables including espinachs, parsley, broccoli and salad purchased from Valencian markets. The presence of *L. monocytogenes* by cultural technique was investigated according to the UNE-EN ISO 11290-1:1997 / A1: 2005 and UNE-EN ISO 11290 -2:2000 / A1: 2005ISO. Tipical colonies on Palcam and ALOA plates were tested for β-haemolysis, catalase, oxidase and gram stain and identified by API Listeria system. Samples were also analyzed by multiplex PCR as previously described (Ballesteros et al., 2010) directly and after secondary enrichment in Fraser broth (ISO 11290). Serotyping was performed with the commercial system *Listeria* antisera set (Denken Seiken.Co, Ltd., Tokio, Japan) according to manufacturers' instructions.

Results: Only 3 samples (2 broccoli and 1 espinach) were positive for isolation of *L. monocytogenes* in both Palcam and ALOA plates. The three isolated colonies showed the same API 6510 profile and serotype 1/2a.

By PCR, 31 samples among the 70 were positive for the presence of the L. *monocytogenes* specific hlyA fragment after secondary enrichment. PCR yielded negative results in all samples without enrichment.

Conclusions: PCR procedure was more eficient than normalized cultural method for detecting *L. monocytogenes* in packaged vegetables and results were available within 8 h. The presence of *L. monocytogenes* in MAP vegetables could find its source in the raw material itself or indicate a possible cross-contamination in the food industry. Therefore, we should emphasize the need of preparation, sanitation and storage good practices, to avoid for *L. monocytogenes* reaching the consumer.

Keywords: *Listeria monocytogenes*, vegetables, detection

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Development of a DNA microarray for the detection of pathogenic and spoilage bacteria in seafood

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Microbial spoilage of seafood is an area of global concern, causing serious foodborne intoxications and resulting in high economic losses in the sector of fishing and aquaculture. The fast and accurate identification of pathogenic and spoilage bacterial species is important to assure seafood quality and safety. Over the past few years, the tools for molecular diagnosis have greatly improved, replacing traditional cultivation methods for the rapid detection of pathogenic and spoilage microorganisms in the food sector. Within the ample area of molecular techniques, gene arrays can hybridize multiple DNA targets simultaneously, and thus, have enormous potential for the detection and identification of pathogens in routine analysis.

The present study describes the development of a method, under the format of a DNA microarray, for the rapid and precocious detection of the main pathogenic and spoilage bacteria, present either in captured fish or in aquaculture products. The applied methodology was based on the use of the ligation detection reaction (LDR) coupled to a universal array. This method requires the design of two oligonucleotide probes specific for each target sequence. In the ligase reaction both probes are ligated to a present template only in the case of perfect match in the discriminating position. Afterwards, the ligation product hybridizes to a certain location on the universal microarray. By this way, sequences can be differentiated by just one polymorphism and the unequivocal detection of the corresponding bacterial species can be carried out, avoiding false positives.

For the probe design, bacterial species with importance in seafood-spoilage and foodborne intoxications were considered, including the genera *Acinetobacter*, *Aeromonas*, *Bacillus*, *Carnobacterium*, *Listeria*, *Photobacterium*, *Pseudomonas*, *Shewanella*, *Staphylococcus*, *Stenotrophomonas*, *Vibrio* and the family of *Enterobacteriaceae*. Furthermore, non-spoilage and non-pathogenic species that are potentially present in seafood were taken into account to ensure the accuracy and specificity of the designed probes. Oligonucleotide probes were designed based on sequences of the 16S rRNA gene, using the oligonucleotide retrieving for molecular applications (ORMA) tool that allows the search for discriminating nucleotides and the design of specific probes. After testing the specificity of the possible candidate oligonucleotides in-silico, four probes were selected and synthesized, being specific for the genera *Aeromonas*, *Pseudomonas* and *Shewanella*, as well as for the species *Morganella morganii*, respectively. For the validation of the probes in-vitro, 16 strains obtained from type culture collections were tested. The results demonstrated high specificity and sensitivity of the synthesized probes.

In conclusion, the designed probes, coupled to the universal array, can be applied for the rapid, efficient and accurate identification of pathogenic and spoilage bacteria potentially present in seafood, as well as in food in general. The application of this sensitive method in the sector of seafood and aquaculture products would result in the accurate analysis of the microbial risk, as well as an increased safety and quality of seafood.

Keywords seafood pathogens; seafood spoilage; bacterial identification; DNA-microarray; Ligase detection reaction; probe design, ORMA

Development of a rapid procedure of real-time PCR to detect *Listeria monocytogenes* in cheese

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Listeria monocytogenes is a ubiquitous foodborne pathogen that causes listeriosis, a fatal disease of public health concern. *L. monocytogenes* can be found in unprocessed foods of animal origin and in some processed and ready-to-eat foods (RTE) by post-processing contamination. Cheese is a RTE food that has been associated with foodborne listeriosis.

Classical cultivation methods for detection of *L. monocytogenes* in foods are time-consuming, but they still remain as official methods. These methods comprise selective enrichments (24–48 h) and isolation on selective media (48 h), followed by serological and/or biochemical identification. The recommended standard method for isolation of *L. monocytogenes* takes 5 days to confirm a negative result and up to 10 days to confirm a positive one (Grady et al., 2008). Alternative methods such as real time PCR have been described (Rodríguez-Lázaro et al., 2004).

The purpose of this study was to optimize a real time-PCR method to detect *L. monocytogenes* in cheese according to a previously described protocol (García et al., 2010). Three DNA extraction methods were tested. The extraction with “Epicentre MasterPure DNA kit” was performed following the manufacturer’s protocol. The second DNA extraction protocol was based on CTAB method (Rodríguez et al., 2011a) with a hot-cold incubation, resuspension on CTAB buffer and proteinase K at 65ºC, and incubation with RNAse at 37ºC. The third was a chelex-based method (Rodríguez et al, 2011b) that included two incubation steps: first with proteinase K and lyticase at 65ºC, and second with RNAse at 37ºC. Sediments obtained with CTAB and chelex-based methods were purified by an EZNA kit according to manufacturer’s instructions. Samples were prepared in triplicate as follows: cheese portions of 5 g were mixed with 10 ml of peptone water (1% w/v) containing serial 10-fold dilutions of an overnight culture of *L. monocytogenes.* Ten ml of homogenate were used for each of the tree extraction methods.

Detection of *L. monocytogenes* in cheese was carried out in triplicate by real time PCR in samples with no enrichment step. Reactions were performed using SYBRGreen and TaqMan protocols. The SYBRGreen protocol was as follows 10 min at 95ºC and 40 cycles of 15 s at 95ºC and 60 s at 60ºC, this included a dissociation melting analysis. The TaqMan protocol was as follows: 2 min at 50ºC, 10 min at 95ºC and 40 cycles of 15 s at 95ºC and 60 s at 60ºC. Primers and probe used were *hlyQF, hlQR* and *hlQP* (Rodríguez-Lázaro et al. 2004).

The minimum level of detection of *L. monocytogenes* in cheese without previous enrichment using the SYBRGreen protocol was 10^3 cfu/5 g with a C_t value of 29.44 ± 0.2. This result was obtained with the DNA extracted with CTAB method. When TaqMan protocol was used with the same DNA extraction method, the minimum level was also 10^3 cfu/5 g, but the C_t value was higher (32.66 ± 0.2). With the other two extraction methods the sensitivity was lower, being the minimum detection level 10^4 cfu/5 g in both protocols, with C_t values of 33.5 ± 0.7 and 37.55 ± 0.37 in SYBRGreen and TaqMan protocols, respectively.

The real time-PCR method assayed can be useful to survey compliance of cheese with UE microbial criteria for *Listeria monocytogenes*.

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Keywords real time-PCR, *Listeria monocytogenes*, cheese

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Development of a Real Time PCR system for detection of ochratoxin A-producing strains of the *Aspergillus niger* aggregate

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Members of *Aspergillus* section *Nigri* are distributed worldwide, being considered as common food spoilage fungi. Some species of this section produce ochratoxin A (OTA), mainly *A. carbonarius* and several members of the *Aspergillus niger* aggregate. While *A. carbonarius* consistently produces large amounts of ochratoxin A, the reported percentages of OTA-producing strains in the *A. niger* aggregate is much lower.

Detection of ochratoxigenic *A. niger* aggregate strains is important to prevent OTA contamination in foodstuffs and biotechnology products. A new Real Time PCR procedure has been developed for the rapid and specific detection and quantification of ochratoxin A-producing strains of the *A. niger* aggregate. Two specific primers delimiting a 120 bp fragment and a probe were designed and directed to a polyketide synthase (PKS) from *A. niger* CBS 513.88 genome. This PKS has a strong similarity to PKS of *A. ochraceus* fragment involved in ochratoxin biosynthesis. Specificity was confirmed by testing primers towards purified DNA from 91 fungal strains, including reference and food isolates representative of OTA producing and non-producing strains of the *A niger* aggregate, as well as other *Aspergillus*, *Penicillium*, and *Fusarium* spp. The SYBR-Green and the TaqMan approaches developed allowed the specific detection only of ochratoxigenic strains of the *A. niger* aggregate. All other analyzed food related fungi gave negative results.

This is the first report on a Real Time PCR system for the detection of OTA producing strains of the *A. niger* aggregate. It can be used for the rapid quality assessment of food products and can be a valuable tool to enhance the detailed knowledge of the expression conditions of ochratoxin A biosynthetic genes.

Keywords *A. niger* aggregate; ochratoxin A; polyketide synthase; real time polymerase chain reaction

Development of a starter culture for production of kefir

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Kefir is a fermented effervescent milk with sweet sour taste and low alcohol content, resulting from the metabolic activity of microorganisms present in kefir grains, a complex and specific mixture of bacteria and yeasts surrounded by a polysaccharide matrix. The traditional method of obtaining the kefir cultures by successive reinoculation grain is simple, but difficult to standardize the inoculum, which results in an irregular production. The aim of this study was to isolate and identify microorganisms in kefir grains from the Lab. Fitofarmacos (Unifenas, MG, Brazil) to be used as a starter culture in the production of kefir in order to obtain a product with standardized characteristics and similar to those produced with kefir grains. The lactic acid bacteria (LAB) were isolated in MRS agar and M-17 suplemented with 100 mg /L cycloheximide. The yeasts were isolated in YM agar suplemented with 200 mg /L chloramphenicol. The plates were incubated at 22°C for 72h under anaerobic conditions for bacteria (anaerobic chamber) and aerobic conditions for the yeasts. The phenotypic characterization of the isolated bacterias were based on cell morphology by Gram-staining, breath test, gas production in the presence of glucose and the research of catalysis. Based on these tests, the LAB was identified using the API 50 CHL kit (Bio Mérieux, Marcyl'Etoile, France) and the results were confirmed with molecular identification by amplified ribosomal DNA and Restriction Analysis (ARDRA) and DNA sequencing. The yeasts were grouped into profiles to biochemical and physiological tests, according to standard method (Yarrow, 1998) and the results were confirmed by DNA sequencing. After the identification of the isolated microorganisms from kefir grain, the starter culture was selected to be applied in the Kefir production. This was formulated with six microorganisms: *Lactococcus lactis* ssp*. lactis*, *Lactococcus lactis* ssp. *cremoris*, *Leuconostoc mesenteroides* ssp. *mesenteroides*, *Lactobacillus casei*, *Torulaspora delbrueckii* e *Kazachstania unispora*. To evaluate the sensory characteristics of the new product, samples of kefir prepared with grain and with the starter culture were submitted to acceptability. Attributes of aroma, effervescence, acidity, texture and overall impression and also purchase intent were evaluated. The data were first analyzed by Analysis of Variance (ANOVA) followed by average tests (Turkey) and Frequency Analysis Distribution, using as sources of variation the samples and the panelists. The results revealed that the kefir produced from the starter culture showed significantly higher acceptability ($p \leq 0.05$) with average scores around 5.0 on 7.0 points on hedonic scale. The kefir prepared from grains received the lowest percentage of positive purchase intent (30%), and also the least acceptable in relation to the effervescency, consistency and overall impression. This results suggest that not only kefir formulation prepared from starter culture was more accepted by consumers, but also shows more positive sensory characteristics than the kefir obtained from grains.

Keywords: kefir, starter culture, microbial identification, sensorial evaluation

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Development of antimicrobial films for microbiological control of packaged salad

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Current lifestyles have caused a major shift in consumption trends which are reflected in an increase in the demand for minimally-processed ready-to-eat products such as salads. Fresh-cut salads, defined as mixed vegetables that have been cleaned and/or trimmed and/or peeled and/or cut into 100% usable product and packaged, offer the consumer nutritious, convenient and tasteful (tasty) products while maintaining their freshness. Modified atmosphere packaging combined with refrigeration is a preservation technique that ensures quality over a short period. Nevertheless, shelf-life is limited mainly due to microbiological growth which can affect food quality and/or produce food-borne illnesses.

The aim of the present work was to develop antimicrobial films consisting of PP/EVOH structures with oregano essential oil and citral. Both substances are known for their antimicrobial activity based on their interaction with the cell membrane. The films developed were used to pack minimally-processed salads combining modified atmosphere technology to extended shelf-life, and active packaging technology to reduce possible microbiological risks. The antimicrobial activity of the films against both pathogenic microorganisms and the habitual microflora was investigated 'in vitro' and also on the food itself. The effect of the release of the antimicrobial agent on the sensory characteristics of the salad was also studied.

The results showed that antimicrobial activity, reducing spoilage flora on salad as well as inhibiting the growth of pathogens in contaminated salads. This effect was greatest against Gram negative bacteria. Sensory studies showed that the package most effective and accepted by customers was that containing oregano essential oil.

Keywords: Fresh cut vegetables, oregano, citral, EVOH, antimicrobial films.

Development of antimicrobial release systems based on EVOH films containing LAE intended for active food packaging applications

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Current concerns in the food industry for reducing the rate of quality loss and spoilage whilst ensuring safety and shelf-life extension of foods has promoted research into active packaging technologies with an emphasis on antimicrobial packaging. Compared to direct addition of preservatives to food, active packaging technologies could stabilize labile preservatives or avoid reaction between incompatible ingredients during food processing and could reduce the amount of active agent required.

Polymer films used in food packaging can act as reservoirs and release systems for active agents. These systems can be tailored to supply the active agent in a sustained manner over a defined period of time by: adjusting the amount of plasticizers and excipients, polymer crosslinking or blending, or modifying the hydroscopic nature of the polymer matrix. Preferably, the active system should have a mechanism for triggering its activation, such as irradiation, pH, humidity or temperature.

The aim of this work has been to develop antimicrobial films intended for food packaging applications incorporating the antimicrobial compound LAE (ethylN-dodecanoyl-L-arginate hydrochloride) in EVOH copolymers of different mol % ethylene content (i.e.EVOH-29 and EVOH-44), and to study the effect of the ethylene molar content of the copolymer on the release kinetics of LAE in aqueous media. The antimicrobial capacity of the resulting films was also tested *in vitro* in liquid media against *L. innocua* and *E. coli.*

EVOH-29 and EVOH-44 films were made by casting incorporating 5% and 10 % LAE in the film forming solution. The release of the compound in the aqueous medium used as a liquid food simulant was studied at 4 ºC and 23   HPLC. To evaluate their antimicrobial activity, a set of films was immersed in liquid medum and stored at 4 ºC for 5 days whilst another set was immersed in liquid medium and stored at 37 ºC for 24 h. After the corresponding storage time the antimicrobial activity of the films was assayed against bacteria.

The results showed that the release kinetics of LAE in the aqueous medium depended on the ethylene content in the EVOH copolymer and the diffusion temperature at which the experiment was carried out. Higher diffusion coefficients were obtained at 23 ºC and for EVOH-29 which has a greater amount of hydroxyl groups capable of retaining water molecules in the polymer structure thus promoting matrix plasticization and hence facilitating the release of the agent. Regard to the antimicrobial activity of the films, all of them showed total growth inhibition in the liquid medium tested.

Keywords: antimicrobial food packaging**,** EVOH, LAE, *L. innocua, E. coli.*

Development of bacteria identification chip to detect the lactic acid bacteria in Thai fermented sausage

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Nham is a Thai style fermented pork sausage, traditionally produced by natural fermentation of lactic acid bacteria. In addition to giving texture and taste to the sausage, the lactic acid fermentation prevents growth of some food pathogens. The lactic acid population commonly found in Nham is composed of *Lactobacillus plantarum*, *L. pentosus* and *L. sakei*, in which *L. plantarum* has been commercially used as Nham starter. Due to the rising concern on food safety, the method to monitor bacteria population in food processing is crucial in order to provide a safe and high quality food for consumers. The 16S rRNA-based traditional methods to characterize bacteria population can be time-consuming and laborious. Alternatively, the microarray technology has been used for a high-throughput screening for different purposes. This approach provides a simultaneous, culture-independent and can be cost-effective than traditional methods.

This work developed the Bacteria Identification Chip (BIC) using the microarray approach. We designed a total of 164 bacteria specific probes based on 16S rRNA sequences for 12 bacteria of interest in which seven are lactic bacteria, two are foodborne pathogens and three are out-group bacteria. The following parameters for BIC construction were optimized for signal intensity and probe-target specificity: i) substrate for fabrication, DNA probe attached better to aminosilane-coated slides than superamine-coated slides, ii) probe length, probes with a minimal length of 40 bases gave stronger signal than the shorter probes, and iii) hybridization condition, hybridization temperature at 57 °C gave strong positive signal intensities with less false positive signals. BIC was validated with an individual pure culture and able to specifically detect 12 bacteria (*L. plantarum*, *L. fermentum*, *L. brevis*, *L. animalis*, *L. casei*, *L. sakei*, *L. delbrueckii*, *L. monocytogenes*, *S. aureus*, *E. coli*, *P. aeruginosa* and *M. luteus*). The multiplex detection was further evaluated by applying BIC to detect bacteria samples containing mixtures of two, three and five bacteria species. This prototype was able to accurately detect multiple bacteria species simultaneously in the tested samples. The feasibility of BIC to detect the target bacteria in food was evaluated in Nham samples. Three groups of Nham sample, natural fermentation, *L. plantarum* started fermentation and *L. brevis* spiked fermentation, were collected on day 2, day 3 and day 7 of the fermentation. The target bacteria population from each Nham sample group was detected by BIC and the population dynamic during the fermentation process was compared. The natural fermented Nham sample contained *L. sakei*, *L. plantarum*, *L. animalis* and *L. delbrueckii*, whereas the *L. plantarum* started Nham sample contained *L. plantarum*, *L. sakei* and *L. delbrueckii*. The *L. brevis* spiked Nham sample contained *L. brevis*, *L. plantarum*, *L. delbrueckii* and *L. fermentum*. Moreover, the natural fermentation and *L. brevis* spiked samples showed lower positive signal levels of *L. plantarum* on day 2, but gradually increasing on day 3 and day 7 of the fermentation. In contrast, the *L. plantarum* started fermentation showed a higher positive signal level on day 2 than the natural and *L. brevis* spiked samples and the positive signal level remained high on day 3 and day 7. Thus, BIC was proven to be useful as an alternative method to detect and monitor target bacteria population during the food fermentation. This work provides a proof of concept for the development of a simultaneous and reliable microarray approach for bacteria detection and monitoring in food samples.

Keywords lactic acid bacteria; nham; microarray; oligonucleotide array

Development of PCR for simultaneously detecting *Clostridium botulinum* types A, B, E, and F

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Clostridium botulinum is an obligate anaerobic, endospore-forming bacterium that produces a lethal neurotoxin called botulinum neurotoxin (BoNT). BoNTs are one of the most potent biological and chemical substances known, and are responsible for a paralytic disease known as botulism, which is characterized by severe flaccid paralysis. BoNTs are divided into seven toxin types, A to G, according to their antigenic properties. Toxin types A, B, E, and F cause human botulism, and belong to groups I or II. Group I consists of proteolytic types A, B, and F, and groups II contains non-proteolytic of B, E, and F types.

The presumptive identification of the toxigenic strains and typing of BoNT were based on an enrichment step lasting and subsequent detection of the toxin by *in vivo* mouse bioassay. This technique is higly sensitive and specific but laborious, time-consuming (5 to 10 days), and costly and raises ethical concerns with regard to the use of experimental animal. Efforts have been made to develop alternatives to animal testing, as recommended by international legislation. Molecular biological methods based on the detection of BoNT genes in any neurotoxigenic microorganisms would be an ideal substitute. Different PCR methods based on the detection of BoNT-producing Clostridia in food and clinical samples have been described. Result obtained using PCR assays to detect neurotoxin gene fragments have shown a high level of agreement with those from mouse bioassay. However, there have been few reports of the use of a single PCR primer set for simultaneous detection of BoNT genes of more than one type causing human botulism.

In this study we report the development of an method for simultaneously detecting *C. botulinum* types A, B, E, and F. With the comparison of the sequences of *C. botulinum* types A, B, E, and F, Biological Defence Unit has developed specific primers for detecting simultaneously the four types.

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Development of volatile metabolites in beef stored in air and vacuum pack

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Metabolic activity of microorganisms in fresh meat results in biochemical changes and formation of metabolites that can contribute to spoilage. The determination of these metabolic by-products can give information about the type and rate of spoilage, and studies on molecular bases carried out through advanced mass spectrometric techniques can be instrumental for this purpose.

The aim of this study was to identify the volatile organic compounds (VOCs) developing in beef during chill storage in air and vacuum pack. Further objective was to look for a possible correlation between microbial development in meat and release of VOCs in the headspace in order to identify some molecules that could work as molecular indicators of meat spoilage.

The total viable counts (TVC) and spoilage-associated microbial populations such as lactic acid bacteria (LAB), *Pseudomonas* spp., *Brochothrix thermosphacta* and *Enterobacteriaceae* were quantified by viable counts performed on meat samples during storage for 1 week in air or 3 weeks in vacuum pack. In addition, at the same time points the SPME-GC/MS analysis was carried out in the headspace of beef samples, monitoring the rate of production of microbial volatile metabolites during storage.

The spoilage-related bacteria generated alcohols, ketones, fatty acids, esters and the complexity in VOC composition showed a tendential increase during storage. Differences of qualitative and quantitative type were observed in the VOCs detected in beef stored in aerobic conditions and under vacuum. The highest concentration of molecules related to the spoilage activity, and known to be responsible for potential meat off-flavour, was detected in the headspace of meat stored in air.

Some compounds were produced only in meat stored in air, such as phenylethylalcohol, ethyl 2-hexenoate, 1-hexanol, 2,3-butanediol, isoamylacetate. Some other molecules were detectable only in meat stored under vacuum, such as octanoic, nonanoic and decanoic acids, acting as molecular tracers of the specific microbial population. Finally, some typical spoiling compounds appeared as suitable indicators of quality degradation of meat in each storage conditions.

In the same storage conditions, although some similar general trend in the VOC production and microbial development was observed, a significant variability of concentrations of both metabolites and microbial loads were observed in the analyzed replicates. This result suggests that there can be a high degree of variability of meat contamination (which can vary from site to site) and consequently of microbial development and metabolite release during storage of different meat chops. Experiments carried out to study in depht the issue showed that this variability could be related also to additional factors which can influence the very complex process of meat spoilage during chill storage such as the initial number of psychrotrophic flora and its growth dynamics at low temperatures, pH, inter-species relations, etc. Consequently, the qualitative and quantitative composition of the microbial population and their spoilage-associated metabolites, can vary, and in some cases largely, in different meat parts.

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Keywords Meat spoilage; volatile metabolites.

Diabetes related enzyme inhibitory *Lactobacillus* species as potential probiotics for diabetes management

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Global projection of diabetes clearly demonstrates that this sugar is not sweet in nature. As a silent assassin, diabetes is affecting nearly 6.6% of world's adult population cost world economy very dearly both in term of life and money loss i.e. $376 billion (11.6% of total world healthcare expenditure). India with nearly one fifth of the total diabetic population is an unchallenged diabetic capital. It is a multi-factorial disease with many unknown risk factors. One of these risk factor is postprandial hyperglycemia (PPG). A person destined to develop diabetes remains in a postprandial hyperglycaemic state for 10-12 years before the onset of diabetes and is regarded as an independent risk factor for CVD in diabetics. Control of postprandial hyperglycemia in early stages has the potential for the treatment of diabetes. Many drugs are use to control PPG but insulins and digestive enzyme inhibitor are the only specific drugs available in market that specifically target postprandial hyperglycemia. A-glucosidase and α- amylase inhibitors have recently taken foremost attention because of their unambiguous action. But due to the various side effects of synthetic inhibitors, identification and characterization of these inhibitors from natural resources have become a common practice in last decade. But most of these enzyme inhibitors are isolated from plant sources and as large scale production from plant is expensive and cumbersome. Also many inhibitors have been screened and isolated from the fermentation broth of microorganism but they also have been reported to exert some negative health effects. So either enzyme inhibition through some food sources or by the help of some food grade microorganism can have a dual advantage of combining nutritional approach with the pharmacological approach. One of these food grade organisms are Probiotics i.e. live microorganisms which when administered in adequate amounts (10^7 cfu/ml) confer a health benefit on the host like increased absorbability, alleviation of lactose intolerance, immuno-stimulation, pathogen exclusion, production of bioactive compounds, anti-carcinogenic activity and de-conjugation of bile acids to lower blood cholesterol and other lipids etc. Therefore, the present study was done to evaluate digestive enzymes (i.e. α-amylase and α-glucosidase) inhibitory activity of food grade Lactobacillus spp. For this, lactobacilli were isolated from human faeces, human milk and various fermented and unfermented milk products like milk, dahi and cheese (Churpi, Gouda, Cheddar and camel milk cheese). A total of 429 colonies were picked up and identified based on catalase, morphological and microscopical caracteristics. Only 258 isolates were found to be Gram positive catalase negative rods, which were then again subjected to α-glucosidase and α- amylase production test and the negative isolates for these enzyme (54) were then screened for enzyme inhibition against yeast α-glucosidase, rat intestinal α-glucosidase and porcine pancreatic α-amylase. The best 10 isolates showing best inhibition against all enzymes includes *Lactobacillus Salivarius* (2), *Lactobacillus plantarum* (4), *Lactobacillus fermentum* (3) and *Lactobacillus casei* (1) were then subjected to a baterry of probiotic tests recommended by WHO 2002. Among all isolate *Lactobacillus Salivarius* and *Lactobacillus plantarum* shows good acid and bile tolerance. *Lactobacillus Salivarius 2* and *Lactobacillus plantarum 3* shows reduction of 2 and 1.5 log cycle at pH 1.5 while only a marginal decrease at pH 2 respectively after 2 h. The marginal decrease in the log (cfu/ml) value at low pH of 2 indicated the good tolerance of *Lactobacillus Salivarius 2* and *Lactobacillus plantarum 3* against acidic conditions prevalent in the stomach. All the strains *Lactobacillus Salivarius* and *Lactobacillus plantarum* were able to grow 1 and 1.5% while shows no reduction in viability at 2% bile which was about 5–6 times more concentrated than bile present in human intestine. These results indicated the survival potential of isolates in the presence of toxic bile salts. All isolates exhibits varying degree cell surface hydrophobicity *Lactobacillus Salivarius 2* and *Lactobacillus plantarum 3* shows 50.61% and 69.48% against n-octane, 45.21% and 32.2% against n-Hexadecane and 47.69% and 67.37% against Xylene. Both isolates exhibit good bile salt hydrolase as well as antimicrobial activity. This work appears to be the first concise report on the diabetes related enzyme inhibitory profile of Lactobacillus species. Hence, these isolates may be use for development of functional antidiabetic food that can target postprandial hyperglycemia.

Keywords probiotics; diabetes; α- glucosidase inhibiors; α-amylase inhibitors

Effect of method of production of kefir in shelflife

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Kefir is a carbonated fermented milk with a slightly acidic flavor. It is traditionally produced by the kefir's grain inoculation in milk. However the amount and species of microorganisms in kefir grains may change after successive productions, becoming difficult to standardize the drink quality. Changes in certain microbiological, physicochemical, and sensory parameters of kefir were studied during refrigerated storage. Kefir batches were prepared using 5% added kefir grains (traditional kefir) and starter culture (starter-culture kefir) containing bacterias and yeasts. Samples for analysis were taken after production and after 2, 7, 14, 21 and 28 days of storage at 4 ± 1° C. The pH of the samples made using kefir grains and starter culture decreased significantly ($p<0,05$) while acidity increased ($p<0,05$) during storage. The viscosity of starter-culture kefir increased during storage. The syneresis of traditional kefir increased significantly ($p<0,05$), while the syneresis of starter-culture kefir remained constant over the period storage. The ethanol concentration of kefir increased significantly ($p<0,05$). The inoculated culture (kefir grains or starter culture) exerted an influence, the starter-culture kefir had the lowest pH and the highest acidity and viscosity, while the traditional kefir had the higher syneresis and ethanol. The counts of lactic acid bacteria on the traditional and starter-culture kefir has changed from 9.19 to 8.37 log CFU/mL and from 9.37 to 9.78 log CFU/mL, respectively, while the yeast has changed from 6.34 to 6.52 log CFU/mL and 5.75 to 6.10 log CFU/mL. Sensory analysis of the kefir revealed that the samples were acceptable until the second week of storage. The storage time has influenced the sensory characteristics: presence of foam, acid and alcoholic flavors and off-flavor in both samples. The lumpiness, acid flavor and creaminess were the most important attributes to distinguish the traditional and the starter-culture kefir.

Keywords: kefir; kefir grains, starter culture; microbiological, physicochemical, and sensory characteristics

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Effect of nitrogen supplementation on yeast fermentation performance and mead quality

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Mead is a traditional drink, containing 8-18% (v/v) of ethanol, which results from the alcoholic fermentation of diluted honey performed by yeasts. However, when it is produced in a homemade way, mead producers find several problems, namely, the lack of uniformity in the final product, slow or premature fermentations arrest, and the production of ‘‘off-flavours” by the yeasts. These problems could be due to several factors, including lack of essential nutrients such as a deficiency in available nitrogen. Additionally, it has been reported that mead fermentation is a time-consuming process, often taking several months to complete, depending on the type of honey, yeast strain and honey-must composition. Since mead production is a time-consuming process, to make its production viable it is necessary to reduce the fermentation time while producing an end product of quality. Thus, the aim of this study was to evaluate the effect of nitrogen addition to honey-must on two active dry wine yeasts (ADWY) fermentation performance, as well as on the mead composition and volatile aroma compounds production.

Honey must was prepared according a recipe developped in our laboratory, and supplemented with potassium tartrate and pH adjusted to 3.7 with malic acid. Then to study the effect of nitrogen adittion a part of honey-must was adjusted with diammonium phosphate (DAP) to achieve the concentration of nitrogen required by yeast to complete alcoholic fermentation. The honey-musts were inoculated in order to obtain a pitching rate of 1×107 viable cells/ml. Several parameters were determined during the fermentation to evaluate the effect of nitrogen addition on yeast growth, fermentation profile, mead composition and mead aromatic profile. For this study as biological material were selected the ADWY Saccharomyces cerevisiae Lalvin QA23 and Lalvin ICV D47.

The supplementation of honey-must with DAP reduced fermentation lenght in aproximadatelly seven days, however sugars were not fully consumed, suggesting that other factors could be interfering with yeast growth. Furthermore, it was verified that for both yeasts the specific growth rate and final biomass were higher in musts supplemented with DAP. Mead final composition was similar in the two experimental conditions, however, even in the honey-must to which DAP was not added about 25 mg/L of assimilable nitrogen remained at the end of fermentation. Some fermentative aroma compounds which contribute to the sensorial quality of mead, including alcohols, fatty acid ethyl esters, acetates, volatile phenols and volatile fatty acids, were identified and quantified. Global analysis of volatile profile revealed that the concentration of fatty acid ethyl esters and volatile phenols was higher in meads supplemented with DAP. The concentration of volatile phenols was below their perception threshold but the levels of acetate and ethyl esters could contribute to enhance fruity character in meads produced. These results are very useful for optimise the mead production and improving its quality.

Keywords mead; assimilable nitrogen, yeast performance, aromatic profile

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Effect of post mortem temperature treatment on microbiology meat quality of suckling lamb

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Chilling processes are employed to preserve the lamb carcasses from microbiological contamination and extend the shelf life. The temperature conditions during the first 24 h post mortem play a great role in the ultimate carcass quality. In this sense, the most common chilling treatment used is transferring the carcasses to a chamber at 0-2ºC immediately after dressing. In order to improve benefits as well as meat quality, other chilling treatments have been studied.

The aim of this work was to evaluate the effect of post mortem temperature treatment on microbiological carcass quality. To carry out the study, 30 Churra suckling lambs were used. Lamb carcasses were randomly assigned to three different post mortem temperature treatments: conventional (2ºC for 24 h), ultra-rapid (-20ºC for 3.5 h then 2ºC until 24 h post mortem) and slow (12ºC for 7 h then 2ºC until 24 h post mortem). Carcass pH and temperature were measured at 0, 2, 5 and 24 h post slaughter in the M. *longissimus lumborum*. Lamb carcasses were examined for total viable and *Enterobacteriaceae* counts as follows: just after dressing and 24 h post mortem, samples were taken from the leg and breast area of left side of each carcass using the excision technique to microbiological testing. A 5 cm^2 sample from each site was aseptically removed using a sterile scalpel and forceps, and both pieces from one carcass were transferred into a labelled, sterile stomacher bag for the microbiological analysis.

Post mortem temperature treatment had not effect on M. *longissimus lumborum* pH immediately after slaughter (0 hours) or at 2 hours post mortem ($p>0.05$). However, a significant effect ($p<0.05$) of post mortem treatment on carcass temperature post mortem was found. As expected, carcasses chilled at -20ºC shown the fastest rate of temperature fall, from 31.2ºC to -0.1ºC at 5 h, whereas conventionally and slow chilled carcasses reached internal temperatures of 3.8ºC and 11.1ºC, respectively. On the other hand, pH values at 5 h post mortem showed significant effect of chilling treatment ($p>0.05$), with ultra-rapid chilled carcasses having a higher pH than the other two treatments.

The total viable counts on lamb carcasses before and after post mortem temperature treatment were satisfactory according to Regulation (EU) 2073/2005. Regarding temperature treatment effect, at 24 hours post mortem ultra-rapid carcasses had significantly ($p<0.001$) lower total viable counts than conventional and slow chilled carcasses. The *Enterobacteriaceae* counts measured just after dressing and after 24 hours post mortem, were dependent on temperature treatment. Ultra-rapid and conventional treatment showed a decrease between samples taken just after dressing and 24 hours post mortem, whereas *Enterobacteriaceae* counts increased from 0 to 24 hours post mortem in slow temperature treatment. Counts after 24 hours post mortem in ultra-rapid and conventional chilling treatments were satisfactory according to Regulation (EU) 2073/2005, whereas slow temperature treatment showed counts above that limits.

Taking into account microbiological analysis results, conventional and ultra-rapid chilling treatments should be chosen, because of the higher *Enterobacteriaceae* counts obtained in slow chilled carcases.

Keywords: carcass refrigeration; suckling lamb, microbiological contamination.

Effect of storage temperature on survival of *Listeria monocytogenes* inoculated on dry cured iberian meat products

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Dry-cured meat products are considered shelf-stable products due to their salt content and low water activity. However, nowadays, these meat products commercially distributed as sliced ready-to-eat products and post-processing manipulation, such as slicing and packaging, can enable cross-contamination and serve as a vector for the spread of pathogenic bacteria, such as *Listeria monocytogenes*. It is noteworthy that this microorganism can persist and grow at high and low pH values, at low water activity and at refrigeration temperatures. Therefore, this study was undertaken to measure the survival and death of *L. monocytogenes* from a high level inoculum in dry cured meat products as affected by storage temperature.

To carry out the study, different commercial dry cured Iberian meat products were used: dry cured ham (pH 5.93, a_w 0.920), dry cured loin (pH 5.99, a_w 0.885) and two dry cured sausages “salchichón” (pH 5.03, a_w 0.828) and “chorizo” (pH 4.99, a_w 0.813). The dry-cured meat products were sliced and inoculated with a *L. monocytogenes* culture to obtain counts that reached 10^4 cfu/g in the products. Then, meat products were vacuum-packed and stored at 4, 8 and 12ºC for up to 365 days. Microbiological analyses (enumeration and detection of *L. monocytogenes*) were performed on two packs taken from each meat product at selected times (0, 90, 180 and 365 days). The experiment involved two complete replications each using a different batch of each dry cured Iberian meat products. Death rates were determined as the slopes of the lines fitted to the linear part of the curves (days 0–90 for all storage temperatures; rapid initial decrease) by simple linear regression. In addition, the lines were fitted to the pathogen death curves for days 180–365, as a secondary death phase at a lower rate was observed.

During storage at 4, 8 and 12ºC, the counts of *L. monocytogenes* decreased in the four dry cured Iberian meat products which may indicate the physico-chemical characteristics of products evaluated, such as low a_w, low pH, and the inclusion of other antimicrobial ingredients in the product (sodium chloride, sodium nitrite, sodium ascorbate and natural spices) plus the storage conditions (anaerobiosis conditions) did not allow the survival of *L. monocytogenes*.

Differences were found among products in the survival of *L. monocytogenes*. At 4 ºC, dry cured loin showed the lower death rate and the sausages the highest. The higher pH of dry-cured loin in comparison with dry fermented sausages probably exerted a lower inactivation of *L. monocytogenes*. In fact, it has been reported that this pathogen was more sensitive to pH 4.0 than 4.5.

However, differences in survival of *L. monocytogenes* on the four dry cured meat products became less pronounced when the temperature increased. At 8 and 12ºC, the death rate of *L. monocytogenes* remained constant or decreased slightly in dry fermented sausages. Conversely, storage of dry cured loin and ham at 8 or 12ºC was more detrimental to *L. monocytogenes* than storage at 4ºC. The observed trends in dry cured loin and ham are in agreement with previous reports which pointed out less survival of *L. monocytogenes* at elevated temperatures (>12ºC). At these temperatures, an effect antimicrobial more pronounced of organic acids and others ingredients present in the formulation could be a reasonable explanation for higher death rate observed. However, further investigations about this effect will have to be done.

This study demonstrates that the dry cured Iberian meat products evaluated does not support the growth of *L. monocytogenes* during a long storage period. Besides, the results suggest an influence of the temperature and the type of dry cured meat products on the death rate of *L. monocytogenes.*

Keywords *L. monocytogenes,* dry cured meat products,

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Effect of toxic *Fusarium moniliforme* on some biochemical component of some date palm cultivars

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The study concentrated on the common associated fungi with date palm (Phoenix dactylifera L.) fruits with special reference to Fusarium isolates. Also, the ability of Fusarium isolates to produce different mycotoxins and their effect on specific biochemical components and quality aspects of date fruits. The pathogenic capability of twenty-six isolates of F. moniliforme (F. verticillioides) isolated from different date fruit cultivars were carried out in vitro. Mycotoxins were estimated by immune-affinity column. Biochemical changes as amino acids, water soluble vitamins and fat soluble vitamins were determined with HPLC in all treatments. The most isolated fungi were obtained from Hayani cv. which were belonging to 10 genera and 23 species from all tested cultivars. Fusarium moniliforme were the most prevalent and recorded the highest frequency percentages compared with other isolated Fusarium species. Fumonisin was the highest toxin in tested date cultivars followed by T-2 and Zearalenone. The high amount of mycotoxins was found in Hayani, Samani and Zaghlol cultivars, respectively. Artificial inoculation by F. moniliforme induced several biochemical changes. This toxic isolate caused a reduction in protein, total sugar, fat and fiber contents comparing with control and non-toxigenic isolate. Moreover, there were differences in the fractions of amino acid and Vitamins content of the tested isolates. Date palm fruits may be attacked by toxigenic Fusarium moniliforme isolates. This contamination led to reduction in quality of date fruits due to loss of their nutrients as the result of biochemical change.

Effectiveness of sodium hypochlorite washing for the reduction of *Listeria monocytogenes* in ready to eat lettuce leaves

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Fresh produce is not a common vehicle for foodborne diseases compared with other types of foods. However, various foodborne pathogenic microorganisms as *Listeria monocytogenes*, which has a psychrotrophic nature that allows its replication at refrigeration conditions, have been linked to cases of foodborne infection in ready-to-eat fresh fruits and vegetables.

On the other hand, chlorination is considered the main way to minimize the transmission of pathogens and is the most commonly used sanitizer method to treat fresh products. In fact, hypochlorites are powerful disinfectants with great oxidizing properties, which are active against a wide spectrum of organisms, and are non-toxic to humans at low concentrations. In this framework, the objective of this work was to study the effectiveness of washing lettuce leaves with sodium hypochlorite at different doses and times for the reduction of *L. monocytogenes* and natural microbiota.

L. monocytogenes (CECT 936) from Spanish Type Culture Collection (Valencia, Spain) was used to inoculate fresh-cut leaves of lettuce (*Lactuca sativa*) bought on a local supermarket. A final transfer of 10 mL of *L. monocytogenes* culture was added into 1 L of sterile deionized water, the fresh-cut lettuce was completely immersed in the inoculum solution and kept under constant agitation for 10 min at room temperature. This dipping inoculation model represents fresh or recent contamination of the product.

Chlorine solutions were made immediately before use by diluting different volumes (0.1 mL; 0.2 mL; 1 mL; 10 mL; 15 mL; 20 mL; 25 mL; 30 mL) of a concentrated solution of sodium hypochlorite in deionized water to achieve concentrations of 4, 8, 40, 400, 800 and 1200 ppm chlorine. Potable tap water was used as a control (0.7 mL residual chlorine). After that, two types of washing were assessed: a) dipping, where inoculated cut-lettuce pieces (25 g) were transferred from inoculation recipe and dipped into 1000 mL of each treatment solution at room temperature for 5, 15 and 30 minutes and b) shower, where cut-lettuce pieces (25 g) were rinsed during 1 minute approximately with sodium hypochlorite solutions. For all treatments three replicates for each analysis were made. Total mesophilic viable microorganisms and *L. monocytogenes* counts were performed according to standard methods.

The results showed that total viable biota and *L. monocytogenes* reduction were different depending of the washing method. However, these differences were not significant (P-value 0.0918 y 0.0552, respectively). In relation to dipped washing, time and dose of chlorination were studied. Results showed that time was not significant for total viable biota (P- value 0.1745) neither for *L. monocytogenes* (P-value 0.0617). Nevertheless, dose were significant (P-value 0.0000) in both cases. In relation to shower washing, the results for both populations of microorganims were similar: a dose from 0.7 to 40ppm allows for a reduction of 1 Log CFU/g and the rest of doses analysed, from 400 to 1200 ppm, reduce until 2 Log CFU/g. Within this framework, results highlight that domestic washing of fresh vegetables prior to its consumption constitutes a fundamental stage to guarantee the consumers safety, since it can reduce small loads that could be present in lettuce due to slight deviations in the control of the food chain.

Keywords Sodium hypochlorite, lettuce, washing effectiveness, *L. monocytogenes*

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Effects of preincubation conditions on growth kinetics and Enterotoxin production of *Staphylococcus aureus* in sliced cooked chicken breast

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Staphylococcus aureus is one of the most important food poisoning agents around the world, being reported as the main cause of foodborne diseases in many countries. The presence of *S. aureus* in ready-to-eat foods is often attributed to inadequate hygiene during handling. Specially, cooked chicken products have been involved in several outbreaks. In this study we evaluated the influence of preincubation conditions (temperature, pH and a_w) on the subsequent behaviour and Enterotoxin A production of *Staphylococcus aureus* in sliced cooked chicken breast, stored at different temperatures. Preincubation conditions were monitored in Brain Heart Infusion Broth (BHI) with an initial inoculum level of ~10^2 cfu/g. After reaching the early stationary phase *S. aureus* was inoculated in sliced cooked chicken breast with an initial concentration of 10^3 cfu/g, and incubated at 10, 15 and 20ºC during 30 days. The *S. aureus* growth was periodically evaluated by plate counting, incubating at 37ºC during 24-48 h. The logarithmic concentration vs time data were fitted to the Baranyi primary model by using the DMfit program (Institute of Food Research, Norwich, England), in order to estimate the kinetics parameters (lag phase and maximum growth rate or μ_{max}). Those conditions presenting a significant increase of the *S. aureus* in cooked chicken slices (> 10^5 cfu/g) were evaluated for Enterotoxin A production by means of an inmunoenzymatic test (MiniVidas, Biomèrieux). The results showed that *S. aureus* was able to reach the exponential growth phase in modified BHI (pH, a_w 0.99) in 8.75 h. Mean μ_{max} values were situated in 0.2 h^{-1}. The subsequent growth in sliced cooked chicken breast was remarkably slower, although at 20ºC an increase in *S. aureus* population was detected from 3.0 to 9.1 log cfu/g in approximately 140 h. This fact have led to Exterotoxin A production at 20ºC, at the end of the storage period. Besides, the estimated lag phase at 20ºC was 10.7 ± 4.6 h, and the with μ_{max} was 0.11 ± 0.009 h^{-1}.

On the contrary, cooked chicken samples stored at 15ºC and 10ºC presented a decrease in microbial population during the whole experimental period (15ºC: 1.1 log reduction in ~570 h; and 10ºC: 2.6 reduction in ~673 h). Also, an inactivation rate was predicted at 15 and 10ºC (-0.0025 and -0.003 h^{-1}, respectively). Other published studies reported that storage temperature is considered among other environmental factors, the most important to limit the growth of *S. aureus* (Lanciotti et al., 2001; Fujikawa and Morozumi, 2006). Valero et al. (2009) did not observe growth of *S. aureus* in Triptone Soja Broth at 8ºC, excepting at optimal of pH and a_w levels. Also, these authors reported an increase of the probability of growth at 13ºC in the pH range 6.0-7.0 (a_w > 0.95). In cooked chicken breast, Castillejo et al. (2002) demonstrated that *S. aureus* was unable to grow at 10ºC although the microbial levels were constant during the storage time. Only at temperatures above 13.5ºC, growth of *S. aureus* was observed.

These results highlight the importance of the maintenance of an appropriate storage temperature in order to inhibit *S. aureus* growth and Enterotoxin production. On the other hand, cell stress produced by preincubation at limiting temperature, pH and a_w conditions did not show a delay in the subsequent growth in sliced cooked chicken at 20ºC. Therefore, prevention of cross-contamination in ready-to-eat foods together with apropriate maintenance conditions before consumption could guarantee food safety against this microorganism.

Keywords *Staphylococcus aureus*; preincubation conditions; sliced cooked chicken breast; Enterotoxin production.

Efficacy of some antimicrobials on the microbial quality of "Armola" cheese stored at 4 °C

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Extending the shelf life of a cheese product could play an important economic role by reducing losses attributed to spoilage as well as introducing the products to new markets. Consumer's demand is currently driven towards foods that are natural but still safe and convenient to use. The search for more natural antimicrobials have led food scientist to investigate their inhibitory efficacy such as bacteriocins, antimicrobial enzymes, essential oil and dried fermented-based products.

"Armola" is one of the traditional Turkish cheeses and is produced mainly in Seferihisar, İzmir. The shelf life of this cheese is very short (3-4 days). The aim of this study was to extend the shelf life of "Armola" cheese by the use of Nisaplin®, Natamax®, Microgard™ 100 and Microgard™ 300 as antimicrobial agents. Nine different treatments were tested: Control sample with no antimicrobials added; samples with Nisaplin® (0.01–0.05%); samples with Natamax® (0.001–0.005%); samples with Microgard™ 100 (0.1–0.5%); and samples with Microgard™ 300 (0.1–0.5%). The cheese samples were stored at 4 °C for 7 days. The microbiological quality was studied by enumerating aerobic mesophilic bacteria, total coliform bacteria, lactobacilli, psychrotrophic bacteria, enterobacteria and molds-yeasts. The pH values of all cheese samples were also determined during storage.

Microbiological analyses showed that all antimicrobial treatments had different responses to microorganisms enumerated during storage. Irrespective of treatments, aerobic mesophilic bacteria reached levels $\geq 10^9$ CFU/g during storage. The spoilage microflora of cheese was dominated by lactobacilli. Antimicrobial agents tested presented a decrease on aerobic mesophilic bacteria of 0.72-1.03 log CFU g^{-1} compared to control at the end of storage. Analysis of "Armola" cheese samples revealed that the mean initial (day 0) population of yeasts was high (6.35 log CFU g^{-1}) and stayed constant up to 7 days of storage for Natamax-treated samples, but the yeast population in the rest of the samples increased to 8.1-8.30 log CFU g^{-1}. Although addition of higher concentrations of antimicrobials had no significant beneficial effects ($P > 0.05$) microbial spoilage of the studied cheese samples at the end of storage period the use of 0.05% Nisaplin® and 0.005% Natamax® showed significantly ($P < 0.05$) more inhibitory effect on enterobacteria & lactobacilli, and psychrotrophs, respectively. Moreover, the initial pH value was 4.61 and a small reduction in pH recorded throughout 3 days of storage, thereafter pH slightly increased on day 7.

The present study provides first insight into the inhibitory effects of the antimicrobials used in "Armola" cheese. The combinational use of tested antimicrobials needs to be investigated to demonstrate their synergistic and antagonistic effects on the microflora of "Armola" cheese.

Keywords: Antimicrobial activity; "Armola" cheese; natamax; nisaplin; microgard

Fermentation of must from black grapes: wine starter role in natural antioxidant power evolution

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Wine antioxidant activity varies considerably depending on grape cultivar, environmental factors in vineyard, and wine processing techniques. Only a few studies have evaluated the effect of winemaking techniques and yeasts on the antioxidant activity of wines. Among the different methodologies proposed to assess the antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) method can be recommended; 50% of the total radical scavenging activity in red wines is attributed to polymeric phenolic compounds.

The aim of this work was to study yeast strain variability to confer high antioxidant activity to red wine using 5 hybrid strains of *Saccharomyces cerevisiae*, obtained during the PRIN 2007 project: *"Wine strain improvement strategies to enhance red wine safety based on parietal adsorption activity"*.

Regarding the wine antioxidant activity, the 5 hybrids: RC029A-1DxRE078C-1C $^{(H1)}$, RC029B-1CxRE078C-1C$^{(H2)}$, RC029B-1CxRC039C-1C$^{(H3)}$, RC029B-1CxNA093B-1C$^{(H4)}$, and RE049B-1AxNA093B-1C$^{(H5)}$ were compared to the commercial strain *S. cerevisiae* F15 Zymaflore (Laffort Œnologie), selected for red wine production. Winemaking trials using black grapes from the Calabrian cultivars *Magliocco*, *Gaglioppo*, *Nerello calabrese* and *Malvasia nera* were performed. Grapes were destemmed, crushed and inoculated with 5% of 48-h precultures of each strain. Wines were analysed for: ethanol content, colour, colour intensity and tint, Folin-Ciocalteu index, % of DPPH inactivation, and total ascorbic acid equivalents (TAA) expressed as mg/mL.

Results demonstrate that yeast significantly modifies wine antioxidant activity in different ways according to the grape cultivar used. In detail, for the *Magliocco* cultivar the TAA content of the wine produced using the F15 Zymaflore strain is significantly higher than the TAA content of the wines produced using 4 out the 5 hybrids. Regarding the *Gaglioppo* cultivar, results vary between the two wineries involved in the study. In the first-one, no hybrid produces wines with a TAA content significantly different from wine produced using the F15 Zymaflore strain. In the second-one, 4 out the 5 wines produced using the hybrids exhibit a TAA content significantly lower than in wine produced using the F15 Zymaflore strain. For the *Nerello calabrese* cultivar the TAA content of the wines produced using the H3 and H4 strains are significantly higher than in the wine produced using F15 Zymaflore strain. For the *Malvasia nera* cultivar the TAA content of the wines produced using the H3 and H5 strains are significantly higher than in the wine produced using F15 Zymaflore strain; in contrast, the TAA content of the wine produced using the H2 strain is significantly lower than in the wine produced using F15 Zymaflore strain.

In conclusion, it seems advantageous to select yeast starter cultures for winemaking in function of their positive correlation with total antioxidant activity.

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Keywords DPPH; natural antioxidant power; red winemaking; wine yeast selection

Fruit juice spoilage by *Alicyclobacillus* and *Zygosaccharomyces rouxii*

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Alicyclobacillus ssp. are gram-positive and endospore forming microorganisms which can survive in low acid and high temperature conditions. Their spores can also grow at a pH of 2.5-6.0 and pasteurization temperature of 95°C for over 2 min. The *Alicyclobacillus*, in fruit juice produces guaicol and halophenols giving rise to unfavorable fruit juice flavor. The spoilage of apple, orange, grape, peach and other fruit juices by these bacteria species has been known. However, other troublesome microorganism in fruit juices and/or concentrates is *Zygosaccharomyces rouxii*. The *Z. rouxii* is xerotolerant and osmophilic yeast which has the growing ability below a_w value of 0.70. This yeast can cause spoilage of honey, fruit juice, fruit concentrate, syrups and dried fruits due to low water activity. Although, *Z. rouxii* frequently occurs in fruit concentrates, it has also been detected in fresh fruit juices. The objective of this review is to evaluate fruit juice spoilage by *Alicyclobacillus* ssp. and *Zygosaccharomyces rouxii*.

Keywords: *Alicyclobacillus*, *Z. rouxii*, Fruit juice.

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Fungal Contaminations of Some Foods, Their Mycotoxin Production and Effects of Antifungal Agents on These Fungi

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Fungal contamination of food may be one of the more pervasive and seldom recognized cause of disease. Fungi produce mycotoxins that are versatile and potent causes of disease. Mycotoxins can cause acute and chronic illnesses, induce cancer, and damage vital organs such as the liver kidney and brain. The purpose of this study was to isolate filamentous fungi from some foods and testing for mycotoxin production and finding the effects of some antifungal agents on to these isolates.

Twenty-one fungal isolates were obtained from some of supermarket foods such as cheese, yogurt and olive. The majority of the isolates identified belong to the genera *Penicillium sp.* and *Aspergillus sp.* and *a few to Geotrichum candidum, Humicola sp., Chrysosporium* and unidentified species. Taxonomical identification was based on the morphological characteristics of the fungi as observed under the microscope by using diagnostic literatures. These isolates were tested for mycotoxin production. Some of the fungal cultures were moderately toxigenic, while several additional cultures were slightly toxigenic. Effects of Nisin, Propionic acid, sodium propionate, sorbic acid, sodium nitrate, potassium nitrate, lysosyme on fungi isolates were tested. These are antifungal agents which can use food production prosess according to Turkish Food Codex.

Key words: mycotoxins, fungi, antifungal agent, *Penicillium sp*.

Identification and characterization of *Bacillus* strains by MALDI-TOF mass fingerprinting and genomic analysis

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The *Bacillus* genus includes species such as *Bacillus cereus*, *Bacillus licheniformis* or *Bacillus subtilis*. These microorganisms are widely considered spoilage and pathogenic bacteria in food products. The present work was aimed at applying Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (MALDI-TOF MS) to the classification of these species. In this study, a collection of 52 *Bacillus* strains either isolated from fresh or processed food or from culture type collection were considered and their mass spectra compiled. The resulting mass fingerprints were compared and characteristic peaks at the species- as well as genus- level were assigned. Peak lists with 10-20 peak masses were extracted and clustered by means of the SPECLUST application to elucidate phyloproteomic relationships among the collection species considered. MALDI-TOF MS fingerprinting proved to be a fast and accurate technique for identifying food-borne *Bacillus* strains with a view to ensuring food quality and safety. Furthermore, genetic analysis based on 16S rRNA nucleotide sequencing was carried out and the phylogenetic tree obtained was compared with phyloproteomic analysis. The results obtained in our study evidenced that MALDI-TOF, as compared to 16S rRNA sequencing, is a more powerful approach for the accurate classification of *B. cereus, B. subtilis* and *B. licheniformis*, also providing valuable information at the intra-specific level.

Keywords *Bacillus*, food safety, MALDI-TOF MS, phyloproteomics, phylogenetics, bacterial identification

Identification of a new lytic bacteriophage against *Lactococcus lactis* from natural whey starter cultures used in the production of the Italian buffalo Mozzarella cheese

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Eighteen samples of whey used as source of natural wild starter cultures for the manufacturing of the water buffalo Mozzarella cheese and implicated in fermentation failure during the coagulation phase of the milk were screened for bacteriophage infection. During this research seventy five starter cultures were isolated, purified and characterized from buffalo milk-whey in order to be used as phage hosts. One phage active against *Lactococcus lactis* was isolated and its phenotypic and genotypic features were investigated. The lytic-cycle of the bacteriophage has been demonstrated by the short time over lysis of the host in special media. The structural proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The genome was double-stranded linear DNA molecules with a *pac*-type system.

A Multiplex PCR protocol was used for detecting phages related to five different groups and the results showed DNA phage in whey belonging to bacteriophages P335 against *L. lactis lactis* and *L. delbruekii* and *S. thermophilus* phages.

This is the first report of the isolation and characterization of a virulent lytic *Lactococcus lactis* phage from buffalo whey starter cultures responsible of the unsuccessful milk curdling of the Italian D.O.P water buffalo mozzarella cheese

Keywords: lytic bacteriophage, lactococcus lactis, water buffalo mozzarella cheese, natural starter cultures

Impact of ecological variables associated to climate change on the growth of *Fusarium graminearun* and *F. culmorum* in wheat grain and on type B trichothecene production

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Fusarium head blight (FHB) or scab, is a serious disease of small grain cereals, caused by a complex of many different species in the genera *Fusarium* and *Microdochium*, and now under the influence of climate change. Climate change will have direct impacts on FHB in wheat crops, since weather factors greatly affect epidemics, the relative proportions of species of ear blight pathogens responsible and the production of mycotoxins. Of these fungi, two species, *Fusarium graminearum* (teleomorph, *Gibberella zeae*) and *Fusarium culmorum* (no known teleomorph) are of concern in the Spain because they produce a range of toxins that can contaminate small grain cereals, particularly type B-trichothecenes. Crop production in Spain varies by up to 20% from year to year, largely as a result of highly variable weather conditions. There is now concern that changes in the climate resulting from anthropogenic emissions of greenhouse gases may have negative effects on crop production in the Mediterranean region.

Type B-trichothecenes are distinguished by the presence of a carbonyl functional group at C8. The most prevalent B trichothecenes are deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON) and fusarenon X (Fus-X)

Strains of *F. graminearum* and *F culmorun* usually express one of three sets of trichothecene metabolites: (i) NIV and its acetylated derivatives (NIV chemotype), (ii) DON and 3-ACDON (3-AcDON chemotype), or (iii) DON and 15-AcDON (15-AcDON chemotype). *Fusarium* isolates that produce both NIV and DON (NIV/DON chemotype) have been described as "unknown" chemotypes. The 15-AcDON chemotype dominates in North America, central Europe and southern Russia, while 3-AcDON chemotype dominates in northern Europe and some parts of Asia, including China, Australia, and New Zealand.

In this work, a full factorial design was applied to study the potential impacts of climate change (influence of temperature and water activity (a_w)) and type of isolate on the growth of isolates of *Fusarium graminearum* and *Fusarium culmorum* and production of DON, NIV, 3-AcDON and 15-AcDON in wheat grain cultures from wheat grown in different climatic regions.

The results showed that a_w of grains, temperature and strains significantly affected growth and trichothecene B production by *F. culmorum* and *F. graminearun* in wheat grain.

Keywords predictive models; changing climate; trichothecenes; wheat

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Improving safety and storability of fresh escalopes packed in modified atmosphere (MAP) using Lactobacillus curvatus dispersions, sodium lactate and EDTA solutions

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The scope of this project was the improvement storage life, hygiene and safety of pork escalopes preserved in MAP, using bacteriocin-producing cultures of L. cruvatus (produces curvacin), or mild hurdles such as lactate and EDTA, which are labeled as acidity modifiers and do not belong to the chemical preservatives, and also do not alter organoleptic characteristics of raw meat. Experimental design is described below. The following solutions M to S were adsorbed into pork escalopes (100g/portion), using a vaccum tumbler, where

M: Distilled water

W: Solution of 0.9%NaCl

L: Dispersion of Lb.curvatus+0.9%NaCl

G: Solution of 5% sodium lactate+0.9%NaCl

E: Solution of 0.5%EDTA+0.9%NaCl

LG: Dispersion of Lb.curvatus+5% sodium lactate+0.9%NaCl

LE: Dispersion of Lb.curvatus+0.5%EDTA+0.9%NaCl

GE: Solution of 5% sodium lactate+0.5%EDTA+0.9%NaCl

LGE: Dispersion of Lb.curvatus+5% sodium lactate+0.5%EDTA+0.9%NaCl

S: Supernatant of Lb.curvatus fermentation broth(containing curvacin)

After the solutions were adsorbed, the samples were packed in MAP (80%N2, 20%CO2), and preserved at 4°C (x) 22d(days). Sensory and microbiological analysis took place at 1,4,9,15,22d. The following spoilage organisms were measured and used as spoilage indicators: Pseudomonas, Brochothrix thermosphacta, Enterobacteriacae, Enterococcus, Yeasts. Also, the following pathogens were analysed in *in vitro* and *in vivo* studies: Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Bacillus cereus, Clostridium perfingens, Staphylococcus aureus, Campylobacter jejuni. *In vitro* studies included the estimation of inhibition zones (well-diffusion assay) and the Minimum Inhibitory and Bacteriocidal Concentration(MIC/MBC). These pathogens were also inoculated (~4log cfu/gr) in escalopes previously treated with solutions M,W,L,G,E *(in vitro* studies). These samples were also packed in MAP, preserved at 4°C(x)20d, and pathogen populations were enumerated at 1d,5d,10d,15d.

The results showed that spoilage organisms grew well until 15d, then became stable or declined. Single/combined treatments lead to significant reductions up to 6log (15d or 22d). The inhibitory effect was clearest at 8d/9d, as the increase in microbial populations after 12d restricted the effectiveness of the solutions. At 22d all samples were organoleptically unacceptable. Enterobacteriacae were reduced by 6log at 22d by adding LGE solution. Also, GE and S solutions were effective in reducing their population. Maximum antimicrobial activity against Brochothrix was achieved with solution S (5,5log reduction at 22d), and solution L. Most effective combination against Enterococci was solution LE. Pseudomonas spp. were in high numbers, despite the presence of CO2, and reduced by 5log (15d) using solution S. Yeasts were less affected by any solution. Maximum anti-yeast activity occurred with solution GE. Overall storability was extended by 2-4 days (~20-30%) for samples containing L. curvatus, or curvasin solution, and especially for sample LGE. As for the pathogens, L. monocytogenes was significantly restricted by L. curvatus cells or curvacin. Similar inhibition was observed for B. cereus and C. perfingens. S.typhimurium and E. coli declined mostly due to lactate and EDTA. C. jejuni was significantly inhibited, not only by lactate and EDTA, but also by L. curvatus. L. curvatus also had a maximal inhibitory effect against S. aureus. The reduction in pathogens populations varied from 1-4log, depending on the microorganism and storage day, indicating a significant improvement of product safety.

Keywords Lactobacillus curvatus, curvasin, EDTA, sodium lactate, antimicrobial

In situ detection of *Arcobacter* cells in chicken samples by use of combined DVC-FISH method

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Species of the genera *Arcobacter* are emerging human pathogens and a standardized reference method of detection has not so far been proposed. Moreover, arcobacters can enter into a viable but non-culturable (VBNC) state, which is a problem for the detection by traditional cultural methods. We used DVC-FISH method, developed and set up by our research group, for the detection of these bacteria in commercial chicken samples since previous studies indicated that *Arcobacter* spp. are present on many poultry products. DVC-FISH is a technique that combines direct viable count (DVC) procedure with fluorescent *in situ* hybridization (FISH) method and allows specific enumeration and rapid discrimination of viable and non viable cells.

Portions of 10 grams of eighteen comercial chicken samples were mixed and homogenized with 90 ml of Arcobacter Broth medium suplemented with cefoperazone, anphotericin B and teicoplanin. Then, we analyzed them with and without a 24 hours period of enrichment. In both types of analysis, we incubated the samples with 0.3 µg/ml of ciprofloxacin, a DNA-gyrase inhibitor, during 6-7 hours. After the DVC procedure, FISH was performed by using the specific *Arcobacter* spp. probe ARC94 (5´TGCGCCACTTAGCTGACA3´) that targets 16S rRNA and a combination of three EUB338 probes used as a positive control for the Eubacteria domain.

The application of the DVC-FISH method allowed us to detect viable and no viable *Arcobacter* cells in 50% of the chicken samples analyzed. Specifically, we had positive results in two comercial samples detecting at least 10^6 cells/ml of potential pathogen *Arcobacter* cells. Moreover, by the enrichment period, we detected also very low concentrations of these bacteria in contamined samples.

Our results show that the DVC-FISH combination is an effective, rapid and culture-independent useful method to detect viable and non viable *Arcobacter* cells in chicken samples. We demonstrated the presence of potential infective *Arcobacter* cells in this type of samples and the possibility of indirect transmission of these bacteria by food.

Keywords *Arcobacter*; chicken; DVC; FISH

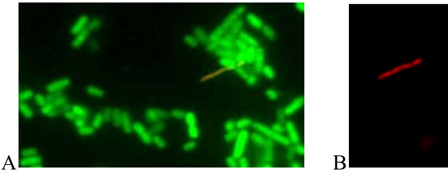
A  B

Figure 1. *Arcobacter* viable cell in one of the analized samples detected in enrichment period by DVC-FISH. A) EUB and ARC94 probe detection. B) ARC94 probe detection.

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Inactivation of some pathogens and conditional pathogens by freezing temperatures during cold storage

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Freezing halts the activities of spoilage microorganisms in and on foods and can preserve some microorganisms for long periods. The process of freezing is less effective in food preservation than are thermal techniques such as boiling because pathogens are more likely to be able to survive cold temperatures than hot temperatures. In fact, one of the problems relating to the use of freezing as a method of food preservation is the danger that pathogens deactivated (but not killed) by the process will once again become active when the frozen food thaws. A number of factors are involved in the selection of the best approach to the freezing of foods, including the temperature to be used, the rate at which freezing is to take place, and the actual method used to freeze the food.

The aim of the study was to determine the effect of minus 18 ^{o}C and minus 72 ^{o}C freezing temperatures on inactivation of some pathogens and conditional pathogens inoculated in ground turkey during storage under freezer for 1 and 2 years.

Methods.
Samples of ground turkey were inoculated with *Rhodotorula rubra, Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Escherichia coli*: the tested cultures, pre-cultivated on slant media, were suspended individually in 10 ml of peptone-buffered solution and a prepared suspension was added to 200 g of ground turkey, mixed well to evenly distribute the culture cells in the sample and 10 g of each sample were packaged in hermetic bags. The initial number of all tested cells was estimated by plate count method and the bags were frozen to minus 18 ^{o}C and minus 72 ^{o}C. The inactivation of individual pathogen or conditional pathogen was estimated by an average of 3 plate counts after freezer storage for 1 and 2 years.

Results.
The number of *B. cereus* was affected by freezing temperatures mostly among all investigated microorganisms: the initial number of this pathogen during the period of frozen storage for a year at minus 18 ^{o}C and minus 72 ^{o}C decreased respectively by 99.98 % and 97.8 %, while 0.7 % and 0.8 % CFU/g of *B. cereus* remained viable after storage under freezer for 2 years. The decrease of the number of other pathogens – *S. typhimurium*, S. *aureus* and *L. monocytogenes* – in the ground turkey samples was found to be lesss pronounced than *B. cereus*. The number of *S. typhimurium* during the period of cold storage at minus 18 ^{o}C and minus 72 ^{o}C for a year decreased respectively by 3 and 1.6 times, but there still remained respectively 6.9 % and 35 % CFU/g of *S. typhimurium* during further storage for 2 years. The number of *S. aureus* after frozen storage at minus 18 ^{o}C for a year decreased 10 times; during further storage for 2 years it still decreased by 95.4 %. The freezing and storage temperature at minus 72 ^{o}C less affected the viability of *S. aureus* culture: 85.7 % and 30.7 % of viable CFU/g survived after frozen storage for 1 and 2 years respectively. *L. monocytogenes* test culture in frozen meat also tended to be destroyed during cold storage. After frozen storage for 1 and 2 years at minus 18 ^{o}C 54.5 % and 0.3 % of viable CFU/g survived, while 100 % and 35.9 % CFU/g respectively was destroyed during frozen storage at minus 72 ^{o}C. The CFU/g of *E. coli* culture were found to be not resistant to freezing and storage at minus 18 ^{o}C: the number of *E. coli* decreased by 12 times after frozen storage for a year and there still remained 100 CFU/g after 2 years. The number of *E. coli* during the frozen storage for 1 and 2 years at minus 72 ^{o}C fell down to a smaller extent, i.e. respectively by 1.1 and 87 times. *R. rubra* test culture in frozen ground turkey also tended to be destroyed during frozen storage. There was an 82.7 % reduction in the number of yeast culture after frozen storage at minus 18 ^{o}C, another 83 % of CFU/g of tested culture were killed after 2 years. The frozen storage at minus 72 ^{o}C for 1 and 2 years resulted in 24 % and 58.6 % reduction in the number of *R. rubra* culture.

Conclusions.
All studied microorganisms were susceptible to freezing at minus 18 ^{o}C and minus 72 ^{o}C because the count of all microorganisms decreased after storage under freezer but was not completely inactivated. Freezing and frozen storage at minus 18 ^{o}C more affected the viability of all microorganims than freezing and frozen storage at minus 72 ^{o}C. All tested microorganisms retained viability even during a long-term frozen storage. The number of investigated microorganisms survived 5 – 120 more times in samples frozen at 72 ^{o}C than in samples frozen at 18 ^{o}C .

Keywords pathogens; conditional pathogen; freezing; inactivation; minus 18 ^{o}C; minus 72 ^{o}C; ground turkey

Influence of abiotic factors on Ochratoxin A production by *Aspergillus niger* on maize kernels

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The mycotoxin contamination of agricultural products is a serious health hazard with an increasing attention worldwide. Because of the toxic potential and high incidence of ochratoxin A (OTA) in cereals, one of the first maximum levels for OTA regulated by the EU in 2002 was specifically for raw cereal kernels and cereal-derived products.

Aspergillus niger is an OTA-producing species commonly isolated from a great variety of food commodities. Due to the high incidence of this specie in maize, it could be a source of OTA in this cereal.

Temperature and water activity (a_w) are the key abiotic factors that influence fungal growth and mycotoxin production. As there are only a few studies on the impact of these parameters in natural substrates, the aim of this study was to determine the effects of temperature and water activity (a_w) on OTA production by *A. niger* on maize kernels. Our results show that maize supports both growth and OTA production by *A. niger*. The studied strain was able to produce OTA in maize kernels in only five days at different temperatures and water availabilities conditions. *A. niger* produced OTA from 15 to 40 ºC, and the highest OTA level was recorded at 15 ºC. The concentration of OTA produced at 0.92 a_w was significantly lower than those at 0.96 a_w and 0.98 a_w. These results will lead to a better understanding of the role of *A. niger* in the OTA contamination of maize.

Keywords abiotic factors; *A. niger*; maize kernels; ochratoxin A

Inhibition of *Campylobacter* by Tunisian chicken cæcum isolates of *Lactobacillus salivarius*

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Campylobacter is the commonest bacterial cause of infective gastroenteritis in the developed world, and frequently causes food borne illness. *Campylobacter* species are common bacterial pathogens that cause gastroenteritis in humans, both in industrialized and developing countries. Within the genus *Campylobacter*, *Campylobacter jejuni* and *Campylobacter coli* are the predominant species isolated from poultry and are the most common species associated with human campylobacteriosis. In order to find new ways to reduce *Campylobacter* in chicken products, 150 lactic acid bacteria (LAB) isolates from cæcum were screened for inhibitory activity against *C. jejuni* and *C. coli*.

The antimicrobial activity of cell-free supernatant and partially purified bacteriocin was determined by well diffusion method. Three LAB isolated from different caecum exhibited a clear anti-*Campylobacter* activity. These isolates were identified as *Lactobacillus salivarius ONIRIS 51*, *L. salivarius MMS122* and *L. salivarius MMS151*; they are active against *C. jejuni* and *C. coli*. It was also shown by PFGE that the strain *L. salivarius* ONIRIS 51 used in this study was different from the strain *L. salivarius* NRRL B-30514 producing bacteriocin OR7 (Stern et al. 2006).

The activity was not affected by catalase. Complete inactivation or significant reduction in activity was observed after treatment with proteinase K, trypsin and α-chymotrypsin. The active peptide from the cell-free supernatant of strain *L. salivarius* Oniris 51 was then purified in three steps. First, the peptide was precipitated with 80% saturated ammonium sulphate. After centrifugation, the pellet was resuspended in ammonium acetate and loaded on a SepPack C_{18} cartridge. This cartridge was washed and the bacteriocin eluted with isopropanol. After drying, this active fraction was used for final purification by reversed phase HPLC on C_{18} column. Its amino acid composition and molecular mass were determined. Molecular mass of this bacteriocin was 5382,23 Da. New bacteriocin Oniris 51 appears potentially very useful to reduce *Campylobacter* in poultry prior to processing.

Key words: *Campylobacter*, lactic acid bacteria, bacteriocin, chicken cæcum

Isolation and Identification of Lactic Acid Bacteria, Partially Purified Bacteriocin and Molecular Characterization Using 16S rRNA from Cacao Fermentation in West Sumatra, Indonesia

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West Sumatra Indonesia has a center plantation of *Theobroma cacao Linn* for Hybride clone of Forastero. The hybride clone of cacao performed good quality of fruits less fat and high antioxidant content. Cacao fermentation is very important to produce specific cacao aroma for chocolate Industries. Cacao pulps have been used for bean fermentation and isolating lactic acid bacteria during 2 to 3 days fermentation. This investigation aim to isolate and identification of one potential lactic acid bacteria, bacteriocin and partially purified bacteriocin also molecular characterization using 16S rRNA. Lactic acid bacteria were isolate in de Man Rogosa agar (MRS agar) medium, morphology and Gram stain were identified. Antimicrobial activities were determined by using well diffusion method as indicator strain used was, *E.coli* , *Staphylococcus aureus and Salmonella typhimurium* (Univ.of Andalas collection) with several antibiotics as control. Growth and bacteriocin production were determined at various pH (2, 3, 4, 5, 6,7 and 8) and temperature range from (35, 40, 45, 50, and 55) ^{o}C. The incubation time varied from (6, 12, 18,24,30,36 and 48) h. partially purifies bacteriocin precipitated with 80% ammonium sulphate saturation and further purification by DEAE-Cellulose at pH 7.0. The molecular weight determination SDS-PAGES were done by polyacrylamide gel electrophoresis. DNA sequence of selected bacteria, were determined using gene of 16S rRNA with universal primer of 27F: AGAGTTTGATCMTGGCTAG and 1525 R:AAGGAGGTGWTCCARCC. DNA characterization using 16S rRNA and amplified PCR products obtains fragment DNA of 1500 bp. The highest total colonies of lactic acid bacteria found in cacao hybrid, as many as 1.5 x 10^9 cfu/mL, the isolate was gram positive and cocus. In this experiment we were focus on potential lactic acid bacteria namely H4 (Hybrid 4) for further studied. The molecular weight of bacteriocin was about 10 kDa, maximum bacteriocin production was 35 ^{o}C at pH 3. Molecular DNA sequence and BLAST analysis conform as 98 % homology with *Pediococcusacidilactici* GL 20. More interesting we found lactic acid bacteria in high total colony after processing and roasting chocolate paste. This study revealed the possibility of using bacteriocin as food preservative and the *Pediococcusacidilactici* GL 20 as probiotic. There is no report so far concerning this topic and potential bacteriocin of lactic acid bacteria in cacao hybrid.

Key Words: Lactic acid bacteria, bacteriocin, *Pediococcusacidilactic*, *Theobroma cacao Linn*

Mehiawah (a Gulf fish sauce) as a Potential Source of Probiotics

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Indigenous fermented food products are considered by many nations as cultural and national heritages. Mehiawah is one of the most popular traditional fermented fish sauce in the basin of the Gulf which includes the Gulf Cooperative Counties (GCC: Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and United Arab Emirates) in addition to Iran.

Mehiawah is still largely produced as a home-made commodity. The main raw material is dried Indian sardines (*Sardinella* gibbosa). Spices, ground wheat/grains and salt are added. Fermentation takes place in the sun for a week. This bio-preservation method produces a sauce-like suspension which is traditionally eaten as a spread on locally baked thin bread.

Previous work from our laboratory and others showed that the fermentation of fish is accomplished by the naturally occurring lactic acid bacteria (LAB) in the presence of a source of carbohydrate. The safety aspects of locally produced Mehiawah was studied by our laboratory. We have previously also shown that, when Mehiawah is prepared under adequate hygienic condition, the LAB outgrow a very potential pathogen *Staphylococcus aureus* and reach a large count (10^7-10^8 cfu/ml). Several strains of LAB have been isolated from Mehiawah and identified by biochemical tests, including API 50 CH. These strains included *lactobacillus acidophilis* (jenseenini), *lactobacillus casei*, *lactobacillus* delbrueckii, *lactobacillus delbruecki* supsp*. Lactis*, and *lactobacillus delbruecki* subsp *Bulgaricus.* Currently, characterization of many of these bacteriocin producers and their products is under investigation. The species composition of LAB in Mehiawah and their count are indications to establish/consider Mehiawah as a potential functional food.

Keyword: fish sauce, LAB, Mehiawah, probiotics,

Microbial load reduction by UV-C application in aubergines

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Vegetables are usually treated with chlorinated water after washing to reduce microbial load prior to packaging. Chlorination is considered the main way to minimize the transmission of pathogens from infested plant produce or debris to non-infested surfaces such as those mechanically injured during harvesting, transportation or processing, wounds, or the natural plant surface openings [1, 2].

The use of non-ionizing, germicidal and artificial ultraviolet light (UV) at a wavelength of 190–280nm (UV–C) could be effective for surface decontamination of fresh products. Treatment with ultraviolet energy offers several advantages to food processors as it does not leave any residue, does not have legal restrictions, is easy to use and lethal to most types of microorganisms [3].

The current work studies the effect of several UV-C doses in decay of whole aubergines (*Solanum melongena*). The fruits were collected from greenhouses in Almeria. The doses tested were 1.04, 2.07 and 4.14 kJ/m2, measured previously with a radiometer equipped with a sensor (220 nm-280 nm). The best results to reduce incidence of decay (due to Botrytis cinerea and other indicator microorganisms) were obtained by the two higher doses, both after 7 days at 10 ° C (shelf life) and after complementary 3 days at 20 ° C (market period). The worst result was obtained with the control fruits (no radiation). Another very important postharvest indicator was the evolution of firmness. In general, radiated aubergines preserve firmness throughout the experiment, without significant differences between the treatments. However, the control treatment suffered a very important decrease in this parameter regarding the initial values.

Key words. sanitization, *Botrytis cinerea,* UV-C, ultraviolet, decay.

Acknowledgments
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Microbiological characteristics of Traditional Turkish Fermented Sucuk and identification of their yeast flora

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Traditional fermented sucuk consumed by Turkish people is one of popular meat products in Turkey. The sucuk is produced with meat (beef, water buffalo meat), fat (beef fat and sheep tail fat), salt, sugar, nitrite, nitrate and spices (black pepper, red pepper, cumin and garlic). Additionally, it is produced with either spontaneous fermentation or commercial starter culture. Generally commercial international starter cultures used in sausage fermentation are also used in the sucuk fermentation. Therefore, lactic acid bacteria (*Lactobacilli*, *Leuconostocs* and/or *Pediococci*), calatase positive cocci such as *Staphylococcus xylosus* and *S. carnosus* and *Kocuria varians* (*Micrococcus varians*) and yeast (*Debaryomyces hansenii*) may be important starter cultures used in the sucuk fermentation. However, traditional Turkish fermented sucuk have distinctive microflora different from commercial starter cultures. So far, lactic acid bacteria and catalase positive cocci have isolated from traditional Turkish fermented sucuk. But, the study on determination of yeasts is lacking in the scientific literatures. Thus, the aim of the study was to isolate and identify of yeasts from traditional Turkish fermented sucuk. Yeasts which have proteolytic and lipolytic, can also affect taste and aroma of fermented meat products. In this study, thirty five different samples from the traditional Turkish fermented sucuk were analyzed for microbiological properties including total mesophilic aerobic bacteria, total coliform, *Micrococcaceae*, yeasts and LAB counts. First of all, yeast counts of the sucuk samples were determined. Then, yeasts from the sucuk samples were purified on Dichloran Rose Bengal Chloramphenicol Agar (DRBC). Two hundred fifty isolates obtained were identified using API ID 32 C kits. Consequently, total different 17 yeasts were identified as *Candida zeylanoides* (38%), *C. famata* (30%), *C. lipolytica* (8%), *C. colliculosa* (6%), *C. parapsilopsis* (4%), *Geotrichum capitatum* (3%), *C. glabrata* (3%), *Cryptococcus albidus* (%2), *Rhodotorula mucilaginosa* (1%), *C. pelliculosa* (1%), *C. rugosa* (1%), *C. kefyr* (1%), *C. lusitaniae* (1%), *C. pulcherrima* (<1%), *C. krusei* (<1%), *C. globosa* (<1%) and *C. tropicalis* (<1%).

Keywords:Traditional Turkish fermented sucuk, microbiological characteristics, yeast identification

Microbiological characteristics of traditionally Turkish fermented European cranberrybush (*Viburnum opulus* L.) fruits

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Fermented vegetables and fruits are one of the most popular foods consumed in throughout the world. The objectives of this research were to determine composition of lactic acid bacteria (LAB) and some other microorganisms in fermented European cranberrybush fruits that have high acidity or low pH value. For this purpose, fresh European cranberrybush fruits were obtained from ten different regions of Kayseri and its surroundings in Turkey, and then these fruit samples were traditionally fermented at approximately 18 ^{o}C for 4 months. Also, ten traditionally fermented European cranberrybush fruits were purchased from ten different regions of Kayseri and its surroundings. Then the total twenty samples were analyzed for some microbiological and physicochemical characteristics. Microbiologic analysis including LAB, yeast-mould, total mesophilic aerobic bacteria, *Alicyclobacillus* spp. *Staphylococcus aureus,* total coliform and *Escherichia coli* counts were conducted in fermented European cranberrybush (*Viburnum opulus* L.). The pH values of the fermented samples were between 3.36 and 4.44. LAB counts of the samples changed from 3.92 to 8.30 log cfu/ml in the fermented fruits however LAB counts were < 6.07 (log cfu/ml) in only four samples. On the other hand, TMAB and mould-yeast counts of the samples were determined from 5.73 to 8.97 and from 5.00 to 7.58 log cfu/ml, respectively. However, *Alicyclobacillus* spp, *Staphylococcus aureus,* total coliform and *Escherichia coli* were not determined in any samples. In conclusion, it might be presumed that fermented European cranberrybush fruits may safely be consumed due to their tested characteristics.

Keywords: European cranberrybush fruits; *Viburnum opulus* L.; Turkish fermented fruit; LAB and microbiological characteristics.

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Microbiological Profile and Chemical Changes of Coconut Palm Sap (*Cocos Nucifera*) During Natural Fermentation

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Palm sap is widely consumed in Asia and Africa. Natural fermentation of coconut palm sap involves the breakdown of carbohydrate materials under anaerobic condition with the reaction of a variety of microorganisms and enzymes present. The purpose of this study was to evaluate the changes of ethanol content together with the microbiological profile in the coconut sap during fermentation. Coconut palm sap (*Cocos nucifera*) was collected from the local manufacturers. Samples were fermented at 30^{0}C for 63 days. Chemical and microbiological analysis were carried out at every seven (7) days interval, starting from day 0 (fresh tapping) until day 63. Ethanol contents were analysed using Headspace Gas Chromatography Mass Spectrometer (GC-MS). The result showed that the initial concentration of ethanol content in fresh coconut sap was 0.17%. Drastic increase in the ethanol content for sample was recorded during the first seven days of fermentation, before beginning to slightly drop after day 21 of fermentation. Overall, the range of ethanol content in coconut palm sap was from 0.17% at day 0 to 7.42% at the end of the fermentation period. The changes of other volatile compounds were also observed during fermentation. The pH value decreased from the initial pH of 6.16 in fresh coconut sap and dropped to 3.16 in the fermented coconut sap. Identification of microbial species was done using the Analytical Profiling Identification system, API. *Saccharomyces cerevisiae* dominated the yeasts species during the fermentation while other yeast species including *Cryptococcus humicola, Cryptococcous laurentii, Candida famata*, and *Stephanoascus ciferrii* were also identified. *Lactobacillus platarum* and *L.brevis* were the dominant lactic acid bacteria, whilst the strain of acetic acid bacteria known as *Acetobacter* and *Gluconobacter* were also identified.

Keywords; coconut palm sap, microbiological profile, ethanol, fermentation, yeasts.

Microbiological profile of maize and rye flours and mother-dough throughout time

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Besides water, maize and rye flours are the main constituents of *broa* – a sourdough bread manufactured following traditional protocol at farm level in Portugal. Additionally, mother-dough – *i.e*. a piece of leavened dough kept aside from batch to batch, constitutes the only starter culture present throughout breadmaking, and thus responsible for fermentation; nowadays mother-dough is frequently kept in the refrigerator. Sample of maize and rye flours, as well as mother-dough were assayed for their microbiological profiles throughout storage time, to characterize variation in the viability of microorganisms: total viable counts, as well as viable yeasts, molds, Gram$^-$ rods, Gram$^+$ rods (endospore-forming and nonsporing), and Gram$^+$ cocci (catalase$^+$ and catalase$^-$). In general, all microbial groups exhibited a good resistance to storage, so use of mother-dough appears effective in this form of breadmaking.

Microbiological, Physicochemical and Sensory Evaluation of Fermented milk from blends of tigernut , soy and groundnut milk

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The study was aimed at producing an acceptable yoghurt analogue from blends of tigernut- , soy- and groundnut milk with a view to producing a beverage that will combine the health benefits of the vegetable sources of protein and a novel product that will be affordable to all in the developing countries.

Following production of milk from tigernut, soybeans and groundnut, different blends (1:1:1; 1:2:1; 1:1:2; and 2:1:1) respectively were formulated and inoculated with 2% (w/v) commercial starter culture containing *Lactobacillus bulgaricus; L. acidophilus* and *Streptococcus thermophilus*. Samples were incubated at 45 ^oC for 6*h* and subsequently stored at refrigeration temperature (5± 2 ^oC) for 21 days. After 6hrs, and on a weekly basis, samples were subjected to microbiological analysis, physico-chemical and sensory evaluation.

Results show that the Lactic acid bacteria (LAB) counts which were initially 3.6×10^5– 9.9×10^6 were 8.4×10^5 – 10.0×10^6 cfu/ml in all the refrigerated samples on 7th day of storage. LABs were still viable in the samples even after 14 days of storage (1.5×10^3 – 3.3×10^4) cfu/ml. pH values of the milk blends decreased from the initial range of 6.12 - 6.67 to 4.71 – 4.80 and the TTA increased from 0.11 -0.16 to 0.56 – 0.83% after 6 hrs of incubation. These values changed to 4.58 – 4.73 and 0.58 – 0.94% for pH and TTA, respectively after 21 days of storage. Responses elicited from tasters reveal that there was no significant difference in the mouthfeel of the prepared fermented vegetable milk samples and the commercial sample ($p>0.05$). In terms of sweetness, the tasters found sample D (TSG, 2:1:1) to be comparable to commercial yoghurt sample ($p>0.05$).

It can be concluded that an acceptable yoghurt analogue could be produced from combinations of tigernut, soybeans and groundnut milk.

Keywords: Tigernut, soybeans, groundnut, yoghurt analogue, refrigerated storage

Molecular Analysis of Bacterial Microbiota Associated with Two Oysters (*Crassostrea gigas and Crassostrea corteziensis*) at Different Sites

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Resident microbiota presumably plays an essential role against pathogen colonization and maintains oyster health. In this study, bacterial microbiota was assayed based on 16S rDNA genes analyses, restriction fragment length polymorphism, and temperature gradient gel electrophoresis for different growth stage in *Crassostrea gigas* and *C. corteziensis*. Comparison of the microbiota profiles were performed using Dice's similarity coefficient (C_s) post-larvae obtained from the hatchery, which were subsequently transported for grow-out at two sites. At the post-larvae stage, microbiota composition on both oysters was similar ($C_s > 90\%$) in most specimens. The bacterial community associated with each species of oyster, especially *C. corteziensis*, at a cultivation facility were very similar, if not identical, and was influenced by the environment in which the oysters grew ($C_s > 70\%$). Profiles of temperature gradient gel electrophoresis revealed low bacterial diversity; the principal belonged to β-Proteobacteria, Firmicutes, and Spirochaetes. In the post-larvae, some bacteria (especially *Burkholderia*) colonize oysters and remain strongly associated throughout the grow-out phase and were not altered by the site of cultivation. However, bacterial composition may partly change when oysters were placed at the growing site.

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Molecular and physiological traits of *Hanseniaspora* spp strains towards application in winemaking

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The use of starter cultures of active dry yeasts of *Saccharomyces cerevisiae* is a common practice in most wine-producing regions since the middle of the 20th century to insure reliable fermentation and to achieve an adequate final quality. Non-*Saccharomyces* wine yeast species have been traditionally associated with the production of off-flavours compounds that depreciate wine quality. Recently, the introduction of mixed starters together with *S. cerevisiae* has gained particular interest, since it has been suggested that these yeasts could impart some beneficial organoleptic characters.

Looking for non-*Saccharomyces* strains that could have oenological potential for enhancing wine characteristics, thirty-three isolates of *Hanseniaspora* spp*.* were used in this study. The yeast strains are indigenous strains from Douro Demarcated Region and were isolated from grapes, must or wines. Also certified yeast strains obtained from the Spanish Type Culture Collection (CECT) were studied in terms of phenotypic traits and used as reference for molecular characterization. The strains include the species more frequently associated with wine ecosystem: *Hanseniaspora uvarum* (CECT1444), *Hanseniaspora vineae* (CECT1471), *Hanseniaspora osmophila* (CECT11206), *Hanseniaspora guilliermondii* (CECT11027), *Hanseniaspora occidentalis* (CECT11341).

All isolates were screened for interesting oenological traits (β-glycosidase, protease and sulphite-reductase activities and resistance to cerulenine, 5,5,5-trifluoro-dl-leucine (TFL), ethanol and SO_2). β-glycosidase activity as well as cerulenine, TFL and sulphite resistance, were the least discriminatory traits. On the opposite a great variability was found regarding protease and sulphite reductase activity and ethanol resistance.

Discrimination of species within the species of *Hanseniaspora* spp*.* was demonstrated by the use of PCR-fingerprinting with the microsatellite oligonucleotide primer $(GTG)_5$ that additionally enabled discrimination between the strains tested. The genetic similarity of individual isolates from the different origins was shown to be comparable, with no correlation between the genotypic affiliations and origin. It appears that musts and wines from a grape variety and/or from a specific location are not preferentially colonized with a distinct group of strains. In the other hand, it was interesting to note a high genotypic and phenotypic variability within strains isolated from the same sample at different stages of fermentation.

The results obtained clearly revealed the potential of selected strains of these non-*Saccharomyces* wine yeasts for being applied usefully in wine-making for diversification of wine styles

Keywords Wine yeasts; Non-*Saccharomyces*; enzymatic activities; PCR-Fingerprinting

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Molecular Characterization of ochratoxigenic fungi associated with raisins

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Fifty raisins samples were collected from different shops and markets in Jeddah city, Saudi Arabia, raisins were analyzed mycologically for present of fungi on two cultural media (MEA and DRBC). *Aspergillus* spp. counts obtained with DRBC culture medium were higher than the mean values obtained with the MEA culture medium, and the black fruit samples showed high contamination in comparison with white fruit samples. Six species of *Aspergillus* spp. isolated from MEA and DRBC were identified and most frequent isolated species were *Aspergillus carbonarius* and *A. niger*. The occurrences and abundance of *Aspergillus carbonarius* and *A. niger* were (38% and 31.4%) and (18% and 11.59%), respectively.The mycotoxin analysis showed that OTA was found in 70% of the raisin samples from black and white raisins samples OTA was detected in 42 and 28% of the total samples. *Aspergillus carbonarius* (31.40%), *A. niger* (11.59) and *A. ochraceus* (1.83%) were potential producers for OTA among the mycobiota isolated in this study. Fourteen isolates of *Aspergillus carbonarius* (73.7% of total *A. carbonarius* isolates) and two isolates of *A. niger* (22.2% of *A. niger* isolates) were ochratoxingenic isolates. Using RAPD-PCR technique showed no correlation between Ochratoxin potential of studied strains and clustering analysis of DNA patterns. Also, for the first time, multiplex PCR assay was used for detecting the contamination of raisin samples with ochratoxin A directly without isolating the producers. This methodology successfully allowed the detection of amplification products from naturally occurring fungi in raisins. The method described in this study represents a much quicker and more reliable detection procedure for the presence of ochratoxigenic fungi found in raisin.

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Multivariate analysis to identify yeast strains with technological applications in table olive processing

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This survey uses a multivariate classification analysis to detect yeast strains with interesting biochemical activities for the processing of table olives among a collection of 32 isolates belonging to different 16 yeast species. Lipase, esterase and β-glucosidase activities (desirable characteristics) were quantitatively evaluated in both extracellular and cellular fraction for all isolates. Three different types of media (1- laboratory medium, 2- laboratory medium + arabic gum + refined olive oil, 3- basal medium + arabic gum + refined olive oil) were used to assess both lipase and esterase activities. In the case of β-glucosidase activity was only used the laboratory medium. The study of the quantitative data by principal component analysis led to the identification of several *Wickerhamomyces anomalus* and *Candida boidinii* isolates with promising characteristics (the best global activity levels), clearly differentiated of the rest of yeasts. Results obtained in this work open new alternatives to the use of this methodology in the study, classification and selection of the most promising yeasts to be used as starters, alone or in combination with lactic acid bacteria, during table olive processing.

Keywords Table olives; principal component analysis; starters; technological characteristics; yeasts

Nitrogen availability of grape juice impacts yeast population dynamics during mixed culture fermentations

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Non-*Saccharomyces* yeasts are an ecologically and biochemically diverse group capable of altering the fermentation dynamics, composition and flavour of wine. In the last two decades, several groups have examined various non-*Saccharomyces* yeasts as potential adjuncts to *S. cerevisiae* to exploit their flavour complexing properties. A few dry yeast of single non-*Saccharomyces* or blends of two or three yeasts are now available in the market for commercial wine production. Despite the increasing interest in the industrial application of non-*Saccharomyces*, work is still needed for understanding their contribution and persistence during alcoholic fermentation.

In this study, the effect of initial nitrogen concentration of grape juice on the population dynamics during mixed culture fermentations with *Saccharomyces cerevisiae* and a selected strain of *Metschnikowia pulcherrima* was investigated. For that purpose a natural grape juice (GJ) obtained by pressing grapes of the cv. Tinta Roriz, clarified and pasteurized was used. Yeast cells, either in pure or mixed culture, were used to ferment this GJ either non-supplemented (GJ) or with diammonium phosphate (DAP) supplementation (GJ_{DAP}), according with legal limit of 1g l^{-1} established by European legislation. Fermentation profiles and yeast growth kinetics as well as the physicochemical and the sensory quality of the wines obtained were evaluated. The results evidenced that co-inoculation strongly affected *Saccharomyces cerevisiae* population dynamics. Moreover, the persistence of *Metschnikowia pulcherima* was highly dependent on the initial nitrogen concentration in grape-juice. The results obtained in this work indicate that yeast strains do affect wine aroma and composition, and, depending on the nitrogen availability of grape musts, co-inoculation of *Saccharomyces* with non-*Saccharomyces* could be a good strategy to respond to the new challenges of the consumer demands for wines with more complexity of flavour and style distinction.

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Nutritional upgradation of animal feed produced by solid- state bioconversion of wheat straw in an industrial scale bioreactor

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Presence of lignin in wheat straw limits its digestibility in ruminants. Thus physical/chemical/biological pretreatment of plant materials improves their utilization as upgraded feed. Among these pretreatments, the biological treatment with white-rot fungi holds more potential. Wheat straw, a poor quality agroresidue, was fermented using a white-rot fungus, *Ganoderma* sp. rckk02, in a 1200 L vertical solid-state bioreactor under optimized growth conditions such as, inoculum density (1% w/w), airflow rate (70 lpm) and temperature (30°C) with intermittent mixing (0.5 rpm). 60 Kg of wheat straw was inoculated with 60 L of fungal inoculum prepared in 150 L bioreactor at temperature 30°C, pH 5.4, agitation speed 100 rpm and aeration rate of 1.0 vvm. The solid-state fermentation (SSF) of wheat straw resulted in to a considerable increase in its crude protein (~16.50 %) with a degradation of lignin and cellulose up to 29.00 and 28.30 %, respectively on 10th day. Noticeably, the maximum efficiency of fermentation of ~31% was achieved on 5th day, where the fungus was observed to degrade maximum lignin with the minimum loss of cellulose and hemicellulose content. *In vitro* gas production analysis using rumen liquor from fistulated bull also revealed that 5th day fermented product possessed comparatively higher fermentable carbohydrate causing increased *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) both almost by12.00 %. To the best of our knowledge this is the first report on the large scale bioconversion of wheat straw by *Ganoderma* sp. rckk02 under SSF conditions in a 1200 L solid-state bioreactor into animal feed.

Keywords: Fermentation; Bioreactor; Animal feed

1200 L Solid- state Bioreactor: Overview & growth inside

Polyvinyl alcohol and rosemary extract as useful antimicrobial polymeric mixture for food purposes

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In the last years, several research efforts have been focused on the preparation of novel substitutes that can replace the traditional packaging materials, with the aim to identify those materials and formulations capable to prolong shelf life of food, not compromising their quality and safety. A wide spectrum of polymeric materials have been developed for this purpose. At present, poly-vinyl alcohol (PVA) is the most widely produced water soluble polymer, and it can represent a very promising candidate for the preparation of bio- packaging. Numerous additives of vegetal source are currently used for the production of ''green'' eco-polymers. Officinal herbs represent a precious font of polyphenols and antioxidant biomolecules with effective activity against pathogen and unwanted microorganisms. This study was focused on the use of rosemary ethanolic extract and PVA to create a polymeric mixture with antimicrobial activity against different pathogen bacteria, for packaging applications. Thus, a series of PVA-dealcoholised rosemary extract polymers, having different vegetal extract amount was prepared. Samples were tested against some pathogen Gram positive and Gram negative microorganisms by the halo test on agar plates. Polymeric mixture revealed a dramatic antimicrobial activity against all microorganisms tested. **Figure 1** shows the activity exhibited by the polymeric mixture against the toxinogenic strain *E.coli* DSM 8579, already using 0.5% of rosemary extract. No activity was observed using only PVA matrix (**NG**) or only rosemary extract (**RE**) in liquid form, at the amount indicated in the figure. Such results let us hypothesise the use of the developed polymer for food applications, which could offer a low cost and highly effective alternative to conventional packaging. Biochemical and microbiological studies are in progress to evaluate the capability of the polymeric mixture to preserve food against microbial contamination.

Keywords antimicrobial, vegetable extract, polymer

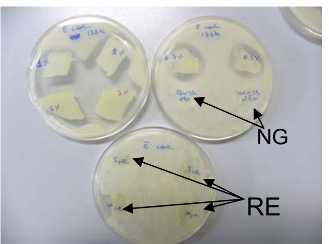

Figure 1

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Prevalence and antimicrobial resistance of *Salmonella* spp. isolated from raw meat and meat products in Algiers (Algeria)

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The aim of our study was to determine the prevalence of *Salmonella* spp. strains in meat and meat products, to identify serovars and to examine the antimicrobial susceptibility of these strains.

A total of 314 samples taken at different retail outlets in Algiers were analyzed. 61 (19.43%) tested samples were positive for *Salmonella*, of which 68 (21.66%) strains were isolated; 64 (20.38%) belonged to the *enterica* subspecies and 4 (1.27%) to *arizonae* subspecies. The most significant occurrences were recorded for the categories of red meat (25.69%) and poultry (17.97%).Serotyping of the *enterica* subspecies strains revealed 21 different serovars and two untypeable strains. The most prevalent serovars were *S*.Anatum (14.6%), *S*.Altona (12.50%), *S*.Corvallis, *S*.Enteritidis and *S*.Typhimurium (each 7.81%).The susceptibility study of 32 selected antimicrobial agents revealed that 56 (90.32%) isolates were resistant to at least one antimicrobial of which 20 (32.26%) showed multidrug resistance. Resistance to sulphonamides (85.48%), nalidixic acid (16.13%), streptomycin (14.52%) and tetracycline (12.90%) was the most frequent. The rate of resistance to pefloxacin was estimated at 4.84%. *S*.Typhimurium has acquired a resistance to 9 antimicrobials and its profile includes an "ASCTSu" pentaresistance type. It showed a reduced susceptibility to amoxicillin/clavulanic acid, but was however susceptible to cephalosporins and fluoroquinolones.

The obtained results have allowed us to confirm the presence of *Salmonella* in meat and meat products, to find a significant antigenic diversity and to emphasize the role of anarchic and continuous antibiotic practices in the appearance of resistances.

Keywords *Salmonella;* meat; meat product; prevalence; serotyping; antimicrobial resistance profile.

Prevalence and antimicrobial resistance of thermotolerant *Campylobacter* strains isolated from poultry in some farms and slaughterhouses in the region of Algiers (Algeria)

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The aims of our study are the evaluation of the prevalence of thermotolerant *Campylobacter* in broilers and turkeys in the region of Algiers, and the study of the antibiotics sensitivity of isolated strains.

A total of 600 samples were analyzed. For each studied species, 100 samples of droppings, 100 of cecal content and 100 of neck skin, were collected from farms and poultry slaughterhouses. 213 out of 300 (71%) turkey samples and 263 out of 300 (87.67%) broiler samples tested positive for thermotolerant *Campylobacter*, which represent a global prevalence of 79.3%. The thermotolerant *Campylobacter* were isolated from 76.5%, 94% and 67.5% of droppings, ceacal content and neck skin respectively. All the tested strains were resistant to at least one antibiotic and 367 (98.7%) were multiresistant. 95.9% (n = 375) strains were resistant to nalidixic acid, 82.9% (n = 324) to tetracycline, 80.8% (n = 316) to ciprofloxacin, 72.1% to ampicillin (n = 282), 22.8% (n = 89) to erythromycin. All isolates were sensitive to gentamicin and chloramphenicol. 21 different resistance profiles were observed; the most common was: AM NA CIP TE.

The results showed that the thermotolerants *Campylobacter* are not only very common in our broiler and turkey farms and poultry slaughterhouses, but also show extremely high levels of resistance to several antimicrobial agents; thus representing a significant risk of contamination for humans through the ingestion of poultry meat and derivatives, generating a direct danger at food poisoning and an indirect danger of cross antibiotic resistance between avian and human strains.

Keywords Thermotolerant *Campylobacter;* turkey; broiler; prevalence; antimicrobial resistance profile.

Prevalence of *Listeria spp* in ready to eat foods (RTE) from Algiers (Algeria)

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The aim of this study was to establish the occurrence of *Listeria spp*., especially *Listeria monocytogenes* in ready to eat RTE food marketed in Algiers (Algeria).

A total of 227 samples were collected from different producers and retailers.

All samples were analyzed using a conventional cultivation method AFNOR V08-055.

Out of 227 samples tested, 21 (9.3%) tested positive for *Listeria spp*. among them, 6 (2.6%) tested positive for *L. monocytogenes. L. innocua* was the most common *Listeria* species found being detected in 11 samples (4.8%), although both *Listeria ivanovii* and *Listeria welshimeri* were detected in 3 (1.3%) and 1(0.4%) food samples respectively.

The study of the *antimicrobial sensibility* of *Listeria monocytogenes* strains was assessed using the disk diffusion assay, according to the criteria defined by the Clinical Laboratory Standard Institute recommended by the World Health Organization. The result showed no resistance.

The study has enabled us to detect these contaminants in a wide range of RTE foods, to suggest that contamination likely occurs after heat treatment, and to assess the danger represented by this category of food for populations at risk.

Keywords: RTE, *Listeria spp*, *Listeria monocytogenes*, prevalence, *antimicrobial sensibility.*

PROBIOLIVES: Table olive fermentation with selected strains of probiotic lactic acid bacteria. Towards a new functional food

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Table olives are one of the most important traditional fermented vegetables in Southern European countries. The annual world production exceeds 2 millions tons, the majority of which (~60%) comes mainly from Spain, Italy and Greece. Most fermented olives currently on the market still result from spontaneous fermentations of conventional raw materials. Sometimes, production remains as a huge conglomerate of handicrafts small industries, using middle century technology with very limited profits. Moreover, the sector is supporting a progressive competition from countries with low production costs. This situation is causing a progressive decrease in the exports to USA, Canada and Puerto Rico of about 2% yearly and losses in the farmers and industries profits.

The European olive sector could take advantage of the markets' opportunities only if the sector is able to find its adequate niche; the adaptation to the new consumers will require a huge effort in innovation, redesign of current processes, adjustment of the traditional products to the new requirements and development of new ones more consumer-oriented products. Today, there is an increasing market for the fermented products in Europe in general, as the consumer's demand for natural and fermented products. Indeed, the consumers are more and more interested in consuming high quality products of which they know the geographical origin and other beneficial characteristics.

Probiotic food products are in general fermented foods containing an amount of viable and active microorganisms, large enough to reach the intestine and exert an equilibrating action on the intestinal microflora. In this way, probiotics are defined as: 'Live microorganisms which when administered in adequate amounts (as part of food) confer a health benefit on the host'. Traditional fermented foods hence constitute a good working base for the development of probiotic-type functional foods. Since health sells at high prices and consumers prefer traditional products, innovation should be targeted to the identification of health-proactive food matrices for use as vectors of delivery of unusual probiotic strains, while keeping a traditional character. In this respect, it has been shown that probiotic strains may be either of intestinal or food origin, and they show identical metabolic and functional properties.

PROBIOLIVES (contract nº 243471) is an European project focused to provide to the SME-AGs and their SMEs new tools to increase their technological level, competitiveness and profits by the production of olives, fermented with probiotic bacteria, preferably isolated among the lactic acid bacteria colonizing the olive surface or its interior in pitted, stuffed and sliced olives. Lactic acid bacteria from the olive microflora are the dominant microorganisms in natural fermentations and in the mentioned project they are studied to determine if some of them possess probiotic properties. This way, the selected probiotic bacteria can be introduced into the brines at the onset of fermentation, to act as starters, to be able to dominate and ensure a proper fermentation inhibiting the growth and survival of undesirable microorganisms. The final goal is the production of a functional product, containing probiotic bacteria in adequate amounts to improve consumer's health, without altering the quality characteristics of fermented olives. Obviously, consumer acceptance studies are essentials for the exploitation and the introduction of this new food into the EU and the international markets. At the same time, a better control of the fermentation process, early detection of faulty fermentation and spoilage, and assessment of the time needed for fermentation completion will be achieved by monitoring microbiological and physic-chemical quality indices.

Most of the presently available probiotics in the market are of animal origin, in particular dairy products, such as yogurt, cheese, desserts, ice-creams. However, dairy product consumption may be limited due to allergies or intolerances to milk (lactose) and derivatives thereof. It should therefore be advantageous to provide food products that allow to administer probiotic bacteria without causing allergies or intolerances and that can be stored for a long time after opening.

Keywords Table olives; functional food; lactic acid bacteria; probiotics

Probiotics: The Star Neutraceuticals for Management of Lifestyle Related Diseases

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With the changing lifestyle modern society has acquired a number of lifestyle related diseases. Following sedentary lifestyle combined with an increase consumption of unhealthy diets, alcohol, tobacco and physical inactivity are all set to blame for these. The global burden of lifestyle related diseases continues to grow in all pockets of world and tackling it constitutes the major health challenge of 21st century. Today, these diseases of civilization represent a leading threat to human health and are radically distressing the world economy as well. The major types of lifestyle related diseases include obesity, diabetes, cancer, osteoporosis, heart diseases, and many others like chronic renal failure, Pulmonary Disease, asthma, Alzheimer's, atherosclerosis, chronic liver disease, Crohn's disease, nephritis, stroke and depression etc. Among all these cardiovascular diseases, cancers, obesity and diabetes are the world's biggest killers, causing an estimated 35 million deaths each year and the number is expected to grow by 65% by 2030. Even worsening the situation, 80% of all above deaths occur in low- and middle-income countries which are also crippled by an ever increasing burden of infectious diseases, and nutritional deficiencies. In India the situation is quite alarming as the disease profile is changing rapidly and has been identified as one of the nation that is going to have most of the lifestyle disorders in the near future. Already considered the diabetes capital of the world with a home to 58 million diabetics, India now is heading towards gaining another dubious distinction of becoming the lifestyle-related disease capital as well. Taking medicines for these diseases has become a fashion in today's world but because of side effects associated with allopathic medicines and socioeconomic burden, use of alternative, natural treatments and functional foods can be an economic and safe substitute to the above problems. Moreover, increase in consumer awareness has also resurged the interest in use of natural and safe alternative therapies. Therefore, inclusion of bioactive dietary supplements and/ or functional foods in daily meal which may pave the way to improve or reduce the risk of these disorders is strongly recommended. These strategies include nutritional therapies, herbal therapies, massage, yoga and meditation. Most important among all therapies is diet management and nutritional therapies like probiotic therapy. As treatment of lifestyle related diseases requires combined efforts from both medical practitioners as well as patients. Modification of sedentary lifestyle, exercise and inclusion of healthy diet can help to manage these diseases and. Many natural therapies without any side effects are being used from ancient times for prevention and the recent concept in this is the inclusion of functional foods containing probiotics. The term 'Probiotic' is derived from the Greek language meaning 'for life' or stimulator and are defined as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance. Probiotics are well reported to exert various gastrointestinal health benefits like improved absorbability, alleviation of lactose intolerance, improving gastrointestinal disorders like diarrhoea, irritable bowel syndrome and constipation by inhibiting the excessive proliferation of pathogenic intestinal bacteria primarily by promoting the proliferation of beneficial gastrointestinal indigenous microflora. Recently probiotics mainly lactobacillus abd bifidobacteria have been documented to have beneficial effects beyond gastrointestinal health as they were found to have antihypertensive, antihypercholestremic, anticancerous, antidiabetic and immune system modulating effects. In short words probiotics have the potential of management of lifestyle related disease like diabetes and its related complication, hypertension, hypercholestremia, oxidative stress, cancer, cardiovascular diseases etc. via positive modulation of several different physiological systems, apart from its conventional benefits for gastrointestinal health. Probiotics have exhibited these beneficiary actions via their antihypertensive, antioxidative potential, improvement of lipid profiles and insulin resistance, immune system modification, acid production, bioactive peptides etc. These positive findings suggested the potential use of dietary alternatives such as probiotics, to alleviate the occurrence of diseases via a fundamental and safe approach as compared to drugs or hormone therapy. Probiotics could also serve as a complementary supplement to enhance the well-being for those already suffering the diseases and taking drugs or hormonal therapy to medicate the condition. Further revelation on the potential of probiotics in future research could lead to a boost in probiotic-fermented food industries–dairy and non–dairy. Nevertheless, more studies are needed to better understand the exact mechanisms, in vivo target sites, stability and safety, prior to using probiotics as an alternative treatment for lifestyle related diseases.

Keywords probiotics; diabetes; obesity; cancer; lifestyle related disease

qPCR assay for detection of human faecal contamination in food samples

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Food and drinking water serves frequently as vehicles for transmission of human enteric viruses like noroviruses. Contamination of produce can take place during production by use of sewage polluted water or inefficient hygienic norms.

Currently, there are no routine testing for viruses in foods and water due to the lack of validated methods and the methods used in research laboratories are still too labour-intensive and expensive to be incorporated in the quality control of most food industries.

In this project we developed at molecular indicator tool to determine the presence of human faecal pollution in relevant food sources and growth environments. A qPCR assay that detects a specific indicator organism, *Bacteroides dorei*, unambiguously linked to human faeces/wastewater was developed.

As extraction of RNA and DNA from mollusks, fruits and vegetables can be challenging, effort was put into optimizing the procedures for extraction, so that both RNA (norovirus) and DNA (the bacteria) was retrieved as efficient as possible. The optimisations steps as well as qPCR results of norovirus and *B.dorei* will be presented.

The impact of the method and examples of its use in mollusks, raspberries and other contaminated foods will also be presented.

Keywords Indicator Bacteria, norovirus, Food-borne viruses, qPCR, extraction optimisation, RNA extraction, DNA extraction

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Quantification of aflatoxin, ochratoxin A and patulin producing moulds by qPCR in dry-cured ham

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The environmental conditions found in dry-cured ham throughout the ripening process favor growth of a mould population where could be found aflatoxin, OTA and patulin producing moulds. The presence of the above toxigenic moulds on the surface of the hams does not always indicate the corresponding mycotoxin occurs. However, in some cases OTA and aflatoxins have been detected in dry-cured ham, probably as consequence of the growth of OTA and aflatoxin producing strains. Although patulin has never been detected in dry-cured ham, some particular strains could produce this mycotoxin throughout ripening process, since strains able to produce this mycotoxin has been isolated in this product (Martín et al., 2004). For this, to protect the consumer health, it would be great interest to know if it is possible an early detection of toxigenic moulds before mycotoxins are produced.

Detection of mycotoxigenic molds by DNA-based techniques, such as real-time quantitative PCR (qPCR), is a good alternative to traditional detection techniques which are time-consuming. qPCR assays have been developed to detect and quantify aflatoxin, patulin and OTA producing moulds based on *sterigmatocystin O-methyltransferase* (*omt-1*), *non-ribosomal peptide synthetase* (*otanpsPN)* and *isoepoxydon dehydrogenase* (*idh)* genes involved in aflatoxin, OTA and patulin biosynthesis, respectively (Rodríguez et al., 2011a,b,c).

The purpose of this work is to study the suitability of specific qPCR assays for early detection aflatoxin, OTA and patulin producing molds to be used as prevent action in HACCP programs and relating to the ability of these assays to quantify the fungal growth throughout incubation period before mycotoxin production. For this purpose, slices of dry-cured ham were placed separately in presterilised orthogonal receptacles and inoculated separately on the surface with spores of six producing strains: two aflatoxin-producing strains, two OTA-producing strains and two both patulin-producing strains at a concentration of 3 log spores per gram. Then, inoculated samples were incubated at humidity controlled during 21 days at 25ºC. Sampling was carried out by triplicate from each at 0, 7, 14 and 21 days of incubation at 25 ºC. Negative controls from non-inoculated dry-cured ham samples were also analyzed.

Fungal growth was stimated by plating in culture media and by qPCR. Accumulation of mycotoxins on ham slices was also evaluated. A high correlations between cfu data obtained by specific qPCR assays and those obtained by plating was observed. In all cases detection of toxigenic moulds was previous to production of mycotoxins in dry-cured ham slices. Thus, qPCR could be used to monitorice in the HAPPC toxigenic moulds throughout the processing of dry-cured ham with the purpose of take corrective measures to avoid contamination by mycotoxins on the hams. In conclusion, specific qPCR assays could be used to prevent the presence of aflatoxin, OTA and patulin producing moulds in dry-cured ham before mycotoxin production.

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Keywords aflatoxin, OTA, patulin, qPCR

Rapid differentiation of *Enterococcus* species by MALDI-TOF mass spectrometry

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Enterococci are Gram-positive bacteria that can survive under severe conditions. The species of the genus *Enterococcus* are common symbiotes of the gastrointestinal tract of warm-blooded animals. Being the most abundant Gram-positive coccus in humans, *Enterococcus* represents an indicator of faecal contamination. Otherwise, *Enterococcus* plays an important role in the food sector. First, some *Enterococcus* species are used as probiotics to improve the microbial balance of the intestine or as a treatment for gastroenteritis in humans and animals. In addition, a beneficial role has been ascribed to *Enterococcus* spp. in food products of animal origin, such as milk, cheese and meat, as well as in fermented sauces, vegetables and plants, due to the positive effect in the ripening and development of flavour in meat and dairy products. However, besides the beneficial effects in food products, the ubiquitous nature of *Enterococcus* spp. can also cause problems in the food sector, since it is not considered as a "Generally Recognized as Safe" (GRAS) organism. In this sense, the genus *Enterococcus* has been described to be frequently present as a contaminant in food products (milk, cheese, fermented sausages), being implicated in food spoilage, as well as in food intoxications. Although, so far, no correlation between the presence of *Enterococcus* strains in food and human infection has been demonstrated, the presence of virulence genes in *Enterococcus* strains used as starter cultures in food has been identified. Thus, an *Enterococcus faecium* outbreak in pigs has been linked with sepsis in humans in China. Furthermore, *Enterococcus* spp. is known to cause nosocomial infections and the possible spreading of antibiotic resistance through the food chain is a problem of global concern. Finally, there is an increased need for rapid and accurate identification of enterococci at the species and subspecies level as a means of effectively assisting infection control and epidemiological studies.

The aim of the present work was the application of MALDI-TOF MS as a fast and accurate method to differentiate species of *Enterococcus* as an alternative to methods based on DNA amplification. In this study, 52 *Enterococcus* strains were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), including 11 different species. The studied strains contained 39 strains isolated from different dairy and meat products, as well as 13 reference strains obtained from the Spanish type culture collection (CECT). Comparing the spectra obtained by MALDI-TOF MS, three peaks with the mass values of *m/z* 4426, 4732 and 6401 were identified as genus specific biomarkers that were present in all species. In addition, characteristic peak masses were determined for the species *Enterococcus faecalis*, *E. faecium*, *Enterococcus gilvus*, *Enterococcus devriesei* and *Enterococcus malodoratus*. However, no biomarker could be found that were related to the diversity of hosts from which the *Enterococcus* strains were isolated.

Furthermore, clustering of the peak mass lists of the spectral profiles obtained by MALDI-TOF MS was carried out as a tool for the classification of all studied strains. At the same time, a fragment of the 16S rRNA gene was sequenced and phylogenetic analysis was carried out and compared to the cluster obtained by the proteomic approach. In both cases, a clear separation of strains of the species *E. faecalis* could be observed. Although the differentiation is not clear for some species, the clustering obtained by phyloproteomic analysis resulted to be more discriminating than the phylogenetic approach, allowing the differentiation of most strains at species level. In conclusion, MALDI-TOF MS and cluster analysis showed to be a competent tool for the rapid classification of *Enterococcus* strains isolated from different food products.

Keywords *Enterococcus*; food quality and safety; bacterial identification; MALDI-TOF MS; phyloproteomics

Real Time quantitative expression study of a polyketide synthase gene related to ochratoxin A biosynthesis in *Aspergillus niger*

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Ochratoxin A (OTA) is a mycotoxin with nephrotoxic effects and has also carcinogenic, genotoxic and immunotoxic properties. To date, there is limited information about the genes involved in the OTA biosynthesis, but it is well known that a polyketide synthase (PKS) is required. In our laboratory, we developed a Real Time PCR procedure for the rapid and specific detection and quantification of OTA-producing strains of the *A. niger* aggregate using as a target a putative PKS gene related to OTA production. The aim of this study is to demonstrate that PKS gene expression appears to correlate with OTA production in the fungus indicating a possible role for the product of this gene in OTA biosynthesis in *A. niger*.

One strain of *A. niger* from our culture collection was grown on yeast extract sucrose (YES) and malt extract agar (MEA) media, which are known to affect the OTA biosynthesis. The strain was incubated at 25ºC for seven days and monitored each day. The transcription of PKS and β-tubulin genes were monitored using a reverse transcription real time PCR approach and relative quantification was the analytical method of choice. OTA production was monitored in parallel by HPLC.

After 2 days of incubation relative expression of PKS gene was 100-fold higher in permissive medium (YES) than in restrictive medium (MEA) This correlate well with the results from HPLC analysis of OTA production, where OTA levels in YES medium were 20-fold more than in MEA. The correlation between PKS gene expression and OTA production will be discussed. This work represents an important first step in increasing our understanding of the genetic mechanism of OTA biosynthesis in *A. niger*.

Keywords *A. niger* aggregate; ochratoxin A; polyketide synthase; real time reverse transcription polymerase chain reaction; relative quantification; gene expression.

Role of Yeast in the Persistence of two Pesticides during the Vegetable Fermentation

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The effect of several yeast strains of genera *Saccharomyces*, *Pichia*, and *Kluyveromyces* on the persistence of two pesticides (pimetrizin and dimetoate) in brine during the fermentation process of vegetable products was determined. The growth of yeast strains in a synthetic brine in presence of the pesticides was tested in a kinetic growth lector Bioscreen C and compared with their growth in a brine control without pesticides. After incubation for 7 days with yeast strains, the concentration of pesticides in the synthetic brine was determined. Previously to pesticide analysis, the brines were centrifugated for removing the yeast pellets. The pesticides were determined in both free-yeast brine and yeast pellets and compared with the control consisting in sterile brine with pesticides. A modified QuEChERS (quick, easy, cheap, effective, rugged and safe) method was used for pesticide extraction that were analysed by LC-MS. The effect of yeast on the concentration of the two pesticides in synthetic brine depend of yeast strain. This effect is clearest on pimetrozin and basically consisted in the retention and not degradation of these pesticides.

Keywords vegetable fermentation; yeast; pesticides; pimetrozin; dimetoate

Salmonella behavior in cocoa fermentation

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Salmonella foodborne outbreaks due to consumption of chocolate have been known since 1960. Even though epidemiologic studies have identified cocoa beans as an important source of contamination of this product, there is no data in the literature on the behavior of *Salmonella* in cocoa pre-processing stages. The aim of this study was to evaluate the behavior of *Salmonella* during cocoa fermentation. The fermentation process was carried out for 7 days, so that in each day one box containing 2kg of cocoa seeds was inoculated with 4 log MPN/g of a pool of 5 strains of *Salmonella* (*S.* Eastbourne IAL 1131, *S.* Enteritidis ATCC 13076, *S.* Oranienburg IAL 1203, *S.* Senftenberg IAL 1235, *S.* Typhimurium ATCC 14028). Daily, 50g of sample were collected from each box to determine the count of yeasts, lactic bacteria, acetic bacteria, *Salmonella,* and pH. The *Salmonella* count was determined by using the most probable number (MPN) method. In the first 3 days of fermentation a pH below 4.0 was observed; from the 4th day on we observed a trend of increasing pH values, reaching 6.5 on the last day of fermentation. Despite the low initial observed count (approximately 2 log CFU/g), the yeast population reached 7.6 log CFU/g on the 2nd day of the process, remaining around 8.0 log CFU/g until the 7th day. The count of lactic bacteria averaged 5.7 log CFU/g on the 2nd day, reaching a maximum count of 8.4 log CFU/g only on the last day. Acetic bacteria was detected from the 2nd day, with the maximum count of 7.7 log CFU/g on the 6th day of fermentation. Samples that were inoculated in the first 5 days showed a *Salmonella* count ≤0.48 log MPN/g after 24 or 48h of the inoculation. However, at the end of the fermentation, with reduction of the microbial metabolic activity intensity, the damage caused by the environment on the pathogen was eased. On the last day growth of *Salmonella* was observed in most samples, with counts of up to 7.5 log MPN/g. Futhermore, *Salmonella* did not affect significantly the growth of the main microorganism groups involved in cocoa fermentation. Based on the results, we conclude that *Salmonella* behavior during cocoa fermentation is closely related to day/stage at which contamination occurs.

Keywords: chocolate, cocoa, *Salmonella.*

Screening of Lactic Acid Bacteria for Antifungal Activity Against Fungi

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Many chemical preservatives that target fungi growth in food have been approved and used for many years. Recently the consumers are looking and demanding for products without chemical preservatives and still maintain good shelf life and safe. The growth of spoilage fungi have been a global concern because of the economy loses and the health hazard of the mycotoxins produced by the spoilage fungi. A total of 22 lactic acid bacteria isolated from Tarhana and Lactic acid bacteria were screened for antifungal activity using dual agar overlay method and well method against *Alternaria alternata, Aspergillus parasiticus, Aspergillus oryzae Penicillium griseofulvum, Penicillium chrysogenum, Penicillium notatu, Penicillium citrinum, Penicillium roquefort, Aspergillus fumigatus*. Ten isolates showed inhibition activity after 72 h incubation at 30°C. Supernatant of 10 isolates with antifungal activity was evaluated by well method and they inhibited the growth of the fungi at 30°C for 72 h. F 2,1 supernatant reduced the mass growth of *Penicillium griseofulvum, Penicillium chrysogenu, Aspergillus fumigatus* and *Aspergillus parasiticus* when incubated for 6 days at 30°C. The isolates were identified using API 50CH as *Enterococcus durans* F2.1. F2.1 isolates studied inhibited the growth of the mycelia and conidia germination of the fungi which indicate the possibility of using LAB isolates as biopreservative.

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Spoilage microbiota during beef storage at 4°C in different conditions evaluated by PCR-DGGE and direct pyrosequencing

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Bone-less beef chops were stored at 4°C in four different conditions: (i) air (A), (ii) modified atmosphere packaging (MAP), (iii) vacuum packaging (VP), (iv) bacteriocin activated antimicrobial packaging (AP). After 0 to 45 days of storage the beef samples were analyzed in order to determine the viable counts of spoilage bacteria. The DNA extracted from the meat samples at each time and condition was analyzed by a 16S rRNA gene-based metagenomic approach via 454-pyrosequencing. The microbial ecology at species level was further investigated by using species-specific PCR assays and 16S rRNA-based PCR-DGGE analysis of DNA extracted from beef samples and bulk cells after viable counts.

The pyrosequencing results showed that the microbial species varied depending on the storage conditions and according to the time of storage. A total of 403 taxonomic units were obtained; however, sequences with no incidence above 1% in at least 1 of the 25 meat samples analyzed (6 samples for each packaging condition plus meat at the time zero) were discarded. Therefore, a final number of 38 operational taxonomic units (OTUs) tracebacks were considered. The initial meat before packaging was found to be contaminated by at least 21 different taxonomic units and this diversity changed dramatically depending on the storage conditions. *Ralstonia* sp. and *Limnobacter* sp. were the most abundant in the meat at time zero. In the first week of storage in A, *B. thermosphacta* developed up to an incidence of 76%; however, after 2 weeks and until the end of the storage the system was dominated by *Pseudomonas* sp. with an incidence between 80 and 95%. *B. thermosphacta* had an incidence above 95% during the first week of storage in MAP, while *Pseudomonas* sp. also occurred in the later stages of storage and *C. divergens* had an incidence of about 35% after 45 days in MAP. More bacteria were observed during storage in VP; after an initial presence of *B. thermosphacta* and *Pseudomonas* sp., other taxa such as *Streptococcus* sp., *Lactobacillus* sp., *Lactococcus* sp. *C. divergens* and *Carnobacterium* sp. developed during storage. The highest variety of species was observed in meat stored in AP. However, while at the early stages microorganisms such as *Ralstonia* sp., *Limnobacter* sp. *Limnobacter thiooxidans, Bradyrhizobium* sp., *Rudaea cellulosilytica* and *Rhodococcus* sp. were found, after 3 weeks of storage in active packaging the abundance of these bacteria dramatically decreased and a high incidence of *C. divergens* up to 95% characterized the AP samples at the final stages of storage.

PCR-DGGE profiles of bulk cells from MRS showed *Lc. pseudomesenteroides* and *C. maltaromaticum* in A and MAP while *Lactobacillus sakei* was found in the later stages of storage in VP and AP. *Pantoea* spp. and *Serratia grimesii*/*liquefaciens* were found in A and VP while they were inhibited in both MAP and AP storage conditions.

The use of different packaging conditions affected both loads and species diversity of spoilage related bacteria in meat. Owing to the significantly higher sensitivity, the pyrosequencing resulted more powerful than the traditional PCR-DGGE approach by allowing detection of a wider number of species.

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Keywords meat spoilage; meat packaging; pyrosequencing

Stimulatory effect of novel polyphenol-based supplements from olive mill waste on the growth and acid production of lactic acid bacteria

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OBJECTIVES: The utilization of polyphenol-based supplements from olive mill waste (OMW) in lactic acid fermentation and in media for the cultivation of lactic acid bacteria (LAB), in order to stimulate biomass growth and lactic acid biosynthesis in LAB used as starter cultures in fermented dairy and meat products.

METHODOLOGY: Polyphenols deriving from OMW were selectively separated after an ultrafiltration process developed in our lab, using macroporous absorbing resins, and concentrated in powder form after lyophylization. The OMW-polyphenol powder was added in liquid culture media (modified MRS and M17 broth, supplemented with additional sugars) or milk, to which several lactic acid bacteria were separately inoculated and grown in incubating shakers, in order to investigate the potential stimulatory effect on LAB growth and lactate accumulation. Polyphenol concentration in the liquid media was 0ppm (blank), 500ppm, 1000ppm, 2000ppm, and 5000ppm. Lactate was measured as total acidity of culture medium, biomass was estimated my measurements of optical density and turbidity of fermentation broth, and sugars were measured spectrophotometrically by the DNS method. Respiratory Quotient (RQ) was measured by a O2/CO2 gas analyser to in order to screen the respiration rate of each culture. Polyphenol concentration was measured by the Folin-Ciocalteu method.The LAB tested in this study were: Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus sakei, Lactobacillus curvatus, Pediococcus pentosaceus, Streptococcus thermophilus, Lactococcus lactis.

RESULTS: Most LAB tested were clearly stimulated by the presence of 500-1000 ppm polyphenols in the culture medium compared to the blank samples (without polyphenol addition). Total biomass and cell growth, as well as lactate accumulation and production rate were significantly higher in cultures supplemented with 500-1000ppm of the polyphenol preparation. Similarly, a higher maximum RQ was also observed in these cases, showing a more intense cell metabolism, and sugars (lactose and glucose) were utilized at higher rate when 500-1000ppm polyphenols were present. A concentration of 2000 ppm improved many but all of the cultures tested, showing a better performance in biomass and acid biosynthesis compared to the blank, but was inferior to the performance of 500-1000ppm added polyphenols. A concentration of 5000ppm was inhibitory for all cultures tested, and caused a decrease in cell growth, sugar consumption and lactate production, showing that after exceeding an optimum stimulatory concentration, polyphenols exert an antimicrobial effect. In all cases, polyphenol concentration in the medium was stable during the fermentation, showing that polyphenols are not metabolized, and was slightly reduced only at the end of each fermentation, probably due to hydrolytic enzymes excreted during cell autolysis. The extend of stimulation was separate for each culture. Lactobacillus casei and Streptococcus thermophilus (good lactate producers) were mostly stimulated by the presence of polyphenols, while in slowly growing LAB, or LAB which do not produce much lactate the stimulatory effect was not so much pronounced. Also, the enhancement of cell growth and acid biosynthesis depended on inoculum concentration. Cultures with higher inoculum levels which were growing faster showed a more pronounced stimulatory effect than those with low inoculum levels. When using milk as a substrate, and S. thermophilus-L. bulgaricus as a starter culture, acidification during yoghurt or feta cheese production occurred much faster when either 500 or 1000ppm polyphenols were added, and this effect was reversed at 5000ppm polyphenols, as observed in the synthetic media with the individual cultures.

CONCLUSIONS: These results, recently patented under a Greek patent application, show a clear stimulation of lactic acid bacteria by the polyphenol preparation tested, which is novel in the literature and can have many industrial applications. A higher growth and acid biosynthesis of LAB cultures is important in industrial fermentations (e.g. lactate production) to improve production rate and operational costs, but also in the production of fermented dairy and meat products, where the enhancement of starter culture growth is crucial for ensuring the fermentation process and avoiding the growth of spoilage microorganisms or pathogens, especially in the first hours/days of the fermentation. This effect probably occurs via a non-specific stimulation of key biosynthetic enzymes, a hypothesis which is under investigation.

Keywords: polyphenols, lactic acid bacteria, lactic acid fermentation

Study of mycobiota in wheat grain grown in different agroclimatic regions of Spain

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The species of fungi occurring in wheat and their enumeration are affected by conditions such as climate, location, time of harvest, and characteristics of the wheat variety. It has been reported that certain fungal species in wheat can produce mycotoxins under suitable conditions. These mycotoxins may pose a health hazard as they can contaminate manufactured products such as bread.

Type B trichothecenes and ochratoxin A (OTA) are acquiring great relevance between the different mycotoxins because it is being found in a great variety of foods and drinks. Although foods that can contain these mycotoxins are very diverse, the highest incidence levels have been generally found in cereals and by-products.

Type B trichothecenes are a family of sesquiterpene compounds related mycotoxins produced by various species of *Fusarium* such as *F. graminearum, F. culmorum*, *F. poae* and *F. equiseti.* Type B trichothecenes are inhibitors of protein synthesis, can cause rapid irritation to the skin or intestinal mucose, feed refusal, immunological problems, vomiting, dermatitis, and hemorrhagic lesions. OTA is a mycotoxin produced mainly by species of *Penicillium* and *Aspergillus,* such as *Aspergillus* section *Circumdati*, A*spergillus* section *Nigri* and *Penicillium verrucosum*. OTA is nephrocarcinogenic, immunosuppressor, teratogenic, nephrotoxic and possibly genotoxic.

In this work, the mycobiota of wheat grain samples from Spanish factories that manufacture that commodity for human consumption was studied. Special attention was paid to the potentially producing species of type B trichothecenes and OTA.

Two hundred and thirty seven wheat grain samples from different agroclimatic regions of Spain were analyzed. One hundred and fifty kernels per sample were surface-sanitized with a 2% sodium hypochlorite solution and incubated on two culture media: 1) potato dextrose agar; 2) potato dichloran agar. The most abundant fungi were species of *Aspergillus, Alternaria, Penicillium* and *Fusarium*, which were present in 100 %, 94 %, 88 % and 51 % of the samples, respectively. *Aspergillus* section *Nigri* and *Aspergillus* section *Circumdati* occurred in 15 % and 27 % of the samples, respectively. In this study, differences in mycobiota were also found related to the geographic origin of the samples.

Keywords *Aspergillus*; *Fusarium*; mycobiota; ochratoxin A; occurrence; wheat; trichothecene; toxigenic fungi

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Study of the extraction of toxins HT2 and T2 in oat grain by Accelerated Solvent Extraction technique

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HT-2 toxin (HT2) and T-2 toxin (T2) are mycotoxins that belong to the group known as type A trichothecenes, a family of related cyclic sesquiterpenoids. Type A trichothecenes are the most common mycotoxins and are produced in different cereals, such as oats, by several species of *Fusarium,* mainly *F. sporotrichioides* and *F. langsethiae*.

The highly toxic T2 and HT2 are of special interest because they have been shown to induce DNA fragmentation, to inhibit protein synthesis and to show immunosuppressive and cytotoxic effects. Moreover, it has been reported that these toxins have extremely toxic effects on skin and mucous membranes. Recent data collected to evaluate the risk of dietary exposure to *Fusarium* toxins by the populations of EU member states showed that T2 and HT2 are quite common contaminants in cereals. In 2001, the toxicity of T2 and HT2 was evaluated by both the Scientific Committee for Food and the Joint FAO/WHO Expert Committee on Food Additives. Thus, the provisional maximum tolerable daily intake for the combination of these toxins or alone was set at 0.06 mg/kg body weight/day.

There are several methods to determine these toxins in food. Usually, methods based on an extraction with acetonitrile/water or methanol/water with a help of an orbital shaker, following by a cleanup step using solid phase extraction or immunoaffinity columns are state-of-the-art in laboratories. The most frequently applied techniques for the determination of these mycotoxins are GC with electron capture or mass spectrometric detection as well as LC with fluorescence detection after derivatisation or with tandem mass spectrometric detection.

The aim of the present study was to develop a new, rapid and easy-to-use method for the extraction of T2 and HT2 in oat grain by Accelerated Solvent Extraction (ASE) technique. Different extraction solvents, different extraction times, and different extraction temperatures were tested to optimise the procedure. The proposed extraction was tested by quantifying the levels of the mycotoxins in oat grain spiked with known amounts of standards.

The preliminary results indicated that the better recoveries are obtained using acetonitrile/water (90:10, v/v) as the extracting solvent during 5 minutes in a single cycle and at a temperature of 80 ºC. The recovery rates obtained under these conditions were 94.2% for HT2, and 100.6% for T2 in a sample of oat grain contaminated with 2.0 mg HT2/kg and 1.0 mg T2/kg.

Keywords trichothecene; ASE; mycotoxin extraction

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Survival of human pathogenic and epiphytic microbiota during storage in refrigerated mango pulp treated or not by high hydrostatic pressure

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Mango fruit is highly appreciated for its taste, freshness and vitamin content. Mango pulp is a convenient processed fruit product for commercialization, but at the same time is highly prone to spoilage by contaminating bacteria from the food and the food processing environment. Contamination with human pathogenic bacteria during processing is a health hazard, and there are very scarce data on the fate of pathogens in this food product and also on the ways to achieve their microbial inactivation. In the present study, pulp obtained from ripened mango fruit (*Mangifera indica* Var. Kent) was artificially contaminated with human pathogenic bacteria (*Escherichia coli, Salmonella enterica, Listeria monocytogenes*) and also with natural microbiota from the fruit surface. Artificially contaminated mango pulp was treated or not by high hydrostatic pressure (HHP, 400 or 500 MPa for 8 min). Survival was determined during storage of mango pulp for one month under refrigeration.

In non-pressurised pulp, *E. coli. S. enterica* and (to a lesser extent) also *L. monocytogenes* survived for at least 15 days during storage under refrigeration, and high levels of epiphytic microbiota were detected as well (4 to 6 log CFU/g). In the pressurized pulps, reductions of viable counts ranged from ca. 5 (*E. coli, L. monocytogenes*) to 7 (*S. enteric*) or 3 log cycles (epiphytic microbiota) after treatment, and only some *E. coli* viable cells were detected during late storage. Surviving epiphytic microbiota increased to ca. 3.5 to 4.5 log CFU/g during storage. Results from this study suggest that HHP treatments are useful to improve the safety of mango pulp against accidental surface contamination with human pathogenic bacteria and to reduce the microbial load of epiphytic bacteria.

Keywords: high-pressure processing; mango; foodborne pathogens

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Temperature and water activity effects on Ochratoxin A production by *Aspergillus carbonarius* on maize kernels

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Ochratoxin A (OTA) is a potent nephrotoxic mycotoxin produced by some *Aspergillus* and *Penicillium* spp. Since the description of OTA production by members of *Aspergillus* section *Nigri* (*Aspergillus niger* and *Aspergillus carbonarius*), they have achieved greater significance due to their potential to contaminate several food commodities with this mycotoxin. Natural occurrence of OTA in maize and maize-based products is a worldwide problem. As *A. carbonarius* has been reported on maize, it could be a source of OTA in this commodity in tropical and subtropical zones of the world.

The aim of this study was to determine the effects of water activity (a_w) (0.92-0.98) and temperature (5-45 ºC) on OTA production by *A. carbonarius* on maize kernels. The studied strain was able to produce OTA in maize kernels from the fifth day of incubation over a wide range of temperatures and water availabilities. *A. carbonarius* produced OTA from 15 to 35 ºC and the maximum concentration was achieved at 15 ºC, although not differing statistically from the concentration detected at 20 ºC. At 0.98 a_w the OTA concentration was significantly higher than those at 0.96 and 0.92 a_w. These results indicate that *A. carbonarius* can produce OTA in maize before harvest and their levels can increase during storage, transport and processing.

Keywords *A. carbonarius*; maize kernels; ochratoxin A; temperature; water activity

The nutritional value of aminoacids from cell walls of Mucoralean strains

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The protein composition of the cell wall depends on environmental conditions and developmental stage. The suitability of an organism for commercial protein production should be based on its protein content, determined as accurately as possible, and its amino acid composition, in addition to the efficiency of converting substrate carbon and inorganic nitrogen into organic nitrogenous compounds. In the present work the cell wall of mycelial species of the Mucoralean fungi strains *Absidia cerulea, Mucor mucedo* and *Rhizopus arrhizus* was studied the content of protein and the comparison with nutritional potential of essential amino acids composition. The strains of *A. cerulea, M. mucedo,* and *R. arrhizus* were grown in Erlenmeyer flasks, was inoculated with 10 discs of 6cm of diameter in the synthetic medium for Mucorales, at 25^0C, in orbital shaker of 150rpm, during six days in the end of exponential phase. The biomass was harvested by filtration, washed with distilled water and was freeze dried, and the biomass determined by gravimetry. The cell walls were extracted by homogeneization and sonicator processes. After the cell walls were freeze dried and submitted to total protein content and amino acid characterization. The protein content was measured by Folin phenol method. A 50-mg amount of the dried mycelial powder was placed into 10-ml ampoules. Five milliliters of 6 N HCl was added. Hydrolysis was carried out in vacuum-sealed ampoules at 110 C for 24 h. Separation of amino acids was accomplished using a Beckman, automatic amino acid analyzer. In general, the protein content of *M. mucedo* and *R. arrhizus* were similar 10.4% and 10.2%, respectively, however, *A. cerulea* showed low protein content (4.5%). In general the amino acid compositions of the Mucoralean strains appears to be the same, except that arginine in *M. mucedo* (2.94%), compared with *A. cerulea* (5.95%), and *R. arrhizus* (5.00%). The essential amino acids leucine and lysine are predominant amino acids in the cell walls corresponding to *M. mucedo* (7.32%), *A. cerulea* (9.35%), and *R. arrhizus* (7.93%). Phenylalanine occurred in *M. mucedo* (3.47%) and *R. arrhizus* (4.21%). However, in the total amino acids the most predominant glutamic and aspartic acids, followed alanine, and appear similar amounts in the fungi used in this study. The results obtained suggest the Mucoralean fungi potential of cell walls in nutritional value of aminoacids which contains adequate proportions of all essential amino acids.

Key words: Mucoralean fungi; essential amino acids; mycoproteins

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The potential of *Aspergillus* section *Nigri* to produce ochratoxin A and fumonisin B_2 in brazil nuts

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Knowledge of toxigenic fungi distribution in food is important because it gives parameters to control and prevent mycotoxin production. Ochratoxin A (OTA) and fumonisin B_2 are two mycotoxins produced by *Aspergillus* section *Nigri* which are of concern to human health. The objective of this study was to verify the occurrence of *Aspergillus* section *Nigri* in brazil nuts and in foods when the presence of these species has been common; and also to evaluate the toxigenic potential of these isolates to produce ochratoxin A and fumonisin B_2. The fungi were isolated using direct plating techinique. A total of 50 units of each sample of brazil nuts were placed onto Dicloran 18% Glicerol (DG18) plates and incubated at 25ºC for 5days. The identification was carried out according to their macroscopic and microscopic characteristics. For testing the potential to produce OTA, the isolates were grown on Yeast Extract Sacarose agar (YESA) and OTA was extracted from agar plug technique, using thin layer chromatography (TLC) plates under UV light. For fumonisin B_2, the isolates were inoculated onto Czapek Yeast Extract 20% Sacarose agar (CY20S) and incubated at 25ºC for 7 days. The toxin was extracted with methanol and identified using High Liquid Performance Chromatography (HPLC). A total of 339 strains of *Aspergillus* section *Nigri* were isolated from brazil nut samples collected from the forest in the Amazon region to market in São Paulo State. Only 3.2% of *Aspergillus* section *Nigri* isolates were able to produce ochratoxin A. Among them, two isolates were identified as *Aspergillus carbonarius* according to their microscopic characters. The other isolates are being identified as belonging to *Aspergillus* section *Nigri* for further molecular identification. Up to now 56 isolates of *Aspergillus* section *Nigri* were tested for fumonisin B2, and 55.4% (31 isolates) were positive for this toxin.

Keywords: *Aspergillus* section *Nigri,* fumonisin B_2, ochratoxin A, brazil nuts

The protective effect of a probiotic agent against shiga toxin-producing *Escherichia coli* (STEC) colonization in sheep

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Shiga toxin-producing *Escherichia coli* (STEC) strains are food-borne pathogens that cause human diseases, and ruminants are the main reservoirs of STEC. The first step of enteric infection is colonization of the host's gut mucosal surface by pathogenic strains of bacteria. Probiotic bacteria can decrease the severity of infection by competing for receptors and nutrients and by synthesizing an acid that creates an unfavorable environment for the growth of several bacterial species. The aim of this study was to determine whether the inoculation of sheep with probiotic strains decreases the colonization of pathogenic bacteria. Sheep that received oral inoculums at a concentration of 2×10^9 per mL of viable bacteria of *E. coli* (STEC) carriers of *Stx1*, *Stx2* and *eae* genes were compared with other groups that did not receive inoculums. When probiotic bacteria were inoculated together with the pathogenic bacteria, the number of pathogenic bacteria in a population was similar to the control. Thus, we conclude that these probiotic bacteria were effective in reducing colonization by pathogenic bacteria and could be used as an alternative method to decrease STEC infection in sheep, thereby reducing transmission to humans.

Key words: *Escherichia coli*, *Stx1*, *Stx2*, *eae,* probiotic, sheep

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The role of *Pseudomonas* and aerobe spore-formers in bacterial spoilage of milk and dairy products

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Aerobe spore-formers belonging to the genus *Bacillus sensu lato* and psychrotolerant Gram-negative rods belonging to the genus *Pseudomonas* are considered the most important spoilage micro-organisms in dairy products. Once they enter the raw milk, their spores or their spoilage enzymes, respectively, cannot be destroyed by conventional heating processes such as pasteurization. Though these spoilage organisms have been subject of many studies and are thus historically well-known, large-scale raw milk isolation campaigns with identification based on current taxonomic insights and coupled to an extensive screening for enzymatic properties, support the need for re-evaluating the dominant species concerning dairy spoilage within these two groups of organisms (*Bacillus s.l.* and the genus *Pseudomonas*).

Operational management throughout the dairy chain can influence species composition and bacterial load of raw milk prior to processing. At the farm, variable feeding and housing strategies of cows, as well as seasonal differences, can influence the microbial quality of milk. Therefore, to determine the predominant aerobic spore-forming microbiota, a sampling campaign for raw milk of both organic and conventional dairy farms was set up in both summer/autumn and in the winter period. An initial screening for spoilage potential was performed using elective media for lipolysis and proteolysis. An assessment of lipolytic and proteolytic activity was measured in quantitative assays (respectively titration of free fatty acids and the TNBS-assay).

The initial screening on elective media revealed that mainly species belonging to the *B. cereus*-group, *Paenibacillus* species, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus pumilus*, *Bacillus clausii* and *Bacillus circulans* could be particularly detrimental for consumer milk quality. Quantitative studies showed strains of *B. cereus*, *B. amyloliquefaciens*, *B. subtilis* and *P. polymyxa* to be strongly proteolytic, along with *B. licheniformis*, *B. pumilus* and *Lysinibacillus fusiformis* to a lesser extent. Though the method for quantification of lipolytic activity is widely accepted in the dairy industry, it turned out to be unsuited for detection of short chain free fatty acids that are mainly produced by aerobe spore-formers. Therefore, significant lipolytic activity could only be demonstrated in a strain of *B. clausii* and *B. pumilus*.

Psychrotolerant *Pseudomonas* bacteria benefit from prolonged cold storage throughout the dairy chain. Another part of this study mapped this outgrowth of the *Pseudomonas* microbiota throughout the dairy chain prior to processing of the raw milk using isolations and molecular monitoring (DGGE) during lab-scale simulations of the cold dairy chain. Predominant milk spoilers were identified and possible contamination sources were identified.

Outgrowth of *Pseudomonas* members occurred from the beginning of the dairy chain (farm tank) under both optimal and suboptimal storage conditions. An even greater risk for outgrowth as indicated by a vast increase of about 2 log colony forming units per mL raw milk existed downstream in the chain, especially when raw milk is stored under suboptimal conditions. This difference in *Pseudomonas* outgrowth between optimal and suboptimal storage already became statistically significant from within the farm tank. The predominant taxa proved to be vigorous milk spoilers. They were identified as *Pseudomonas gessardii*, *Pseudomonas gessardii*-like, *Pseudomonas fluorescens*-like, *Pseudomonas lundensis*, *Pseudomonas fragi* and *Pseudomonas fragi*-like. At the farm-level, feed concentrate and maize silage as wel as biofilms in the milking equipment appeared to be possible contamination sources for important milk spoiling *Pseudomonas* species along with storage tanks at the dairy plant.

Keywords raw milk; spoilage

Transformation of monoterpene alcohols, such as nerol and geraniol, with *Aspergillus niger* in Yeast-Malt medium

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Monoterpene alcohol formation mechanism during the imoshouchuu (Japanese traditional sweet potato spirit) making has been presented[1]. The monoterpene alcohols, such as geraniol, nerol, linalool, and α-terpineol, were existed as the β-glucosides in sweet potate. Geranyl and neryl β-glucosides were hydrolized by *Aspergillus kawachii* to produce geraniol and nerol. During the distillation, linalool was produced from geraniol and nerol, on the otherhand, α-terpineol was mainly produced from nerol. Linalool was also produced from linalyl β-glucoside by the hydrolsis during the distillation. However, α-terpinyl-β-glucoside was remained after distillation.

We are interested in the possibilities to produce useful compounds from geraniol and nerol by *A. niger*.

Therefore, transformation of nerol with *A. niger* investigatedinpre-cultured in Czapek-Dox[2] was investigated at first. The first main products from the transformation of nerol by *A. niger* in Czapek-Dox media were α-terpineol and linalool. After 8 days from the addition of nerol, the amounts of α-terpineol and linalool was started to decrease and the amounts of furan type of diastereomeric linalool oxides were increased.

In this research, transformations of nerol and geraniol with *A. niger* in Yeast-Malt (YM) medium will be reported. *A. niger* was pre-cultured in 300 ml of YM medium for 3 days. After that, 0.4mmol nerol or geraniol was added to the culture and the culture was continued. The formation rate fromnerol was far faster in the Czapek-Dox medium than that in YM medium, though the growth rate of *Aspergillus niger* was faster in YM medium than that in Czapek-Dox medium. Therefore the transformation rate was faster in the nitrogen restrict culture.

The transformation of nerol with *A. niger* in the YM medium 1 day, ω-hydroxynelol was produced at first. After 10 days, the amount of ω-hydroxynerol was decreased and ω-hydroxylinalool was produced. On the other hand, in the case of the transformation in the Czapek-Dox medium[2)], ω-hydroxylinalool was produced, whereas ω-hydroxynerol was not produced. Therefore, the ω-hydroxylinalool production pathway from nerol with *A.niger* were different from the cases in these two media.

The first main product from the transformation of geraniol by *A. niger* was linalool. A small amount of α-terpineol was also produced. The produced amounts of linalool and α-terpineol was increased and decreased several times, ω-hydroxylinalool was produced with decreasing the amount of linalool and α-terpineol in both YM and Czapek-Dox media.

In the cases of the transformation from nerol in Czapek-Dox medium and those from geraniol in both media, it seems that ω-hydroxylinalool was produced via linalool. Therefore, the transformation of linalool with *A. niger* was examined in YM medium. From the result of this transformation, ω-hydroxylinalool was also mainly produced.

Keywords transformation, monoterpenealcohol, nerol, geraniol, linalool, ω-hydroxylinalool, ω-hydroxynerol

T. Ohta *et al*, *Agric,Biol Chem*., 55, 1811-1816, 1991. T.Tsuruta, MICROORGANISMS IN INDUSTRY AND ENVIRONMENT From Scientific and Industrial
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Upward or Downward Dehiding of Beef Carcasses – which gives a cleaner product?

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The hide is the primary source of beef carcass contamination. Preventing contamination during dehiding is challenging. Superior skinning methods are paramount. In Ireland two skinning methods are employed - Upward (UHP) and Downward Hide Pulling (DHP). A definite shift from UHP to DHP usage is occurring as the latter is believed to give a more hygienic product. This study investigates the scientific basis for this hypothesis. An abattoir that was changing its dehiding method was selected. Cattle with a Clean Livestock score of 3 were chosen for the study. $100cm^2$ areas on eight carcass sites were sampled using the wet-dry double swab technique on 72 carcasses divided into two groups of 36 skinned by each technique. Total viable count and Enterobacteriaceae count for each site were determined using ISO methods 4833:2003 and 21528/2:2004. No significant difference was observed in total carcass contamination between the methods. Significant differences ($p<0.05$) in contamination were observed at the flank, shin, brisket and neck. Critical observation of each technique attributed these site specific differences to identified deficiencies in the abattoirs pre-requisite programmes (PRPs) and were not attributable to the hide-puller type per se. Sound PRPs are more critical than hide puller type in hygienic carcass production. This novel comparative study facilitates an abattoir's decision making if considering changing their skinning method.

Use of genetic screening to evaluate the lactobacillaceae survival to low pH and bile salts

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Lactobacilli species have been extensively used as probiotics. One prerequisite property is their ability to survive trough the gastrointestinal tract. Most of the studies based on extensive analysis of phenotypic traits were performed to identify strains able to survive to low pH and bile salts *in vitro*. In addition, many functional studies have been performed, generally based on a single gene to get a better comprehension of the survival mechanism. For this purpose, we screened 152 lactic acid bacteria isolates belonging to the Lactobacillaceae representing the microbiota of an African pearl millet fermented slurries for presence/absence of 21 genes coding for this function. This molecular screening was completed by a functional analysis by testing *in vitro*, the survival of the isolates to low pH and bile salts.

Only four genes out of twenty-one were common to all isolates: the house keeping gene *groel*, but also other non essential genes such as LBA1272, *dltD* and LBA0493. This genetic screening showed a high prevalence of genes related with the low pH and bile salts survival as 82% of our bacterial collection harbored 14 genes out of 21. We selected 38 isolates differing by their genetic equipment to study *in vitro* their low pH survival ability and their bile salts tolerance. Among them, 55% survive at least one hour in synthetic gastric juice at pH 2, 34% survive for two hours, 18% for three hours and 11% for four hours while most of the isolates were resistant to tolerant to bile salts. On the one hand, no relevant genes were found to mediate the low pH survival ability. On the other hand, the frequent detection of genes involved in bile resistance is accompanied by a good tolerance to bile salts of the isolates and the most discriminating gene was *bsh* encoding for a bile salt hydrolase.

Many isolates composing the microbiota of the investigated traditional cereal-based food have the potential to survive to the GIT conditions. This study forms the basis for the selection of potential strains at genetic level to further evaluate them as potential probiotics.

Keywords lactic acid bacteria, low pH survival, bile salts survival, genetic screening

Virulence genes and clonality in *Campylobacter jejuni* isolates from human and poultry at Portugal

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Campylobacter is a leading cause of food-borne bacterial illnesses and poultry meat is pointed out as the principal source of infection. The last EFSA report shows that the notification of campylobacteriosis has increased in 2009 and remains the most common zoonosis in the European Union, with a total of 198,252 confirmed human cases. Furthermore, campylobacteriosis is also the most frequent antecedent infection before the onset of the post-infectious peripheral neuropaty Guillain-Barré syndrome (GBS). Several studies evaluated the prevalence of virulence associated genes, such as the ones implicated in the adhesion and invasion of intestinal cells; the production of toxins, such as cytolethal distending toxin (CDT), lipooligossacharide (LOS) and glycosylation system.

The aim of this study was to evaluate the presence of several virulent related genes in *Campylobacter jejuni*, such as the CDT genes, the *cgtB* and the *wlaN* gene, present in LOS locus and involved in the biosynthesis and variation of LOS structure, which is implicated in the development of GBS in humans. The presence of *flaA* gene, involved in the codification of flagellar apparatus was also assessed. A RLFP (Restriction Fragment Length Polymorphism) of the *flaA* gene was performed in order to evaluate the genetic heterogeneity among the isolates.

The samples analyzed were isolated from the cecum, carcass neck skin and breast of poultry (n=36) from different production systems (intensive, extensive indoor and organic), and from five different flocks. Samples isolated from human clinical cases were also studied (n=50). The detection of the *cdt* genes (*cdtABC*), *cgtB* gene and *wla*N gene were performed by PCR according to Samosornsuk *et al.* (2007), Müller *et al.* (2007) and Parker *et al*. (2005) respectively. The *flaA* gene was detected by PCR, followed by RFLP with restriction enzyme *DdeI* (Ertas *et al*., 2003).

The results obtained revealed that both the isolates collected from poultry and humans were all positive for the presence of three *cdt* genes (*cdtABC*) and *flaA* gene. On the other hand, the *cgtB* gene was detected in 8.3% of isolates from poultry (n=3) and in 2% of isolates from humans (n=1). The *wla*N gene was detected in 14% of all isolates analyzed. The presence of the three *cdt* genes in all isolates might indicate potential capacity for production of a fully active toxin and consequently appearance of symptoms in humans. *Campylobacter jejuni* strains with similar genetic profiles by RFLP-*flaA* could be observed in isolates from human and poultry origin.

Although the percentage of positive isolates for *cgtB* and *wlaN* genes was low, it remains important to evaluate the presence of this and other genes from the LOS locus, in order to understand the prevalence of possible GBS inducing strains.

Keywords: *Campylobacter*, virulence**,** poultry, Campylobacteriosis, *cdt*ABC**,** *fla*A,

Wild Irish Pheasant -Establishing Processing Hygiene Microbiological Criteria

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Microbiological criteria provide guidance on the acceptability of foodstuffs and their HACCP-based manufacturing processes. Regulation (EC) 2073/2005 establishes process hygiene criteria (PHC) for carcasses of domestic fowl. No such criteria exist for pheasant. It is thus appropriate to establish PHC. The processor selected for the study procures pheasants hunted from protected reserves, which are stocked with 18-week-old pullets from a rearing unit 3-4 months prior to the shooting season. In season 1 on each of 10 processing days 4g of the neck skin (NS) were aseptically harvested from 35 pheasants selected at random post-chilling. The NS from 7 carcasses were pooled to create 5 x 25g final samples. Samples were analysed for the presence for *Salmonella* using ISO method 6579. One sample revealed the presence of *Salmonella.* This procedure was repeated in seasons 2 and 3 with identical results. PHC for pheasant were determined thus: **n** = number of units compromising the sample = 50 derived from 10 consecutive sessions; **c** = number of samples where *Salmonella* is detected = 1; **m = M** = absence in 25g of a pooled NS sample. Ongoing performance exceeding these criteria prompts the establishment to implement timely corrective action to its processing procedures and to review disease control and bio-security measures on the rearing farm. In the absence of legally mandated PHC for pheasant, it is recommended that processors follow the protocols outlined to establish their own PHC.

Wild Irish Venison - Establishing Process Hygiene Microbiological Criteria

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Microbiological criteria can be used to verify HACCP and other hygiene control measures. Regulation (EC) 2073/2005 establishes the process hygiene criteria (PHC) for carcasses of domesticated species. No such criteria exist for wild venison. This study proposes to establish PHC for wild venison. A single processor was selected for the study. On each of 16 days, 5 carcasses were selected. On each carcass a 100cm^2 area was abrasively swabbed in 4 locations (haunch, saddle, neck & shoulder) post-dressing and pre-chilling. Samples were pooled prior to analysis for Aerobic Colony Count (ACC) and Total Enterobacteriaceae Count (TEC) using ISO methods 4833:2003 and 21528/2:2004 respectively. Results for both criteria were reported as daily mean log values CFU/cm^2. The daily mean log ACC ranged from 0.12-3.42log$_{io}$CFU/cm^2 with a mean (x) of 1.76log$_{io}$CFU/cm^2 and a standard deviation (s) of 1.22log$_{io}$CFU/cm^2. TEC values ranged from 0.00-0.86log$_{io}$CFU/cm^2 with x= 0.28log$_{io}$CFU/cm^2 and s=0.37log$_{io}$CFU/cm^2. The value for the lower control limit (m) was determined as $m = x + 1/2s$ while the upper control limit (M) was calculated as $M = x + s$. The PHC for wild venison are therefore m=2.37log$_{io}$CFU/cm^2 and M=2.98log$_{io}$CFU/cm^2 for ACC and m=0.47log$_{io}$CFU/cm^2 and M=0.65log$_{io}$CFU/cm^2 for TEC. Comparing ongoing performance to these criteria allows the establishment to implement timely corrective action to its procedures. In the absence of legally mandated PHC, it is recommended that each processor follow the protocols outlined in this study to establish their own PHC.

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^{13}C-Metabolic Flux Analysis of *Amycolatopsis mediterranei* S699: Rifamycin B Producing Actinomycete

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Amycolatopsis mediterranei is an industrially important actinomycete producing polyketide antibiotic rifamycin B, which shows pronounced antibacterial activity gram-positive bacteria, particularly mycobacterium. It therefore used to treat tuberculosis (TB), leprosy, and other mycobacterium infections. Improvement in the yield of rifamycin is specifically relevant in Indian perspective because India has the largest total TB prevalence in the world at 3 million contributing one-fifth of the global TB cases. Cost of rifamycin production is very critical because it is a low value drug and strategies that would increase the yield even slightly will significantly affect profitability. The need of the hour is to use a systematic method rather than random mutagenesis to identify highly probable targets for genetic manipulation to further improve stains productivity. Quantification of the intracellular carbon flux distribution provides an ideal platform for systemic understanding and targeted manipulation of cellular systems for improvement of product yield and strain performance. Thus the key task of the present work is to quantify the intracellular metabolic flux distribution in the carbon assimilating and the secondary metabolite synthesis pathways of rifamycin B producer actinomycete *Amycolatopsis mediterranei* S699. In the present study *A. mediterranei* was grown in a defined medium containing 100% 1-^{13}C-glucose as the isotopic tracer in a microtiter plate operated in batch fermentation mode to investigate the flux distribution. The labeling pattern of the proteinogenic amino acids in the hydrolysate of the biomass harvested in the mid exponential phase of the culture were analyzed using gas chromatography and mass spectrometry (GC/MS) and expressed as mass isotopomer distribution (MID). MID's were used to quantify the intracellular flux distribution by iteratively minimizing the difference between simulated and measured MID's & between simulated and measured flux distributions. From the estimated flux distributions, it was found that among all the carbon assimilating pathways, glycolysis quantitatively plays the most important role in the synthesis of precursor molecule of rifamycin B antibiotic. S-Methyl-malonyl-COA contributes 73% (molar basis) to the starter molecule of rifamycin B and is synthesized through the TCA cycle. It thus seems probable that suitable genetic modifications to increase the flux in the TCA cycle can improve rifamycin B yield. It was also observed that the relative flux distribution values for *A. mediterranei* were much lower than those reported for other *Amcolatopsis* species and other antibiotic producers, a characteristic probably representative specifically for the species *mediterranei* within the genus *Amycolatopsis*.

Keywords: ^{13}C-MFA, *Amycolatopsis mediterranei* S699, rifamycin, secondary metabolite, antibiotic, mass isotopomer distribution

A new process of total conversion of whey into a lactic fermentate and its possible environmental applications in fungi biocontrol

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In the present work we have developped a fermentation process in the conversion of whey in a fermented medium, rich in lactic acid, highly bioavailable protein and lactic acid bacterias. It has also been assessed its potential use in biocontrol of phytopathogenic fungi.

Whey is the liquid obteined from the coagulation of milk during the cheese making process after separation of proteins and fat. That liquid is approximately 90% of the volume of milk and contains most of its hydrosoluble compounds. The whey is very dilute since only contains between 6 – 7 % of dry matter, mainly lactose, for this reason it has been long considered a waste.

Spanish production of whey is approximately 1,3 million tons. Currently it is been used about 50% of whey as a supplement in animal feed in liquid form or in the food industry by separating its components. However, its use is limited by the difficulty of transportation . The rest of the whey should be treatment by conventional techniques, presenting a serious challenge to its high COD (90.000-95.000 ppm), which lactose is the main culprit.

Through a fermentation process, we have optimized the total conversion of lactose to lactic acid, as well as obtained an increase in the bioavailability of present proteins. During the optimization we have evaluated parameters such as pH, temperature, alkali used for pH control and addition of bacterial extracts.

After identify the optimal parameters of fermentation, we obtained a virtually complete conversion of lactose to lactic acid. Consequently, we favor its use in animal feed and agricultural fertilization, because lactose is a disaccharide that causes various diseases in the digestive system of adult animals and is extremely recalcitrant in the environment, meanwhile the lactic acid is wide used by practically all the organisms.

Finally, we tested its possible potential in biocontrol of phytopathogenic fungi, such as the oomycete Phytophthora cinnamomi, one of the organisms that cause the syndrome of dry trees in the Quercus genus, obtaining encouraging results for future use in the treatment of this disease.

Keywords: whey; lactose; lactic acid; lactic acid bacteria; biocontrol; Phytophthora.

An Efficient Protocol to Obtain Axenic Culture of Oil Rich *Neochloris pseudoalveolaris* and Its Adaptation to Photoheterotrophic Cultivation

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Microalgal lipids are the oils of future for sustainable biodiesel production. Therefore, screening of different microalgae species with a high biomass and lipid productivity is gaining importance. In addition to that, growing of microalgae under different cultivation conditions like photoheterotrophic and/or heterotrophic offers extra production capacity to microalgal cultivation industry.

In this study, biomass and lipid productivity of *Neochloris pseudoalveolaris* which was obtained from our microalgae culture collection isolated from local resources were investigated under photoautotrophic conditions. Before adapting it to photoheterotrophic conditions, *Neochloris pseudoalveolaris* was made axenic with an improved protocol, and then potential use of various carbon (glucose, glycerol, sodium acetate and ethanol) and nitrogen sources (yeast extract, KNO_3, NH_4Cl) for culturing *Neochloris pseudoalveolaris* in photoheterotrophic batch cultures were studied.

Photoautotrophic growth provided a biomass productivity of 0,011 g/l.d and cellular lipid content (15-20% of dry biomass). The alga was subjected to antibiotic treatment with a mixture of penicillin, streptomycin sulfate, gentamycin sulfate. Antibiotic mixture was spread over BBM agar plates and then alga culture was strike on it. Colonies growing on antibiotic agar plates was collected with toothpicks. Axenic status was confirmed after subculturing three times in sterile BBM medium with glucose (1g/l) and also on nutrient agar. For culturing microalgae in photoheterotrophic conditions, different glucose (1g/L, 3 g/L and 6 g/L), glycerol (1g/L, 3 g/L and 6 g/L), sodium acetate (1g/L, and 3 g/L), ethanol (1g/L, 3 g/L and 6 g/L) concentrations and yeast extract, KNO_3 and NH_4Cl at concentrations of 1g/l were used. The highest biomass productivities were obtained at 6 g/L glucose and 3g/l ethanol. It was found that *Neochloris pseudoalveolaris* preferred yeast extract as nitrogen source.

Keywords Axenic culture; *Neochloris pseudoalveolaris*; photoheterotrophic cultivation; biodiesel

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An innovative growth strategy for propagation and bacteriocin production of *Lactobacilli*

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Lactobacilli belong to the group of Lactic Acid Bacteria (LAB), extensively utilised in the contemporary food industry. These bacteria are mainly used as natural acidifiers, for the inoculation of bulk quantities of milk and vegetables, in order to produce a variety of fermented products. As such, large quantities of their biomass, produced in cost effective enviromentaly friendly nutrient media, are necessary. Furthermore, during their growth, they naturally produce antimicrobial substances, called bacteriocins or lantibiotics. Due to the constantly developing need for natural food preservatives, bacteriocins deriving from Lactobacilli metabolism, function and activity has been extensively investigated. The possibility of producing these substances in mass quantities was investigated through several techniques. Three known bacteriocin producing strains of *Lactobacilli*, were carefully selected, *L.plantarum NCIMB 8014*, *L.casei NCIMB 11970* and *L.lactis NCIMB 8586.* They were grown into simple batch cultures without pH control, where their physicochemical needs were determined. Through the determination of the optimum nutritional conditions for their propagation an optimised growth medium occurred. A simple, liquid turbidometric method was developed to test the bacteriocin productivity of these strains, on the developed media. The antimicrobial activity and potency of the bacteriocins produced, were tested against the target strain *L.delbruckii subsp.lactis NCIMB 8117.* In an effort to facilitate the extraction of bacteriocins from the fermented broths it was decided , to fabricate a nutrient medium , that would contain, low molecular weight nitrogen sources and equally support high production of bacteriocins and large yields of biomass.

In order to achieve that, the optimised medium was then filtrated via ultrafiltration membrane modules of 30 and 4 kDa MWCO , in an effort to simplify the medium and facilitate the extraction of the produced substance. The medium's efficiency in supporting the growth and the bacteriocin production of the bacilli was tested and comparative studies between filtrated and unfiltrated media were done.The filtered medium, containing low molecular weight nutrient sources, was proven to to successfully support growth and bacteriocin production.

Keywords: LAB, Bacteriocins, Target strain, Ultrafiltration, Growth rate, DT.

Application of fructan and sucrose hydrolysing enzymes in ethanol production by *Zymomonas mobilis*

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A bacteria *Zymomonas mobilis* has some advantages for ethanol production (higher ethanol tolerance, less biomass formation, higher ethanol yield on glucose substrates, non-requirement of controlled addition of oxygen) as compared with traditionally used yeasts. The factors limiting its industrial application are the narrow substrate range (only glucose, fructose and sucrose) and the biosynthesis of number of by-products (polifructan levan, oligofructans, sorbitol, gluconic acid) on sucrose medium, which decrease the ethanol yield. The inulin containing Jerusalem artichoke is attractive carbohydrate source for ethanol production. However, *Z.mobilis* cannot directly utilize the inulin.

In the present study the application of inulinases in two various way for ethanol production by Zymomonas mobilis is presented

The co-fermentation of *Zymomonas mobilis* with fructan hydrolyzing enzymes was used for ethanol production from inulin containing substrates. Co-fermentation of the enzyme preparation Fructozyme L (Novozyme) containing exo- and endo- inulinases with bacteria *Zymomonas mobilis 113S* on different Jerusalem artichoke based substrates (mashed pulp and juice) was studied. The saccharification activity of Fructozyme L for Jerusalem artichoke substrate at different temperatures was tested. It was found that the saccharification rate at 30°C is sufficient for applying this enzyme for co-fermentation with *Z.mobilis*. Higher ethanol yield was obtained in fermentation of Jerusalem artichoke juice as compared with mashed pulp.

Other studied application of fructan hydrolysing enzymes was an increase of ethanol yield by *Zymomonas mobilis* on sucrose containing substrates. It is reported, that levan and oligofructan biosynthesis can be decreased by using co-fermentation with invertase. However, the action of invertase increase the fructose concentration. It is known that *Z.mobilis* utilize the glucose first and therefore accumulation of free fructose can lead to increase of sorbitol and gluconic acid production. We propose to use the co-fermentation with enzyme which hydrolyse not only sucrose, but also levan and oligofructans. This will allow to better control the free fructose concentration by adding this enzyme at different fermentation stages. In present study the enzyme preparation „Fructozyme L" was used for polifructan levan as well sucrose cleaving during the co-fermentation processes to increase ethanol yield. The kinetic parameters of Fructozyme L activity was determined for *Zymomonas mobilis 113S* levan at the two temperatures 60 °C – optimal temperature for Fructozyme L, and at 30°C – temperature for fermentation process by *Z.mobilis*. The pseudo first order kinetic behaviour was observed for the *Z.mobilis* levan at the both temperatures. The influence of yeast invertase and „Fructozyme L" on the ethanol yield and sorbitol biosynthesis by *Zymomonas mobilis* on sucrose medium was compared. Two strains - *Z.mobilis 113S* and *Z.mobilis ATCC 29191* was used in our study. Different concentrations of added enzymes were tested. Both invertase and „Fructozyme L" added at the start of fermentation decreased the levan biosynthesis and increased the ethanol yield (up to 80% from theoretical value). In both cases the increase of sorbitol production was observed. The addition of „Fructozyme L" at later fermentation stages allowed to reduce levan yield without significant increase in sorbitol biosynthesis.

Acknowledments.
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Keywords Zymomonas mobilis; Ethanol; Levan; Sorbitol; Fructan; Inulinases

Application of ultrasound to control of *Aspergillus flavus* in cosmetics

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The effect of ultrasound on organic compounds in living tissue and are often related to the phenomenon of cavitation, a term used to describe the formation of cavities or bubbles in a liquid medium containing varying amounts of gas or vapor that are dissolved in the middle. In medicine it is suggested that ultrasound of high intensity is able to cause some reduction in certain infectious agents and microbiology, mechanisms of inactivation of cells appear to be associated with cavitation.

Due to the high power of fungal contamination of cosmetics, it is important to develop new techniques to preserve it to rapid fungi deterioration and subsequent consumer health hazard. On the present work it was probed the efficiency of ultrasound in decreasing the growth of *Aspergillus flavus* in cosmetic.

Thus contaminated samples with the above mentioned fungus, were irradiated at constant temperature (25°C) and power (600W/cm^2), for a variety of time exposure: 0 (control), 12, 16, and 20 minutes. The ultrasound generator model VCX- 600 was utilized.

It was possible to show that the use of ultrasound is efficient in decreasing the growth of microrganisms and thus preserve cosmetic which went from 35,000 CFU / mL to 50 CFU /mL. Ultrasound is a excellent biocide agent in preparation and preservation of emulsional cosmetic products.

Twenty minutes of continuous irradiation yelded an almost complete depletion of microrganisms.

Keywords *Aspergillus flavus,* cosmetics, ultrasound, contamination, cavitation.

Azo-dye orange II biodegradation by bacterial culture in a packed column at laboratory level

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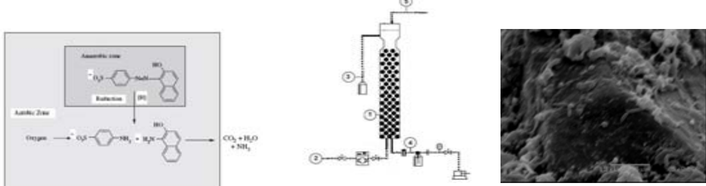
Introduction. The industrial wastewaters containing dissolved dyes constitute a great problem of environmental pollution, whose effect not only affects the water body which is the final receiver and to infer in the aquatic life processes avoiding the free light flow, but also affect the operation of domestic wastewater plants in a detrimental way. The azo-dyes are the greatest group of synthetic dyes in variety and produced amount, representing 70% of the total manufactured colorings, more than 2000 dyes are registered in the Colour Index. It has been demonstrated that the azo-dyes and the nitro-compounds can be reduced into sludge in the animal gut resulting in the corresponding toxic amines.

Objective. The purpose of this study was to implement a packed column with activated coal in order to biodegradate the azo-dye Orange II from synthetic wastewaters, operating in a continues flow using an immobilized culture of Enterobacter sp. and to determine the best conditions to improve the formation of micro aerobic niche suitable for its reduction and the further oxidation up to the mineralization of the products.

Methods. The bidegradation of the azo-dyes was reached in anoxic conditions and the removal of the amines produced in such a treatment were given in oxic conditions (left figure). This is why those conditions were set up in order to favor the experimental treatment (right figure).



The physical and hydraulic characterization of the column was established: a) the color extinction by absorption, b) the presence of byproducts by thin layer chromatography and it was proved the support colonization by scanning electron microscopy. Different speeds of gas and liquid in flow for dye concentrations of 50 and 100 mg/l were proved as process variables.

Results. The biodegradation results (color extinction, subtracting the support saturation) are shown in the following Table.

Run	Entry	Flow (ml/min)	Aeration rate (L/L per min)	Biodegradation volumetric rate (mg/L-h)	Removal (%)
1	100	0.47	127.66	10.41	98.38
2	50	0.47	127.66	4.91	92.72
3	50	0.94	63.83	8.85	83.5

Keywords biodegradation; colors, azo-dye, column

Biodegradation of pomegranate ellagitannins

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The ellagitannins (ETs) are water-soluble hydrolysable polyphenolic compounds with molecular masses ranging between 300 and 20,000 Da. These amorphous and astringent flavor molecules do not contain nitrogen and act as weak acids. ET’s are considered secondary metabolites and they are found in plant cytoplasm and cell vacuoles. ETs are nutrients of high relevance in human diet and there is evidence of their key activity in the prevention of degenerative diseases such as cancer and cardiovascular diseases due their antioxidant properties. When the ETs are exposed to acids or bases, the ester bounds are hydrolyzed and the hexahidroxydiphenic group (HHDP) is released, however, this is a non-stable group requiring a lactonization step to move on to a stable form, leading to the formation of monomer with high antioxidant activity, ellagic acid. The ET’s biodegradation has been poorly explored due to their complexity and diversity, however the available information is scarce and confuse, therefore in this work the biodegradation of pomegranate husk ET’s was evaluated under conditions of solid state fermentation using *Aspergillus niger* GH1 to contribute to understanding of the biological degradation of this complex molecules. The ET’s were extracted from dehydrated and ground pomegranate husk and purified through liquid chromatography using Amberlite XAD-16. These ET’s were used as substrate of solid state fermentation on polyurethane foam as support of the growth of *Aspergillus niger* GH1. Enzymatic extracts were obtained and several enzymatic activities (xylanase, β-glucosidase, cellulase, polyphenol oxidase, tannase) were assayed during the kinetic growth of the culture. A putative enzyme is proposes in this study, ellagitannase. The ellagic acid accumulation was measured during the fermentation and was observed that the maximum ellagic acid accumulation appeared at 12 h. It was observed the xylanase activity appeared at 30 h of culture time (42.9 U/L), β-glucosidase activity at 24 h (525.5 U/L), cellulase activity at 6 h (24.4 U/L), tannase activity at 18 h (164.1 U/L), polyphenol oxidase activity was not found and finally ellagitannase activity at 12 h (200 U/L), this last activity was clearly associated to ellagic acid accumulation during the fermentation.

Keywords: ellagitannins, ellagic acid, solid state fermentation

Bioemulsifier/biosurfactant production by *Candida lipolytica* UCP 0988 in acid or alkaline seawater with low oxygenation

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In this work, an bioemulsifier/biosurfactant production process by *Candida lipolytica* UCP 0988 in acid or alkaline seawater with low oxygenation, supplemented with nitrogen and phosphorus sources, using corn oil as the sole carbon source was investigated. A 2^3 full factorial design with four central points was carried out to evaluate the effects of the initial pH (6, 10 and 14) and the concentrations of ammonium sulphate and potassium dihydrogen phosphate on the biomass concentration, the emulsification activity and the surface tension of the cell- free culture broth filtrates. The experiments were carried out at 28 ° C and 150 rpm during 96 hours in 1000 mL Erlemeyer flasks with a working volume of 750 mL. Corn oil was added to the flaskes after the yeast inoculation, creating an environment with limited oxygen. The initial salinities of the biosurfactant production media ranged from 40 to 73 ‰. Reduced surface tension and high emulsification activities were obtained over a wide pH range, specially at pH 14. The pH increase was the factor that more favored the biomass concentration increase and the surface tension reduction. The increase of the potassium dihydrogen phosphate concentration was the factor that more favored the increase of the emulsification activity. High emulsification activities (up to 6UAE) were obtained for emulsions water-in-oil corn, water-in-canola oil and water-in-soybean oil and also for motor oil–in-water and burned motor oil-in-water emulsions. The highest emulsification activities for water-in-oil diesel (5.71 UAE) and water-in-n-hexadecane (3.89 UAE) emulsions were obtained in experiments with initial pH 14. The yeast grew best at alkaline pH values (10 and 14). The higher production of biomass (17.6 g / L) was observed in medium with initial pH 14 and surface tension of 31.72 mN/m.. The results show that the yeast and its bioemulsifiers/biosurfactants are suitable candidates for bioremediation applications in marine environments.

Keywords biodegradation, kerosene, biosurfactant, seawater, *Candida lipolytica*, extremophile

Bioethanol production via nonisothermal simultaneous saccharification and fermentation processes using carob industry wastes

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Typically, the main types of feedstocks for bioethanol production are raw materials containing fermentable sugars, polysaccharides that can be hydrolyzed for obtaining fermentable sugars and lignocellulosic biomass [1]. Carob (*Ceratonia siliqua L.*) is a perennial leguminous tree and an important component of the Mediterranean vegetation. It has an important role in the economy of the south of Portugal (Algarve) where 50,000 tons of carob fruit are produced each year, making the region the third largest producer in the world [2]. The global amounts of carob pod production are nearly 400,000 tons per year from about 200,000 ha. This raw material contains fermentable sugars and, to take all benefits of the fruit, in a first step all soluble sugars are extracted and the fibers are treated. These lignocellulosic materials are composed of carbohydrate polymers (cellulose and hemicellulose), lignin and a remaining smaller part comprising extractives and minerals in a complex structure. The cellulose and hemicellulose typically involve up two thirds of the lignocellulosic materials and are the substrates for second generation ethanol production [3]. By hydrolysis of cellulose and hemicelluloses fractions, bioethanol can be produced from lignocellulosic material, by fermentation of the obtained sugar monomers [4]. The so-called 'Simultaneous Saccharification and Fermentation' (SSF) process, has several advantages, performing hydrolysis and fermentation in a single step [4]. However, enzyme activity is forced to be far below its potential, because the enzymatic hydrolysis reaction in SSF process is operated at a temperature lower than the optimum level of enzymatic hydrolysis. A Nonisothermal Simultaneous Saccharification and Fermentation process (NSSF) was suggested, in order to overcome this problem [5]. In NSSF process, saccharification and fermentation occur simultaneously but in two separate shake flasks at different temperatures [5]. The lignocelluloses is retained inside a hydrolysis shake flask and hydrolyzed at the optimum temperature for the enzymatic reactions (i.e. 50ºC). The supernatant from the hydrolysis shake flask is recirculated through a new shake flask to ferment sugars from hydrolysis, which runs at its optimum temperature (i.e. 30ºC) [5]. In this work, tests were performed with different pretreatment solutions, to choose the best one. The best result was obtained with diluted acid at 100 ºC with a total sugar recovered of 25.54g/l. This pre-treatment will increase the sugar concentration in the medium as well as preparing the lignocellulose material for cellulase activity. After the hydrolysis reaction using Celluclast 1.5L and Novozyme 188 enzymes, an increase of 2.34 g/l of total sugar was obtained. In order to find out if there are compounds that inhibit the growth of fermenting microorganisms, all supernatants obtained through acid and enzymatic hydrolysis were tested. The fermentation with pretreatment supernatant had a yield of 49 % (ethanol/fermentable sugars) and the mixture of both supernatants mentioned above had also approximately 50 % of ethanol production. Since no inhibition occurred, SSF and NSSF were tested in different specifications: i) SSF with pretreated pellet; ii) NSSF with enzymatic hydrolysis followed by fermentation; iii) Final pretreated mixture in a SSF system and iv) the final pretreated mixture in a NSSF system. The best production of ethanol (73.2 g/l) was achieved by the NSSF process (iv), with a product/substrate yield of 38%. The low value of total sugar obtained by enzymatic hydrolysis is compensated by sugars from acid hydrolysis. It is reported that the existence of inhibitors after hydrolysis can interfere in ethanol production, however, in our case, it is suggested that the initial high sugar concentration caused this inhibition. Therefore, we can reach values very close to the theoretical yield which is very promising for 2nnd generation bioethanol production at large scale.

Keywords: Enzymatic hydrolysis, ethanol, carob pulp, SSF and NSSF.

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Bioleaching of precious metals from low-grade copper ores using mixed consortium in air-uplift bioreactors: performance evaluation under single and two stage configurations

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Bioleaching processes are considered to be more attractive, economical and environmental-friendly for solubilizing precious metals from low-grade mineral ores than the conventional chemical methods. The key to enhance the bioleaching efficiency is to provide suitable microbes, either suspended or attached to ore particles, with a conducive environment for their growth and survival. Considering this requirement, the present research work aimed to investigate the effectiveness of suspension leaching and percolate leaching methods for the extraction of precious metals from low-grade copper ores using a consortium of chemolitho-autotrophic bacterial cultures in an air-uplift bioreactor. Air-uplift bioreactors have several competitive advantages over stirred tank reactors and rotating column bioreactors in bioleaching studies, for example, simplicity, ease of operation, excellent heat transfer capacity, favorable interphase mass transfer, and good mixing property/shear rate.

For this work, a 5L capacity, custom-designed air-uplift bioreactor equipped with automatic pH, temperature and air flow rate controllers was employed in single and two-stage configurations. In the single stage configuration, the ore samples were suspended directly into the bacterial consortium maintained in air-uplift bioreactors. On the other hand, in the two stage system, the ore samples were packed in a column and the bacterial consortium was percolated and collected back into the air-uplift bioreactor in a repeated sequences. The chalcopyrite ore sample, collected from a mining industry (KGHM Polska Meidz, Lubin, Poland) was ground to the particle size of less than 2 mm and characterized using acid digestion, sequential extraction and SEM - EDX (Scanning Electron Microscope - Energy Dispersive X-ray Spectroscopy) methods before use. A mixed consortium of *Acidothiobacillus caldus, Leptospirillum ferriphilum, Leptospirillum ferrooxidans* and *Sulfobacillus thermosulfidooxidanse* was prepared (each 25% v/v) and used under controlled experimental conditions for bioleaching over a time period of 10 days. The following experimental conditions were maintained during the course of the experiment: total pulp density - 2%; pH -1.5; temperature - 40°C; and air flow rate - 100 L/hr. The time-series trends in the leaching pattern of Copper (Cu), Silver (Ag) and Iron (Fe) from the ore samples were studied by periodically analyzing their corresponding concentrations in the bioleach solution using ICP-MS (Inductively Coupled Plasma – Mass Spectrophotometry). The bacterial attachment onto the chalcopyrite ore surface was characterized by SEM-EDX. Appropriate bioreactor configuration identified for the mixed consortium to achieve maximum bioleaching efficiency for Cu, Ag and Fe from low-grade copper ores will be discussed.

Keywords Bioleaching; Chacopyirte ore, Air-lift bioreactor; Copper; Mixed consortium, Single and two stage

Bioleaching of zinc from low-grade complex sulfide ores by *Pseudomonas aeruginosa* UTMC 01404

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Nowadays, because of high expenses of chemical extraction of minerals from low-grade ores, bioleaching is of interest. Bioleaching is commonly done by use of bacteria such as *Thiobacillus* genus. However, high enzymatic ability of *Pseudomonas* aeruginosa makes it a good candidate for bioleaching reactions.

In this study, Zinc extraction has been investigated in a synthetic media using *Pseudomonas aeruginosa* UTMC 01404. Zinc extraction has been evaluated in the presence of different glucose concentrations (2, 4 and 6 g/l) at 220rpm and 37C during 6 days. The total number of bacteria and the media pH of flasks have been estimated every 12 hours of the incubation as the signs of bacterial growth and activity. The amount of extracted Zinc has been assayed daily. The highest amount of Zinc has been extracted after 5 days of incubation from the flasks containing 6 g/l glucose.

Keywords Bioleaching, *Pseudomonas aeruginosa*, Zinc

Biomass and lipids production by *Cunnighamella elegans* UCP 542 using glycerin as carbon source

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The lipids are found in fungi as components of membranes, cell wall reserve material and in some cases extracellular compounds. The proportion of membrane lipids and reserve material and types of lipids found in fungi are determined by the conditions of growth and development. The main of this work was to evaluate the biotechnological potential of *Cunninghamella elegans* related to biomass and lipids productions using alternative substrates glycerin, as carbon sources. *C. elegans* was grown in the media synthetic medium for Mucorales and modified (substitution of glucose by glycerin obtained from biodiesel production), pH 6.0, and inoculums of spores suspensions 10^7 /mL. The Erlenmeyer's flasks were incubated at 28^0C in orbital shaker of 150 rpm, during 5 days. A factorial design 2^2 with four central points, and variable response was biomass and lipids productions. The lipids were extracted from biomass using methanol/chloroform, and the amount of lipids was determined by gravimetric method. The best condition was observed using the inexpensive medium (glycerin) for its growth of *C. elegans* developed in this study. The results showed the biomass yield of 1.7g/L, and the higher total lipids obtained were 8.0% in the same condition. From these results it was found that the glycerin in the synthetic medium did not affect the morphological and microscopic aspects of the fungus. The inexpensive medium (Synthetic medium for Mucorales supplemented with glycerin) for biomass and fungal lipids were dominated by palmitic and linoleic acids, corresponding to a 100 and 500 times comparing with the synthetic medium for Mucorales.The agro industrial residue glycerin could be indicated as alternative source of carbon and nitrogen in order to facilitate the biotechnological production of biomass and lipids, and contributed with the reduction of industrials residues accumulation.

Keywords: Total lipids, Mucorales, Oleaginous microorganisms

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Bioscouring of jute fabric with thermostable xylanase from *Bacillus pumilus* ASH

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A cellulase free, alkaline, thermostable xylanase produced by *Bacillus pumilus* ASH has been proven to be a cost effective bioscouring agent for the jute fabric. Bioscouring is an eco-friendly process in comparison to the chemical based scouring process. An enzyme dose of 5 IU/g of oven dried jute fabric resulted in release of 112% more reducing sugar and weight loss of 98% as compared to control when incubated at 55 ºC. An incubation time of 120 min is sufficient to increase the whiteness and brightness of fiber up to 3.93 and 10.19% respectively and decreases the yellowness to about 5.57%. Addition of chelating agents such as ethylenediamine-tetra-acetic acid (EDTA) and wetting agent as tween-20 greatly enhances the enzyme action. Bioscouring of jute fiber with xylanase enzyme in addition with EDTA and tween-20 resulted in an increase of 9.63, 4.28 and 10.71% of reducing sugars, whiteness and brightness respectively as compared to conventional process. Further bleaching of jute fabric improves the various jute properties like tenacity, brightness, yellowness and whiteness.

Keywords: xylanase, bioscouring, jute, brightness, whiteness, yellowness

Biosurfactant production by *Rhodotorula glutinis*: emulsifying property and stability

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Biosurfactants are amphipathic molecules with hydrophobic and hydrophilic portions that are distributed preferentially at the interface between fluid phases through different degrees of polarity and hydrogen bonding. These properties give these surfactants the ability to reduce surface tension and promote the formation of microemulsions for the solubilization of hydrocarbons. In addition those characteristics of emulsification are employed to use renewable sources for biosurfactant production. These characteristics confer excellent detergency properties and emulsifiers that make biosurfactants which one of the most versatile functionality under extreme conditions. However, the microbial production of biosurfactants is limited to cost and microorganisms producer. In an attempt to reduction of these costs agro-industrial wastes have been used for surfactants production. The production of biosurfactant was carried out using the medium composed by 25% post frying oil and 2% of corn steep liquor. The fermentation for biosurfactant production by *Rhodotorula glutinis* UCP (1555) was conducted during 96 hours, under agitation of 150 rpm and at 28°C. After the fermentation process, the net metabolic liquid free cells were obtained and the biosurfactant production was determined by two methods: surface tension, and emulsification. The ability of *R. glutinis* grow and produce biosurfactant on substrate containing waste as the likely sources of carbon (post frying oil) and nitrogen (corn steep liquor) was demonstrated. The maximum reduction in surface tension of the water 72 to 34.01 mN/m, and the emulsifier index was 100% to post oil frying. The biopolymer showed significant results for the interfacial tension (14.7 mN/m). The stability results showed to be effective, whereas for NaCl concentrations of between 2 to 20%, using the net metabolic liquid containing the biosurfactant to surface tension. However, the stability determined by emulsification index showed effective results to all sodium chloride concentrations, and all temperatures and pH 10 tested. The results showed higher biotechnological potential of *R. glutinis* UCP 1555 to biosurfactant production as effective tensio-active and emulsifier agent, demonstrating potential for application in bioremediation processes.

Keywords stability; emulsification index; biosurfactant; *Rhodotorula glutinis*

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Biosurfactant production by *Cunnighamella elegans* UCP 542 using corn steep liquor and soybean oil as substrates

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Biosurfactants are amphiphilic compounds produced on living surfaces excreted extracellularly, and contain characteristics hydrophobic and hydrophilic moieties, that reduce surface tension and interfacial tensions between the surface and interface, respectively. The biosurfactant exhibit emulsification properties are often categorized as emulsifiers may not lower surface tension. The biosurfactants are classified by their chemical composition and microbial origin. These can be classified according to their molecular weight, being mostly produced by bacteria and yeasts and filamentous fungi. The aim of this study was to evaluate the *Cunninghamella elegans* UCP 542 to produce biosurfactant using agro industrial substrates corn steep liquor and soy bean oil, as nitrogen and carbon sources. The biosurfactant production was carried out using Erlenmeyer's flasks of 250ml of capacity containing 100 mL of the medium [corn steep liquor 7% and soy bean oil 5%], incubated at 28 º C, under orbital agitation at 150 rpm, during 48 hours. The fermentation was performed in triplicate. The isolated biosurfactant determination of surface tension was performed using the cell-free metabolic liquid being measured in an automatic tensiometer. The free cell metabolic liquid was used to biosurfactant extraction and was precipitated using acetone as solvent, and maintained in refrigerator until precipitation. After this period the precipitated was separate by filtration, and was evaporated at room temperature.The isolated biosurfactant was characterized as polymeric structure containing proteins, carbohydrates and lipids. The results showed that the biosurfactant was able to reduce the water surface tension of 70 to 28 mN/m, with 48 hours of growth. The filamentous fungi *C. elegans* UCP 542 showed ability of bioemulsifier activity using burned engine oil corresponding to 94.2%, followed the use of waste vegetable oil from fast food corresponding to 83.13%. However, the emulsification activity do not was observed too gasoline and diesel oil, respectively. These data suggest the biotechnological potential of biosurfactant produced *C. elegans* and possible applications of this compound to bioremediation process to remove hydrophobic pollutants.

Keywords: Bioemulsificants, *Cunninghamella elegans*, chemical properties.

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Biosurfactant production by *Mucor circinelloides* Using Apple peel, vegetable oil and corn steep liquor as Substrate

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Surfactants are amphipathic molecules composed of a hydrophobic portion and a hydrophilic portion, and have a propERTY OF surface tension REDUCTION and have high emulsifying capacity. The biosurfactants are compounds of microbial origin that exhibit surfactant propertY, low toxicity and high biodegradability. In this study, biosurfactant production by Mucor circinelloides was studied, using a factorial design of 22, to evaluate the influence of the MEDIUM components AND THE COMBINATION OF: apple peel (5 g, 12.5 g, 20 g) and soybean oil post -frying (5%, 7.5%, 10%) associated with corn steep liquor (2%), pH 6.5. The tests were kept at room temperature for 144 hours and static. The results showed that in all conditions tested there was a reduction of the surface tension of 44.9 mN / m to 33mNm, however, the biosurfactant produced emulsifier showed great promise. The indices were evaluated using emulsification: diesel, motor oil, soybean oil after frying and motor oil. The best results were seen emulsification index at the centralpoint (12.5g of apple peel, 7.5% soybean oil after frying and corn steep liquor 2%) with soybean oil after frying (93.6%.), engine oil burning (80%), motor oil (78%). We observed an increase in pH (8.0) in all trials.The results indicate the feasibility of production of biosurfactant withemulsifying properties from low-cost way formulated with apple cores and vegetable oil.

Keywords: Apple peel, Biosurfactant, *Mucor Javanicus*.

Biosurfactant production by *Pantoea sp*. in submerged fermentation using pineapple peel as an alternative medium

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The biosurfactants are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces, or excreted extracellularly and contain hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively. The biosurfactants are molecules produced by bacteria, filamentous fungi and yeasts when grown in different carbon sources as a result of their metabolism. The aim of the present study was to investigate the biotechnology potential of *Pantoea* spp. for biosurfactant production. Tests were carried out to detected hemolytic activity by *Pantoea* spp. The presence of the halo produced in blood agar suggested the biosurfactant production. Then submerged fermentation was carried out using a factorial design 2^2 to evaluate the influence of the components of the medium: corn oil, corn steep liquor and pineapple juice obtained from the bark for biosurfactant production by the bacteria. The Erlenmeyer's flasks were incubated at 30°C, at 150rpm during 72 hours. The liquid metabolic of free cells was used to evaluate te biosurfactant production by the reduction of surface tension, emulsification index, toxicity test, isolation and chemical characterization of the biosurfactant. The results showed the bacteria *Pantoea sp*. water reduction of surface tension of 69.97 ± 0.12 to 40.05 ± 0.12 mN/m. The emulsification activity rates were observed with motor oil, diesel, gasoline, kerosene, soybean oil, corn and sunflower oils. The toxicity test used to *Artemia salina* showed lower toxicity. The chemical composition of the biosurfactant indicated a polymeric structure containing lipids, proteins and carbohydrates. The biotechnological results showed higher potential of *Pantoea* spp to biosurfactant production and suggest the use of pineapple peel waste for tensio-active agent production as a low-cost medium.

Keywords: Biosurfactant, *Pantoea spp.,* Pineapple peel.

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Biosurfactant production by *Rhizopus arrhizus* using agro industrials substrates as alternative medium

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Surfactants are compounds that lower the surface tension of a liquid, between two liquids or between a liquid and a solid. Surfactants act as **emulsifiers, wetting and foaming agents, dispersants** and **detergents.** Structure composed of the hydrophobic and hydrophilic groups, also known as amphiphilic substances. Interest in microbial surfactants has increased considerably in recent years, especially due to their alternative substrates considering the cost of production represents approximately 50% of the final product. The aim of this work was investigated the biotechnological potential of *R. arrhizus* to produce biomass and biosurfactant using soluble agro industrials substrates as rice bran husks and corn steep liquor, as alternative sources of energy (carbon) and nitrogen, respectively. The study was used a factorial design (2^2), using four central points, and having corn steep liquor and rice bran husks according the factorial design, and the biomass and biosurfactant production as the variable response. Biosurfactant concentration was estimated by two methods, the indirect estimation was based on the fact that the surface activity is dependent on the concentration of the active compound and emulsification index. The biosurfactant production was analyzed using liquid metabolic free cells measuring surface tension water reduction and emulsification index (E_{24}) using ten oils [soybean oil, canola oil, corn oil, motor oil, engine oil burned, palm oil, cottonseed oil, soybean oil after frying, kerosene and rice bran oil]. The biomass dry was determined by gravimetry after 96 hours cultivation. The best result was obtained in the assay 4 [8% of corn steep liquor and 3% of rice bran husks], biomass production of 9.10g/L. The liquid metabolic of free cell showed a water surface tension reduction of 26.5 mN/m in the assay 6 [6% of corn steep liquor and 2% of rice bran husks], and the best emulsification index of 63.8% in palm oil in the assay 5 [6% of corn steep liquor and 2% of rice bran husks], both at the central point of the factorial design 2^2. In addition, *R. arrhizus* strain used in this study produced biosurfactant when grown in batch cultures with corn steep liquor and of rice bran husks as nitrogen and the energy source. In view of the possible use of biosurfactants, our goal was to develop a continuous production process. Such a process has several advantages as compared with the production of biosurfactants in batch cultivations the exact control of the culture conditions which is essential for high biosurfactant formation by the cells is accomplished.

Key words: rice bran husks; biosurfactant, *Rhizopus arrhizus*

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Biosurfactants productions by *Candida glabrata* strains using industrial wastes as carbon and nitrogen sources

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The industrial waste, once generated, it needs proper destination, in consequency the environmental problems, represent a loss of raw materials and energy, requiring significant investments in treatments to control the pollution. In this sense , the aim of this work it is the use of the industrial waste in biotechnology process, mainly biosurfactant production, considering the low cost. The biosurfactants are microbial compounds that have the ability to reduce the surface tension as due to their amphiphilic characteristics. They have several important industrial and environmental applications, particularly as humectants, surfactants, in cosmetology, in therapeutic preparations, in control of environmental pollution systems by crude oil and its derivatives. The strains of *Candida glabrata* UCP 1002 isolated from mangrove sediments (Rio Formoso city, PE, Brazil) and *C. glabrata* UCP 1556 isolated from soil of semi-arid region located in the interior of Pernambuco state, Brazil were used for biotechnological production of biosurfactants using industrial wastes as substrates. The studies were carried out using the medium composed by yeast extract (1 g), peptone (1 g), glucose (2 g), $CaCO_3$ (0.12 g) and $MgSO_4$ (0.03 g)/litre as control medium. This medium was modified replacing the carbon by whey (carbon source) and nitrogen source by corn steep liquor (nitrogen source) for biosurfactant production. The fermentations were carried out according to conditions established by factorial design Central Composite Rotational Design (DCCR) of 2^2 during 72 hours, under shaker at 150 rpm, at 28°C. The production of biosurfactant was determined using net metabolic free cells, were submitted to determination of surface tension and emulsification index. The growth was estimated by biomass determined gravimetrically. The results showed that the strain of *C. glabrata* UCP 1002 showed higher potential to produce biosurfactant as due to reach the lower surface tension reduction of 72 to 33.2 mN/m) in medium composed by 25% whey and 11.2% of corn steep liquor. For the rate of emulsification, the best results were obtained under the same conditions of the factorial design, with values of 25% whey and corn steep liquor 4% for both strains of *C. glabrata*1002.However the best efficiency was observed in the strain UCP 1556 removal the hydrophic pollutant removing 90% with showed that strains, higher emulsifying properties.

Keywords: Corn steep liquor; whey; *Candida glabrata*; biosurfactant

Brazilian biodiversity in a Culture Collection devoted to the identification and preservation of microorganisms useful in Environmental, Industrial and Applied Microbiology

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The Andre Tosello Foundation was created in 1971 and its Tropical Culture Collection is the big one of Latin America with 7570 strains, where 4004 are Bacteria, 2121 fungi and 1445 yeasts. These strains come from research 4452, from industry 1229 and 1889 from other culture collections. Those strains are representative of the brazilian biodiversity. In the future the Foundation intends to create a microalgae collection because of the importance of these microorganisms mainly for the Food Industry, Pharmaceutical, Medical, third generation energy (biocombustibles) production, and so on. Brazil and the South America countries have many reasons to be involved with their big diversity, and to use then to the welfare of the population.

This paper will treat the Tropical Culture Collection to show how it is, the areas it is involved: Applied microbiology, Culture and preservation methods, Environmental protection, Industrial microbiology, Fermentation, Freeze drying and so on, and also about the services and training courses it offers: Management of a culture collection, Culture and preservation methods, Propagation, Preservation, Storage services (Bacteria, Yeasts, Fungi), Distribution (Bacteria, Yeasts, Fungi). Its strains can be consulted in the catalogue in the site www.fat.org.br. This collection is registered at WFCC, in the WDCM number 885. Now by developing a project with the financial aid of the Foundation of Research and Development of S. Paulo State - FAPESP the Tropical Culture Collection is having its infrastructure modernized, with novel equipments, and it is implanting the identification of the strains by molecular biology, complementing the techniques in use of biochemical identification. So, it is the same as a Biological Resource Center and use its facilities in infrastructure for innovation in science and technology.

Keywords: FAPESP, culture collection, bacteria, fungi, yeast, resource center, biodiversity

Cellulase production by *Penicillium* sp. strain IS-07 using agro-industrial by-products

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Cellulases are enzymes with a high potential of biotechnology and is currently being applied as biocatalysts in a series of industrial processes and can be produced by different fermentation processes. Brazilian tropical environments show a rich biodiversity, making it promising to search for new microorganisms with biotechnological applications, especially lignocellulolytic fungi and bacteria, which are extremely important. Thus, this study aimed to evaluate the production of cellulase (CMCase and FPase) by the strain *Penicillium* sp. IS-07 in submerged fermentation using sugarcane straw as a carbon source and corn steep liquor as a nitrogen source, using the methodology of factorial design 2^{5-1}, varying temperature, agitation, inoculums concentration, carbon and nitrogen source concentration. The measurement of enzyme activity was determined by quantification of reducing sugars by DNS method. The best enzymes production of CMCase (2,778.1 U / L) and FPase (104.2 U / L) was detected after 4 days of culture, in assays 16 and13, respectively, and the data statistically significant at 10%. This study demonstrates that the strain *Penicillium* sp. IS-07 features high production of cellulase and potential for optimization of enzyme production, in order to develop products with biotechnological potential.

Keywords: *Penicillium* sp. IS-07, cellulase, corn steep liquor, sugarcane straw

Characterization of new xylanases from *Penicillium canescens*

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In present time the great attention is paid to understanding the role of "silent" genes in organism. This knowledge may allow finding out the evolutionary ways or metabolic pathways or discovering "old" enzyme with a new function or properties. In this study we investigated family of endoxylanases of *Penicilliun canescens*. Endoxylanases (EC 3.2.1.8) depolymerize the major chain of xylan formed by β-1,4 linked residues of D-xylose into short oligosaccharides. Xylanolytic enzymes widely used in pulp and paper, textile, and fodder industries; they are also used for utilization of agricultural wastes. Fungi of the genera *Aspergillus, Trichoderma*, and *Penicillium* are currently the main producers of fungal endoxylanases.

Four novel genes coding for endoxylanase family were described in the mycelial fungus *Penicillium canescens* have been cloned. The *xylB, xylC*, and *xylD* genes encode endoxylanases of glycosyl hydrolase family 11; the *xylE* gene, those of family 10. In the promoter region of the *xylB, xylC*, and *xylD* genes, the binding sequences for the transcriptional activator of xylanolytic genes have been found.

Since the TATAA sequence, which is an element of the minimal eukaryotic promoter, has not been found in the promoter region of the *xylC* gene, in contrast to those of the *xylB* and *xylD* genes, it may be assumed that this gene is silent. Comparative phylogenetic analysis has shown that the cloned genes are highly homologous to some endoxylanase genes of mycelial fungi of the genera *Penicillium* and *Aspergillus*. However, within the species *P. canescens*, they exhibit a low homology both within and between families, and they diverge into different branches of the phylogenetic tree, which suggest divergence of the genes of this group at an early stage of evolution.

The *xylB, xylC*, *xylD* and *xylE* genes were homologically expressed in *P. canescens*. All plasmids contained xylanase's gen under control of strong promoter - *bgaS P. canescens*. We obtained four strains PCE-7/pPCGXD, PCE-7/pPCGXB, PCE-7/pPCGXE and PCE-7/pPCGXC that produced xylanases D, B, E and C respectively. Unfortunately the level of enzyme production was sufficient for obtaining of only Xyl E and Xyl C homogeneous enzyme preparations that were purified and characterized. Purified enzymes belonged to family 10 (Xyl E) and 11 (Xyl C) of hydroxyl hydrolases. Mw of Xyl E and Xyl C were 40 kD and 25 kD respectively. pH and temperature optima were 5.0 and 50°C for Xyl C; and 6.0 and 70°C for Xyl E. *K*m и *V*m for Xyl C were 1.34 g/l and 8.2 mkM/(mg·s); and 0,52 g/l and 1,25 mkM/(mg·s) for Xyl E. Thus both enzymes demonstrated properties common for its families.

Also we carried out DSC-analysis of enzymes. According obtained data Xyl E was a very stable enzyme with melting point of the protein globule 73°C in contrast with Xyl C (53°C) . For understanding of nature of stability of Xyl E its 3D structure was solved with resolution 1.5 Å. The 3D structure of xylE had folding of TIM-barrel typical for xylanases of this family. Analyzed enzyme didn't contain carbohydrate binding domain, and the presence of three disulfide bonds explained the relatively high stability of xylanase xylE. We hypothesize that changing of environmental conditions including extreme ones caused the divergence of the genes encoding enzymes with properties that help to survive under extreme conditions. These enzymes can be used as a model for design of biocatalysys with target properties.

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Keywords mycelial fungus; endoxylanase; genes; heterologous protein expression; recombinant protein; enzyme properties; 3D structure

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Chitin and chitosan produced by *Cunninghamella elegans* using alternative medium- coconut water

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Chitin and chitosan hold a great economic value as due to their versatile biological activities and chemical applications, mainly in medical. Recent advances in fermentation technologies suggest that the cultivation of selected fungi can provide an alternative source of chitin and chitosan. The amount of these polysaccharides depends of the fungi species and culture conditions. Filamentous fungi have been considered an attractive source of chitin and chitosan for industrial applications because their specific products can be manufactured under standardized conditions. However, to optimize the production of chitin and chitosan from fungi, it's usually used complex or synthetics cultures media, which are expensive. It's becomes necessary to obtain economic culture media that promote the growth of fungi and stimulate the production of the polymers. Economic microbial culture media normally use vegetables components. Microbiological process was studied for production of chitin and chitosan by *Cuninghamella elegans* grown in Coconut water. A laboratory assay was carried out to evaluate the *Cunningamella elegans* growth using Coconut water during 96 hours, under agitation (125 rpm) at 28°C. The mycelial biomass was determined following lyophilization. Chitin and chitosan were extracted by alkali-acid treatment, and the polymers were characterized by Infrared spectroscopy and viscosity. A higher production of biomass can be verified in 96 hours of growth corresponding to 2,19g/L. In addition, the best yield of chitin (389mg/g) and chitosan (129mg/g) are obtained in 48 hours and 96 hours of growth, respectively. Chitin and chitosan showed degree of deacetilation, respectively of 23% and 80% and viscosimetric molecular weight of chitin and chitosan 2.39 $_x$ 10^4 g/mol and 3.5 $_x$ 10^4 g/mol, respectively. The results suggest that Coconut water improves chitin and chitosan production and the mycelial biomass of *C. elegans* may be used as an alternative source of these polymers.

Keywords Coconut, biopolymers, polysaccharides

Comparison of Lovastatin Synthesis using Glucose and Lactose by Metabolic Flux Analysis

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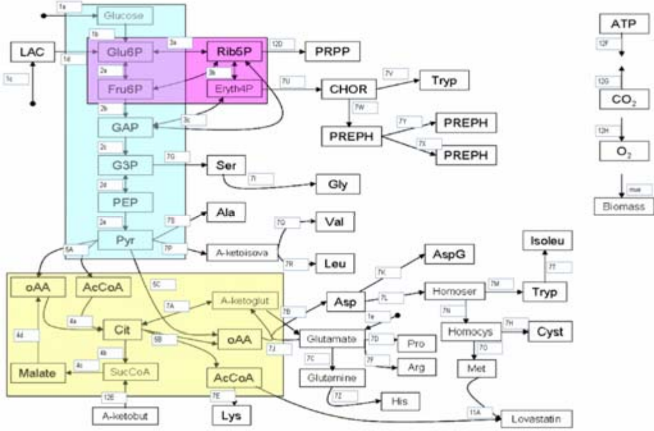
Lovastatin is a natural statin produced primarily by Aspergillus terreus and Aspergillus flavipes. It competitively inhibits HMG-CoA reductase and prevents the synthesis of mevalonate, a committed precursor in the cholesterol biosynthetic pathway. The mechanism of inhibition and the use of lovastatin and its many derivatives, as a drug for the treatment of hypercholesterolemia have been extensively studied. However, the synthesis of lovastatin through the polyketide biosynthetic pathway has remained relatively uninvestigated. In this work, we present the metabolic flux analysis of lovastatin biosynthesis and examine the differences that occur when two different carbon sources, glucose and lactose, are used. Lovastatin was produced by submerged cultivation and macroscopic variables such as, the carbon-source uptake rate and oxygen uptake rate was measured. A detailed stoichiometric model was developed and the internal metabolite fluxes were calculated based on the flux analysis framework. The theoretical maximum yield under given operating conditions and energy audit was determined. The critical P/O ratio was also determined. Our results showed that while the carbon source influences the relative fluxes in the pathways driving growth of cells and lovastatin production, the metabolic state is defined by the oxygen uptake and energy status of the microorganism.



Keywords Lovastatin, metabolic flux analysis, polyketide pathway, theoretical yield, bioreactor.

Detection *Streptococcus mutans spaP* Gen in Dental Plaque Sanples and Its Association With Early Childhood Caries

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Introduction
Tooth decay is one of the most common infectious diseases in humans. The infectious nature of this entity, shows a group of microorganisms isolated mainly human dental plaque, in which stress, bacteria such as mutans streptococci, especially S. S. mutans sobrinus

Objective
The purpose of this study was quantified by polymerase chain reaction (real-time RT-qPCR), the SPAP gene S. mutans in dental plaque samples and correlate the data found with early childhood caries.

Materials and Methods
Comprised of children attending the Child Development Center ALFA Gady (Monterrey-Nuevo. Leon, Mexico). The total number of children between 12 months and 46 months of age during the 2009-2010 academic year was 92. Of these, 83 met the inclusion criteria, but in the end only took 80.

Results
Amplification with the primer for the SPAP gene nucleic acids extracted from S. mutans was positive for 91.3% of cases, all children with caries were positive for the SPAP and only 8.75% were negative, placing these within the group of children without caries.

Conclusion
There is an association between the presence of S. SPAP gene mutans and patients diagnosed with early childhood caries. There is a close association between the prevalence of early childhood caries and the percentage of S. mutans of total bacteria detected by real time PCR.

Development of silage fermentation by using of *Lactobacillus casei* and *Lactobacillus bulgaricus*

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Lactobacillus casei and *lactobacillus bulgaricus* have been tested according to their acidifying capacity, because the end fermented silage pH should be 4 or less to perform the two main objectives of the fermentation. *Lactobacillus bulgaricus* was most acid tolerant than *lactobacillus casei*. After 10 days of incubation time at 30ºC, results indicated that, pH level was decreased up to 2.8 in case of using *Lactobacillus bulgaricus*, up to 3.0 in case of using *Lactobacillus casei*, and up to 2.8 in case of using mixed culture of them. Results of the acidifying capacity test may suggest that the inoculums whether, alone or in mixed culture can tolerates low levels of acidity that obtained from the silage fermentation process and may suggest that the use of the two organisms in a mixed culture is the favored. bulgaricus and *L. casei* have been tested as successful inoculum to poultry and rumen silages. The two organisms can be used as a silage inoculum, but they fermented the poultry silage quickly more than the rumen silage. This difference in the acceleration of the fermentation may be back to the difference in the chemical structure of the two silages. The poultry silage needs no additives (beside the inoculum) to accelerate the fermentation process, when lactobacillus casei and lactobacillus bulgaricus have been used as fermenters alone or in combination. On the other hand, rumen silage needs additives (beside the inoculum) to accelerate the fermentation process. Also, production of lactic acid in poultry silage was higher than in rumen silage at the same times of the fermentation. This result may suggest that the available sugar amount was higher in poultry silage than in rumen silage. The results emphasizes that, Using of *Lactobacillus casei* and *Lactobacillus bulgaricus* as microbiological silage additives improve the silage fermentation process

Diversity of AOB communities in aerobic granular sludge at different SBR cycle length and wastewater composition

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Ammonia-oxidizing bacteria (AOB) communities in an aerobic granular sludge were investigated in three constantly aerated column sequencing batch reactors (SBRs) treating anaerobic sludge digester supernatant. The SBRs differed in the length of a working cycle that was 6 h, 8 h and 12 h in reactors 1, 2 and 3, respectively. In series 1, a 1:1 mixture of supernatant and municipal wastewater was introduced to the reactors that resulted in Total Kjeldahl Nitrogen (TKN) concentration in the influent of 280 mg N/L. In series 2 the reactors were fed only with supernatant and TKN in the influent averaged 570 mg N/L. The efficiency of nitrification in series 1 varied between 68-84%. In series 2 nitrification efficiency increased and, regardless of the length of the working cycle, averaged 93%. Diversity and species composition of AOB were determined using PCR-DGGE targeting *amoA* gene and sequencing. The diversity of AOB communities in granular sludge depended on both the TKN concentration in the influent and the length of the SBR cycle. At the given length of the cycle, a higher AOB diversity was observed in series 2, at TKN concentration in the influent of 570 mg N/L. In both experimental series, lengthening of the SBR cycle was negatively correlated with AOB diversity. In series 1 the AOB diversity, expressed by a Shannon-Wiener index of diversity, decreased from 1.76 in reactor with a 6-hour cycle mode to 1.53 in the reactor with a 12-hour cycle mode while in series 2 it lowered from 2.64 (6-hour cycle mode) to 1.92 (12-hour cycle mode). The highest AOB diversity characterized the granular sludge from the reactor with the 6-hour working cycle treating anaerobic sludge digester supernatant. Comparison of DGGE patterns using cluster analysis showed that the main factor selecting for different AOB communities was wastewater composition. AOB communities characterizing granular sludge samples from each series had a high level of similarity and created separate clusters. Sequences of isolated DGGE amplicons grouped with the sequence of *Nitrosospira* sp. REGAU.

Keywords aerobic granules; biodiversity; ammonia-oxidising bacteria,anaerobic sludge digester supernatant

Docosahexaenoic Acid Production by Heterotrophic Microalga, *Crypthecodinium Cohnii* in Whey and Corn Steep Liquor Medium

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The therapeutic significance of ω-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) has recently been a resurgence of interest since these compounds are now recognised as having a number of important nutraceutical and pharmaceutical applications. Microalgae are the primary producers of ω-3 polyunsaturated fatty acids and much effort is being devoted to developing a commercially feasible technology to produce EPA or DHA directly from microalgae. As it is well-known, economic feasibility of a bioprocess could be improved by using cheaper substrates, such as industrial by-products or wastes. Therefore, in this study; *Crypthecodinium cohnii*, a marine heterotrophic microalga used for the commercial production of docosahexaenoic acid ((DHA,22:6n-3) was cultivated in whey and in media containing corn steep liquor (CSL). Moreover, biomass productivity, lipid content and DHA proportion of the total fatty acids in both media were compared to those obtained in synthetic medium.

In preliminary experiments, different proportions of whey and CSL supplemented with sea salt were tested separately. CSL was added to the sea salt medium in a range of 0.5- 20g/100ml and whey was used at various concentrations between 25-100% (v/v). The maximum biomass productivity, lipid content and DHA proportion of the total fatty acids were found to be 0.4 g/l.d, 25-35% and 35-45%, respectively. The highest biomass productivity (1.6 g/l.d) was achieved at 20 g/100 ml CSL with 11% lipid content of dry biomass and 25-30% DHA proportion of the total fatty acids. Decreasing amount of CSL resulted in an increase in lipid content of biomass while the biomass productivity was still higher than the value obtained in the complex medium. The addition of glucose into CSL medium increased the biomass productivity up to 3 g/l. d. *C. cohnii* cultivated in whey had also higher biomass productivity (0,6 g/l.d) than that of the complex medium with almost the same 21-26% lipid content of dry weight (21-26%). In the following experiments, statistical screening of medium components for the lipid production by *C. cohnii* using both whey and CSL was carried out separately by Taguchi design. Significant components affecting the biomass productivity and lipid content of *C. cohnii* were optimized by using response surface methodology. Thus, this optimized medium conditions which was provided a unique opportunity to utilize large quantities of whey and corn steep liqour through fermentation of the alga *Crypthecodinium cohnii* on these by-products to produce docosahexaenoic acid (DHA,22:6n-3).To our knowledge, this is the first report about DHA production by *C.cohnii* using whey and CSL.

Keywords Docosahexaenoic acid; heterotrophic microalgae; *Crypthecodinium cohnii*; whey; corn steep liquor

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Effect of Dibenzothiophene on the Ultrastructure of *Cunninghamella elegans* UCP 542

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Polycyclic aromatic hydrocarbons (PAH) and heterocyclic aromatic compounds (HAC) are of environmental problem which concern their recalcitrant and carcinogenic behavior. Xenobiotic compounds induced changes in the biochemistry, physiology and morphology of eukaryotic and prokaryotic cells, causing cellular alterations. Thus, information on ultrastructure is important tools employed in elucidating adjacent mechanisms to cellular adaptation in the presence of recalcitrant agents. The aim of this work was to analyze the ultrastructure of the *Cunninghamella elegans* grown in the presence of dibenzothiophene (DBT) using the routine and cytochemistry catalase methods observed by transmission electron microscopy (TEM). The results obtained using the technique of routine showed variation in fine structure of hypha, variations in texture, electron density cytoplasm, electron density number of bodies and mitochondria. Additionally, the cytochemistry method for catalase showed variations in the intensity and distribution of products of reaction in the cell wall, in the cytoplasm and mitochondria. In the mitochondria markers are quite evident when is compared with the control (without DBT treatment). The variations observed by TEM are related to the increase of concentration of DBT. The results showed important information's about the effect of xenobiotic DBT on the fine structure of the fungus *Cunninghamella elegans*.

Keywords: Catalase; Cytochemistry; *Cunninghamella elegans;* Dibenzothiophene; Ultrastructure.

Effect of different physicochemical parameters on the Kefir grains production using Taguchi design

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Kefir is manufactuered by inoculating milk with kefir grains. These grains contain lactic acid bacteria (Lactobacillus, Lactococcus, Leuconostoc), acetic acid bacteria and yeast mixture coupled together with casein and complex sugars by a matrix of polysaccharides denominated kefiran.

Taquchi design and Mini-fermentation system was used to optimize the kefir grain production.

The effects of different parameters including percentage of fat in milk, skim milk proportion, incubation temperature, incubation time, tryptose, glucose, CO_2 concentration and amount of milk on the percentage yield of kefir grains were investigated. The results showed that best media for kefir grain production were skimmed milk and low fat milk. Results of Taguchi design L-18 showed that the effects of percentage and volume of skim milk were 46 and 24%, respectively. Effect of temperature (37°C) was only 5.9% and although fat inhibits kefir grain production but its effect is only 1.8%. It was concluded that the Taguchi design is a promising technique to evaluate effect of multi variables simultaneously and economically.

Key words: Taguchi, Optimization, Mini-fermentation, Kefir grain manufacturing

Encapsulation of yeast for efficient 2nd generation bioethanol production: Finite element modeling of concentration profiles in encapsulated yeast

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With the increasing energy demands and pressures on the environment, 2nd generation ethanol, produced from lignocellulosic raw materials, appears as an economically and environmentally viable alternative to oil-based fuels. The lignocellulosic materials have a recalcitrant structure, and must be pretreated before hydrolysis to fermentable sugars. Unfortunately, many inhibitory compounds are formed during this process.

The inhibitory properties of lignocellulosic fermentation media usually lead to reduced rates of ethanol production or even complete absence of fermentation. Encapsulation of yeast cells in 3-4 mm large membrane capsules of Ca-alginate and chitosan has been shown to improve the inhibitor tolerance of the yeast and lead to improved production rates. A likely explanation to this is that the outer layers of cells can detoxify some of the inhibitors, thereby protecting the cells situated deeper in the capsule.

Another issue with lignocellulose materials is the sometimes high pentose content. Xylose is the most abundant pentose. Recombinant xylose-fermenting *Saccharomyces cerevisiae* strains have been constructed, but the xylose uptake rate is hampered by competitive inhibition by glucose. Our hypothesis is that concentration gradients in the capsules may cause favourable conditions for simultaneous uptake and metabolism of both glucose and xylose even in the presence of lignocellulosic inhibitors.

To investigate this we simulated the concentration profiles for glucose, xylose, furfural and HMF by finite element modeling using COMSOL Multiphysics 4.1. An example of such concentration profiles is shown in figure 1. Xylose consumption was found to always be improved by encapsulation of cells. For glucose, encapsulation may be beneficial if the combined inhibition effect is strong enough.

The model formulated in this project was validated by comparison to pulished experimental values. Generally, the model fitted the experimental data reasonably well, after an adaptation of the maximum glucose consumption rate. Additional experimental work to validate these simulation results are underway.

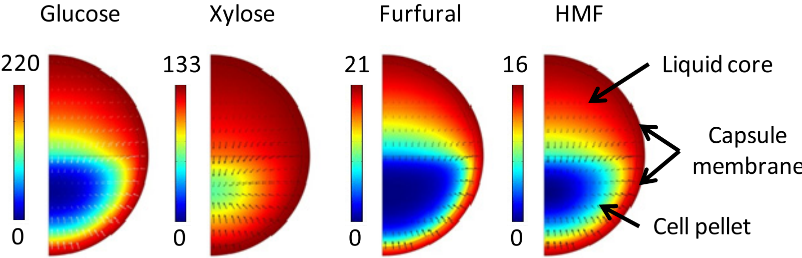

Figure 1. Simulated steady state concentration profiles of glucose, xylose, furfural and 5-hydroxymethylfurfural (HMF) in a liquid core capsule half filled with yeast. The yeast cell pellet is the lower half of each semicircle, the liquid core is the upper half, and the capsule membrane surrounds the circumference of the sphere. The capsule is rotationally axisymmetric around the vertical left boundary of each figure. The scale bars indicate the simulated concentrations in mM, the highest values represent the external concentration of each compound. The used model included competitive inhibition of glucose on xylose uptake and vice versa, and non-competitive inhibition of the furan aldehydes on uptake and metabolism of the sugars.

Keywords biofuel production; mathematical modeling; lignocellulose; inhibitors; *Saccharomyces cerevisiae*

Evaluation of some aeration conditions in *Erlenmeyer* flasks for toxin production by *Bacillus thuringiensis* grown on glycerol

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Bacillus thuringiensis var. *israelensis* synthesizes a toxin called δ-endotoxin that has lethal action against larvae of mosquitoes transmitting diseases like dengue, malaria and elephantiasis. This bacterium is able to metabolize various substrates, including glycerol, a carbon source of low cost and abundant, due to the huge increase in biodiesel production worldwide. Glycerol is a byproduct of the transesterification reaction of oils and fats made to produce this biofuel. Among many factors that influence the production of this toxin are the presence of an organic and inorganic nitrogen source, adequate concentrations of some cations and oxygen supply. Many studies for the development and / or optimization of this process, especially those involving the formulation of culture medium for bacteria cultivation, are performed in *Erlenmeyer* flasks. The conditions for oxygen transfer to the fermentation medium in this type of flask can vary significantly depending on factors such as flask volume, medium volume by flask volume ratio, type of agitation and shaking frequency. The presence or absence of baffles has also been found to influence the growth characteristic of bacteria that promotes the synthesis of the mentioned toxin. In the present study it was evaluated the influence of some aeration conditions in flasks, on the growth and toxin production by *Bacillus thuringiensis* var. *israelensis*, in medium containing glycerol derived from biodiesel production as a substrate. The composition of the medium, for all tests was (in g/L): glycerol (10), yeast extract (12), $(NH_4)_2SO_4$ (3), $CaCl_2.2H_2O$ (0.12), $MgSO_4.7H_2O$ (1.5), $MnSO_4.H_2O$ (0.09), K_2HPO_4 (1.5) and KH_2PO_4 (1.5). The initial pH was equal to 7.0. The following aeration conditions were tested: the volume of the bottle (500 mL, 100 mL and 1000 mL of medium with 200 mL of medium), type of bottle (with baffles and conventional), type of agitation (orbital and reciprocal) and frequency of agitation (86 rpm and 96 rpm to 180 rpm and reciprocating motion to the orbital motion). The cell growth, before the beginning of of lumps formation by the bacteria was measured by optical density and, after this period, by the dry mass of cells. The concentrations of glycerol were determined by HPLC. Toxin production was determined indirectly as larvicidal activity, by exposure of larvae of the mosquito *Aedes aegypti*, in the fourth instar of development, to different volumes of fermented broth in 6 mL of water. The results were expressed as percentage of death of the larvae over a period of 24 hours of exposure. The profiles of growth and substrate consumption for the tests in 500 mL and 1000 mL flasks were similar, but the larvicidal activity was much higher in 1000 mL flask, with 100% of mortality of larvae with 10 µL of medium against 80% mortality with 50 µL. For the frequency of agitation in a reciprocal shaker, 96 rpm showed higher growth rate and substrate consumption, and in this case, the larvicidal activity was also greater than the smallest agitation (86 rpm). The agitation-type orbital (bottle with baffles) led to a growth rate and substrate consumption considerably higher compared with the reciprocal type (standard bottle). However, the larvicidal activity was higher in the second case, with 100% death of larvae with 200µL of medium, compared to 40% death with orbital agitation. It was concluded that the best condition for aeration is the use of reciprocal agitation-type, conventional *Erlenmeyer* flask and shaking frequency equal to 96 rpm. It was also noted the lack of association between growth and toxin formation by the bacteria under the studied conditions.

Keywords *Bacillus thuringiensis*, oxygen transference, larvicidal activity, δ-endotoxin

Extremophilic bacteria as a new source for nanoparticles and urease production

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Recently, microbial biosynthesis of nanoparticles (NPs), due to the unique properties of produced materials and the worldwide tendency to use eco-friendly systems, has been developed. However, this technology is limited by the problems of the products toxicity for the microorganism used and high cost of the process. Application of extremophilic microorganisms, due to high tolerance to toxic and stress conditions, is considered as one of the most efficient solutions in this respect. In the present study, the microflora of Ramsar geothermal hot springs located in Mazandaran province, Iran, was screened for native thermophilic bacteria capable of biosynthesis of silver and gold nanoparticles. Two strains, identified as "*Ureibacillus thermosphaericus*", showed high potential for silver and gold nanoparticle biosynthesis with extracellular mechanism, and selected for the biosynthesis optimization. Biosynthesis reactions were conducted using the culture supernatant at different temperatures (60-80 °C) and silver and gold ion concentrations (0.001-0.1 M). After 24 hrs, the color of culture media turned to brownish-yellow and red, indicating the presence of silver and gold NPs, respectively. The highest absorbance values, measured by UV-vis spectrophotometer, were 530 nm for gold NPs and 420 nm for silver NPs, which shows that the strains could biosynthesize more concentration of Ag & Au NPs in comparison with the previous works. The produced NPs were characterized by TEM, XRD & FT-IR, and it was shown that the strains could produce spherical NPs in the range of 10-100 nm. The results obtained showed that pure spherical nanoparticles in the range of 10-100 nm were produced, and the maximum nanoparticle production was achieved using 0.01 M Ag-NO3 and 0.001 M HAuCl4 at 80°C. In addition, the selected strains showed high urease activity, and can be used a source for urease production. In conclusion, the findings of this study confirmed the great biocatalyzing potential of the extremophilic *U. thermosphaericus* supernatant for intensified biosynthesis of silver and gold nanoparticles and urease at elevated temperatures and high silver and gold ion concentrations.

Key words: Biosynthesis, Silver, Gold, Nanoparticles, Extremophilic microorganisms, urease, *Ureibacillus thermosphaericus*

Formulation of the media of waste ice cream for the production of lipase by *Bacillus licheniformis* (UCP 1014)

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Enzymes catalyze chemical reactions with great specificity and rate enhancements. These reactions are the basis of the metabolism of all living organisms, and provide tremendous opportunities for industry to carry out elegant, efficient and economical biocatalytic conversions

Lipases are ubiquitous in nature and are produced by several plants, animals, and microorganisms. The lipases of microbial origin represent the most widely used class of enzymes in biotechnological applications and organic chemistry. The fats present in the waste of ice cream have different types of fatty acids: saturated, monounsaturated and polyunsaturated. The nutritional requirements for microbial growth are fulfilled by several alternative media as those based on defined compounds (synthetic medium) like sugars, oils, and complex components such as peptone, yeast extract, malt extract media, and also agro-industrial residues containing all the components necessary for microorganism development. A factorial design is often used by scientists wishing to understand the effect of two or more independent variables upon a single dependent variable. Factorial experiments allow subtle manipulations of a larger number of interdependent variables. Whilst the method has limitations, it is a useful method for streamlining research and letting powerful statistical methods highlight any correlations. This work study was conducted to select an alternative means for lipase production by *B. licheniformis* (UCP 1014) and then the formulation of an alternative means of production, using waste from ice cream industry media through a factorial design 2^4 in conditions variables. The assays occurred during 96h, 150 rpm, 37°C. The results showed that the media C (glucose 1.0%, peptone 2.0%, 0.5% yeast extract, olive oil, 1.0%, $NaNO_3$ 0.1%, 0.1% KH_2PO4, $MgSO_4.7H_2O$ 0.05%), indicating a lipase activity of 256 (U/mL) / min. Tests of the factorial design, the test indicated that 11 had the highest activity of 480 U/mL for lipase production. The results suggest the reuse of waste oil from the ice cream industry for the development and production of microbial lipase.

Keywords: lipase; ice cream waste, *Bacillus licheniformis*

Genetic modification of ergot alkaloid producing fungus, *Claviceps purpurea*, for increasing of alkaloid production

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Claviceps purpurea is an organism, producing ergot alkaloids (EA), the compound interesting in pharmaceutical industry for its biological activity resulting from structural similarities with neurotransmitters.

Costs of EA based medicaments production could be significantly reduced with use of genetically modified *Claviceps purpurea*.

Three genes of EA biosynthetic cluster were selected for overexpression in *C. purpurea* industrial strain because of possibility to affect quantity of produced alkaloids. Constructs with these genes under the control of strong fungal promoter for glyceraldehyde 3-phosphate dehydrogenase were prepared. Industrial mutant strain with high level of EA production was transformed with two of three of these constructs, presence of transgene was detected by southern blot method and higher level of transgene expression was confirmed by both, northern blot method and real time PCR. Transformed *C. purpurea* was inoculated on rye and mature sclerotia were screened for changes in EA production.

Project of *Claviceps africana* genom sequencing was initiated.

Inner parts of genes of interest were amplified with degenerated primers in *Claviceps gigantea* and compared with known sequences of these genes in other *Claviceps* species.

Growth and Productivity Impacts of Periplasmic Nuclease Expression in an *Escherichia coli* Fab' Fragment Production Strain

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Host cell engineering is becoming a realistic option in whole bioprocess strategies to maximise product manufacturability. High molecular weight (MW) genomic DNA currently hinders bioprocessing of *E. coli* by causing viscosity in homogenate feedstocks. We previously showed that co-expressing Staphylococcal nuclease and human Fab' fragment in the periplasm of E. coli enables auto-hydrolysis of genomic DNA upon cell disruption, with a consequent reduction in feedstock viscosity and improvement in clarification performance. Here we report the impact of periplasmic nuclease expression on stability of DNA and Fab' fragment in homogenates, host-strain growth kinetics, cell integrity at harvest and Fab' fragment productivity. Nuclease and Fab' plasmids were shown to exert comparable levels of growth burden on the host W3110 *E. coli* strain. Nuclease co-expression did not compromise either the growth performance or volumetric yield of the production strain. 0.5 g/L Fab' fragment (75L scale) and 0.7 g/L (20L scale) was achieved for both unmodified and cell-engineered production strains. Unexpectedly, nuclease-modified cells achieved maximum Fab' levels 8-10 hours earlier than the original, unmodified production strain. Scale-down studies of homogenates showed that nuclease-mediated hydrolysis of high MW DNA progressed to completion within minutes of homogenisation, even when homogenates were chilled on ice, with no loss of Fab' product and no need for additional co-factors or buffering.

Keywords Cell engineering, periplasm, nuclease, growth kinetics, productivity

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How industrial microbiology could be used to teach biotechnology

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In Spain, secondary school (high school) students have to carry out a research work to complete their studies. Connecting this research work to some aspects of Biotechnology could be an opportunity for the students to make contact with the world of research. The Department of Biochemistry and Molecular Biology, and the Fermentation Unit of the University of Barcelona have been actively involved with the development of activities addressed to secondary students.

Designing a simple research work for secondary school students related to biotechnology could be difficult because it is an interdisciplinary subject difficult to relate toa specific subject, such as chemistry, biology, technology, etc.

We have designed experiments based in alcoholic fermentation which could increase the students' knowledge of biotechnology and could be added to students' research work. Alcoholic fermentation is frequently used in school lectures and experiments, but rarely these experiments involve any aspect beyond ethanol production. For this reason, cellular morphology and metabolism were studied and ethanol tolerance was compared in two yeast strains: *Kloeckera apiculata* and *Saccharomyces cerevisiae*, whichwere isolated from early and final stages of wine production, respectively. Determination of ethanol tolerance was carried out in YPD media with ethanol content ranging from 0% to 20%. The assessment of carbohydrate utilization pattern was done using an API 50CH gallery with an isolated *S. cerevisiae* with 12% ethanol tolerance. Substrate utilization and biomass growth were monitored at different pH and temperature to optimize the ethanol production. In addition, the Crabtree effect was detected during the experiments.

Experiments were carried out in university laboratories with secondary school students (16-17 years old). Each student spent one month at least to perform these experiments. Working together, secondary school and university can improve the general knowledge of microbiology and biotechnology , thus contributing to modify the general negative opinion that society has about these sciences.

Keywords Ethanol tolerance, research work and high school students.

Immobilization of xylanase using the production waste agro-residue as support and its application in chromophore removal from newspaper pulp

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The immobilization supports like agarose, gelatin, alginate, chitosan and polyacrylamide gels get damaged during their reuse due to high temperature of enzyme assay. Although various supports which are stable at extremes conditions are available, yet these are costly and unavailable easily making the process uneconomical. A cheaper support for immobilization of xylanase stable at higher temperature was developed. Xylanase was produced under solid state fermentation using wheat bran by *Bacillus pumilus* SV-85S. Residual wheat bran after the isolation of xylanase following fermentation, was collected, thoroughly washed, air dried, autoclaved and used as support for immobilization. The support was moistened with crude xylanase and incubated at 37°C for 75 min. The activity of bound and unbound enzyme was determined. The immobilized xylanase was characterized and investigated its potential in removal of chromophores from newspaper pulp. The use of residual wheat bran as support makes the immobilization process cost effective. The optimization of process parameters through response surface methodology gave an immobilization yield of 83.6%. The pH and temperature stability was enhanced after immobilization. The bound enzyme displayed a higher V_{max} and it could be reused for 15 cycles still retaining 70.0% of its initial activity. There was no leaching of enzyme after 2 months storage at 4°C. The chromophores were removed efficiently up to 10 cycles with immobilized xylanase. The immobilization protocol proposed here is apparently cost-effective as it reuses the wheat bran. A considerable stability and reusability of the bound enzyme may be advantageous for its industrial application.

Keywords: Immobilization; Xylanase; Xylan; Response surface methodology; Wheat bran

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Improvement of upper limit of thermotolerance in *Saccharomyces cerevisiae* for cost-effective ethanol production by over-expression of *RSP5p* ubiquitin protein ligase

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Using thermotolerant yeast strains to conduct simultaneous saccharification and fermentation (SSF) process at temperatures closer to optimum for commercial cellulases can be a solution to achieve higher ethanol production. Our previous genetic study about the thermotolerant *S. cerevisiae* strain C3723 isolated in Thailand suggested that most plausibly six genes designated *HTG1* to *HTG6* are responsible for this phenotype. Here, we describe Htg^+ strain exhibiting confluent growth at high temperature (41°C) and resistant to heat shock besides its ability to tolerate to ethanol, osmotic and oxidative stresses and hydroxyurea, an agent which induce DNA damage. *RSP5* encodes E3 ubiquitin ligase was cloned as *HTG6* gene. We found that *RSP5-C*, a new allele of *RSP5* gene in Htg^+ strain which possessed five base changes located in promoter, one silent base change in the open reading frame and two base changes in the terminator region is associated with its high temperature resistant phenotype in *S. cerevisiae* thermotolerant strain C3723 and its derivatives. Transcription level of *RSP5-C* allele from Htg^+ strain was higher than that of designated *RSP5-BY* allele originated from the *htg6* host strain (Htg^-) due to the base changes existed on promoter region of *RSP5-C*. We also revealed that increased ubiquitination of proteins in Htg^+ strain was higher than that in Htg^- strains after exposure to temperature up-shift (41ºC). Over-expression of the wild-type *RSP5* allele in Htg^- confered thermotolerance at 41ºC as in the case of *RSP5-C* allele. Moreover, we found that an Htg^+ strain bearing an over-expressed *RSP5-C* exhibits more ability to tolerate higher temperature (43°C). This research illuminated that over-expression of *RSP5* has much potential as a simple technique to develop thermotolerance in *S. cerevisiae* strains which nowadays are used in industrial fermentation.

Keywords Thermotolerance, *S. cerevisiae*; Bioethanol

In-vivo evaluation of fibersol-2 desalted by yeast and calcium chelated fibersol-2

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Bangoura A.O et al. 2006 studied the preparation of fibersol-2 by applying brewing yeast *Saccharomyces Serevisiae* for removing free glucose liberated during enzymatic hydrolysis of the pre-treated cornstarch (this method is called yeast application for desalting fibersol-2); followed by assessing the chelating capacity of this new product to calcium ions.

In the present work, 36 mice were fed within 3 days to study the physiological effect of fibesol-2 desalted by yeast and calcium chelated fibersol-2. The feed types were as follows; stock feed: 6.977 mg/g of calcium content; fibesol-2 desalted by yeast: 0.462 mg/g of calcium content; calcium chelated fibersol-2: 9.274 mg/g of calcium content; and $CaCl_2$: 11.1mg/ml of calcium content.

The results indicated that there is statistically insignificant difference of the blood glucose levels of the two products as analyzed by Duncan's test. Also the blood glucose levels of the two products are between the normal ranges (3.85-6.2mmol/L), which ranges is also specific for human. The test insulin content after feeding mice indicated that, the insulin secretion levels of mice fed with fibersol-2 desalted by yeast and calcium chelated fibersol-2, were also between the normal ranges (1.9-23mmol/L). These results indicated that fibersol-2 desalted by yeast, as well as calcium chelated fibersol-2 improved glucose tolerance and no delay of calcium absorption in the digestive tract.

The results in this study also indicated that faecal volume were increased for the mice fed with fibersol-2 desalted by yeast and calcium chelated fibersol-2, compared to the references groups. These results were also the same for the weights of the urine from mice fed with fibersol-2 desalted by yeast and calcium chelated fibersol-2, which were also increased compared to the other groups with the same feed consumption.

The consumption of fibersol-2 desalted by yeast and calcium chelated fibersol-2 are good ways to help the digestive system clean and healthy, to improve glucose tolerance and to control calcium deficiency disease.

Key words: Fibersol-2, Yeast for desalting fibersol-2, Chelated Calcium, Blood glucose levels, *Saccharomyces Serevisiae*.

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Influence of carbon and nitrogen source on the chitin and chitosan production by *Rhizopus arrhizus* - Factorial design

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Chitin and chitosan hold a great economic value as due to their versatile biological activities and chemical applications, mainly in medical. Recent advances in fermentation technologies suggest that the cultivation of selected fungi can provide an alternative source of chitin and chitosan. The amount of these polysaccharides depends of the fungi species and culture conditions. Filamentous fungi have been considered an attractive source of chitin and chitosan for industrial applications because their specific products can be manufactured under standardized conditions. This research describes an experimental study of the influence of the glucose, thiamine and asparagine concentration on chitin and chitosan production by *R. arrhizus*. The effect of these factors or the interaction effects between these will be observed by factorial design analysis. For chitin and chitosan production suspensions of 10^8 sporangioles/mL of *R. arrhizus* was inoculated in Erlenmeyer flask containg Hesseltine and Anderson medium, varying glucose, thiamine and asparagine concentration. These parameters were varied symmetrically around the central point according to the 2^3 factorial design (Glucose 20.0, 40.0 and 60.0 g/L; Tiamine 0.002, 0.005 and 0.008mg/L; Asparagine 1.0, 2.0 and 3.0g/L). An estimate of pure experimental error was calculated from four replicates run corresponding to a central point of the complete factorial. The response recorded were chitin and chitosan yield. The flasks were incubated at 28ºC, 150 rpm, during 72 hours. The mycelia were harvested, washed and submitted to lyophilization process. The process of extraction of chitin and chitosan involved deproteination with sodium hydroxide solution, separation of alkali-insoluble fraction, extraction of chitosan by Acetic acid. The degree of deacetylation for chitin and chitosan were determined by infrared spectroscopy. The data were analyzed for significance by the Student´s t-test and chi-square test, using the Statistic program, version 6.0 of Statsoft Inc., USA. The best yield of biomass was obtained in experimental condition 6. (glucose 60.0g/L, Tiamine 0.008 mg/L and asparagine 1.0g/L). On the other hand the best yiels of polysaccharides were obtained in experimental condition 4 (60.0g/L glucose, 0.003mg/L thiamine and 3.0g/L asparatine) for chitosan (96 mg/g) and in experimental condition central point (40.0g/L glucose, 0.005mg/L tiamine and 2.0g/L asparagine) for chitin (202mg/g). There was no statistical difference, 95% significance, between the parameters studied for chitin and chitosan production. The obtained results suggested that the carbon and nitrogen source of the culture medium influence the chitin and chitosan production.

Keywords: Factorial designer, biopolymers, polysaccharides

Influence of fermentation media on bacillus licheniformis strain protein excretion: A proteomic approach

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Nowadays, one of the major bottlenecks for industrial enzyme producers is the high cost of the enzyme production. Some 30-40 % of the production cost of many industrial enzymes is estimated to come from the cost of the growth substrate. The use of the low-cost growth substrates for the production of industrial enzymes is expected to reduce production cost greatly. This is especially important for alkaline proteases, which account for over 25 % of the total industrial market. Agro-alimentary industry by-products could serve as inexpensive fermentation sources.

In the present study the influence of different fermentation media on the enzyme excretion by *Bacillus licheniformis* ATCC 21415, in two ways:

a) Measuring the enzyme activities (protease and lipase)

b) Characterization the protein excretion profile by a proteomic approach (LC-Ms-Ms) .

The chosen fermentation media are by-product, whose main feature is the high insoluble nitrogen content in form of proteins supplying of carbon, nitrogen and sulphur,

This media are feathers, are an important by-product of the poultry industry. We have used two types of feather, the difference between both is the lipid content, and finally like a control rich media (LB) was used.

Many bacteria belonging to the genus Bacillus excrete large amounts of enzymes and other proteins into the culture medium. The most effective keratin-degrading strains in the Bacillus genus belong to the *B. licheniformis* species. The alkaline serine protease subtilisin Carlsberg, one of the most important enzymes, is excreted into the medium by strains of this specie. In addition, these microorganisms are able to produce other enzymes of industrial interest, such as lipases, chitinases, etc.

In the current work we have optimised the production of alkaline protease by *B. licheniformis* ATCC 21415 using as fermentation medium feathers from the local poultry industry. This process has taken into considerations physicochemical parameters such as temperature, substrate concentration and initial pH of fermentation.

As a result the enzyme excretion depends on two parameters, the fermentation media, and the substrate concentration. Feathers as substrate induce a high protease and lipase excretion, but only a low substrate concentration (below 2%, w/v), the LB is a very poor media for enzyme excretion and good for biomass production.

In addition lipase production is directly related with the lipid content present in feathers.

All these data have been checked using a proteomic approach based on LC-Ms-Ms, thus we have obtained the sequence of the excreted proteins.

Keywords: Bacillus licheniformis; feather; enzymes; protease; lipase; proteomic.

Influence of light and cassava wastewater (manipueira) in the production of astaxanthin by *Mucor circinelloides*

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Astaxanthin is a high-value carotenoid which is used as a pigmentation source in fish aquaculture. Additionally, a important role of astaxanthin as a food supplement for humans has been suggested. The astaxanthin can be make use of natural sources or synthetic, the preference exists in if to use astaxanthin of natural sources, extracted by microbiological saw. A brief exposure to light, results in a substantial synthesis of carotenoids for microorganisms. In this way, the aim of this work was to analyze the potential of cassava wastewater (manipueira) in the production of astaxanthin using *Mucor circinelloides* (UCP-69) exposed to blue or white lights. The filamentous fungi *Mucor circinelloides* (UCP-69) belongs to the Culture Collection of Catholic University of Pernambuco, registered in Federation of Culture Collection-FCC. For the production of astaxanthin, the fungi were inoculated in Erlenmyers flasks containing 100mL of the economic medium [manipueira in the concentrations of 4%, 7%, and 10% and distilled water], pH 6.5 during 96hours, using tree conditions: blue light, white light and in total absence of lights (dark). After that period the biomass were collected by centrifugation. For the extraction of astaxanthin the biomass was disrupted using acetone as solvent. The astaxanthin was separated by centrifugation at 4000*g*/10 min. The content of astaxanthin was determined at wavelength of 470 nm in Spectrophotometer. The best conditions obtained was manipueira 7% with blue light (150 µg/g of biomass) in comparison with dark (23.6 µg/g of biomass), and white light (88.70 µg/g of biomass). The use of cassava wastewater 4% showed 55.3 µg/g of biomass using white blue light, and 27.98 µg/g of biomass with white light, and 8.64 µg/g of biomass in the dark. However, the use of the higer level of cassava wastewater 10% was 50µg/g of biomass with blue light, and 35.90 µg/g of biomass with white light and 8.90 µg/g of biomass in the dark. The results obtained in this work contributed for introduce a new component of the production medium as cassava wastewater. That waste demonstrated special components for nutrition of *M. circinelloides,* and cheap source for astaxanthin production.

Keywords: cassava wastewater, carotenoids, agro-industrial waste, *Mucor circinelloides*, astaxanthin

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Influence of simultaneous factors on chitosan production by *Syncephalastrum racemosum* (UCP/WFCC 0148) in corn steep liquor culture media

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The natural synthesis of biopolymers chitosan by microorganisms has been related to the different variables, which may cause an increase in the production or an improvement of the physical-chemical characteristics of these biopolymers. Chitosan, β-(1→4) D-glucosamine, is a partially deacetylated form of Chitin, β-(1→4) N-acetyl-Dglucosamine, by thermo-chemical deacetylation in concentrated sodium hydroxide. Chitin and chitosan, as a biopolymer, are used in a wide range of fields such as biotechnology (enzyme immobilization), food and nutrition (emulsifying, thickening and stabilizing agent, packaging membrane, antioxidant and dietary supplement), water engineering (flocculant, chelating agent for metals), and medical applications (artificial skins, drug-delivery systems blood anticoagulant and recently in gene therapy). Since chitosan is usually insoluble in water, it is necessary to protonate its NH2 groups to obtain the soluble acidic form.). This study investigated for the first time the simultaneous influence of different factors (substrate concentration, incubation temperature, pH of culture medium and inoculum size) in the production of chitosan from the growth of the filamentous fungus *Syncephalastrum racemosum* in culture medium containing only agro-industrial residue (corn steep liquor) as sole source of nutrition. Corn steep liquor - CSL - was used as sole substrates for growth of the fungus. The growing conditions were evaluated according to 2^4 factorial design to determine the important factors for maximum yield of chitosan in submerged culture. The results showed that the yield of chitosan was 7.8 g kg-1 of substrate when S. racemosum was grown in CSL. FT-IR and X-ray of microbial chitosan showed characteristic peaks of a chitosan pattern, which confirmed the extracted product as chitosan-like. The high percentage of nitrogen on CSL suggests this residue as a good source for production of a chitosan with high physicochemical qualities to be applied in biotechnological areas as pharmaceutical and medical.

Keywords chitosan; corn steep liquor; *Syncephalastrum racemosum*

Investigation of polyhydroxyalkanoate production kinetics in *Bacillus subtilis* strain isolated from dairy waste factory

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Polyhydroxyalkanoate and their copolymers are more than 40 compounds that exist among the degradable plastics. They are more desirable in comparison to other biodegradable polymers because of their properties such as higher degradability, plasticity, resistance against water and also the simplicity of production.

In this research work Polyhydroxybutyrate production versus fermentation duration was investigated using *bacillus subtilis* strain isolated from dairy waste factory. Glucose and yeast extract were used as carbon source in concentration of 30 g/L and 7.5 g/L respectively. The amount of produced Polyhydroxybutyrate was analyzed with gas chromatograph. Optimum fermentation duration for Polyhydroxybutyrate production was 72 hour and the Polyhydroxybutyrate content was 0.59 g/g.

Key words: polyhydroxyalkanote; isolation; *bacillus subtilis*; production of biopolymer; biodegradable

Isolation of Polyhydroxybutyrate (PHB) producing bacteria, *Bacillus subtilis* from dairy waste factory

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Polyhydroxyalkanoates are a group of intercellular product which are produced by a variety of microorganisms under nitrogen and phosphate limitations. In this study several bacterial species which were able to produce polyhydroxyalkanoate were isolated from the active sludge of a dairy, plant oil and beverage factory. Gas chromatography showed that *bacillus subtilis* which was isolated from the activated sludge of the dairy factory is the best species for producing Polyhydroxyalkanoate among the isolated microorganisms.

Key words: biopolymer; Polyhydroxybutyrate; isolation; gas chromatography; *bacillus subtilis*

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Isolation of thermophilic actinomycetes producers of thermostable proteases

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Actinomycetes are well-known for their antibiotic synthesis rather than their enzyme production. That is why the ability to produce variety of enzymes could be another potential advantage. In this respect, 58 thermophilic actinomycetes strains were first isolated from different natural sources: manure, soils, water, etc and were screened for proteolytic activity on skimmed milk agar. The isolates were cultivated in Kosmachev medium at 45°C for 48h. The 11A strain originally isolated from a soil sample collected from Antarctic areas that showed highest proteolytic activity was chosen for further analyses. The accomplished observations on the microbial growth in continuous shake flasks confirmed the strain’s thermophilic nature and identified its maximum protease synthesis at 42°C for 48h. The pH profile for enzyme production was investigated as well. The observed pH range was from 6.5 to 8.5 and the optimal value for the maximal enzyme synthesis was determined at pH 8.0. Extracellular enzyme activities of crude supernatant fluid samples were studied and partially characterized in terms of their thermostability. The influence of different temperatures on the enzyme activity was observed for period of 2 days. The enzyme retains more than 90% from its initial activity during incubation at 50°C for 48h. It was also found that the enzyme is relatively stable up to 80°C. The nutrient medium substances play a significant role in the fermentation process. For biosynthetic purposes the essentials are carbon and nitrogen sources. The influence of different concentrations of carbon and nitrogen nutrient sources on the level of enzyme production was also investigated.

Keywords thermophilic actinomycetes, thermostable proteases

Kinetics of batch and fed-batch fermentations using carob pod extract by an ethanol-tolerant strain of *Saccharomyces cerevisiae* on aerated STR

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In the latest years, the quest for new and renewable energy sources has greatly increased due to the depletion of fossil fuels reserves. Agricultural waste appears as a cheap and renewable energy source that can contain great amounts of carbon to be transformed in biofuel. Ethanol is one of the products that can be biologically produced and can be used as biofuel additive to gasoline. Industrial wastes of carob pod have a large content in carbohydrates that can be ferment into bioethanol. *Saccharomyces cerevisiae* yeasts have been widely used in fermentation processes for bioethanol production because of its considerable tolerance to high concentrations of ethanol and sugar content and low pH values [1]. The scope of this study was to evaluate kinetics of an ethanol-tolerant strain *S. cerevisae* F13A growth and ethanol, apparently ethanol-tolerant, when fermenting carob pod extract with high sugar concentration in batch and fed-batch fermentations. Batch fermentations were carried out in an aerated stirred tank reactor with 2.4 l of carob syrup with 250 g/l in sugar content and supplemented with peptone and yeast extract at low concentrations, with two different aeration rates in order to verify the positive influence of different aeration flux. Results showed that at a higher aeration rate, such as 0.63 vvm, ethanol production reaches its maximum of 70.7 g/l with a yield of 0.3 g of ethanol per g of substrate and *S. cerevisae* growth figured a specific growth rate of 0.1 h^{-1}. This production fell short of the expected and theoretical yield of 0.51 g ethanol/g substrate, while at 0.13 vvm of aeration rate ethanol production reached 110.6 g/l showing a yield of 0.45 g ethanol/g substrate. It is worth to refer that was verified that ethanol concentration lower than 50g/l did not inhibit sugar consumption or transport. However at ethanol concentrations of 80-90 g(l growth was completely inhibited , but fermentation process continues showing more tolerance to ethanol inhibition

Carob extract fed-batch fermentation was carried out, at 30 ºC, 250 rpm and 0.13 vvm of aeration rate, to improve ethanol production by addition of fresh medium and alleviate ethanol toxicity due to the dilution of the medium. At the first stage of this fermentation ethanol content reached 67.0 g/l with a yield of 0.48 g ethanol/g substrate and a cellular growth with a specific growth rate of 0.226 h^{-1} was noticed. After 20 hours of fermentation 0.75 l of carob extract medium were added providing more carbon source for ethanol production. Cells continued to grow at 0.079 h^{-1} and ethanol concentration reached 99.6 g/l after 50 hours of fermentation with a yield of 0.47 g ethanol/g substrate. After a second addition at 50 h, ethanol concentration increased and reached its maximum of 126.7 g/l at 120 hours with a yield of 0.50 g ethanol/g substrate. In this third stage, cellular growth was observed with a specific growth rate of 0.011 h^{-1}. During these three stages total sugar consumption increased progressively from 47.6 % to 52.0 %, reaching 61.8 % at the last stage, while at the batch fermentation 89.0 % of the available sugar was consumed. Ethanol productivity at the batch fermentation was 2.04 $g.l^{-1}.h^{-1}$ however, at fed-batch fermentation ethanol productivity reached 3.64 $g.l^{-1}.h^{-1}$ at the first stage, decreasing harshly within the next two stages achieving values of 0.65 $g.l^{-1}.h^{-1}$ and 0.69 $g.l^{-1}.h^{-1}$. These results show that carob pod was successfully used to produce bioethanol and major production occurs during exponential growth phase, but higher values of ethanol content it's possible at stationary phase. Although fed-bath fermentation has lower ethanol productivity, fresh medium addition showed to be an excellent way of enhancing ethanol production from 110.6 g/l to 126.7 g/l. due to the decrease of ethanol toxicity and higher availability of total sugar

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Keywords Bioethanol; batch; fed-batch; carob

Lactobacilli : Their Role and Importance in Contemporary Food and Pharmaceutical Industry

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A review article

Biofermentation of otherwise called biotransformation process has been a heavily exploited and vastly researched field of biochemical engineering science. Throughout the the years, especially during the last decades, a great number of of microbial groups has been tested for the production of commonly used chemicals. Due to the forecasted scarcity of petrol, an effort has been done to replace the production of chemicals deriving from petrochemical feedstocks, with fermentation. A group of bacteria that has being widely investigated due to their abilities are Lactic acifd Bacteria (LAB), especially *Lactobacilli* . These microorganisms have been widely used in today's food, chemical and pharmaceutical industry.

These bacteria have numerous features, which are based on their main ability to ferment complex carbohydrates such as the production of acids, enzymes and natural antimicrobial substances called bacteriocins. Currently, they are principally used as natural acidifiers for the inoculation of bulk quantities of milk and vegetables in order to produce a variety of fermented products. some of the most important uses of *Lactobacilli* in the industry will be reviewed. The production of lactic acid and lantibiotics through the usage of modern fermentation technology, shall be highlighted

Keywords:Lactobacilly, LAB, fermentation science, biochemical engineering, industry, bacteriocins, lactic acid

Media optimization for glycopeptide antibiotic balhimycin production in batch fermentation process using genetic algorithm and decision tree technique

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Media optimization has long been considered as key to achieve economical industrial production of secondary metabolites. Problems associated with media optimization include raw material variability and often introduction of new strain. Various approaches such as statistical methods (Box-Wilson and Box-Behnken method) have been used to optimize the media for secondary metabolite production. These methods are constrained by their capacity to handle only a limited number of variables at small levels, usually only two. The increase in the number of variables demands exponential increase in the number of experiments. As an alternative approach, genetic algorithm (GA) can optimize a large number of variables at wide range of levels. In this study, we applied two independent approaches based on GA and decision tree algorithm (DTA) to optimize media for a glycopeptide antibiotic balhimycin production by *Amycolatopsis balhimycina* DSM 5908. GA was used as a global search algorithm and successfully tested for the medium optimization of balhimycin production. The fermentation medium for balhimycin production consisted of fifteen components in which five were chosen as variable components. Using GA, a large number of different medium compositions were possible by variation of concentration of each of these five variable components at five levels. This presents a large combinatorial search space (5^5). Optimization was achieved within four generation via GA. These four generations consisted of 120 shake flask experiment which is the small fraction of search space. The maximum productivity was found to increase by eight-folds in second generation as compare to zero generation. We obtained six best media compositions with variability in ingredients but comparable productivity. Existence of more than one solution can be useful to achieve high productivity having an economic media composition. The DTA provided the rule for media-media interactions in the form of a set of rule for media composition that give high as well as low productivity. Other than optimization of media for balhimycin productivity, these techniques can also be applied to other important industrial product fermentation.

Keywords optimization; balhimycin; secondary metabolites; genetic algorithm; decision tree analysis

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Multiplicity of β-(1,4) endoxylanase in Jonesia denitrificans BN13

Boucherba Nawel; Francis Duchiron, Estelle copinet, Benallaoua Said

Jonesia denitrificans BN-13 was isolated from a sample collected from Algerian soil. it is the seventh strain discovered in the Jonesia kind, This strain produced six extracellular endoxylanases named Xyl 1, Xyl 2, Xyl 3, Xyl 4, Xyl 5 and Xyl 6 with have molecular weight of 250 ; 150 ; 70 ; 42 ; 40 et 26 KDa on birchchwood xylan medium. Analysis of the native zymogram from the filtrat of orange peel containing the strain, showed that *Jonesia denitrificans BN13* produces 5 major xylanases : Xyl a, Xyl B, Xyl C, Xyl D and Xyl E with very closer molecular masses. A thermostable extracellular xylanase 4 was purified and characterized from *Jonesia denitrificans* BN-13, after two consecutive purification steps using ultrafiltation and Q sepharose column by using equipment AKTA purifier. The xylanase specific activity was found to be 77U/mg and the purification fold was 15. Estimated molecular masses were 42 and 40 KDa for SDS-PAGE and gel filtration. xylanase activity was enhanced by β-mercaptoéthanol and DTT.. The Xyl 4 is active at pH 7.0 and 50°C, the xylanase is also activated in the presence of the reducing agents like the DTT, β-mercaptoéthanol, the time of half-life of the xylanase 4 is of 9.34 H with 50°C in the presence of the substrate and of 7 in the absence of the substrate. After 1month, 2 months and 5 months of conservation, the relative activity is respectively of 100%, 99,67% and 98,43%. The Study of the products of hydrolysis reveals that the enzyme is an endo β- xylanase. The Evaluation of the properties of binding to the carbohydrates show clearly that the xyl4 strongly binds to cellulose what suggests the presence of a binding domain to cellulose (CBD), the binding domain to xylan is not present in the enzyme.

Optimization of Biodesulfurization (BDS) process of gasoil by the addition of biosurfactants to the immobilization cell systems

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Introduction: The Biodesulfurization (BDS), a biocatalytic process performed by microorganism that remove selectively sulphur from hydrocarbon fractions, is considered an environmental friendly process due to the mild reaction conditions required and because it is not necessary the application of additional technologies to remove toxic molecules generated during the reaction. The principally limitation of the BDS process is the restricted access of microorganisms to organic substrates, by low solubility of these compounds in aqueous phases. The use of cells immobilization is viewed as a solution in order to increase the interaction between reactants, being the bacterial immobilization by adsorption using inorganic materials considered an improvement of the entrapment cells method that reduces masses transference and the steric effect. Despite to the works that have been developed in this field, this methodology requires complementary technologies to scale up the BDS. The addition of surface active compounds to the bacterial immobilization process should be increases the bioavailability of the sulfur substrates for the bacterial cells.

Metodology: In this study the *R. rhodochrous* IGTS8 (ATCC 53968) was used. This organisms was grown on sulfur-free Medium A, supplemented with sodium succinate and citrate as the only energy and carbon sources. DBT 0.1 mM was used as the only sulfur source. The cultures were grown at 30 ºC and 250 rpm in a rotary shaker. Cells were collected by centrifugation at 4000 x *g* and 4 ºC for 30 min. The obtained pellets were suspended in 10 mL of saline solution (0.85 % NaCl). For immobilization process the bacteria cells numbers were adjusted by measuring the turbidity at 600 nm (OD_{600}). Obtained bacterial cells were contacted with 1,0 g of support (Silica, Alumina and Sepiolite) during 24 h. The amount of immobilized cells was measured by turbidity at 600 nm (OD_{600}). The polyethylene glycol sorbitan monooleate (Tween 80) and a biosurfactant (Bs) from a marine strain were used as surface active compounds. For determination the effect of the surfactant on the BDS reaction, differents loading (0.1 – 0.6 %) with values below and above of the Critical Micelle Concentration (CMC) (CMC_{Tween}: 0.35 %; CMC_{Bs}: 0.4 %) were studied. For the biocatalytic reaction of BDS the bacterial cells adsorbed and the optimal loading of surfactants were placed in a 10 mL of Medium A sulfur-free. One milliliter of Dibenzotiophene (DBT) or 4,6 Dimethyl Dbenzotiophene (4,6 DM-DBT) or gasoil (4700 mg/L of sulfur) was added for its desulfurization [1].

Results: The results show that the added of Tween 80 and the Bs to the free cells systems increase the BDS activity of DBT, 4,6 DMDBT and gasoil to different loading of surfactants. The maximum BDS activity of DBT was found at about 0.5 % and 0.3 % for Tween 80 and Bs, while for 4,6 DM-DBT was at about 0.1 % and 0.3 % for Tween 80 and Bs. In the case of gasoil the maximum activity of BDS was at about 0.3 % in both surfactants. The comparison of both surfactants shows a higher effect of the Bs on the BDS activity in all sulphur substrates. A chemical nature different (Hydrophile Lipophile Balance (HLB)) between both surfactants and a non-toxic effect of the Bs can be explained these results. On the other side, was observed an increase on the values of the BDS activity of Gasoil, when the surfactants are added to the cell immobilized systems.

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Acknowledgements: Financial support from the FONDECYT (Chile) Project 1110724

Keywords Biodesulfurization, Immobilization cells, Biosurfactants

Optimization of medium composition for extra cellular alginate lyases of a marine bacterium

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Among five marine isolates grown on minimal medium, an isolate A4 was chosen based on its high alginase lyase specific activity (0.56 mg/ml), in comparison to other isolates. The isolate A4 was identified as *B. subtilis* partial sequence, based on 16S rDNA gene.A 2^n factorial design was applied to elucidate media compositions that significantly affect enzyme formation. Sixteen trials were investigated and trial 16 with positive levels of yeast extract, peptone, sodium alginate and sodium chloride showed a 15 fold increase in specific activity. The F ratio revealed that both factors; sodium chloride and alginate, whether separately or combined are positively effective at 1% level of significance.

Keywords alginate lyase, 2^n factorial design

Polygalacturonases from plant pathogenic fungi and their role in fruit juice factories

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Pectinolytic enzymes work by hydrolyzing the ester bond between galacturonic acid and methanol (pectin esterases) or by cleaving the glycosidic bonds of specific polymers (polygalactrunases, pectin and pectate lyases). The enzymes are being synthesized by plant and microorganisms. Thus, polygalacturonases are pectinolytic enzymes that hydrolyze polygalacturonic acid chains in pectins present in cell wall structure. It seems feasible to obtain pectinolytic enzymes suitable for both agriculture and industry via screening the endogenous microorganisms. The aim of the project was to isolate polygalacturonases that are active at acidic pH. These enzymes are suitable in fruit juice industries; some 5-10% of fruit juice is usually trapped in pectic polysaccharides, depending on the fruit type and how pectin rich they are. Moreover, eliminating the excess pectins via enzyme treatment may alleviate the problems of clogging the filters used in the factories.

Our results demonstrated amongst 8 fungal species that put under screening, Macrophamina Sp. showed the highest polygalacturonase activity under low pH.

Keywords: Polygalacturonases, Macrophamina, Juice Industry

Production and Characterization of Chitosan by *Rhizopus oryzae* Using Media Agribusiness (manipueira Supplemented with corn steep liquor)

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Chitosan is a natural polysaccharide originated from the deacetylation of chitin. It is soluble in acid medium due to the presence of amino groups in the form of free radicals along the polymer chain. In this sense, the chitosan has been studied successfully in a wide variety of applications because it shows characteristics of biocompatibility, biodegradation and also presents antimicrobial and antitumor properties, emulsifier, and chelating metals. It is also used in wastewater treatment and training gels. The fungus Rhizopus oryzae belongs to the order Mucorales, class Zygomycetes, whose species have chitin and chitosan in their cell walls, factor, a physiological characteristic used as a significant character in the taxonomy and phylogeny of these organisms. In this work we analyzed the production and characterization of chitosan by R. oryzae substrate using low-cost manipueira supplemented with corn steep liquor. The strain of Rhizopus oryzae (UCP 1506), was courtesy of the Culture Collection of the Catholic University of Pernambuco (UCP), and maintained on Potato Dextrose Agar at 5 ° C. The experiment was performed using the manipueira (10%, 7.5% and 5%), supplemented with corn steep liquor (6%, 4% and 2%) at pH 5.5 to 6.0, incubated in an orbital shaker at 150 rpm, at 38, 33 C and 28 ° C for 96. After this period the cultures were collected through a nylon filter (Silkscreen, 120F). The biomass was washed with distilled water, dried and kept in a desiccator until constant weight. Chitosan was extracted with 1% sulfuric acid (40:1 v / g) in an autoclave at 121 ° C for 15 min then the samples were centrifuged (4000xg - 15min.), Neutralization and precipitation at pH 10 overnight at 5 ° C. The chitosan copolymer obtained were washed with distilled water, dried and kept in a desiccator until constant weight. The characterization of chitosan was performed by Infrared Spectroscopy (FTIR). The production of chitosan was better provided manipueira 10% and corn steep liquar at 28°C providing a yield of 44,67 mg/g dry biomass.The degree of acetylation of chitosan was 67%. The medium supplemented with corn steep liquor manipueira demonstrated that it can be a viable alternative for the production of the biopolymer (chitosan) reducing production costs as well as giving more noble destiny to waste.

Keywords: Chitosan, Rhizopus oryzae; manipueira and corn steep liquor.

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Production of a novel alkaline protease by *Micrococcus* sp grown on cassava and bambara waste

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There is a continuous search for novel proteases from microorganisms for application in numerous industrial processes. *Micrococcus* specie was isolated from fermented African oil bean seed and tested for its ability to produce protease. The ability of the organism to grow and produce protease when utilizing various local wastes was evaluated. Impact of cultivation pH on enzyme production was also tested. Protease was concentrated with 70% ammonium sulphate precipitation and characterized. Cassava waste combined with bambara nut waste (1:1) gave the best protease yield. Optimal growth of the *Micrococcus* sp was at pH 6 while maximum enzyme production was attained when production medium was adjusted to pH 8. Highest protease concentration in the culture fluid was recorded at 20 h during the exponential phase of growth. Enzyme was optimally active and stable at 95°C. Optimum pH for its activity was pH 12 with optimal stability at the alkaline range (pH 7-11) after incubation for 1 h in appropriate buffers. Enzyme was inhibited by EDTA (40.2%), Hg (45.6%), and Zinc (20.0%) but not inhibited by Pb^{2+} and slightly stimulated by Cu^{2+} (10%). The properties of this novel protease make it a promising candidate for further studies and possible applications in processes involving extreme conditions of pH and temperature.

Keywords Protease, Micrococcus sp, Waste material, Alkaline

Production of bacitracin by *Bacillus licheniformis* (UCP 1016) using media with different concentrations of milk serum

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Bacillus species occur mainly in the soil, and because of spore forming the bacteria have the ability for survival in soil environment, the species produce many kinds of antibiotics which share a full range of antimicrobial activity such as bacitracin, pumulin and gramicidin. Bacitracin is produced by *Bacillus licheniformis* which is a mixture of at least 5 polypeptides, functioning as an inhibitor of cell wall biosynthesis. Milk serum is the liquid remaining following the precipitation and removal of milk casein during cheese-making. This byproduct represents about 85-95% of the milk volume and retains 55% of milk nutrients. Among the most abundant of these nutrients are lactose (4.5-5% w/v), soluble proteins (0.6-0.8% w/v), lipids (0.4-0.5% w/v) and mineral salts (8-10% of dried extract). Therefore, milk serum whey represents an important environmental problem because of the high volumes produced and its high organic matter content, exhibiting a BOD = 30000-50000 ppm and 80000 ppm .This work aimed to study the potential of biotechnology of *B. licheniformis* (UCP1010), isolated from soil contaminated by oil, through the production of bacitracin, using mediums formulated by whey basis, replacing the glutamic acid by milk serum and using glucose as a additional source of carbon. It uses a 2^{3} factorial design, varying conditions of temperature, concentrations of glucose and whey, having the response variable diameter of the halo produced by the microorganism. The production was detected by disk diffusion test, using *Micrococcus flavus* as microorganism test. The results showed the presence of halos with diameters ranging from 11mm and 27mm, at all temperatures studied. However, the best production occurred at 37 ºC, at alkaline pH, after 72 h of microbial growth. According to the experimental design used, tests performed under the conditions of the central point showed best potential for production of bacitracin. This fact indicates that the whey concentration of 40% (v/v) and 20 g/L of glucose were more efficient for the production of the antibiotic.

Keywords: bacitracin, milk serum, *Bacillus licheniformis.*

Production of Bio-ethanol from Agricultural wastes

Muhammad Imran

Lignocelluloses material is abundant renewable energy resource for the production of bio-fuel from fermentative organism (*Sacchromyces cervesiae*). Rice polish, rice husk and sugar cane bagass are cheapest and abundant lignocelluloses resource and have vast potential to produce Bio-ethanol. The main steps for the conversion of biomass into glucose required dilute acid pretreatment, but it also released inhibitory compounds which reduced the ethanol yield. So, the present study was designed to minimize the effect of inhibitory compounds as well optimized the condition like glucose recovery, xylose solubility and lignin degradation during dilute acid pretreatment. The 0.75 mL enzymatic loads for 72 hours give 4.27- 6.45 mg/mL glucose and ethanol yield was 9-13%. After Distillation product of ethanol became 89-93%.

Keyword: Lignocellulosic Biomass, rice polish, Bio-ethanol, enzymatic hydrolysis

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Production of chitin and chitosan by *Absidia corymbifera* using industrial waste as alternative medium

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Chitin and chitosan are copolymers consisting of units *N*-acetil-D-glucosamine and D-glucosamine in varying proportions, with the first type of these units are predominant in the case of chitin, while chitosan is composed predominantly of D-glucosamine units. The extraction of polymers from the fungal biomass is simultaneously easy and economically viable, shows no contamination by proteins, independent of seasonal factors, large-scale production, with easy control of pH, and the production is controlled by carbon and nitrogen concentrations. However, the search for low cost and alternative substrates becomes necessary to replace the synthetic media for chitin and chitosan productions. In this investigation were used the industrial wastes (from industries of candies effluent and corn steep liquor) as an alternative culture medium for growth and chitin and chitosan production by *Absidia corymbifera*. A factorial design of 2^3 was used, with four central points, having as independent variables candies effluent (0 - 5.0%), corn steep liquor (4.0 - 8.0%) and pH (5 -7). The fungus was maintained through Yeast Malt Extract Agar (YMA), the inoculum was standardized to 10^6 spores / mL in 500 mL Erlenmeyer flasks containing 150 mL of the alternatives culture media. The flasks were incubated at 28°C, under agitation of 150 rpm during 96 hours. After the incubation period the biomass was quantified and submitted to extraction of chitin and chitosan. The extraction process involved deproteinization using 1M NaOH solution, followed by treatment with acetic acid 2% solution. The condition 5 (without effluent, 8% corn steep liquor, pH 5.0) showed higher biomass production (12.68 g/L). The best yields of chitin (12.89%) were obtained in condition 10 (2.5% effluent, 6% corn steep liquor, pH 6.0). However, the condition 2 (5% effluent, 4% corn steep liquor, pH 5.0) showed the best performance to chitosan production (52.71%). The promising results show that industrial wastes such as corn steep liquor and candies effluent demonstrated biotechnological potential as an alternative and low cost medium for biomass, chitin and chitosan production by *Absidia corymbifera*.

Keywords: *Absidia corymbifera*; low cost medium, production of chitin and chitosan

Production of Dextran using *Leuconostoc sp.* isolated from fermented wheat flour

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Leuconostoc species were isolated from fermented wheat flour used to prepare indigenous snack popularly known as 'Jalebi'. Jalebi is prepared from fermented dough of fine wheat flour fermented with *Saccharomyces cerevisiae*. The dough is allowed to ferment for 3-12 h. Isolation of the dextran producing microorganisms was carried out from this fermented dough on MRS agar supplemented with sodium azide (0.005%). A total of seven isolates were obtained and identified as *Leuconostoc sp.* using an array of biochemical tests. The dextran production by the isolates was carried out in the sucrose rich (15%) media in triplicates. After incubation of 48 h, two volumes of ice cold isopropanol was added to the culture flasks. The whole content was centrifuged 5000 rpm for 5 min. After centrifugation, the supernatant was discarded and the pellet was dried. The dry weight of pellet was used to determine the dextran production potential of the isolates. Among the seven isolates, the dextran production ranged from 0.35 to 0.49 g per incubation flask. Further work is in progress to immobilize the potential isolate and to evaluate production of dextran from agro wastes.

Keywords Dextran, *Leuconostoc*, fermentation

Production of itaconic acid by Solid State Fermentation on wheat bran with *Aspergillus itaconicus*

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Itaconic acid ($C_5O_4H_6$) is an unsaturated dicarboxylic organic acid. It is produced by two strains of fungi: *Aspergillus itaconicus* (Kinoshita, 1931) and *Aspergillus terreus* (Calam *et al*, 1939) via the Krebs cycle by decarboxylation of cis-aconitic acid by the key enzyme CAD (Cis-Aconitic acid Decarboxylase).

In 2004, itaconic acid has been classified, by the Department Of Energy, among the twelve building-block chemicals from biomass since it is regarded as a substitute for petrochemical-based acrylic or methacrylic acid.

It is mainly used in the industrial synthesis of plastics and polyesters but also in detergents and cleaners or in medicine and pharmacy sectors.

Nowadays, itaconic acid is produced by *Aspergillus terreus* by liquid fermentation.

In a context of sustainable development and economic circumstances, it is necessary to develop techniques which allow using cheap substrates.

Thus, the Solid State Fermentation (SSF) gaining interest since this technique is well suitable for the culture of fungi. It permits to use agricultural co-produced (wheat bran) as support and substrate.

So, the aim of this study is to produce itaconic acid by SSF thanks to the mold: *Aspergillus itaconicus*.

SSF is characterized by: initial moisture, pH, temperature, nitrogen source, carbon source…

In this work, we have studied the effect of pH (2 to 6), temperature (25 to 35°C), initial moisture (50 to 70%) and substrate (wheat bran, beet pulp and a mix) and we have demonstrated that the best production is 4.08 mg/g of dry wheat bran added with sucrose, at pH3, with an initial moisture of 60% after 3 days of fermentation.

We have shown, for the first time, the production of itaconic acid by *Aspergillus itaconicus* by SSF.

Keywords: itaconic acid; solid state fermentation; *Aspergillus itaconicus*; wheat bran

Production, extraction, purification and antibacterial activity of cyclacidine antibiotic

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A great number of anthracyclinic antibiotics have been produced by the *Steptomyces* genus. A particular characteristic of *Streptomyces* anthracyclinic antibiotic produced has been associated to a red pigment appear during the growth. Cyclacidine is an anthracyclinic antibiotic produced by *Streptomyces capoamus* (UFPEDA 3123) in the batch fermentation process. The maximum production of the complex was obtained at 72 hours of fermentation (4 g of crude product) in pH 7.2, and > 5000 U/mL of antibiotic activity. The cyclacidine-cyclamicine complex was purified in cellulose and sepahdex LH 60 colums. The pure cyclacidine recovered 1.7 g (42.5%) was characterized by long red-orange crystals with meting point at 214-242 ºC and Rf is 0.71. The activity against Gram positive bacteria showed the Minimal Inhibitory Concentration (MIC) of 0.8 μg/mL to *Bacillus subtilis, B. cereus* and *B. mycoides*, 0.4 μg/mL to *Micrococcus citreus, B. anthracis* and *B. licheniformis* and 1.6 μg/mL to *Staphylococcus aureus*. Furthermore, the survivants numbers against the Minimal Bactericidal Concentration (MBC) were equal to 0.1% of survivants for *B. subtilis* and *B. cereus*, and 1.0% for the other tested microorganisms.

Keywords: *Streptomyces capoamus*, antharacyclinic antibiotic, antibacterial activity

Purification and Characterization of Leucyl aminopeptidases from Lactobacilli isolated from Algerian camel milk

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Intracellular leucyl aminopeptidases of two indigenous strains of *Lactobacillus, Lb brevis* CHTD27 and *Lb plantarum* BH14 were studied. The cells hydrolysed differentially the chromogen leucyl substrate and weak extracellular activities were noted. Enzymes were solubilized with glycin /lyzozyme treatment of cells and purified by ammonium sulphate splitting up followed by Sephadex G100 and DEAE ions exchange chromatography's separation. The SDS PAGE electrophoresis of the purified enzymes and Sephadex G100 chromatography results allowed to estimate the relative molecular weight of the native enzymes to 100kDa and to suggest that these enzymes were composed by two subunits. The purified enzyme from *Lb* CHTD27 extract showed maximal activity at pH 6.6 and at temperature 40°C, enzyme activity was partially inhibited by EDTA and Cu^{+2} ions but increased by Na^{+2} and Co^{+2} ions. The one extracted from *Lb* BH14 showed to be inhibited by EDTA and also PMSF, it showed maximal activity at pH 7.5 and temperature 40°C.

Key words: Camel milk, *Lactobacillus*, Proteolysis, L-Leucyl Aminopeptidase activity, Electrophoresis, Chromatography.

Purification and characterization of novel laccase from basidiomycete *Steccherinum murashkinskiy* 1963

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Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) representing the largest subgroup of blue multicopper oxidases, use the distinctive redox ability of copper ions to catalyze the oxidation of a wide range of aromatic substrates including p-diphenols and aromatic amines with concomitant reduction with the reduction of molecular oxygen to water. These enzymes are glycoproteins containing unique complex of four copper ions in active center. Laccases could be considered as "ideal" catalyst in green chemistry due to their catalytic properties, especially to broad substrate specificity. They might be used in food industry (decolouring and stabilizing of fruit juice, beer and wine; baking), pulp and paper industry (degradation of lignin in wood pulp and modification of wood fibers), textile industry (textile bleaching and decolorize textile effluents), nanobiotechnology (in biosensors for clinical and environmental analysis), cosmetics industry (in cosmetic and dermatological preparations for skin lightening and as an oxidising agent in the hair dye formulations) also soil bioremediation and synthetic chemistry. However, nowadays industrial application of laccases is limited: no effective natural or recombinant producers are available; the mechanism of catalysis as well as mechanism of lignin degradation by laccases remains unclear, and the production of different isoenzymes / isoformes and their role in lignin degradation has to be elucidated. The aim of the present study is to characterize laccase isoenzymes: biochemical and physico-chemical properties, molecular organization and 3D structures.

The laccases from white-rot basidiomycete *Steccherinum murashkinskiy* 1963 (Komarov Botanical Institute Basidiomycetes Culture Collection, Russia) have been investigated. Three genes encoding laccases have been revealed in the mycelial fungus *S. murashkinskiy* 1963: *lac*1, *lac*2 and *lac*3. It should be mentioned that level of homology between gene *lac*1, *lac*2 and *lac*3 encoding laccase isoenzymes was rather low comprising 67 %. The low level of homology (less than 65%) was also observed with other laccases from order *Polyporales*.

The highest amounts of laccase were produced by *S. murashkinskiy* when growing for 21 d at 28 ºC on glucose-peptone medium with addition $CuSO_4$ as an inducer. The laccase preparation was purified to homogeneity from the culture filtrate of *S. murashkinskiy* mycelia. Three laccase isoforms were detected in cultural broth using immunoblotting technique. The MALDI-TOF-TOF mass spectrometry analysis of laccase isoenzymes allowed to identified them as expression products of *lac*1, *lac*2 and *lac*3 genes. The gene *lac*2 encoded the major laccase isoenzyme – Lac2. The molecular mass of Lac2 was 63 kDa and the isoelectric point - 3.8, according to the results of SDS-PAGE and analytical isoelectric focusing respectively. The spectroscopic characteristics (UV–visible and CD spectrum, EPR spectra) of Lac2 were typical for classic "blue" laccase. The Lac2 substrate specificity was examined using various phenolic (2,6-DMP, guaiacol, hydrochinon, pyrocatechol and sinapinic acid) substrates, syringaldazine and nonphenolic compounds, such as ABTS and $K_4[Fe(CN)_6]$. The apparent Km and Vmax values of the enzyme for substrates were determined from a Lineweaver-Burk plot. The enzyme showed the highest level of catalytic efficiencies for 2,6-DMP, sinapinic acid and syringaldazine: *k*cat/Km were 95.3×105, 165.2×105 and 162.5×105 $s^{-1}\cdot M^{-1}$ respectively. The optimum pH for the enzyme activity was identified as 3.0-3.5, 4.5-5.0 and 2.5 for pyrocatechol, syringaldazine and ABTS used as substrates, respectively. The temperature optimum for ABTS oxidation by Lac2 was 60 ºC. The thermal stability of the enzyme was determined at 50 ºC and the semi period of inactivation (τ½) comprised 37 h.

The structural analysis of Lac2 from *S. murashkinskiy* 1963 using the structure with 1.1 Å resolution, revealed the significant differences in substrate-binding pocket organization in comparison with known laccase structures.

The study of other two isoenzymes produced by *S. murashkinskiy* 1963 is under the progress.

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Keywords white-rot fungi, lignin degradation, laccase

Purification, Characterization, Kinetic Properties, and Thermal Behaviour of Extracellular Chitinase Produced by a Native Microorganisms *Serratia marcescens* B4A

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Chitin a linear homopolymer of β-1,4 –linked N-acetyle-D glucosamine residues is the most abundant nitrogen containing organic compound found in fungi, animals and plants. Chitinase are classified as glycosyl hydrolases that catalyze the hydrolysis of β-1,4-glcosidic bonds of chitin. They are found in bacteria, fungi, higher plants ,insects and some vertebrates. Chitinases have received increasing attention because of their board applications in the fields of biological control and protecting plants against pathogenic fungi.

The purpose of this study was to purify, characterize, kinetic and thermodynamic studies of *serratia marcescens* B4A chitinase. For the first time , the native bacteria was isolated from shrimp ponds and isolated and incubated at 30C° in an orbital shaker at 160 rpm for 48h. The enzyme was purified by ammonium sulfate precipitation and one step ion-exchange chromatography and had an apparent molecular mass of 55 kDa. Its optimum activity was at pH =7 and 50 C° and the K_m and V_{max} values of this enzyme were 0.038 (mg/ml) and 6.53 µmol/min. We studied the kinetic properties and thermal inactivation of chitinase. This enzyme exhibited a $t_{1/2}$ of 78.7min at 60 C° and its specific activity was 27.26 $(u.mg^{-1})$. The activation energy $(\Delta E^{\#})$ for heat inactivation was 5.341$(Kj.mol^{-1})$ and the thermodynamic activation parameters $\Delta H^{\#}$, $\Delta G^{\#}$ and $\Delta S^{\#}$ were also calculated, revealing a potential application for the industry.

Key word: purification,bacteria,chitinase

Removing radionuclide deposits from dry metal and concrete surfaces by fungal lignocellulose mats: The start of a green technology in decommissioning of nuclear facilities

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Unlike the ashes we produce with the flames of Prometheus, the residues of nuclear fuels and the radwastes from nuclear power installations made to alpha, beta, and gamma emitters by thermal-neutron activation [1] or surface contamination retain their hazardous properties for hundreds to millions of years [2]. Research projects accompany the long-term management of the high-level radwastes, spent fuel and fuel reprocessing residues [3] and the identification of dry geological formations for their final storage. Low-level wastes whose radiation diminishes to natural activities in < 500 yr are stored in shallow disposal sites [4] or in envisaged 600-m deep repositories by accepting contact with groundwater [5]. They contain long-lived nuclides such as ^{55}Fe, ^{63}Ni, ^{60}Co, and ^{3}H within the steel of reactor vessels [6] and surface contaminants such as AmBaCClCmCsEuIPuSrTcThU radioisotopes [7] from material impurities, chain reaction, and fuel rods. In concrete, ^{41}Ca is generated [6]. Walls and installations of the reactor hall but also buildings and equipment contaminated with nuclides dispersed by waste handling or accidental events may constitute the main portion of low-level radwaste. Its partial decontamination by scavenging with water and lye, by sand blasting, (electro)chemical treatment with mineral and carboxylic acids and synthetic chelants [8] cause secondary waste streams with further disposal problems or washes portions of the nuclides deeper into porous materials.

It is well documented that natural mats of (pileate) fungi accumulate radioisotopes of CsISr as well as an unlimited bulk of trace metals [9-11]. Solubilization, precipitation, sorption, and uptake of metal cations as well as erosion and dissolution of metallics are common features of bacteria and fungi and could be employed to separate near-surface nuclide deposits on a dry way from metal and brickwork elements to reduce the bulk of potential radwaste from decommissioned nuclear power plants (e.g., 40000 metric tons from Rheinsberg plant, Germany). The citrate and oxalate releasing ascomycete, *Aspergillus niger*, the oxalate producing brown-rot basidiomycete, *Fomitopsis pinicola* as well as several wood-destroying basidiomycetes with the tendency to expand into soil were therefore tested for their ability to acquire, and translocate uranium and to reduce the weight of metal plates by carboxylic acids production. The nature of the water-soluble and -insoluble reaction products was not determined. The recalcitrant uranium was taken up and translocated through the 100-mm long hyphal systems. In addition, all fungal mycelia were able to reduce the weight of the pure or alloyed metals, Fe > Zn > Cu, CuZn > AlMgSi > Cr significantly when added as plates to liquid and solid fungal substrates for 50 to 180 d. The dominating fungal carboxylic acids, malonic > citric, malic > oxalic in realistic solutions of 1 g L^{-1} were able to reduce the weight of Fe > Ni > Zn > Cu > Al disks upon the formation of soluble and insoluble compounds. The mineral acids, H_2SO_4 > HNO_3 > HCl in solutions with a comparable initial pH transformed Fe > Ni > Al primarily to insoluble precipitates. Plates of Portland cement increased their dry weight in the solutions of carboxylic acids apparently by the incorporation of the acids' carbon whereas the mineral acids reduced the dry weight of the samples. Calcium was solubilized by all acids but oxalate. It is concluded that trace metals such as U spread in entire fungal networks which can thus serve as repositories of critical nuclides. In addition, fungal carboxylic acids dissolve (potentially contaminated and corroded) metal surface layers in the µm range by the formation of soluble and insoluble compounds most of the latter can be brushed off. Further tests comprise the placement of plastic-net stabilized lignocellulose mats overgrown with trace metal accumulator fungi on dry structured surfaces contaminated by critical nuclides and the subsequent ashing of the mats by air filtering. Respective *in-situ* tests with contaminated construction elements are inevitable to confirm economy and efficacy of microbial versus acid- and surfactant-based cleaning procedures.

Keywords alpha beta gamma neutron radiation; radioactive waste; waste repository; nuclear power plant; decommissioning

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Screening of antifungal activities of *Bacillus* strains growing on agro-industrial wastes

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Biological control through the use of natural antagonistic microorganisms has been extensively studied, and some yeast, fungi and bacteria have been shown to be effective against various plant pathogens. Gram-negative bacteria, especially *Pseudomonas* strains have been intensively investigated with regard to the production of antimicrobial metabolites. However, Gram-positive bacteria, members of the *Bacillus* genus produce variety of antibacterial and antifungal metabolites among which are biosurfactants - lipopeptides of the surfactin, iturin, fengycin families.

The aim of the study was to evaluate antifungal activities of *Bacillus* strains and their culture supernatants growing on brewery wastes and molasses. The *Bacillus* strains and their culture supernatants were applied to test the *in vitro* antagonistic effect. Our early investigations confirmed that three *Bacillus* strains (named T-1, T'-1 and I'-1a) were able to grow and produce biosurfactants in brewery effluents and molasses media at 30°C under aerobic condition during the stationary growth phase. The agro-industrial wastes replace synthetic media for supporting the *Bacillus* strains growth and biosurfactant synthesis. The *Bacillus* spp. were identified by 16S rRNA gene sequences, BIOLOG system and fatty acid analysis. The following fungal plant pathogens were used: *Botrytis cinerea* A 258, *Rhizoctonia solani* W 70, *Sclerotinia sclerotiorum* K 2291, *Phomopsis diachenii* K 657, *Phomopsis viticola* W 977, *Septoria carvi* K 2082, *Colletotrichum dematium* K 425, *Colletotrichum gloeosporioides* A 259, *Phoma complanata* A 233, *Phoma exigua var. exigua* A 175. The phytopathogens were isolated from the herbs, mainly from cumin, angelica and vines. *Bacillus* strains growing on brewery wastes and molasses and their culture supernatants were tested for the ability to inhibit the growth of fungal plant pathogens in Petri dishes on PDA medium. Mycelium plugs of the fungi were inoculated in the plate middle, 3.5 cm from the bacterial colonies or sterilized discs on which 10 µl supernatants extracts were dispensed. Fungal growth inhibition was evaluated after plate incubation for 3 or 10 days, depending of the organisms at 22°C and compared with abiotic control plates. Results here demonstrate the ability these *Bacillus* strains growing on brewery wastes and molasses to inhibit select fungal growth. Thus these *Bacillus* species have the potential for application in biocontrol of plant diseases. The results also indicate that the biosurfactants produced by *Bacillus spp*. growing on agro-industrial wastes have antifungal activities. Further investigations are currently conducted to isolate the biosurfactants and find out their efficacy as antifungal agents. Natural bacteria like *Bacillus* capable of suppressing pathogens and maintaining their population by competing against deleterious microorganisms can be successfully utilized as biopesticides for sustainable organic farming.

Keywords *Bacillus*, biological control, biopesticides, agro-industrial wastes, biosurfactants

Screening of Halophilic Microorganisms Producing Extracellular Hydrolyses from Different Hypersaline Environments of Turkey

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There are a lot of saline/hypersaline environments of marine and continental origin in different part of Turkey. These hypersaline environments are salt lakes, solar salterns, rock salterns and spring salterns. It has been extensively reported that members of the Halobacteriaceae family constitute the dominant microbial population, especially in those environments where the NaCl concentration ranges from 20% (w/v) up to halite saturation (<32% w/v)). In this study, extremely halophilic microorganisms isolated from Tuz Lake, Acı Lake, Çamaltı Saltern were screened for their hydrolyses activities. Isolates produced lipases, amylases, proteases and DNases. According to their partial 16S rRNA sequence analysis, the halophilic strains were identified as members of the genera: *Halomonas*, *Halobacillus*, *Virgibacillus*, *Halorubrum*, and *Haloarcula.*

Screening of medium for biosurfactant production by *Geobacillus stearothermophilus* UCP 0986

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Surfactants constitute an important class of chemical compounds used in various applications. The most of the available surfactants are synthesized from petroleum, and considering the environmental problems concerns these molecules, actually, led to the demand for natural surfactants. Biosurfactant compounds are molecules produced by microorganisms are active on surfaces has received increasing interest in recent decades due to advantages over the chemical surfactants, such biodegradability, low toxicity and production from renewable source. The biosurfactants are produced by microorganisms and have applications based on the functional properties that include emulsification, solubilization, demulsification, and surface and interfacial tension reduction. The use of alternative substrates, such as industrial waste and vegetable oils, can contribute positively to the reduction of the costs for biosurfactant production. The aim of this work was the production of biosurfactant by *G. stearothermophilus* using Central Composite Rotational Design (CCRD), to screen the culture media using the following components: corn steep liquor, palm oil, coconut oil, vegetable fat and ice cream waste, on the variable response surface tension reduction. The Erlenmeyer's flasks were incubated at temperature of 45ºC, 150rpm, tested in 32 and 72hours. The results showed lower values of tension 25.30 mN/m and 31.50 mN/m in central point of the factorial design (7.5% coconut oil and corn steep liquor 4.5%). The results obtained with vegetable fat and ice cream effluent showed the lowest values for surface tension (28.75 mN /m and 26.71mN/m) assay 5 and 7. The higher emulsification indexes (E24) were observed for engine oils, engine burned oil, post-frying vegetable fat, post-frying soybean and diesel oils. The pH do not showed variations. The results suggest that the best interaction of oils and waste showed in the central point and shown a great biotechnological potential the biosurfactants production.

Keywords: Biosurfactant; *Geobacillus stearothermophilus,* vegetable oils; industrial waste.

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Size distribution of airborne microorganisms at workplaces in metal industry

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Introduction

Metalworking fluids are used to reduce the heat and friction in metal cutting processes. Among different fluids, the most common are emulsions, which are usually a mixture of concentrated mineral oils, various additives, and water. The presence of water and organic substrates creates conditions supporting microbial growth. In numerous industrial processes (such as grinding or cutting), a rapid rotation of metalworking tools may result in a release of oil droplets and, by that, in an emission of biological particles into the air. These particles, if inhaled, can be responsible for numerous adverse health outcomes in the exposed workers. As the respiratory tract penetration efficiency of bioaerosol particles plays a major role in inhalation exposure, the aim of the study was to assess size distribution of bacterial and fungal aerosols at workplaces in metal industry.

Material and Methods

The bioaerosol sampling was carried out at one industrial plant. Four workplaces with metal-finishing machines were chosen for this study. The bioaerosol samples were taken using 6-stage Andersen impactor. During measurements, sampling instrument was placed at the height of 1–1.5 m above the ground level to simulate aspiration from the human breathing zone. The flow rate and sampling time were 28.3 L min^{-1} and 5 min, respectively. After sampling, all impactor plates were incubated and the number of bacterial and fungal colonies growing on appropriate agar media was counted. All isolated microbial colonies were subsequently identified to the genus and/or species level based on their morphology, microscopic structure and biochemical reactivity. The final bioaerosol concentration was expressed as colony forming units (CFU) per cubic meter of the air.

Results

The concentrations of bacterial and fungal aerosols at workplaces ranged from 2.1×10^2CFU/m^3 to 5.0×10^4CFU/m^3 and from 2.8×10^1CFU/m^3 to 4.4×10^2CFU/m^3, respectively.

Qualitative composition of bacterial aerosols showed a clear domination of Gram-positive bacteria, i.e. cocci from genera *Staphylococcus* and *Micrococcus* as well as rods from genus *Bacillus*. There Gram-negative bacteria form genera: *Pantoea*, *Pseudomonas*, *Brevundimonas* and *Chryseobacterum* were also frequently isolated. Among fungi, the most prevalent were molds of *Aspergillus* and *Penicillium* genera as well as yeast from the *Geotrichum* genus. All these species were classified to hazard groups I and II according to Directive 2000/54/EC.

The size distribution analysis revealed that bacteria were present in the air mainly in the form of bacterial-oil aggregates (with aerodynamic diameter of 3.3–7.0µm). Fungi were naturally dispersed in the form of single cells and/or small spores (with aerodynamic diameter of 2.1–3.3µm). Based on these results, it can be assumed that the studied bacterial aerosols, when penetrate the human respiratory tract, may be deposited within oral cavity, thorax and primary bronchi while fungal aerosols may be deposited within secondary bronchi and bronchioles. This can result in numerous adverse health effects in exposed workers from throat irritation onwards, through asthmatic reactions, to allergic inflammation.

Conclusions

Oil mist released into the air during different industrial processes contains numerous viable microbial particles. As it was shown in this study, majority of them were within respirable size and, as such, may freely penetrate upper and lower parts of respiratory tract. Hence, the monitoring of bioaerosol size distribution in this type of occupational environment can be an efficient tool to control the hygienic conditions at workplaces and to select the adequate personal protective measures for workers exposed to airborne microorganisms.

Keywords: metalworking fluids, occupational exposure, bacteria, fungi, size distribution

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Solid state fermentation of Mexican oregano wastes

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Mexican oregano (*Lippia berlandieri* Schauer or *Lippia graveolens* HBK) is recognized in many countries for their aromatic characteristics and flavor quality. Principal products obtained from the plant and marketing are the leaves and the essential oil; however the extraction of the essential oil generates large amounts of agro industrial wastes; these wastes have been used as feed, but the consumed amount is lower than the produced. In this study, a fungal bioprocess for use of these residues was developed.

For solid state fermentation, oregano wastes were mixed with Pontecorvo culture medium at an initial moisture level of 70%, and inoculated with 1 x 10^7 spores per gram of support of *Aspergillus niger* PSH. An aqueous extract was obtained from each flask, at 0 h (EAF 0h) and 120 h (EAF 120 h) of fermentation time and from the wastes without fermentation (EAB). Antioxidant activity, total and reducer sugars, total phenolics, condensed tannins and catechin content was measured in every extract. After that, the extracts were fractionated by column chromatography packed with Amberlite XAD-16.

The content of total and reducer sugars was a significant difference in EAF 0 h (97.16 and 66.52 mg/g dry matter respectably) than EAB (53.63 and 19.47 mg/g dry matter respectably) and EAF 120 h (19.72 and 5.40 mg/g dry matter respectably). Total phenols and condensed tannins had the higher concentration in EAB (13.47 mg gallic acid equivalents /g dry matter and 34.86 mg catechin equivalent/g dry matter, respectably), meanwhile EAF 0h (4.57 mg gallic acid equivalents /g dry matter and 17.34 mg catechin equivalent/g dry matter, respectably) and EAF 120 h (5.57 mg gallic acid equivalents /g dry matter and 8.52 mg catechin equivalent/g dry matter, respectably) had a significant difference. The principal molecule of condensed tannins, catechin was founded only in EAB and EAF 0 h extracts (2.34 and 2.61 mg catechin equivalents/g dry matter). The antioxidant activity was attributed to phenolic contents, being the EAB and EAF 0 h the main extracts with this activity measured by DPPH and ABTS methods.

Extracts fractionation allowed the separation of 5 fractions. Polysaccharides were concentrated in F 0 and the phenolic compounds in F 4. These fractions can permit a specific characterization of the main compounds.

The bioprocess allowed the accumulation and extraction of different compounds as polyphenolics and polysaccharides that can be used to supplement culture media and growth lactic acid bacterias and/or probiotics. These results could give an add value to Mexican oregano wastes.

Keywords antioxidant activity; *Aspergillus niger*; solid state fermentation; polyphenols

Solvent-free Synthesis of Octyl Acetate by Transesterification Catalyzed by Immobilized Lipase

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Synthesis of octyl acetate by transesterification using immobilized lipase from *Rhizopus oryzae* NRRL 3562 was studied in solvent-free condition. The effect of different tansesterification variables namely alcohol molarity, reaction time, temperature, agitation, addition of water and enzyme amount, on molar conversion (%) was investigated. A maximum molar conversion of 92.35% was obtained with the transesterification variables of 2 M octanol in vinyl acetate, with the addition of 0.2 % water, using 20 U immobilized lipase at 36 ^{o}C and 200 rpm in 12 h. The immobilized lipase retained more than 90% relative activity upto six recycles.

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Stabilization of raw starch digesting amylase by multi-point covalent attachment on glutaraldehyde and polyglutaraldehyde activated amberlite beads

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Raw starch digesting enzymes (RSDA) have enormous potential in modern day biotechnology due to their ability to saccharify the starch molecule while by-passing the gelatinization step. However, its application is limited due to poor activity and stability. To stabilize the RSDA from *Aspergillus carbonarius*, the enzyme was immobilized on amberlite beads by conjugation, spontaneous crosslinking and adsorption using glutaraldehyde and polyglutaraldehyde. Effect of immobilization on enzyme stability and catalytic efficiency was evaluated. Immobilization conditions greatly influenced the immobilization efficiency. Optimum pH values shifted from pH 5 to 6 for spontaneous adsorption and crosslinking (AmbRSDA-RET), and adsorption followed by crosslinking (AmbRSDA-CROSS), pH 6-8 for RSDA covalent attached on polyglutaraldehyde activated amberlite beads (PGAmb-RSDA) and pH 7 for RSDA on glutaraldehyde activated amberlite (GAmb-RSDA). GAmb-RSDA had no loss of activity after 120 min storage at pH 9; PGAmb-RSDA lost 9%, while soluble enzyme retained only 35% of its initial activity. Soluble enzyme lost 50% initial activity after 180 min incubation at 60°C, while GAmb-RSDA lost only 7.7% initial activity. RSDA derivatives retained over 90% activity after 10 batch re-use at 40°C. Apparent K_m of enzyme reduced from 0.35 mg ml^{-1} to 0.32 mg ml^{-1} for GAmb-RSDA but increased to 0.38 mg ml^{-1} for PGAmb-RSDA. Covalent immobilization on amberlite beads promises to address the instability and contamination issues which impede the industrial use of RSDAs. Moreover the cheap, porous and non-toxic nature of amberlite, ease of immobilization and high yield makes it more interesting for the immobilization of this enzyme.

Key words: Glutaraldehyde, Amberlite, immobilization, Raw starch digesting amylase, Stabilization

Statistical optimization of medium components for the production of pyocyanin by *Pseudomonas aeruginosa* RS11

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The nutritional requirements for pyocyanin (PYO) production using *Pseudomonas aeruginosa* RS11 were optimized statistically in shake flask experiments. Based on a single-factor experiment design; we implemented the two-level Plackett–Burman (PB) design with 8 variables to screen medium components that significantly influence PYO production. pH, yeast extract, and K_2SO_4 were identified as the most effective variables. Response surface methodology based on the Box-Behnken design was applied to determine these factors' optimal levels and their mutual interactions between components for PYO production. The results showed that 8 µg/ml of PYO production was obtained after a 6 days fermentation period, with optimal concentrations of K_2SO_4 22 gL^{-1}, yeast extract 1.4 gL^{-1} and at pH 5.8. The validity of the model developed was verified, and the optimum medium led to a maximum PYO concentration 17.7 µg/ml, an early three fold increase compared to that in the basal medium.

Key words; Pyocyanin - *Pseudomonas aeruginosa* RS11- Plackett–Burman design

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Study of culture media water activity for *Bacillus thuringiensis* production by solid state fermentation

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Bacillus thuringiensis, an entomopathogenic bacterium used in biological control, can be produced by fermentative process using agro industrial residues as substrates. During the rice processing some residues are produced: rice straw, husk, bran and grits, which have a good potential to be used in fermentation.

The culture medium in solid state fermentation (SSF) is composed by some nutrients heterogeneously distributed with some moisture content. In this process the microbial growth on humid substrate resembles the microbial growth on natural habitat. The water is a limiting factor in the SSF, differently from that in submerged fermentation where water is abundant. The solid culture medium must have a minimum moisture content to allow microbial growth but the concept of water availability in the substrate is best explained in terms of water activity (Aw). The relationship between moisture content and water activity is represented by isotherms.

This work studied the water activity influence on solid state fermentation of *Bacillus thuringiensis* using rice husk, bran and grits as substrates.

Three culture media were tested: (i) rice grits; (ii) rice grits and rice husk; (iii) rice bran and rice husk. In order to study hygroscopic characteristic for each medium, isotherms were constructed. The results show differences for each culture medium probably because their chemical composition. That suggests the importance of to know the relationship of Aw and moisture content.

For each culture medium, two processes were carried out: one with low Aw and the other with high Aw. The fermentations with *Bacillus thuringiensis* were done in plastic bags containing the culture medium.

It was observed differences in productivity. The best results were obtained medium composed by rice grits. The productivity [(spores / g medium) / h] for the process using initial Aw of 0.620 was 3.0 $x10^4$ and the for initial Aw of 0,965 was $1.0x10^6$.

Keywords *Bacillus thuringiensis*; water activity; solid state fermentation; rice residues

Substitution of centrifugations for ethanol precipitations removal by hollow fiber for the polysaccharide purification of *Haemophilus influenzae* type b

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The capsular polysaccharide (PSb) produced by *Haemophilus influenzae* serotype b is a surface antigen that is the main virulent agent of this Gram- negative bacterium. The PSb is currently purified, conjugated to a protein in order to produce an efficient vaccine in adult and even in children aged less than two years.

The classical polysaccharide purification process included: five ethanol precipitations, phenol extraction and several continuous centrifugation/ultracentrifugation to remove the insoluble materials. The industrial installations require areas with protection against fire hazards, toxic and corrosive fumes and the capital cost investment in continuous centrifuge/ultracentrifuge and energy are very high. The cost of production of this vaccine makes difficult for developing countries obtain the auto sufficiency in immuno biological products.

Our laboratory developed a simplified purification method that reduce the ethanol precipitation to two, phenol is eliminated by hydrolytic enzymes, nucleases and proteases, and tangential ultrafiltration with membranes of 100 kDa in the presence of detergent and chelating agent. The purpose of this work is to set up a purification process which can substitute the use of centrifugation during the ethanol precipitations steps by using hollow fiber in order to make easier and cheaper the large-scale purification.

The supernatant from *H. influenzae* type b culture broth is diafiltrated and concentrated through tangential ultrafiltration membrane of 100kDa cut-off (1CTUF100). The concentrated fraction is precipitated with ethanol to 30% final concentration and submitted to hollow fiber 0.2 µm tangential microfiltration (TMF). The precipitated of 30% ethanol was discarded and the microfiltrated 30% ethanol fraction was recovered (1TMF0.2) and concentrated with a tangential ultrafiltration of 50 kDa, to reduce the volume, and precipitated with ethanol to 80% of final concentration. Ethanol was removed by tangential microfiltration with hollow fiber 0.2 µm and the microfiltrated solution of 80% ethanol was discarded. The precipitated of 80% ethanol was solubilized with water and recirculated in hollow fiber system with the permeate line closed. Extensively diafiltrations with water was introduced to recover the maximum of PSb in microfiltrate fraction (2TMF0.2). The 2TMF0.2 was concentrate in 50kDa tangential ultrafiltration membrane to reduce the working volume and submitted to enzymatic hydrolysis, and finally concentrates in 100 kDa tangential ultrafiltration membrane to remove the residual contaminants, obtaining the PSb purified in the final fraction (2CTUF100).

The purified PSb reaches the required purity, that is a minimum of 100 times, weight by weight, more PSb that protein (Prt) or nucleic acids (NA). The relative purity were $RP_{protein}$ =196 mgPSb/mg prt±32% and $RP_{Acid\ Nucleic}$ =1747mg PSb/mg AN±24% based on protein and nucleic acids respectively.

This PSb established purification process is a simple, cheap and easy method for scale-up and it can be easily applied to others vaccine and industrial polysaccharides.

Keywords: insoluble removal, centrifugation substitution, hollow fiber, tangential ultrafiltration

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Sulfate Reducing Bacteria -an important agent for Bio-fouling in cooling water system of fertilizer industries

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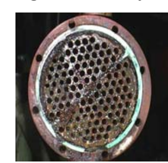
Water is widely used as a cooling media in the industrial heat transfer to cool product or a process. Cooling water is generally contained all the naturally occurring living organisms and nutrients are responsible for uncontrolled multiplication of the living organisms.Biofouling is the process whereby biological material accumulates over time on a surface exposed to water or moisture (fig. A). The biofouling may include combination of bacteria, algae, protozoa, fungi and invertebrate organisms. This biological layer covering the surface formed the biofilms. Biofouling due to microbial contamination is known as 'slime' or biofilms. Algae, fungi & bacteria found in biofilms. The results in bio-fouling of pipelines; developed Microbial Induced Corrosion (MIC) (Fig.B) on heat exchangers surfaces of Open recirculating cooling water system which reducing efficiency.Open recirculating cooling water system provides a very good environment for the survival of microorganisms likes Sulphate Reducing Bacteria (SRB); because of the system pH 6.5-7.5 and temperature 30 °C to 45°C are the most favorable for SRB. These SRB generates energy by reducing sulphate to sulphides. **[$4Fe + SO_4 + 4H_2O \rightarrow FeS + 3Fe(OH) \rightarrow 2(OH)^-$].** SRB are initiating the pitting on both stainless steel, mild steel metals; by producing H_2S (Fig.B). SRB attack on metal is characterized by formation of black sulphides (FeS); so the metal shows black shiny. This growth accumulates chemical & biological fouling in heat exchanger surfaces. It was essentials to isolate using selective medium of Postgate' by standard method (Fig.-C). The spices Desulfovibrio, Desulfomonas & Desulfobacillus are commonly found in recirculating system (Fig.D). It can concluded that dominant population of SRB take part in the formation & stabilization of Biofilms and thereby Microbiological Induce Corrosion (MIC), which reduces the cross sectional area of heat exchangers. The problem of MIC and Biofilm formation can be control by using proper oxidized and non-oxidized biocides.

Keywords: Biofouling, MIC, Desulfovibrio, Desulfomonas & Desulfobacillus.

Figure-A & B. ***Biofouling by SRB on Heat Exchanger*** **Figure-C** Colonies ***of SRB*** **Figure-D** Morphology ***of SRB***



Synthesis of branched chain amino acids in *Escherichia coli* at oscillating conditions in a scale down two-compartment reactor

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In industrial fermentation processes, inhomogeneities are observed due to insufficient mixing. In the feeding zone in the top of a large scale fermenter, areas of high substrate concentration combined with low dissolved oxygen levels occur. In *E.coli* these conditions (especially sudden increase in substrate concentration) favour the accumulation of intracellular pyruvic acid. This accumulation was found to affect the synthesis of norvaline, an irregular branched chain amino acid (BCAA), when cells were exposed to oscillating substrate and oxygen levels.

The presence of (irregular) BCAA is likely influencing the composition of proteins. To study their synthesis, a scale down reactor (SDR) concept was established, in which inhomogeneities in industrial scale fed-batch processes are simulated in lab scale. It consists of a stirred tank reactor (STR) connected to a plug flow reactor (PFR). The PFR is equipped with static mixer modules, five sample ports, DO and pH sensors. The culture is fed when entering at the bottom of the PFR module. Hence, while the STR represents a zone characterized by evenly distributed glucose and oxygen, the PFR mimics a zone with a high glucose gradient (representing the feeding zone of an industrial scale reactor).

In this study, the synthesis of BCAA was investigated in *E. coli* fed-batch cultivations at oscillating (SDR) and non-oscillating conditions (STR). We show that the applied strategy is suitable to observe the short-term and long-term impact of oscillations on *E. coli* when used as host strain for protein expression.

Keywords Oscillation; Norvalin; Scale-down reactor

Textile dyes removal in an activated sludge system by using mineral sorbent

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The low biodegradability of textile dyes indicates that biological treatment is not always successful in the treatment of textile wastewater, in terms of color removal. The use of low-cost and ecofriendly sorbents has been investigated as an alternative to the current expensive methods of removing dyes from textile wastewater. Bentonite is such mineral sorbents which is naturally available in many countries. Organo-Bentonite can be prepared by contacting bentonite and a cationic detergent.

In this study, Municipal type wastewater was used in the activated sludge laboratory pilot plant system. The optimum sludge and hydraulic retention time were determined as 20 days and 20 h respectively. Activated sludge system operated until steady state condition. In this condition, the average concentration of DO and MLVSS in the system was 5.5 and 3200 mg/L respectively. In the first stage, textile dyes including Cibanone Yellow, Basilene Orange, Pigment Blue and Disperse Blue were added separately in to the activated sludge system. The color removal efficiency for individual dye was determined by spectrophotometric measurement in the filtered samples. In the second stage, mixed dye was added in to the activated sludge system and color removal efficiency was determined by COD measurement in the filtered samples.

Before dosage, the average mixed color removal was around 25%. In this study, ordinary clay, bentonite and organically modified bentonite were added in to the activated sludge system. Clay soil, Bentonite and Organo–Bentonite were added at 10 gr/L dose rate separately. Addition of clay soil did not change color removal significantly at different dose rate. The average mixed color removal efficiency by addition of Bentonite in the activated sludge system was 45%. Increasing dose rate of Bentonite did not increase color removal efficiency meaningfully. The most effective sorbent for color removal was found organically modified bentonite. Textile color removal efficiency in the activated sludge system by addition 10 g/L of Organo-Bentonite was 100%. When Bentonite was added to the system, the effluent turbidity was increased, but addition of clay soil and organo-bentonite had little effect on effluent turbidity. For individual dyes, the removal efficiency of Organo-Bentonite for each dye was 100%.

Organo-Bentonite is an excellent dye sorbent in the activated sludge systems.

Key words: textile dyes, organo-bentonite, activated sludge

The Effect of Olive Mill Wastewater on Growth of Heterotrophic Microalgae and Removal of Total Phenol Concentration

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Process of olive oil extraction produces a dark-colored wastewater which characterized by high pollution load because of high concentrations of phenolic and organic compounds. Incorrect disposal of this wastewater represents major environmental problems. Microalgae cultures may serve as a solution to emerging environmental problems such as olive oil mill wastewater (OMW) treatments.

In this study, two heterotrophic microalgal strains (*Galdieria sulphuraria, Crypthecodinium cohnii*) were selected in order to evaluate their capability to grow in, and to degrade the phenolic components of OMW. Olive mill wastewater with different dilution rates (%5,%10,%20 and %50) were used in these two microalgal growth medium. Microalgae growth was also supported by adding extra nitrogen and carbon sources in growth medium containing OMW. The cell concentration and the total phenol concentrations were measured during 20 days growth period. *Crypthecodinium cohnii* growth in % 50 (v/v) dilute OMW medium provided cell dry weight of 0,4 g/l and removing 40% of total phenol concentration in shake flasks. *Galdieria sulphuraria* growth in % 50 (v/v) dilute OMW medium provided 0,95 g/l cell dry weight and 25% removal of total phenol concentration in shake flasks. Addition of extra nitrogen and carbon sources to OMW favoured only cell growths and dry weights and caused a slight increase on removal of phenol concentration compared with the control flasks for both microalgae strains. The results showed that the medium containing OMW could be used for growth of *Crypthecodinium cohnii* with a high phenol removal rates.

Keywords Olive oil mill wastewater, Microalgae, Growth rate, Bioremediation

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Ultrasonic waves: bioeffects on yeast cells

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Applications of ultrasound were starting from 1912 with the primary objective the detection of icebergs on prevention of maritime accidents. Algae, fish deaths and destruction were observed in the vicinity of sonar that equipped ships and submarines during the First World War.

The evolutions of research and studies with ultrasound have big advances following the discovery of piezoelectric transducers in science and technology. As an example we can mention its application in microsurgery, fatigue detection in aerospace mechanics, catalysis sonochemical, biotechnology and others.

The work presented here aims to demonstrate the application of ultrasonic beams in biotechnology with the aim of improving the fermentation of a culture broth containing biological agents. In these experiments we used as ultrasound equipment and oscilator Sonics VCX-600 (20KHz), probe type wave guide. The experiments were conducted in a glass reactor of 200 mL of biomaterial containing cane juice and *Saccharomyces cerevisiae* in suspension. The parameters analyzed were related to the content Alcohlic (FID gas chromatography), and cell viability (Neubauer chamber), TRS (refractometry). Analysis of results showed that the total production exceeded in irradiated samples compared to normal fermentation (without ultrasound), suggesting additional advantage of ultrasound activation. Lastin Trials 1400 min, showed ethanol production systems 12% more than non-enabled systems. In this context alternatives for ethanol production, bio fuel and many other byproducts of the alcohol industries and chemicals could benefit from the use of ultrasound beams in this range of frequencies.

Keywords Ultrasound, *Saccharomyces cerevisiae,* sonochemical catalysis, fermentation, ethanol.

Use of agroindustrial waste for the production of industrial enzymes

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Agro-industrial waste is a renewable energy source, which is some countries can be obtained in large quantities at low cost. Within these wastes can be found the lemon peel and coffee waste and these are produced in large amounts in Mexico. The objective of this work was the use of these agro-industrial residues for production of industrial application enzymes (cellulase, xylanase, amylase and laccase), produced by native white rot fungi (basidiomycetes) from the Northeast region of Mexico using submerged fermentation. Three fungal strains were selected from the Institute of Biotechnology's collection based on results from previous work. In the three strains was detected activity of xylanases, cellulases, amylases and laccases in submerged fermentation with 5% lemon peel and coffee waste as substrate, respectively at day 16. Table 1 shows the activities detected in lemon peel, observing that the RVAN 2 and CH5 strains showed the highest activities for the three enzymes followed by RVAN12. Otherwise, Table 2 shows the activities obtained with the coffee waste and again RVAN 2 and CH5 obtained the highest values; however these were lower than lemon peel, mainly cellulases.

Table 1.

Rot Fungi tested	Lemon peel		
	Enzimatic Activity (U. g^{-1})		
	Xylanases	Cellulases	Amylases
RVAN 2	197,76	532,95	242,93
RVAN 12	68,96	262,6	79,69
CH5	148,18	273,33	171,22

Table 2.

Rot Fungi tested	Coffee waste		
	Enzimatic Activity (U. g^{-1})		
	Xylanases	Cellulases	Amylases
RVAN 2	51,02	57,89	97,28
RVAN 12	39,28	76,64	76,06
CH5	51,11	58,21	80,43

The laccase activity at day 16, for both substrates, is shown in Table 3. The highest values were observed with coffee waste as a substrate. For this enzyme activity, the RVAN 12 showed the highest values on both substrates, followed by RVAN 2, and finally CH5, which did not show activity in lemon peel.

Table 3.

Hongos	Lemon peel	Coffee waste
	Enzimatic Activity (U.L^{-1})	
	Laccase	
RVAN 2	123,58	282,97
RVAN 12	365,18	613,06
CH5	0	46,59

Both residues can be used as a substrate for the production of enzymes of industrial importance, however for hydrolytic enzymes (xylanases, cellulases, and Amilasess lemon peel is better, while for peroxidase enzymes (laccases), the coffee waste gave higher values, this could be because of the effect of inducers which are present in this complex substrates, mainly for the laccase activity.

Key words: Agroindustrial, xylanases, cellulases, amilases, laccases, coffee spent, lemon peel.

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Vat dyeing with woad: implementation of an eco-friendly biotechnological process

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Isatis tinctoria L. is an indigo-bearing plant species referred to as woad. The traditional process for vat dyeing with woad dates back to the Middle Ages and basically relies on the microbial reduction of indigo to its soluble form, leucoindigo, through a fermentative mechanism. The cultivation and use of woad for the production of indigo dye went definitely into decline in the 19th century, when the use of synthetic indigo dye and chemical reduction agents was established, with a negative impact on the environment, due to the release of polluting waste waters by the dyeing industry. However, the ever growing demand for eco-friendly dyeing technologies has recently led to a renewed interest towards eco-friendly textile traditions. In this context, our study was aimed at developing a biotechnological process for vat-dyeing with woad. To this end, two minimal broth media, differing in the nitrogen source, namely fresh yeast extract (YE) and corn steep liquor (CSL), were formulated and evaluated for their capacity of sustaining the growth and reducing activity of the reference strain *Clostrium isatidis* DSM 15098, which was isolated in 1999 from a woad vat prepared and maintained according to the Middle Ages tradition. The ability of this strain to maintain a sufficiently reducing environment inside CSL minimal medium added with 140 g/L of woad powder was further evaluated by performing batch fermentations in laboratory bioreactors. Woad powder was manually extracted following the traditional procedure and its content in indigo dye was determined by measuring the absorbance at 664 nm ($A_{664\ nm}$) of a 0.02% solution in glacial acetic acid. During batch fermentations, pH was set at 9.0 ± 1.0 whereas temperature was maintained at 47°C ± 1°C. The effect of air and oxygen-free N_2 atmospheres on the growth and reducing-activity of the test strain was comparatively evaluated. Both biotic (viable counts of clostridial vegetative cells and thermoresistant spores) and abiotic (redox potential and base consumption) parameters were monitored up to 9 days of fermentation. In parallel, blue dyeing assays were daily performed and 5 x 10 cm cotton cloths or alternatively ~2 g wool yarns were gently entered the indigo dyebaths held stationary in the bioreactors at either 47°C or room temperature. After a residence time of ca. 15-20 s, these were pulled from the bioreactors and exposed to the air, soaped, manually washed, squeezed, and air dried to ensure complete removal of any residual water. The level of *in situ* oxidation of leucoindigo was first estimated by visually evaluating the intensity of the blue colour and further quantified with a spectrophotometric assay. The main results of the study are briefly summarized. The comparative evaluation of the two minimal media revealed that both of them were able to sustain the growth of the test strain and its reducing activity. However, higher cell counts and lower redox potentials (Eh < -600 mV) were recorded when CSL minimal medium was used; hence, this was selected to carry out the further laboratory blue dye assays. As expected the composition of the bioreactor headspace showed a great influence on both the biotic and abiotic parameters monitored, and a neatly higher dyeing capacity was shown by the dyebath maintained under strict anaerobiosis, which started to dye as early as after 24 h. This latter finding was quite surprising, if compared with dyebaths prepared and maintained according to the medieval tradition, which generally take several days to get started.

Keywords Indigo, *Isatis tinctoria* L., *Clostrium isatidis*, batch fermentation

The present study was financed within the National Programme for the reorganization of the sugar-beet cultivation area, Marche Region Action Plan Reg. CE no. 320/2006 – Misura: "Studi, ricerche e sperimentazione" managed by ASSAM, Project title: "Valorizzazione e rilancio della coltivazione del guado (*Isatis tinctoria* L.) nel territorio marchigiano".

Zinc bioleaching by *Pseudomonas aeruginosa*

Sevim Sedighzadeh, Sara Masoudi, Maede Mousavi

Through this research, the extraction of Zinc by using the *Pseudomonas aeruginosa* bacteria was investigated.

Zinc's extraction , were investigated in presence of different concentrations of glucose. The number of bacteria and the pH of these concentrations was measured as well. In ٦% glucose medium, pure zinc was the most extracted material.

Medical Microbiology

Pharmaceutical Microbiology

Antimicrobial agents and chemotherapy

A formulation of olive oils (oHo) shows potent antimicrobial activities *in vitro* and in patients with atopic dermatitis (AD) colonized by *S. aureus*

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Background. It is now clear that mixtures of extra virgin olive oils (EVOOs), or their polyphenols, show more potent antimicrobial and antioxidant activities than each particular EVOO. EVOOs antiinfectious activities are not only due to polyphenols but to some fatty acids too. Sebum of the stratum corneum of the skin contains fatty acids that are essential for the antiinfectious host resistance. Bearing in mind these features, we have elaborated a standardized formulation of at least 3 organic EVOOs (oHo®, Bioaveda, Spain). In 3 different clinical trials, the intake of oHo was able to avoid the dyslipidemic profile that defines cardiovascular risk. Moreover, oHo the intakes ameliorated skin xerosis in these patients. **Objectives.** To evaluate the antiinfectious activities of oHo *in vitro,* and *in vivo* in patients with AD colonized by *S. aureus*. **Methods.** The agar diffusion test was used for the *in vitro* assays. Different oHo concentrations were tested against pathogenic forms of *S. aureus*, *Psudomonas areuginosa*, *Candida albicans* and *Aspergillus niger*. In the *in vivo* study 17 patients (8 children, 6 with asthma) with AD were included. All patients had been unsuccessfully treated with oral and/or topic corticosteroids, topic inhibitors of calcineurin (TIC), and/or emollients for at least 2 years before the entry to this study. Eight patients showed skin infections due to *Staphylococcus aureus*. oHo was given daily at doses depending on age and weight. Two topic preparations, a gel and an emulsion made with oHo, were also applied twice a day in all patients. **Results.** Addition of oHo to agar plaques resulted in complete disappearance of all germs tested. Itching disappeared in all patients in a mean of 15 days, and the complete resolution of eczema was observed in a mean of 45 days after oral plus topic treatments. Five children with allergic asthma to olive pollen did not show asthma during the following year. Rhinitis and *S. aureus* colonization disappeared in all cases. A young adult with generalized AD, *S. aureus* colonization, and graft vs host skin disease (GvHSD) due to semiallogenic bone-marrow transplantation for leukemia, showed CR after 45 days of the combined treatment (see figure, up). Three adult patients who voluntary stopped treatments relapsed, but the re-start of treatments resolved the eczema again. Due to the absence of side-effects, and the high degree of acceptation, all patients continue the treatments just to now. **Conclusions.** oHo is a safe nutrition and topical intervention in severe AD. The *in vitro* results corroborate those obtained in trials in humans.

Keywords: Olive oils, oHo, *S.aureus*, atopic dermatitis

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A functional food additive from marine bacteria for the replacement of antibiotics in aquaculture

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The world-wide problem of fishery, due to the overexploitation of marine resources, has positioned aquaculture as an expansive and productive activity. This situation has placed Chile as the second largest producer and exporter of salmonids (*Salmo salar*). To consolidate the development and growth already reached, Chile is facing important challenges in order to make aquaculture an sustainable activity, environmental friendly and sure for the human health. On this context, the strategies for the control and treatment of infectious diseases in fish farming are, paradoxically, the mayor complication, due to a great quantity and variety of chemicals are used to avoid the enormous productive impact generated by infectious diseases outbreaks. For these reasons, it is necessary to decrease the use of antibiotics and generate different options for the prevention and treatment of microbial diseases. The goal of the present work is to develop alternatives based on the remarkable marine microbial diversity. In oceans, the heterogeneity of physical-chemical and biological conditions represents selection forces that have droved the marine microbial evolution towards a great biological diversity with a cognate chemical variety. This association helps to explain the microbial interactions -among microorganisms or with eukaryotic hosts- and the assimilation of nutrients, frequently scarce at the marine environments. Thus, by the great microbial diversity present in oceans, it is rational to considerer marine microorganisms as sources for the explorations and discovery of compounds with industrial or health applications. In this study, there are presented the physical and biological properties of surface-active compounds obtained from marine hydrocarbonoclastic bacteria (MHB) isolated from inter-tidal ponds of Valparaíso shore. The compounds are biosurfactants (BS) produced during growth of MHB (on sulphur heterocyclic hydrocarbons as the only carbon source), which were selected by their ability to modulate microbial cells communications and the fish immune response. A total of 37 strains of MHB were isolated, characterized and identified by physiological, metabolic, biochemical and molecular assays. Surface-active properties were evaluated by surface tension and emulsifier stability. In accordance to chemical approximations of BS produced, by Gas chromatography–mass spectrometry (GC-MS) and NMR spectroscopy, microbe-microbe and microbe-fish interactions were evaluated using Quorum Sensing biosensors, cells fight-challenges against fish pathogens and by the immune response of *Oncorhynchus mykiss*. The effects against the pathogen *Aeromonas salmonicida* were evaluated by qRT-PCR of virulence transcripts obtained from *A. salmonicida* exposed to different concentrations of BS or viable cells of MHB selected. Immune response of *Oncorhynchus mykiss* was by Tnf-α and IL-1β. Results obtained show that two strains of MHB produce extra cellular BS with emulsifiers and biological features able to affect on *A. salmonicida* the expression of virulence factors and induce, on trout (*Oncorhynchus mykiss),* the immune response by the IL-1β. Strain of MHB that produce the functional bioemulsifiers was identified as member of *Cobetia* genus and was deposited at the Colección Española de Cultivos Tipo (CECT Nº 7764). The BS selected is an emulsifier with suitable properties to its incorporation in solid fish food as a potential functional additive for the control and treatment of microbial diseases in aquaculture. Project F-D07I1061 funded by Fondef Conicyt, Government of Chile.

Keywords hydrocarbonoclastic; biosurfactants; functional-foods; fish-pathogen

Activity methanolic extracts of *Azadirachta indica* (A. Juss) on *P. Gingivalis*

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Periodontal disease is an oral disease that has a high incidence worldwide, plus it is considered the leading cause of tooth loss in adults. This condition is characterized by an infectious disease, painless and slowly progressive, being one of the main etiological agents *Porphyrormonas gingivalis* bacteria. This paper analyzes the antibacterial activity of methanol extracts different parts of the plant *Azadirachta indica (A. Juss)* planted in the northeastern region of Mexico, and currently used empirically in the development of toothpastes people of the region. To do this we collected samples of plant material, these were identified and cataloged in the Herbarium of the FCB UANL. Extracts were prepared by soxhlet method using 30 g of each sample dried and crushed in 500 ml of methanol yields were obtained. Extracts were made from leaves, branches and stems. Was activated strain ATTC 33277 in environment induced by the anaerobic chamber, and preserved 8 days in an incubator at 37 ° C. Subsequently the methanol extracts were prepared at a dilution of 100 mg / ml in bidistilled water, given as a positive control 0.12% chlorhexidine and distilled water with DMSO as negative control. It took 100 ml of each tube with bacterial growth and seeded by the technique of closed groove on petri dish with culture medium sheep blood agar. Sensidiscos Place four sterile filter paper Whatman # 1 moistened with 10µl of each extract to test, are 4 repetitions. They guard the petri dishes in airtight bags to preserve the anaerobic environment and kept in an incubator at 37 ° C for seven days. Subsequently measured the inhibition halos of obtaining the extracts tested were more active on *P.gingivalis* leaf extracts of Neem. According to preliminary results is recommended to continue tests with the extract bioautografía activity and subsequently tested cytotoxicity and mutagenicity tests. The data obtained from this analysis will be crucial to assess the feasibility and safety of its therapeutic use.

Keywords: *Azadirachta indica,* Neem, Periodontal disease

Adaptive proteome changes in population of erythromycin resistant *Escherichia coli* during continuous cultivation in the presence of low concentration of the antibiotic

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The emergence and spread of antimicrobial resistance are complex problems driven by numerous interconnected factors, many of which are linked to misuse of antimicrobials both in terms of wrong dosage and improper specificity. It is a general notion that the resistance to antibiotics is associated with the cost represented by lowering the fitness of bacteria. These costs can be compensated, usually without loss of resistance, by second-site mutations during the evolution of the resistant bacteria. We studied this process on erythromycin resistant *E. coli* grown in non-limited chemostat. In continuous bacterial culture grown for over 120 generations in the presence of sublethal concentration of erythromycin (Ery) we observed a subpopulation of bacteria with higher antibiotic resistance and increased fitness, measured by growth and translation rate. We followed this adaptation of the culture on the level of proteome changes. Comparison of proteomes of erythromycin challenged culture and the control culture from 43h, 68h and 103h of cultivation revealed three practically not overlapping groups of proteins with changed expression. The bacterial population after 43h (roughly 45 generations) of growth in Ery experiment contained small number of clones with higher Ery resistance, it grew with doubling time value nearly 40% higher than the control culture and required nearly 50% more of elongation factor Tu to run the translation system. This was reflected by major changes in compared proteomes. We identified 26 up-regulated and 6 down-regulated proteins in Ery experiment compared to the control. Up-regulated proteins belonged mostly to carbohydrate, amino acid and nucleotide metabolism. Bacterial population after 68h cultivation (roughly 70 generations in control) showed higher number of highly resistant clones, the doubling time was the same and EF-Tu demand was even higher than in 43h population. Comparison of Ery and control proteomes showed much less differences. There were only 7 down-regulated proteins in Ery experiment from different parts of bacterial metabolism. After 103h of cultivation the bacterial population showed important changes in physiology. The proportion of Ery highly resistant clones in the population considerably increased, the doubling time shortened to the control level (60min) and the demand for EF-Tu lowered to the value found in the control (12% of all protein synthesized). These changes were reflected on the proteome level by up-regulation of 19 proteins and down-regulation of two proteins, all identified proteins (except for 2) were different from those found in younger bacterial populations (43h and 68h). Identified proteins were, unlike those in young populations, mostly not involved in energy metabolism. Measurement of translation accuracy, using plasmid borne bacterial luciferase gene system, showed different level of nonsense suppression of TGA stop inserted in *luxB* gene in the control and Ery treated culture. Final population in Ery experiment tended to lower translation accuracy compared to the control population. The changes in the physiology of Ery treated *E. coli* population reflected by distinct proteome changes in chemostat showed a clear evolutionary tendency leading to the selection of more fit subpopulation of bacteria resistant to a higher concentration of Ery. These results support the hypothesis that a long time application of selective pressure, by a sub-lethal concentration of the antibiotic on resistant bacteria, can generate subpopulation with increased resistance. At the same time its handicap of being less fit is eliminated by accumulation of adaptive changes (mutations) increasing fitness of the population, which is demonstrated by concerted changes in protein expression profiles.

Keywords: *Escherichia coli*, antibiotic resistance, adaptive changes, fitness, proteome, chemostat,

An investigation of disinfectant resistance gene in hospital isolated gram negative bacteria

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Biocides have an important role in hospital infection control. The widespread use of biocides may lead to microbial resistance, in particular cross-resistance to antibiotics. The aim of the present study is to determine the *qacEΔ1* gene, which has been known as a determinant of resistance to quaternary ammonium compound (QAC), by polymerase chain reaction (PCR) in clinically isolated multi-drug resistant (MDR) gram negative bacteria. All the isolates were tested for antibiotic susceptibility by disc diffusion method according to the Clinical Laboratory Standard Institute (CLSI).Totally 40 strains of *Acinetobacter baumannii* and 20 strains of *Pseudomonas aeruginosa* were enrolled in the present study. Of the *Acinetobacter baumannii* and *Pseudomonas aeruginosa* strains, 52.5% and 20.0% were positive for the *qacEΔ1* gene, respectively. Specific primers were used to amplify a 300 bp fragment of *qacEΔ1* gene. To the best of our knowledge, this is the first study investigating the distribution of *qacEΔ1* gene in our hospital.

Keywords: QAC, *qacEΔ1,* gram negative bacteria, multi-drug resistant

Antibacterial activity against multi resistant bacteria strains of alkaloid extracts of two Algerian *Fumaria* species

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Antibacterial activity of alkaloids of two medicinal plants *Fumaria bastardii* and *Fumaria capreolata* was evaluated by two diffusion methods, wells method and discs method, against four bacterial stocks resistant to antibiotics isolated from infected patients in hospital. The tested strains are *S.aureus S56*, *K.pneumoniae E47,P.aeruginosa 604 and E.coli).* The contents of alkaloids were 2,42% and 1,17% in *Fumaria bastardii* and *Fumaria capreolata* respectively . Both alkaloid extracts showed a high antibacterial activity against the four strains tested. However *P.aeruginosa* was the least susceptible strain. Extract of *F.bastardii* was the most active one showing a higher activity excepted against *P.aeruginosa* which was most susceptible to *F.capreolata* extract. A synergy effect was observed between alkaloids of *F.bastardii* and the SXT on *K.pneumonia* and between alkaloids of *F.capreolata* and the SXT on *P .aeruginosa 604*.

Key words: Alkaloïds, antibacterial, Fumaria, S.aureus , K.pneumoniae , P.aeruginosa, E.coli,resistant,synergy.

Antibacterial activity of galls of *Quercus infectoria* olivier against oral pathogens

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Objective: To evaluate the antibacterial activity of galls of *Quercus infectoria* Olivier against oral bacteria that has a role in pathogenesis of dental caries and periodontitis.

Materials and Methods: The galls were extracted through cold extraction in order to obtain methanol extract. For acetone extract, it was obtained from the prepared remaining sample. Both extract were screened against two Gram positive bacteria which cause dental caries (*Streptococcus mutans* ATCC 25175 and *Streptococcus salivarius* ATCC 13419) and two Gram negative bacteria which act as causes of periodontitis (*Porphyromonas gingivalis* ATCC 33277 and *Fusobacterium nucleatum* ATCC 25586). The screening test of antibacterial activity was done by using agar well diffusion method. Subsequently, minimum inhibitory concentration (MIC) was determined by using twofold serial microdilution method at a concentration ranging from 5 mg/ml to 0.01 mg/ml. Minimum bactericidal concentration (MBC) was finally obtained by subculturing microtiter wells which showed no colour changes of indicator after incubation.

Results: Methanol and acetone extracts showed inhibition zones which were not differ significantly against each tested bacteria. Among all tested bacteria, *S. salivarius* was the most susceptible. The MIC ranges for methanol and acetone extracts were the same, ranging from 0.16 – 0.63 mg/ml. For the MBC value, methanol and acetone extracts produced MBC value in the range of 0.31 – 1.25 mg/ml and 0.31 – 2.50 mg/ml respectively.

Conclusion: The methanol and acetone extracts of *Q. infectoria* galls exhibited similar antibacterial activity against oral bacteria. Thus, the galls are potentially a good source of antibacterial agents which may be beneficial in development of effective phytotherapeutic agent for prevention of oral disease.

Keywords: *Quercus infectoria*, galls, oral bacteria, antibacterial activity, MIC, MBC

Antibacterial activity of protein preparations from *Moringa oleifera* seeds

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Moringa oleifera (Lam.) seeds are able to reduce turbidity and improve water quality, making it more suitable for human consumption. The seeds contain the water-soluble *M. oleifera* lectin (WSMoL) with coagulant and insecticide activities. Lectins are proteins widely distributed among plants. The lectin binding to carbohydrates of plasmatic membranes promotes erythrocyte network resulting in the agglutinating phenomenon. Lectins have been considered as participants in plant defence mechanisms against phytopathogen microrganisms. Interaction of lectin with teicoic and teicuronic acids, peptidoglycans and lipopolysaccharides present in bacterial cellular walls result in antibacterial activity. The objectives of this study were to examine the antibacterial activity of protein preparations from *M. oleifera* seeds containing or not WSMoL. Hemagglutinating activity was assessed using suspension (2.5% v/v) of rabbit erythrocytes. Seed extract (SE) was treated with 60% saturated ammonium sulphate and chromatography of precipitated protein (0-60 fraction) on chitin column separated non-hemagglutinating components (NHC) from WSMoL. Antibacterial activity of SE, NHC and WSMoL was evaluated on *Staphylococcus aureus* (WDCM 00034) and *Escherichia coli* (WDCM 00013). Milli-Q® water was the negative control. Stationary cultures were maintained on Nutrient Agar (NA) and stored at 4 ºC. Bacteria were cultured in Nutrient Broth (NB) and incubated at 37 ºC for 3 h. The culture concentrations were adjusted turbidimetrically at a wavelength of 600 nm to 10^5-10^6 colony forming units (CFU) ml^{-1}. Two hundred µl of SE (10 mg ml^{-1} of protein), NHC (1 mg ml^{-1} of protein), WSMoL (1 mg ml^{-1}) or Milli-Q® water were added to 200 µl of each incubation medium. The mixtures were shaken and incubated at 37 ºC for 24 h. NA medium (20 ml) was distributed to sterile Petri plates (90 x 15 mm) and allowed to solidify. From each of the incubation mixtures, 50 µl were withdrawn from either near the surface (top) or from the sediment and smeared on NA plates. The plates were incubated at 37 ºC for 24 h, after which bacterial growth was observed. The antibacterial activity of seed preparations was also evaluated using *Cavouco* lake water. Aliquots (0.5 mL) of SE, NHC, WSMoL or Milli-Q® water were added to lake water, diluted 1:4 with Milli-Q® water and incubated at 37 ºC. After 14 h of incubation, 50 µl of each mixture were smeared on NA plates containing NA, and incubated at 37 ºC for 24 h. The minimal inhibitory concentration (MIC) was determined for *E. coli* and *S. aureus*. A 1/1000 dilution in NB of a 10^5-10^6 CFU overnight culture was prepared. Samples of SE, NHC and WSMoL (0.5 mg ml^{-1}) were diluted 1:2 in NB and submitted to a series of 10 double dilutions, to a final ratio of 1:2048. A 180 µl aliquot of each dilution was dispensed into a microtiter plate well. All wells were inoculated with 20 µl of the 1/1000 bacterial inoculum and incubated at 37 ºC for 24 h. After incubation, the optical density at 605 nm (OD_{605}) was measured using a microplate reader. MIC was determined as the lowest concentration at which there was ≥50% reduction in optical density relative to the control well OD_{605}. To determine the minimum bactericide concentration (MBC), inoculations from the wells of treatments that were found to inhibit bacterial growth were transferred to a NA plate and incubated at 37 ºC for 24 h. The lowest concentration showing no bacterial growth was recorded as the MBC. Amoxicillin (1 mg ml^{-1}) was used as the positive control. *S. aureus* MICs were 7.8 µg ml^{-1} (WSMoL), 62.5 µg ml^{-1} (SE), and 625 µg ml^{-1} (NHC). WSMoL had the lowest MIC (250 µg ml^{-1}) for *E. coli* while the NHC and SE values were much higher (500 µg ml^{-1} and 5000 µg ml^{-1}, respectively). MBC assays revealed bactericidal activity only in WSMoL (*S. aureus*, MBC of 300 µg ml^{-1}). In lake water, SE and NHC were not effective in reducing the growth of bacteria; in contrast, WSMoL was effective. In conclusion, WSMoL, a chitin-binding lectin, is a biomaterial of potential antibacterial application.

Keywords lectin; *Moringa oleifera*; antibacterial activity; *Staphylococcus aureus*; *Escherichia coli*; lake water bacteria.

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Antibacterial and antifungal activities of Saharian spontaneous plants

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Nowadays Saharian spontaneous plants are frequently used in Folk medicine. In order to inventory and develop these phytogenetic resources, antibacterial and antifungal activities of three plants were investigated. The plants tested are *Matricaria pubescens, Ammodaucus leucotrichus ,Cymbopogon schaenanthus.* Extracts by maceration in solvents with different polarities were tested. All crude extracts showed strong antibacterial activity against *S.aureus* and *E.Coli* and also antifungal one against *Candida tropicalis.*

Key words: Sahara,Matricaria pubescens, Ammodaucus leucotrichus, Cymbopogon Schaenanthus, S aureus , E.coli, C.tropicalis

Antibiotic resistance and incidence of *Enterococcus faecalis* Conjunctival Swab from Diabetic Patients

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Enterococcus faecalis is an emerging etiologic agent of hospital infections, exhibiting high rates of antibiotic resistances. Enteroccoci have to produce biofilms on intraocular lens materials, further highlighting their potential virulence for the eye. 50 eyes of diabetic patient were analysed for the presence of E. *faecium*, and presumptive isolates were identified by morphological, cultural and biochemical tests and confirmed by the VITEK system (BioMerieux). Automated EcoRI Ribotyping was performed with a RiboPrinter® Microbial Characterization System (Dupont Qualicon). Among the 26 isolates of *Enterococcus* spp., 24 isolates were *E. faecalis*, 2 isolates were *E. avium*. The resistance of the isolates to 10 different antibiotics which are use as ophthalmic drop was determined by the Kirby–Bauer disc diffusion test. Resistance to vancomycin was found in these enterococci, and resistance to vancomycin was found in 16.67 % of *E. faecalis* isolates. The most effective antimicrobials were Moxifloxacin (96.15% of isolates inhibited), Gentamycin (92.3% of isolates inhibited) and Gatifloxacin (92.3% of isolates inhibited). Post operative endophthalmitis might be formed if *E. faecalis* is presented on ophthalmic flora of diabetic patients. *E.* faecalis has potential of forming biofilm on intraocular lens materials. For this reason, it has great benefit to know bacterial flora of the conjunctivas from diabetic patients, who goes under ophthalmic surgery. To know the conjunctival flora giving us a great chance to choose suitable antibiotics for prophylaxis and postoperative use.

Antibiotic resistance in oral *Streptococcus spp.* isolated from healthy children, Turkey

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Mutans streptococci are microorganisms associated with the development of caries and *Streptococcus mutans* is the most frequently isolated member of this group in humans. Because of its heterogeneous colonization of the oral cavity, it may be detected on some teeth and surfaces but not on others in the same mouth. A strong association between salivary levels of *S. mutans* and its colonization of dental surfaces is well established,with a greater number of colonized surfaces at higher salivary levels.

This study aims to identify the predominant streptococcal species in the mouths of healthy children and investigate the antibiotic susceptibility of oral *Streptococcus* spp..

The mouth subjects were healthy school children aged between 4 and 16 years. The oral flora was sampled saliva, gingival crevicular fluid, dental plaque, and dental caries. Spread- plated onto Mitis salivarus agar, 5% defibrinated sheep's blood agar and M17 agar (Oxoid). The plates were placed in an anaerobic flask (Oxoid) in the presence of a gas-generating kit (Anaerobic System BR0038B, Oxoid) and incubated at 37°C for 2-3 days. Presumptive isolates were identified by morphological, cultural and biochemical tests and confirmed by the BIOLOG system. Automated EcoRI Ribotyping was performed with a RiboPrinter® Microbial Characterization System (Dupont Qualicon). The resistance of the isolates to 12 different antibiotics was determined by Kirby–Bauer disc diffusion test with commercial disks.

The predominant species were *Streptococcus mutans*, *S. oralis.* Resistance to Linezolid was found in these *Streptococcus* spp. The presence of resistant bacteria in the mouth can be the major cause of dental antibiotic prophylaxis failure. Particular attention should be paid to antibiotics that are most frequently used in dental practise.

Antibiotic Resistant *E. coli* from water samples of Malir: An issue of public health concern in Pakistan

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Introduction: Gharo is a city in Thatta District, Sindh. The people often complain and greatly suffer with non-availability of civic facilities including drainage system, potable water, sanitation etc. The scarcity of water is a main issue of the inhabitants of this area as just rely upon limited water supply harboring predominantly with water borne diseases as well as gastro and diarrhoea as they are drinking unsafe drinking water since last many years.

Material and Methods: In this study, a team of SCRC visited and collected around 60 drinking water samples (1 Litre) from various spots of Gharo and immediately transported to IIDRL-KU. Microbiologically, all the samples were analyzed by Membrane Filtration Technique (MFT) on different selective and differential microbiological media. All these potential pathogens were identified by conventional and rapid (QTS 10) methods. Antibiotic susceptibility was determined by Kirby Bauer disc diffusion method and Minimum Inhibitory Concentration (MIC) was also determenined by Micro dilution method.

Results: Almost all the samples tested were found positive for potential gram-negative particularly enteric microorganisms including *Escherichia. Coli (60%), Enterobacter aerogenes (40%), Proteus vulgaris (10%), Pseudomonas aeruginosa (28%), Shigella dysenteriae(25%), Salmonella typhi(20%), and Aeromonas hydrophila (2%)* and gram- positive include *Staph. aureus (35%), Staph.epidermidis (30%*). Moreover, a very high resistance pattern was observed against a panel of a dozen of antibiotics like Cephalexin (80%), Erythromycin and Tetracycline (48%), Ampicillin(66%), Novobiocin(70%) Doxycycline(99%), Amoxicillin(41%), Ceftrizoxime (95%), Chloramphenicol (40%) , Gentamicin (60%), Ofloxacin (30%) and Ciprofloxacin(20%). Minimum Inhibitory Concentration (MIC) was also determined variable ranges against the above mentioned antibiotics.

Conclusion: Presence of *E.coli* in water as well as other serious pathogens is a strong indication of sewage or animal waste contamination, which may cause many a wide range of disease and entirely unfit for human consumption. Plus, high level of antibiotic resistance among potential pathogens is a matter of great concern for public health.

Antifungal activity of *Coriandrum sativum* essential oil, its mode of action against *Candida* species and potential synergism with amphotericin B

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The increasing incidence of drug-resistant pathogens coupled with the toxicity of existing antifungal compounds has drawn attention towards the antimicrobial activity of natural products. Essential oils are one of the most promising groups of natural compounds that might be used in the prevention and treatment of fungal infection, namely of *Candida* species that are known as the most common cause of invasive fungal infections in humans. Coriander (*Coriandrum sativum L.*) essential oil is among the most used essential oils worldwide being referred as presenting antimicrobial activity against bacteria, namely foodborne bacteria.

The aim of the present study was to evaluate the antifungal activity of coriander essential oil on *C. albicans* ATCC 90028 and ATCC 24433 and *C. tropicalis* 750.

Antifungal susceptibility of coriander essential oil was assessed according to classical microbiological techniques for minimum inhibitory concentrations (MIC) and minimum lethal concentration (MLC) determination and for study of the effect of essential oil upon germ tube formation. The checkerboard assay was used for the evaluation of the potential synergistic effect between this oil and amphotericin B. Moreover, the effect of coriander essential oil on cellular functions was evaluated by flow cytometry using bis-1,3-dibutylbarbutiric acid (BOX) for membrane potential evaluation, propidium iodide (PI) for membrane permeability assessment and deep red-fluorescing bisalkylaminoanthraquinone number five (DRAQ5) for the analysis of intracellular DNA content.

Coriander essential oil has a fungicidal activity against the tested *Candida* strains with MLC values equal to the MIC value and ranging from 0.05-0.4% (v/v). Flow cytometric evaluation of BOX, PI and DRAQ5 staining indicates that the fungicidal effect is a result of cytoplasmic membrane damage and subsequent leakage of intracellular components such as DNA. Also, concentrations bellow the MIC value caused a marked reduction in the percentage of germ tube formation for *C. albicans* strains. A synergetic effect between coriander essential oil and amphotericin B was also obtained for *C. albicans* strains, while for *C. tropicalis* strain only an additive effect was observed. This study describes the antifungal activity and mode of action of coriander essential oil on *Candida* spp, demonstrating that the use of this oil can be an appealing alternative in the design for new formulations to treat candidosis.

Keywords Coriander essential oil; *Candida* spp.; flow cytometry; germ tube; synergism; Amphotericin B

Antimicrobial activity and anticancer potential of a new *Nonomuraea* sp. strain PT708 originated from Thai cave soil

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Strain PT708 was isolated from cave soil at Pha Tup Cave Forest Park, Nan, Thailand. Phylogenetic analysis indicated that it belongs to genus *Nonomuraea* with relatively high similarity value (98.28%) of 16S rRNA gene sequence to *N. roseola* AJ278221 and *N. dietzii* AJ278220. However, its unique morphology to form single spore is different with these two species compared. Consequently, we suppose that the strain should be nominated to be a new species of genus *Nonomuraea.* Strain PT708 showed ability to produce antimicrobial compounds against *Bacillus cereus*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Paenibacillus larvae* when it was grown in AMHU-5 medium. MRSA was the most susceptible for the antagonistic activity followed by *B. cereus*. MIC values of the crude extract from this strain against *B. cereus*, MRSA and *P. larvae* were 80, 80 and 175 µg/mL, respectively. Moreover, this crude extract showed anticancer potential against human small lung cancer cell (NCI-H187) and oral cavity (KB) with IC_{50} values of 3.48 and 16.11 µg/mL, respectively, but no inhibition was observed for breast cancer (MCF7) at the concentration up to 50 µg/mL.

Keywords *Nonomuraea* sp.; rare actinomycetes; cave soil; antibiotic; anticancer activity

Antimicrobial activity and biocompatibility of chitosan hydrochloride against *Candida* species

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Chitosan, a cationic amino polysaccharide, essentially β-1,4 D-glucosamine (GlcNAc) linked to N-acetyl-D-glucosamine residues, is naturally present in the cell wall of certain fungi, and can also be obtained by chitin deacetylation. Chitosan is a weak base and is insoluble in water and organic solvent; however water-soluble chitosan can be obtained through a chemical modification. In food technology chitosan is important due to its several functional properties and can be used as antimicrobial agent. The aim of this study was to investigate, in vitro, the antifungal activity and the biocompatibility of chitosan hydrochloride, against three pathogenic *Candida* species. Chitosan from crabs (Sigma®) with 65% of deacetylation and viscosimetric molar weigh of 7.59×10^5 g/mol was modified for chitosan hydrochloride, soluble in pure water. The effectiveness of chitosan hydrochloride in inhibiting the growth of *Candida albicans, Candida guillermondi, Candida glabrata* and *Candida krussi* was tested. Chitosan hydrochloride solutions at concentrations ranging from 10.0 to 0.002 mg/mL were prepared in distillated water (v/v), pH adjusted to 7.0. The antifungal activity was assessed by determining the minimum inhibitory and fungicide concentration using broth dilution method in Sabouraud medium. Chitosan hydrochloride was replaced with sterile distilled water in the positive control. The chorioallantoic membrane of chick embryo (CAM) was used to evaluate biocompatibility and toxicity of chitosan hydrochloride. The parameters vasoconstriction, hemorrhage and coagulation to evaluate the potential for irritation according to the method of HET-CAM, signs of inflammation, edema or neovascularization were observed. Microbial growth was observed in the positive control, and the viability of the *Candida* strains was confirmed by growth in Sabouraud agar without chitosan hydrochloride. Chitosan hydrochloride showed identical minimum inhibitory concentration (CIM) and minimum fungicide concentration (CFM) for all *Candida* tested, 0.3125mg/mL and 1,25mg/mL, respectively. The exact mechanism of the antimicrobial action of chitosan hydrochloride is still unknown, but different mechanisms have been proposed, in reference to its chemical and structural properties. Signs of vasoconstriction, hemorrhage, coagulation, inflammation, edema or neovascularization were not observed by application of Chitosan hydrochloride. The results demonstrate biocompatibility and antifungal potential by chitosan hydrochloride against the *Candida* species tested.

Key-Words: Polymer, Antimicrobial activity, Hydrochloride

Antimicrobial activity and biocompatibility of chitosan hydrochloride against *Penicillium* species

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Chitosan, a cationic amino polysaccharide, essentially β-1,4 D-glucosamine (GlcNAc) linked to N-acetyl-D-glucosamine residues, is naturally present in the cell wall of certain fungi, and can also be obtained by chitin deacetylation. Chitosan is a weak base and is insoluble in water and organic solvent; however water-soluble chitosan can be obtained through a chemical modification. In food technology chitosan is important due to its several functional properties and can be used as antimicrobial agent. The aim of this study was to investigate, in vitro, the antifungal activity and the biocompatibility of chitosan hydrochloride, against five pathogenic *Penicillium* species. Chitosan from crabs (Sigma®) with 65% of deacetylation and viscosimetric molar weigh of 7.59×10^5 g/mol was modified for chitosan hydrochloride, soluble in pure water. The effectiveness of chitosan hydrochloride in inhibiting the growth of *P. expansum; P. fumigatus; P. citrinum; P. funiculosum; P. pinophilum* was tested. Chitosan hydrochloride solutions at concentrations ranging from 10.0 to 0.002 mg/mL were prepared in distillated water (v/v), pH adjusted to 7.0. The antifungal activity was assessed by determining the minimum inhibitory and fungicide concentration using broth dilution method in Sabouraud medium. Chitosan hydrochloride was replaced with sterile distilled water in the positive control. The chorioallantoic membrane of chick embryo (CAM) was used to evaluate biocompatibility and toxicity of chitosan hydrochloride. The parameters vasoconstriction, hemorrhage and coagulation to evaluate the potential for irritation according to the method of HET-CAM, signs of inflammation, edema or neovascularization were observed. Microbial growth was observed in the positive control, and the viability of the *Penicillium* strains was confirmed by growth in Sabouraud agar without chitosan hydrochloride. Chitosan hydrochloride showed lower minimum inhibitory concentration (CIM) against *P. fumigatus; P. citrinum* and *P. pinophilum* (0.3125mg/mL) than *P. expansum* and *P. funiculosum* (0.625mg/mL); and identical minimum fungicide concentration (CFM) for *P. citrinum* and *P. pinophilum* (0.625mg/mL) and *P. fumigatus* , *P. expansum* and *P. funiculosum* (1.25mg/mL). The exact mechanism of the antimicrobial action of chitosan hydrochloride is still unknown, but different mechanisms have been proposed, in reference to its chemical and structural properties. Signs of vasoconstriction, hemorrhage, coagulation, inflammation, edema or neovascularization were not observed by application of Chitosan hydrochloride. The results demonstrate biocompatibility and antifungal potential by chitosan hydrochloride against the *Penicillium* species tested, and *P. fumigatus* was the sample that demonstrated higher resistance.

Key-Words: Polymer, Antimicrobial activity, Hydrochloride

Antimicrobial activity of oils from extracts of *Gongronema latifolium (Endl) Decne* on bacterial isolates from blood stream of HIV infected patients

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Oils from *Gongronema latifolium* leaves *(Endl*.) *Decne* was obtained by hydrodistillation and analyzed using Gas Chromatography/Mass Spectrophotometry (GC-MS).The Oils were characterized by high percentage of Phthalic acid (18.61%), stearic acid (4.63%), Palmitic acids (2.72%), Oleic acids (5.2%), arachidic acid (2.34%), and fumaric acid (2.22%). Monoterpenes including camphor, β – Cymene, and phytol as well as Sulfonamides, quinoline and carboxamide were also present.The essential oils as well as aqueous and ethanolic extracts of *Gongronema latifolium* leaves were evaluated for antimicrobial activity against bacteria isolated from blood streams of HIV patients in Lagos. Using agar diffusion method, the essential oil and the extracts showed moderate inhibitory activity against all the *Staphylococcus spp, Escherichia coli, Shigella* spp , *Salmonella* spp*, Klebsiella pneumonia , Pseudomonas aeruginosa, Onchrobactrum anthropi and Candida albicans*. The zone of inhibition values recorded were comparable to control antibiotic ampicillin but less than that of Ciprofloxacin and Chloramphenicol. The MIC for the fatty acids ranged between 5µg/ml– 40µg/ml, while MBC also ranged between 5µg/ml – 40µg/ml, the MIC and MBC for ethanol extract ranged between 3.125mg/ml - 12.5mg/ml and 3.125mg/ml – 25.0mg/ml,while aqueous extract MIC range between 6.25mg/ml – 25.0mg/ml and MBC also ranged between 6.25mg/ml – 25.0mg/ml respectively. Phytochemical screening revealed the presence of compounds such as Saponin, Alkaloids, Pyhlobatinnins, Glycosides and Flavonoids. Extracts of *Gongronema latifolium* may be useful in ethnomedicine and in the treatment of blood stream infections in HIV patients.

Keywords: *Gongronema latifolium*; Oils; Phytochemical screening; HIV related diseases; antimicrobial activity.

Antimicrobial potentials of Marine Algae *Halimeda opuntia* and *Sarconema filiforme* collected from Red Sea Coast

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Marine algae *Halimeda opuntia* and *Sarconema filiforme* is known to produce a wide range of chemically interesting secondary metabolites. Antimicrobial bioassay against some human pathogenic bacteria and yeast were conducted using disc diffusion method. *Halimeda* extract exhibited antibacterial activity against six species of microrganisms, with significant inhibition against *Staphlococcus aureus*. While *Sarconema* extract was better potent as antifungal against *Candida albicans*. Comparative antibacterial studies showed that *Halimeda* extract showed equivalent or better activity as compared with commercial antibiotic when tested against *Staphlococcus aureus*. Further tests conducted using dilution method showed both extracts as having bacteriostatic mode of action against the tested microorganisms. The screening results confirm the possible use of marine algae *Halimeda opuntia* and *Sarconema filiforme* as a source of antimicrobial compounds.

Keywords Antimicrobial; *Halimeda opuntia*; *Sarconema filiforme*; Red Sea Coast.

Antimicrobial susceptibilities of Porphyromonas and Prevotella species isolated from periodontitis infections in north of Portugal

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Objectives: The goals of these studies were to identify Porphyromonas and Prevotella species from periodontal pockets of Portuguese adults suffering of periodontitis infections and to test in vitro β-lactamase production. Their susceptibility to five antibiotics commonly prescribed as treatment or prophylaxis in odontology in Portugal were studied.

Methods: 43 isolates of black-pigmented gram negative strict anaerobes rods were identified by the Rapid ID 32 A (bioMériuex, France) and confirmed by PCR analysis for Porphyromonas gingivalis and Prevotella intermedia. β-lactamase production was determined by nitrocefim dryslide (BBL, EUA) and confirmed by disk diffusion synergy with amoxicillin/clavulanic acid. The susceptibility to antibiotics was performed with amoxicillin, amoxicillin/clavulanic acid, metronidazole, clindamycin and tetracycline impregnated disks (Oxoid) by disk diffusion method.

Results: The Prevotella intermedia represented 44% and Porphyromonas gingivalis 20% of total isolates, with a good correlation between employed methods. The remaining 36% strains belong to other black-pigmented species. Only 2% of Prevotella sp. showed β -lactamase production with resistance to amoxicillin and susceptibility to amoxicillin/clavulanic acid disks. In remaining strains, although results from the disk diffusion method were confirmed by nitrocefim disks, no β-lactamase production was detected. These strains showed susceptibility to all tested antibiotics.

Conclusion: The most frequently isolated anaerobic species from periodontal pockets was Prevotella intermedia. A low number of β-lactamase producing strains was detected. The majority of P. intermedia and all P. gingivalis isolates were susceptible to all the antibiotics tested. These results are not in accordance with most studies of other countries that showed high levels of resistance among anaerobes. Further studies will be required where antibiotic resistance genes will be screened.

Keywords Antimicrobial susceptibilities, Porphyromonas sp. , Prevotella *sp.*

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Antimicrobial susceptibility and quinolone resistance mechanism of *Arcobacter butzleri* isolates from sewage samples in Spain

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Arcobacter butzleri is considered to be an emerging human pathogen that can cause severe diarrhea and bacteremia. The association of *A. butzleri* with human gastroenteritis has been reported in many countries, and such gastroenteritis is considered to be increasing in incidence. Water is a potential source of *Arcobacter* spp. The direct connection between consumption of *Arcobacter* contaminated water and human diseases has not been established yet, although it is likely that water may play an important role in the *Arcobacter* transmission to humans which strongly suggest a faecal-oral route. Fluoroquinolones such as ciprofloxacin and levofloxacin are potential drugs to treat infections. However, there is evidence of increasing resistance to these antimicrobial agents. This resistance may occur due mutations in a quinolone resistance-determining region (QRDR) of the *gyrA* gene. Therefore the goal of this study was to study the quinolone susceptibility of *A. butzleri* isolates and the mutations associated with quinolone resistance.

In this study, sixty *A. butzleri* isolates obtained from sewage samples and the reference strain *A. butzleri* DSM8739 were included. *Arcobacter* susceptibility to ciprofloxacin and levofloxacin was determined by disc diffusion tests (BD, USA) and E-test® strips (AB BIODISK, Sweden). The samples were suspended in Arcobacter broth (Oxoid, England) to a 0.5 McFarland turbidity scale. This suspension was spread with a sterile cotton tip over the entire surface of Chocolate agar plates (Difco, Spain). Antimicrobial susceptibility test discs (5 µg) and E-tests® (0.002 to 32 µg/ml) strips were applied onto the agar surfaces and incubated in microaerophilic conditions for 48 h at 30ºC. As there is not any recommendation concerning the antibiotic resistance of arcobacters, the disc diffusion breakpoints and the minimum inhibitory concentration (MIC) values were adopted from those that were recommended by the Clinical and Laboratory Standards Institute (CLSI®) for campylobacters. In addition, to elucidate the mechanism of quinolone resistance we carried out sequence determination and analysis of the QRDR of their *gyrA*. For that purpose, all the isolates were analysed by PCR using F-QRDR (5'-TGG ATT AAA GCC AGT TCA TAG AAG-3') and R2-QRDR (5'-TCA TMG WAT CAT CAT AAT TTG GWA C-3') primers. The optimal PCR conditions were established by testing different annealing temperatures, $MgCl_2$ and dNTPs concentrations. Then, the resulting PCR products were purified and finally sequenced on both strands by Sistemas Genómicos S.L. (Valencia, Spain).

Among the 60 isolates tested, 46 were sensitive and the remaining 14 were considered to be resistant to both antibiotics by disc diffusion tests and E-test® strips. A disc diffusion zone of <=6 mm and a MIC value >=4 µg/ml indicates resistance. The MIC values with respect to ciprofloxacin and levofloxacin ranged from 0.002 to 0.38 µg/ml and 0.023 to 0.5 µg/ml, respectively for the sensitive isolates. The 14 resistant isolates presented MICs ranging from 8 to 32 µg/ml for both antibiotics. Subsequently, a 344-bp fragment of *gyrA* gene was amplified from all *A. butzleri* isolates. The sequencing of the PCR products revealed a mutation in position 254 of the *gyrA* gene in the 14 resistant *A. butzleri* isolates. This C-254 to T mutation could be the cause of quinolone resistance as this change was absent in all the susceptible isolates.

This study shows high rates of quinolone resistant in *A. butzleri* isolates obtained from sewage samples. Quinolone-resistance was always associated to one mutation in the QRDR region of the *gyrA* gene. The increasing resistance to this class of antibiotics could be a major public health concern as ciprofloxacin has been reported to be one of the most commonly used and best performing fluoroquinolones against arcobacters and water may play a key role in the transmission of infection.

Keywords *A. butzleri*; sewage; quinolone resistance

Bacterial study of groundwater supply in the valley of Assif El Mal (Marrakech area), and treatment trials

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In rural areas, the majority of the population has neither a supply of clean drinking water or proper sanitation. This is the case of the valley Assif El Mal, a tributary left bank of the Tensift Riversituated at 80km south-west from the Marrakech city. It drains a watershed of 517 square kilometers, where people resort to archaic method as the only source of water for any purpose. The groundwater is one among of the principal water supplies available in the region.

The objective of the present study is to assess the bacteriological and physical-chemical quality of water (in reference to the Moroccan standards) in 13 sampling sites (7 wells and 6 sources) located along the River of the valley Assif El Mal and usually used for drinking water. Comparison between winter and summer seasons was established. In addition, a correlation between bacteriological and physical-chemical variables was determined to reveal the process and the origin of possible levels of contamination.

Thus, results show that ground water studied reveals a light chemical contamination. They are rich in $Mg2+$ and $Ca2+$, which makes them very hard. Generally, for all water resources investigated, concentrations of chemical elements analyzed were lower to acceptable limit for drinking water according to Moroccan standards. But, the bacteriological results showed that in one hand, these waters has bad microbial quality because of their load in fecal indicators bacteria such as, fecal coliforms, fecal streptococci and other pathogenic bacteria like Salmonella sp, Staphylococcus aureus, Clostridia and Pseudomonas aeruginosa. On the other hand, there are significant quantitative increases of germs during summer in comparison to the winter period.

Therefore, easily used individually methods for disinfecting the water in these areas are required. Two simple and inexpensive methods were applied to limit the risks associated with consumption of these waters; it is the disinfection by exposure of plastic containers to full sunlight and disinfection by chlorination.

Keywords: ASSIF EL MAL, groundwater, Quality, physicochemical, Bacteriological, Disinfection.

Candida species isolated from human infections from Tunisian and Portuguese patients: molecular identifications and *in vitro* antifungal susceptibility

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The increased awareness about the morbidity and mortality associated with fungal infections caused by resistant strains in various groups of patients concerns mainly *Candida* infections, which affect both humans and animals worldwide. Epidemiological studies have identified risk factors associated with antifungal drug resistance, like hematological malignancies, prolonged neutropenia or immunedepression.

The goal of this study is to quickly identify, with a high sensitive and specific method, different *Candida* sp. clinical isolates, both from human patients from different countries (Portugal and Tunisia) and from different hosts, humans and animals, and compare their susceptibility profiles to fluconazole and voriconazole.

We report the application of a rapid PCR-based technique using a one-enzyme restriction fragment length polymorphism (RFLP) for the identification of the most important infecting *Candida* species and a simple and standardized method for sensitivity testing.

The molecular identification of the 168 pathogen-suspected isolates from human and animal infections in Portugal and from human infections in Tunisia was performed via PCR followed by RFLP analysis, being the results compared with those from conventional methodologies used for clinical microbiology laboratorial diagnosis.

The triazoles voriconazole and fluconazole were tested against all the isolates through a disk diffusion test (Kirby-Bauer method for susceptibility testing) through the CLSI (former NCCLS) M44 disk method. Inhibition diameters were determined by electronic image analysis, using a BIOMIC Plate Reader System for image processing and recording. The results were compared with those of the three groups of isolates.

From all the 168 isolates of *Candida* spp., 154 were from *C. albicans*, 6 from *C. glabrata*, 4 from *C. lusitaniae,* 2 from *C. tropicalis* and 2 from *C. krusei*. From Portuguese human patients, 100 isolates were studied: 87 from *C. albicans*, 5 from *C. glabrata*, 4 from *C. lusitaniae*, 2 from *C. tropicalis* and 2 from *C. krusei*. From Tunisia, 50 isolates were studied: 49 from *C. albicans* and 1 from *C. glabrata*. From Portuguese animals, 18 isolates from *C. albicans* were studied. In all cases, the molecular method allowed correct identification of the isolates in a much faster way than by conventional identification.

For voriconazole and fluconazole, the determined ranges of MIC values were <0.01 to ≥ 6.1 µg/mL and 0.25 to ≥ 165 µg/mL, respectively. Resistance to fluconazole was observed in 10 *Candida* isolates (5.95%) and to voriconazole in 7 *Candida* isolates (4.17%).

In conclusion, this PCR assay allowed specific and quick identification of all *Candida* species isolated from both human and animal infections, an important asset for an optimal outcome of the patients, by allowing timely and appropriate treatment and by reducing the risk for development of drug resistance. Antifungal susceptibility testing is essential to guide physicians in the selection of adequate antifungal therapy.

Keywords: molecular identification; susceptibility testing; antifungal resistance

Cell wall scanning electron microscopy of *Candida albicans* treated with essential oil of *Melaleuca alternifolia*

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Melaleuca alternifolia is also known as tea tree and its essential oil (TTO) has a variety of therapeutic uses. The most important and studied is related to its antimicrobial activity. TTO is active against bacteria and fungi, especially *Candida albicans* and it is been used to treat candidiasis topically.

The objective was to analyze the cell wall morphology of *C. albicans* treated with TTO by scanning electron microscopy (SEM).

C. albicans cells ATCC 64548 ($2.5x10^3$ cells/mL) were inoculated onto agar Sabouraud plates for 12 h at 35 ºC. Then 0.25% TTO was added and the plate was incubated for 24 h at 35 ºC. Control cells were incubated without TTO. One colony of treatment and control were harvested, centrifuged at 13,000 rpm for 1 min, washed twice with PBS and after, 200 µL of 2.5% glutaraldehyde solution in phosphate buffer (pH7.1) were added to the pellets and left for 20 min. The cells were washed and 5 µL added to the top of slides allowing drying completely for 24 h at 37 ºC and after were covered by gold and examined at 10 kV in a scanning electron microscope (Topcon SN300).

The results showed that control cells exhibited smooth surface. However, TTO treated cells presented loss of internal material. TTO is known by its property to kill *C. albicans* cells and one of proposed mechanism is the plasmatic membrane permeability alteration due to the interference of the oil monoterpenes. Analysis of *C. albicans* cell wall by SEM is one more tool to defend the TTO mechanism of action on its plasmatic membrane.

Keywords: *Melaleuca alternifolia*; *Candida albicans, SEM* , Tea tree oil

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Characterisation of volatile components of “Epa-Ijebu”- A native “wonder-cure|” recipe

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Essential oil from Epa-Ijebu, a native cure-all concoction was extracted by hydrodistillation and analysed using Gas Chromatography/ Mass Spectometry (GC-MS). Thirteen organic compounds were identified of which Fatty acids were most prevalent (35.52%). This was followed by normal alkanes such as nonadecene, hexadecane, heptadecane, octadecane and heneicosane (constituting 26.5%). 4-Methyl-2-trimethylsilyloxy-acetophenone (6.39%), Quinoline (5.96%), Alcohol (2.48%), Benzothiazole (1.89%) and 2-p-nitrophenyl oxidiazol 1,3, 4-one-5 (0.85%).

Keywords: Epa-Ijebu; Volatile compounds; Quinoline; Medicinal properties.

Characterization of Antibiotic Resistant Soil Bacteria Adjacent to Swine Production Facilities

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In this study, the environmental factors affecting the increase of erythromycin and tetracycline resistance were investigated. Samples have been collected from manure and soil, before and after manure application at four sampling times. All of the 213 bacteria were belonged to 4 different phylum, *Proteobacteria, Bacteroidetes, Actinobacteria*, and *Firmicutes*, identified based on BLAST results. Class-specific primers were used to amplify each of the six erythromycin resistance genes [*erm*(A), *erm(*B), *erm(*C), *erm*(F), *erm(*G), and *erm(*Q)] and seven tetracycline resistance genes [*tet*(C), *tet*(H), *tet*(Z), *tet*(M), *tet*(O), *tet*(Q), and *tet*(W)] for 46 tetracycline and 18 erythromycin resistant isolates. The majority of the tetracycline resistant (Tc^r) isolates were present in the *Proteobacteria* in soil. Twenty of isolates, representing 14 different genera, were positive for Tc^r genes. Only two isolates possessed *erm*(Q) gene. This study is the first to describe the presence of tet genes in members of the genera *Simplicispira* and *Agrococcus.* Further, nine genera that possessed new tetracycline resistance genes which were not documented before and new combinations of tetracycline genes in *Simplicispira* and *Burkholderia* were identified as well. DNA sequence analysis of a selected tetracycline resistance genes revealed that tet(Z) genes were identical. Thus, the demonstration of the diversity of tetracycline and erythromycin resistant bacterial isolates, and identical resistance genes in both soil and manure isolates in soil and manure constitutes the initial phase of linking the antibiotic use with the spread of resistance to the environment.

Keywords antibiotic resistance, erythromycin, soil, susceptibility, swine manure, tetracycline

Characterization of *Bacillus thuringiensis* strains under the umbrella of a Brazil-Cuba cooperation on bioinsecticides

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Bacillus thuringiensis (Bt) is a sporogenic bacterium that produces characteristic crystalline inclusions (Cry proteins), during sporulation. Each strain shows its specific activity against larvae of different insect and also some nematode pests. Because of its specificity, *Bt* has been widely recommended for biological pest control. Even with the development of genetically modified plants, that could produce Bt-activated toxins, the screening of new toxic *Bt* species is still needed, looking especially for active species against the most resistant pests. A technical cooperation program Brazil-Cuba, funded by the Brazilian Council for Scientific and Technological Development (CNPq), supported studies in order to characterize new *Bt* strains of Brazil and Cuba interest.

In this work, the crystal morphology and Cry protein composition of twelve *Bt* strains, isolated from Cuban environment and maintained at INISAV Culture Collection, were analyzed. Their biological activities were evaluated *in vitro* against *Spodoptera frugiperda* (a very aggressive insect and also very resistant to the *Bt* toxin), *Anticarsia gemmatalis* (very sensitive to *Bt* toxin), and eggs of the nematode *Meloidogyne incognita*. Brazilian and Cuban methodologies for bioassay were utilized, integrating efforts and expertise of both countries.

The insect bioassays showed that two *Bt* strains were highly active against *S. frugiperda* and *A. gemmatalis* (100% mortality within 48h), while eight showed activity just against *A. gemmatalis,* and two showed no activity at all.

Regarding nematicidal effects, six strains interrupted the development of egg masses, some of which appeared necrotic. There were different behaviors regarding nematode infectivity after treatment with each *Bt* strain, but all treatments resulted in nematostatic and disorientation effects with a marked reduction of the nematode infectivity, under the experimental conditions.

Regarding the Cry protein profile by SDS-PAGE, most of the strains showed proteins of 130 kDa and 70kDa, and one of them showed just proteins of 130kDa.

The scanning electron micrographies as well as the morphology, size and number of the inclusions showed variations amongst the different *Bt* strains, as expected. Literature shows that the morphology of *Bt* crystals is not an absolute indicator of its insecticidal activity but, instead, represents an important criteria for characterization of a new strain. Bipyramidal and cubic inclusions were observed in the strains tested.

Based on the results obtained the two most virulent strains against insects and the most promissory nematicidal (at least two) will be further studied for mass production and formulation as a biopesticide. Further studies will be developed in order to clarify if the nematicidal activity could be attributed to a specific fraction (supernatant/precipitated; thermostable / thermosensitive) of the fermentation broth.

Characterization of Endophytic *Streptomyces* SUK 06 and its Methyl Benzoate Compound

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New drugs, especially antibiotics are urgently needed to counter and reverse the spread of antibiotic resistant pathogens. Emerging, reemerging pathogens and occurrences of superbugs are increasing throughout the world. In this research we have isolate *Streptomyces* SUK 06, an endophyte of *Thotthea grandiflora*. Isolation of the endophytes was carried out using surface sterilization methods and identified by Gram stains, colony morphology, light microscopy and scanning electron microscopy (SEM). Matured SUK 06 colonies showed a smooth, folded, granulated, dry and chalky texture. The arial mycelia were brown while the substrate mycelia were grey. The SEM observation revealed the filamentous and the hyphae were coiled while the spore arrangement was *Spiral* type. The 16S rRNA gene sequence of SUK 06 revealed that its 96%-98% similar to *Streptomyces misawanensis*. The result of disk diffusion methods showed inhibition against *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus fecalis, Pleisimonas shigelloides* ATCC 27853, *Staphylococcus aureus* ATCC 25925, *Bacilus subtilis*, *Bacilus cereus* ATCC 6464, dan *Methicillin resistant Staphylococcus aureus* ATCC 700699. SUK 06 isolate was then growth in A3M media and extraction of active metabolites using ethyl acetate in which 12 liter culture yielded 2-3g of crude extracts. The crude extracts were fractionated using radial chromatography. Fractions were collected and pooled together based on their thin layer chromatography (TLC) profiles. The pooled fractions were tested against *Bacillus cereus* ATCC 6464 and further purified before pure substance subjected to minimum inhibitory concentration (MIC) and 600 MHz nuclear magnetic resonance (NMR) analysis. The pure substance which has 9.181×10^{-9} molar MIC value is identified as methyl benzoate by NMR analysis.

Key words: endophytes, *Streptomyces*, methyl benzoate, NMR, SEM

Characterization of *Staphylococcus aureus* Isolated from Healthy Children in Portugal

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In order to understand the prevalence and the diseases caused by MRSA isolates to provide better diagnosis and treatment, the prevalence of *Staphylococcus aureus* carriage among healthy preschool children in the North of Portugal was evaluated. Material and Methods: Nasal swabs were collected from 3 to 6 year-old healthy children who were attending kindergarten. Of the 283 children, 135 were carriers of *S. aureus*; 15% of the isolated strains were Multidrug Resistant *Staphylococcus aureus*. Resistance to gentamicin, chloramphenicol, rifampicin, oxacillin, nitrofurantoin, tetracycline, erythromycin, ampicillin and penicillin were determined to be 1.5, 2.2, 2.2, 6.7, 19, 34.1, 73.3, 84.4 and 91.1%, respectively. All the strains were sensitive to vancomycin.

Our study reveals a high prevalence of healthy children carrying *S. aureus* in the nasal cavity. This study reports the nasal carriage of *S. aureus* and MRSA and specific resistant forms of these microorganisms among healthy children and identifies risk factors associated with these pathogens.

Keywords: *Staphylococcus aureus*; susceptibility to antibiotics; MRSA; characterization

Chlamydia trachomatis in periodontal desease in population of northeast Mexican

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Introduction: Chronic periodontitis (CP) is one of the most frequent diseases in adult oral cavity. People over the age of 35 are the commonest affected in Northeast Mexico. Among the various agents associated with CP, the A. Actinomy Cetemcomitans, P. Gingivalis, P. Intermediate, T. Forsythensis, and T. Denticola are included. Chlamydia Trachomatis (CT) is one of the most prevalent bacteria in the world. As an intercellular germ, it tends to cause chronic infections. CT enters the body by way of moist mucous membranes and some of its serotypes are installed in conjunctival, nasal, and pharyngeal mucous membrane. Objective: Determine the frequency in which CT appears in the periodontium of patients with or without CP. Methods: 91 patients attending the periodontal clinic with active CP, >4mm probing depth, presence of bleeding on probing, and loss of insertion were selected at random. Periodontal biopsies and CT pharyngeal secretion were performed through the application of a direct immunofluorescence test (DIT) with monoclonal antibodies against the germ, marked with fluorescence Results: Evidence of PC (control group: GE) was not found in 41 of the patients (average age 49.05+_10.99). According to DIT test, 53.6% of GE cases and 52% of GC presented CT in gingival furrow tissue, but the difference was not statistically significant. The analysis of pharyngeal exudate was positive for IFD in 53.6% of GP and 50% of GC; however, only 14.2% of GE and 20% of GC presented conjunctival or pharyngeal infection syndrome. Conclusion: Our data agreed with the literature published so far, concluding that CP is more frequent in older people, and CT is present when there are no associated symptoms. Even though an association between CT and CP was not found, it is concluded that gingival furrow epithelium is a reservoir for CT, an important factor that spreads the germ.

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Comparative study of the microbiota detected in areas which are close and away from hospital facilities in Barcelona

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GRUPO DE INVESTIGACION EN MICROBIOLOGIA APLICADA Y MEDIO-AMBIENTAL.FACULTAD DE VETERINARIA DEPARTAMENTO DE SANIDAD Y DE ANATOMIA ANIMALES. CAMPUS BELLATERRA 08193 BELLATERRA (BARCELONA).

The main aim of this study is to know the possible influence of the hospital environment on the microbiota of Barcelona. In order to do so, the micro-organisms of these areas will be identified, and their resistance to antibiotics according to the area of isolation will be defined.

The hospital facilities chosen for this study were: close areas to Hospital Clínic (city centre), Hospital de Ntra. Sra. del Mar (seaside) and Hospital de Sant Pau (north of the city). The area chosen as a contrast was Plaça Catalunya.

Once the areas to be analyzed were selected, air samples were taken by means of opening Tryptone Soy Agar plates (TSA, for detection and isolation of bacteria) and Sabouraud + antibiotic (ASab, for detection and isolation of mycobiota). In addition, urban furniture, doors and fences samples were taken by means of sterile swabs. The swabs were spread on TSA and ASab plates. After incubation of all plates under the right conditions for each group, counting, isolation and identification of the colonies were carried out.

The results showed that there is an abundant presence of Gram positive cocci in those areas closer to hospital facilities. In the selected contrast area (Plaça Catalunya), however, the predominant bacteria group was Gram positive sporulated rods.

As for moulds and yeasts, there were no differences among the main genera isolated in the sampled areas, although it was observed that in areas closer to the mountains, the presence of fungi was much higher.

In order to evaluate the possible resistance of the isolated strains to antibiotics of common use in hospitals, axenic cultures of both *Streptococcus* spp. and *Staphylococcus* spp. (which were the most frequently isolated in the study) were used to perform antibiograms. The results indicated that some of the strains isolated close to Hospital Clínic were are resistant to Cephalotin whereas the strains isolated in other areas were not. On the other hand, a strain of *Staphylococcus* isolated from urban furniture near Plaça Catalunya, is resistant to Erythromycin, whereas the rest of the isolated strains showed intermediate resistance to this antibiotic.

Taking into account the results, we can indicate that the distribution of the strains in the environment, as well as their ability to be resistant to antibiotics, increases slightly as we approach to hospital facilities. However, areas with high visitors density (Plaça Catalunya) the concentration of micro-organisms is high and the their resistance to antibiotics may be, in some cases, elevated.

Key words: *Staphylococcus, Streptococcus*, Hospital facilities, Resistance to antibiotics.

Complementary and alternative medicine approach for treating topical bacterial infections

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Background: Our capacity to properly address the worldwide incidence of infectious diseases lies in the ability to detect, prevent, and effectively treat these infections. With increasing overuse and misuse of antibiotics, infectious bacteria have become increasingly antibiotic-resistant. Therefore, identifying and analyzing inhibitory agents is a worthwhile endeavor since few new antibacterial compounds have been produced in recent decades. The use of natural nanominerals to heal skin infections has been evident since the earliest recorded history, and specific clay minerals may prove valuable in the treatment of bacterial diseases, including infections for which there are no effective antibiotics, such as Buruli ulcer and multi-drug resistant infections.

Results: We have subjected two iron-rich clay minerals, which have previously been used to treat Buruli ulcer patients, and additional clay mineral mixtures to in vitro antibacterial assays against a broad-spectrum of bacterial pathogens, revealing three clay mineral mixtures that exhibit bactericidal activity. Further investigations into the mechanism of action revealed that antibacterial activity was dependent on chemical, not physical, mineral characteristics. Characterization of the physicochemical enviroment indicate that soluble metal ions, pH, and reactive oxygen species contribute to bactericidal activity. Animal experiments to evaluate the ability of hydrated minerals to treat topical bacterial infections in mice are currently underway.

Conclusions: These results demonstrate that specific mineral products have intrinsic antibacterial properties that are generated by the chemical environment created upon hydration. Application of a consistently antibacterial mineral product could be an inexpensive and complementary method for treating topical infections that are recalcitrant to antibiotic therapy.

Keywords: antibacterial, clay minerals, complementary medicine, treatment

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Computational Designing of Anti Tuberculosis Drug against Cytochrome P450 Mono-oxygenases Enzyme of *Mycobacterium tuberculosis*

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The cytochrome P450 mono-oxygenases enzyme from *Mycobacterium tuberculosis* catalyzes the oxidation of organic compounds such as lipids and steroidal hormones therefore remain as potential drug target. Based on their effectiveness towards inhibiting the growth of *Mycobacterium tuberculosis* and causing toxicity in the human body, the drugs were classified into different categories such as first line, second line and third line. In this study we are focusing on first line anti-tuberculosis drugs.However, due to their toxic effects in the body and resistance development by *Mycobacterium* against first line drugs, thereoccurs the necessityfor finding new drugs against this bacterium. Therefore, we propose here a structure based computational method to find a new potential anti-tuberculosis drug from the ligand database. The molecules were docked against the functional sites of the protein 2UUQ (A) through standard GEMDOCK v2.0 and AUTODOCK4.0 molecular docking tools. The commercially available chemical compound ZINC00004165 (5-[3-(2-nitroimidazol-1-yl) propyl] phenanthridine) comes out at top rank with lowest interaction energy of -113.2 (via GEMDOCK) and lowest docking energy of -9.80Kcal/mol (via AUTODOCK) as compared to first line anti TB compounds. The Z score and normal distribution analysis verified that the ZINC00004165 compound has more affinity towards 2UUQ in comparison to a large number of compounds. Further, the identified compound ZINC00004165 was in good agreement with drug likeness properties of Lipinski rule of five without any violation. Therefore, our finding has concluded that the chemical compound ZINC00004165 can act as a potential new drug designing model compound for *Mycobacterium tuberculosis.*

Keyboard*:* cytochrome P450 mono-oxygenases; Tuberculosis; Ligand database; Docking; Gemdock; Autodock

Construction and immunobiological evaluation of a novel aromatic-dependent *Bordetella pertussis* using the pertussis mouse model system

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Identification of the *aroQ* gene, encoding 3-dehydroquinase, was confirmed by complementation of the *aroD* mutation in *Escherichia coliaroD*mutant, sequenced, insertionally inactivated using a kanamycin resistance cassette via a conjugative suicide plasmid vector by allelic exchange. The *Bpertussisaro*Q mutants grew in laboratory media supplemented with aromatic compounds but failed to grow on an un-supplemented medium. The *aro*Q*B pertussis* mutants were found to persist in the trachea of mice for 6 days and in lungs of mice for 12 days post-administration by the intranasal route. Low levels of interleukin-4 and moderate level interleukin-5 production were observed following one dose vaccination. However, substantial levels of serum interleukin-12, and interleukin-2 with relatively low levels of interferon gamma, were produced by antigen-stimulated splenocytes of mice vaccinated with the *aroQ B pertussis*. Neither of these interleukins was produced by the antigen-stimulated splenocytes of DTaP-vaccinated mice. Mice vaccinated with the *aroQ B pertussis* were protected against an intranasal challenge infection with the virulent parent pathogen. Furthermore, the serum antibody titres and interferon gamma levels produced by antigen-stimulated splenocytes were significantly or substantially increased over the pre-challenge levels. Therefore, the constructed *aroQ B pertussis* could form the basis of developing a non-reverting, non-toxic, live attenuated whooping cough vaccine with potential to impart long term protection and disrupt the transmission cycle of whooping cough from adolescents and adults to infants.

Contribution to the study of antibacterial activity of acetonic extracts of *Pulicaria odora* L. on pathogenic bacteria

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Pulicaria is a genus of the Compositae, tribe Inuleae, containing 80 species with a distribution from Europe into North Africa and Asia.

In our work, we studied the antibacterial activity of acetonic extracts of *Pulicaria odora* L., collected in the region of Tizi-ouzou (Algeria), against four pathogenic bacteria *Listeria inocua*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Results indicated that all extracts showed antibacterial activity, Gram negative strains are more resistant than Gram positives, for standards gallic acid showed the best antibacterial activity followed by tannic acid and quercetin. Minimum Inhibitory Concentrations of the extracts are ranged between 0.3 and 2mg/ml.

Staphylococcus aureus is the most sensitive bacteria with inhibition zones ranged between 9 and 20mm and MIC of 0.3Mg/ml the most resistant is *Escherichia coli.*

Key words: *Pulicaria odora* L., pathogenic bacteria, acetonic extracs, MIC.

Cranberry syrup changes the surface adherence of *E. coli*

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Aims. Cranberry has been shown useful in the prevention of urinary infection by *E. coli*

To determine the changes in the hydrophobicity of P fimbriated *E. coli* and biofilm formation after incubation with commercial cranberry syrup extract at various concentrations.

Methods and Results. 13 strains of P fimbriated *E. coli*, were grown in TSB and CFA culture medium. After incubating a bacterial suspension with cranberry at dilutions of 1:100 and 1:1000, a haemagglutination inhibition test, surface hydrophobicity and biofilm formation were carried out. The surface hydrophobicity *E. coli* decreases significantly after incubation with cranberry and this effect is not modified by the culture media. The biofilm formation it is inhibited after incubation with cranberry syrup and this effect depends on the culture media.

Conclusions. Depending of the culture media, cranberry can modify nonspecific adhesive properties of *E. coli*.

Significance and Impact of Study. Cranberry far only been implicated in the inhibition of P-fimbriae of *E. coli*, our observations show that it acts by modifying adhesive properties under P-related fimbriae.

Cranberry syrup is effective in the prophylaxis of recurrent urinary tract infection. Results of a clinical trial

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Aims: Trimethoprim prophylaxis for recurrent paediatric urinary tract infections (UTI) has been shown to be effective in reducing the recurrence of such infections. As an alternative, the present study evaluates the effectiveness of cranberry syrup in treating paediatric recurrent UTI.

Methods: A controlled, double-blind clinical trial was carried out on infants aged more than one month and older children. The initial hypothesis was that the results obtained from cranberry syrup treatment would be equivalent to those achieved with trimethoprim, in children with a history of recurrent UTI. The outcome was evaluated in terms of UTI recurrence. The statistical analysis was performed using the Kaplan Meier method.

Results: Of the 201 patients eligible, 192 were included to receive either cranberry syrup or trimethoprim. Urinary tract infection observed in 47 patients, 17 of whom were male and 30 female. We recruited 95 patients diagnosed with recurrent UTI at entry. During subsequent follow-up, 26 of these patients presented UTI (27.4%, CI 95% 18.4%-36.3%). 6 of them (6.3%) were male and 20 (21.1%) female. 18 of the patients (18.9%, CI 95% 11%-26.3%) receiving trimethoprim had UTI, versus 8 of the patients (8.4%, CI 95% 2.8%-13.9%) given cranberry syrup.

Conclusions: Our study confirms that, for the paediatric population, cranberry syrup is a safe and non-inferior alternative treatment to trimethoprim (European Clinical Trials Registry EuDract 2007-004397-62) (ISRCTN16968287).

Current Antibiotic Sensitivity Pattern of HVS Clinical Isolates in Karachi

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Objective: To evaluate the frequency of vaginal irritants and to determine their culture sensitivity pattern to most commonly used antibiotics in Karachi, Pakistan.

Methods: This was an observational study conducted at local diagnostic laboratory and its branches in key areas of the city from 1st February, 2010 to 31st January, 2011. 354 high vaginal swabs (HVS) obtained from women of fertile age during a period of twelve months were inoculated on blood agar, CLED and CHROMagar and isolates identified by routine procedures. These strains were assayed for antibiotic sensitivity by disc diffusion technique employing multidiscs, and MIC's by E-test. Ensuing data was sorted and subjected to statistical analysis on SPSS version 17.

Results: Out of 354 specimens, 194were gram-negative rods, and 160 gram-positive isolates. Staphylococcus aureus (39.8%), however, was most frequent followed by gram-negative Klebsiella (22.9%) and E. coli (21.2%). Pseudomonas, Enterococci, Enterobacter, Proteus, Candida and Strep. agalactiae were less prevalent. In vitro sensitivities illustrated that Imepenem (Tienam) was most effective of the drugs tested, covering 98.9% of the gram-negatives and 95% of gram-positive isolates. This was followed by Amikacin (82.47%) and Fosfomycin (75.77%) on gram-negative strains, and Augmentin (81.25%) and Amikacin (78.75%) on gram-positive species. The gram-negatives were resistant to Amoxil (100%), whereas gram-positive strains were mostly unaffected by Cefixime (93.75%).

Conclusions: Alternations in the healthy vaginal ecosystem of a woman place her at an increased risk for the development of clinically significant ordeals, such as pelvic inflammatory disease. Antibiotic sensitivity testing determines the susceptibility of the isolates obtained from mucosal membrane of vagina to a range of conventional antibiotics. Our study recommends culture/sensitivity testing before the administration of antibiotics to satisfactorily treat vaginal infections.

Keywords: Specimen, Gram-positive strain, Gram-negative strain, Sensitivity.

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Design of a PCR for the diagnosis of Respiratory Syncytial Virus

Alberto Tenorio-Abreu; Alberto Tenorio-Abreu, Bárbara Gómez-Alonso, Luis Arroyo-Pedrero, María Lecuona.

Objetive

Design of a conventional multiple RT-PCR for the diagnosis of respiratory syncytial virus (RSV) in pediatric patients.

Material and methods

A set of primers for diagnosis of RSV was designed using the software Primer Express 3.0. Conserved gene sequences of the nucleocapsid N available in the GenBank database was used as pattern . The size of the generated amplicon was 76 bp. Below is showed the set of primers designed:

RSV:

Forward Primer (VRSG+): 5´-TCCAGACTGTGGGATGATAATACTG-3´

Reverse Primer (VRSG-): 5´-CCTGATCTGTCTCCTGCTGCTA-3´

For RT-PCR, we used the Master Mix AgPath-ID One-Step RT-PCR Kit (Applied Biosystem, USA) and the thermocycler 7500 Fast (Applied Biosystem, USA), with the following temperature program: 1x (50 ° C, 30 '), 1x (95 ° C, 10'), 40x (95 º C, 15") and 1x (55 ° C, 30"). The reading was obtained by agarose gel electrophoresis in 2%, so that samples that appeared with a band corresponding to 76 bp in size was considered specific to RSV. Nucleic acid extraction was performed by automatic extractor Maxwell ® 16 System (Promega ®, USA), from 200 ul of nasal aspirate. The technique was able to reproduce results in 3 hours. The sensitivity was determined by the minimum number of copies was able to detect. The specificity was determined by external controls delegated by the microbiology department of the Clinic University Hospital of Valladolid and internal controls diagnosed from positive samples by immunochromatographic capillary (Binax Now ® RSV, Inverness Medical).

Results

The detection limit for primer sets corresponded to a dilution of 1 to 10 copies/µl. Regarding specificity, 100% agreement was obtained with a panel of 10 test samples received since the University Hospital of Valladolid (6 VRS and 4 negative samples). There were also 100% concordance with another 72 positive samples diagnosed by rapid test Binax Now ® RSV.

Conclusion

Data regarding sensitivity, specificity and speed, suggests that this technique can be considered for use in clinical practice. However, further testing with larger series and compared to other standard technique should be needed to confirm these results.

Detection of IgM and IgG antibodies against Mycobacterium tuberculosis in students Faculty of Dentistry, University of Nuevo Leon, Mexico

PhD A. M. Garza-Garza, PhD J. C. Llodra-Calvo, Dr. C A. Y. Arce-Mendoza, PhD M.A.de la Garza-Ramos, PhD M. Á. Quiroga-García

Introduction:
Tuberculosis is a disease widely found in the world. The purpose of this study is determine how many students of the faculty of dentistry in the city of Monterrey, Nuevo Leon Mexico, by the test that analyzes the presence of antibodies IgM and IgG

Material and Methods
Two tests took, the Mantoux Test, inoculating .1cc of DPP in order to give a reading 48 hours later, taking as positives those that presented an indurate zone of 10 mm or more that means previous contact with this bacillus and obtaining from them 5 cc of venous peripheral blood in order to carry out the ELISA Test to detect latent Tuberculosis in blood, test that analyzes the presence of antibodies IgM and IgG against extracellular proteins of Mycobacterium Tuberculosis. Among the study groups, those pertaining to 2 semester showed more contact with M. tuberculosis than those of 9 and 10 semesters w

Results: From groups F6, F7 and F8 where there are more students positive to the studied tests, this is equivalent to 29.3% and it indicates present infection. In conclusion, the students of recent entrance who gave positive to the tests, had previous contact in their environment which means they are immune resistant before Tuberculosis infection. Therefore, since there is no increase of positive IgM in the ninth and tenth semesters this indicates there does not exist any danger of infection during their professional development.

Detection of microorganisms in patients with Brackets MBTY Alexander

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Introduction
Orthodontics as a science can not be isolated from the dental and cultural evolution of the world. The application components can cause alterations in microbial flora due to reduced pH and increased retention areas for microorganisms.

Objective
Determined by multiplex PCR, the presence of Streptococcus mutans, Porphyromonas gingivalis, Streptococcus intermedius and Streptococcus sobrinus in patients with orthodontic treatment techniques in the MBT and Alexander Orthodontics Clinic UAS

Materials and Methods
It tomron 80 patients of both sexes attending the Clinic of Orthodontics and Orthopedics at the UAS of between 12 and 25 years old with fixed appliance orthodontic techniques Alexander (0.18) and MBT (3M), with a minimum of 3 months placing the brackets, all samples were applied Multiplex PCR.

Results
In 41 patients with brackets Alexander, Streptococcus intermedius, 8 (19.5%) compared with 39 patients with MBT brackets Porphyromonas gingivalis in brackets Alexander 5 (12.2%) cases versus 1 (2.6%) case in MBT Brackets, Streptococcus mutans was (p = 738) in brackets Alexander 6 (14.6%) versus 4 (10.3%) MBT. Streptococcus Sobrinus was (p =. 433) with Alexander Brackets 5 (12.2%) and 2 (5.1%) with MBT brackets.

Conclusion
Is to identify microorganisms associated with dental caries and periodontal disease in patients with brackets attached (Alexander) and twin brackets (MBT). The increased presence of microorganisms in number and species was attached (Alexander) compared with twin brackets (MBT). There were no organisms analyzed in this study for age, sex and design of brackets.

Detection of plasmid-mediated quinolone resistance determinants in extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated in Chilean hospitals

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The plasmid-mediated quinolone resistance (PMQR) has been reported worldwide, mainly among Gram negative bacteria belonging to Enterobacteriaceae in Europe, Asia and North America and some countries in South America. However, little is known about the prevalence of PMQR determinants among nosocomial strains isolated in Chile.

In this work we investigated the presence of PMQR determinants *qnr* (variants A, B, S, C and D), *qepA* and *aac(6')-Ib-cr* among 200 ESBL-producing strains of *E. coli* and *K. pneumoniae* (100 each of one) isolated in 10 Chilean hospitals, located from north (Iquique) to the southern (Puerto Montt) of the country during 2008-2009. Determinants were investigated by the Polymerase Chain Reaction (PCR) using specific primers.

The isolates were collected mainly from urine, surgical wounds, sputum, and blood, and exhibit a phenotype of multiresistance including third-generation cephalosporins, aminoglycosides, chloramphenicol, tetracycline and most of them are resistant to nalidixic acid (NAL), ciprofloxacin (CIP) and levofloxacin (LEV). The MIC50 for *E. coli* and *K. pneumoniae* of NAL was 1024 µg/mL and of CIP was 64 µg/mL. The MIC50 values of LEV were 16 µg/mL and 8 µg/mL for *E. coli* and *K. pneumoniae*, respectively. All isolates were susceptible to imipenem and meropenem; however, only 95% were susceptible to ertapenem in *E.* coli and 93.6% in *K. pneumoniae*. The determinant *qnrS* was only amplified in one isolate of *E. coli*, and the *qnrB* was found in 7 isolates of *K. pneumoniae*. In this case the alleles identified were *qnrB1* and *qnrB19*. The genes *qnrA*, *qnrC* and *qnrD* were no found in any isolates. Among *E. coli* isolates, seventy-nine (79%) were positive for *aac(6')-Ib*, of which 75 (94.9% of all) had the *-cr* variant. A different result was observed with *K. pneumoniae*, because 74 isolates amplified the *aac(6')-Ib*, of which 54 (73%) belong to the *–cr* variant. The PMQR determinant *qepA* was not found in none of the isolates studied.

We conclude that the gene *aac(6')-Ib-cr* is the most frequent plasmid-mediated quinolone resistance determinant among isolates of ESBL-producing strains of *E. coli* and *K. pneumoniae* isolated in Chilean hospitals.

This work was supported by the grant DIUC 207036032 from Universidad de Concepción and a grant from Merck Sharp & Dohme-Chile

Keywords Chile; plasmid-mediated quinolone resistance determinant; *E. coli*; *K. pneumoniae*

Determination of antimutagenic properties of Rosmarinic acid, a phenolic compound isolated from Mentha longifolia ssp. longifolia with yeast DEL assay

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Genus Mentha is a well-known species that has a variety of biological properties and abundantly available throughout temperate regions. Member of Mentha genus have been used as a folk remedy for treatment of nausea, ulcerative colitis and liver complaints due to its anti-inflammatory, carminative, stimulant and antioxidant activities. This study was designed to evaluate mutagenic and anti-mutagenic activities of rosmarinic acid (RA), a phenolic compound isolated from Mentha longifolia ssp. longifolia. The possible anti-mutagenic potential of RA was examined against mutagens ethyl methanesulfonate (EMS) and acridine (AC) in Saccharomyces cerevisiae RS112. The test is a simple, reliable method for measuring the frequency of intrachromosomal'deletion' recombination between two partially deleted his3 alleles, separated by a LEU2 marker gene sequence. The results showed that RA has different inhibition rates against EMS and AC-induced mutagenicity. The anti-mutagenic effect of RA might be partly due to protection against the entrance of the mutagen into cells or the modulation of the effect of the mutagen on DNA or by inducing enzymes that will detoxify the mutagen before it reaches the cell's DNA. The properties of RA are of great pharmacological importance and might be useful as a plant origin drug due to its anti-mutagenic effect.

Key words: Antimutagenicity, yeast DEL assay, rosmarinic acid

Determination of chemical composition and antibacterial properties of essential oil of *Mentha longifolia* ssp. longifolia against phytopathogenic bacteria

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In the present study, the chemical composition of the essential oil of *Mentha longifolia* L. Hudson ssp. *longifolia* and the antibacterial activity of the essential oil against phtopathogenic bacteria were determined. The essential oil of *Mentha longifolia* L. Hudson ssp. *longifolia* which grown in Eastern Anatolia was isolated by the hydrodistillation method and analysed by GC-MS. According to GC analysis of the chemical composition of the essential oil of *Mentha longifolia* L. Hudson ssp. *longifolia,* it consisted of 12 identified components. Major components of the oil were cis-Piperitone epoxide (26.52%-GC), Piperitenone oxide (26.40%) and Pulegone (15.66%). The antibacterial activities of the essential oil was also tested against 18 phtopathogenic bacteria. In general, the oil had antibacterial activity at a wide spectrum on the growth of phtopathogenic bacteria. In conclusion, the results revealed that the essential oil from *Mentha longifolia* L. Hudson ssp. *longifolia* have significant antibacterial activity, and the findings of the present study are valuable for further investigations, focus on controlling plant pathogenic bacteria that cause crop loss.

Keywords*:* Antibacterial activity, *Mentha longifolia* L. Hudson ssp. *longifolia*, biopesticide

Development of QSAR Model to Predict the Antimalarial Activity of fosmidomycin derivatives

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We designed and evaluated the biological activity of fosmidomycin derivatives as inhibitors of DOXP reducto-isomerase enzyme. A total of 25 chemical descriptors were considered to calculate the various physico-chemical and steric properties of training data set compounds. Out of 25, only five descriptors showed higher correlation with the biological activity. After forward stepwise multiple linear regression analysis, the QSAR approximation model with significant regression coefficient (r^2) 0.873648 (i.e., 87.36% of relationship between known compound's properties and their respective experimental activity) and significant cross validation coefficient (rCV^2) 0.76637 (i.e., 76.63 % of prediction accuracy of model was developed. The molecular docking studies of fosmidomycin derivatives namely, **11 fosd**, **15 fosd**, **20 fosd**, **23 fosd** and **25 fosd** are comparable with binding poses of fosmidomycin. In addition, it revealed the possibility of inhibitory activity due to higher binding affinity against DOXP Reductoisomerase with docking score -88.878, -63.499, -65.134, -51.332, and -65.125 kcal/mol respectively. The fosmidomycin derivatives **11 fosd**, **15 fosd**, **20 fosd** and **25 fosd** showed good binding affinity than parent compound Fosmidomycin (-61.113 kcal/mol docking score).

Diagnostic yield of the granada medium for Detection of *Streptococcus agalactiae*

Alberto Tenorio-Abreu; Alberto Tenorio-Abreu, Asma Aloui-Sosse, Zaida Díaz-Cuevas, Bárbara Gómez-Alonso, Teresa Mendoza, María Lecuona.

Introduction

Prevention of neonatal sepsis caused by group B Streptococcus (GBS) includes intrapartum antibiotic treatment of colonized women. Detection of GBS colonization is based on culture of vaginal and rectal swab specimens, from all pregnant women between 35 and 37 weeks' gestation, using different selective, enriched and / or chromogenic media.

Objective:

To assess the diagnostic effectivity of chomogenic GranadaTM bifásico broth (bioMérieux® España S.A) for detection of GBS in pregnant women.

Matherial and methods

Vaginal and rectal samples were collected, using the same single swab, from women at 35-37 weeks of pregnancy. Swabs collected at hospital setting were directly immersed in Granada™ bifásico broth (bioMérieux® España S.A) whereas swabs collected in the primary healthcare centres were conserved in Amies media, submitted to the reference laboratory and then immersed in Granada liquid media cited above. From the suspension liquid of all the Granada vials, an aliquot of 1 ul was taken (prior to incubation) using disposable loops and then cultured on blood-agar plates. All plates and media were incubated at 37ºC in a 5% CO2 atmosphere for 24 and 48 hours. All the colonies displaying beta-hemolisys in blood-agar plates were tested for GBS identification by using an specific agglutination kit and subsequently confirmed by the automated sytem Vitek2(biomerieux). Granada media was considered positive when the vials displayed orange colour. GBS isolation on blood-agar plates was considered the reference method.

Results

A total of 251 samples were prospectively included in the study, including 140 (55.7%) collected in the primary healthcare centres and the remaining 111 (44.3%) collected in the hospital setting. According to the standard method, 26 samples were positive for GBS colonization. The Granada medium only detected 21samples as positive. Four out of the 5 false-negative samples were collected in the area. Performance data are shown in the following table.

	Valor	CI (95%)	
Sensibility (%)	80,77	63,7	97,84
Specificity (%)	100	99,78	100
Validity index (%)	98,01	96,08	99,94
Predictive valor + (%)	100	97,62	100
Predictive valor - (%)	97,83	95,72	99,93
Prevalence (%)	10,36	6,39	14,33

Conclusion

In this study, the Granada medium displayed an excellent especificity. In contrast, the sensitivity reported was low to be considered for screening purposes. According to this study, this medium should be complemented with additional medium. Since the false negative results were mainly reported in samples collected in the area (using Amies medium as transport media), the low sensitivity reported could be related to delayed inoculation of the Granada medium.

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Direct molecular identification of fungi from onychomycosis

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Onychomycosis is a fungal infection on hands and feet’s nails, and is caused by fungi that primary invade the healthy nail blade. Although bacteria may also cause nail infections, those provoked by fungi are more frequent. Thus, fungi must be considered the main etiologic human onychopathies. Around 80-90% of the cases of fungal nail’s infections are caused by dermatophyte fungi, 5-17% by yeasts and 2-12% by filamentous non-dermatophyte fungi. Conventional diagnosis based on direct examination and culture of clinical samples is time-consuming and only moderately specific. The aim of this work was to implement a specific and sensitive molecular assay allowing the identification of the etiologic agents of onychomycosis directly in infected nail samples. Fifty four samples collected from unhealthy nails with clinical suspicion of onychomycosis were used in this study. The respective etiological agents were isolated and identified using both conventional and molecular methodologies. Conventional laboratorial diagnosis was carried out on the basis of direct examination of nail fragments in 20% KOH and culture. Crude DNA was extracted from the same samples and the fungal Internal Transcribed Spacers (ITS) region of the ribosomal DNA was amplified and sequenced using universal primers. From the 54 nail samples, 25.9% and 70.1% were obtained from male and female patients, respectively, with an age range from 10 to 70 years-old. About 72% of the samples were obtained from toes nails. Conventional laboratorial analysis evidenced that 35% of the infections were caused by dermatophyte fungi (*Trichophyton rubrum* being the most frequent), 28% by yeasts (with emphasis for *Candida parapsilosis*) and 8% of the infections were caused by other filamentous fungi such as *Scopulariopsis* species. About 9% of the samples contained mixed infections of two or more fungal species. The amplification of the ITS region directly from DNA extracts of infected nail samples, and the respective nucleotide sequence analysis, allowed the rapid and specific identification of most species grown in culture, mainly those from infections caused by only one fungus. Albeit a clear sequence determination was hampered in mixed infections, this molecular approach revealed to be promising for the diagnosis of most onychomycosis, towards the targeted rather than empirical therapy for dermatophytoses.

Keywords onychomycosis; fungi; dermatophytes

Effect of chlorhexidine upon bacterial isolates from colonized intravenous catheters from companion animals

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Bacterial colonization of intravenous catheters may cause nosocomial infections and septicaemia in critical and hospitalized patients. These bacteria may produce biofilms, a known virulence factor involved in microbial evasion to the action of antimicrobial and disinfectant compounds. This situation represents the ultimate example where "prevention is better than cure". The aim of this study was to compare chlorhexidine to other commonly used disinfectants (alcohol and iodopovidone) in terms of their ability to inhibit in vitro growth of bacteria isolated from intravenous catheters, relating these data with the isolates' biofilm forming ability.

The study population comprised a group of 40 animals (28 dogs and 12 cats) hospitalized in the teaching hospital of the Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal. Venopunction sites were disinfected with 4% chlorhexidine and allowed to dry for 10 minutes; all animals under study had been subjected to peripheral intravenous catheterization for a minimum of 6 hours. Catheters' tips were processed immediately after asseptic removal, using standard microbiological techniques for aerobic bacteria identification. Bacteria susceptibility to the different disinfectants tested was assessed by a modified agar diffusion protocol, using chlorexidine (4% and 2% v/v), iodopovidone (10% and 5% v/v) and alcohol (70% and 90% v/v). Biofilm forming ability was evaluated using a Fluorescent In Situ Hybridization (FISH) protocol.

It was possible to isolate bacteria from 9 catheters (22%), 2 of which bore a mixed population of 2 isolates and the remaining 7 yielded just one bacterial species. Staphylococcus was the most frequently isolated species. Bacterial susceptibility to chlorhexidine was higher than to iodopovidone and alcohol, regardless of the concentrations tested, for all isolates. Eight isolates (73%) produced biofilm in a time dependent kinetic: 2 isolates were able to produced biofilm at 24h, 6 at 48h and 8 at 72h.

Prevention of intravenous catheter colonization, particularly due to biofilm producing bacteria, is of major importance in critical patients. Our results show a higher antimicrobial susceptibility to chlorhexidine than the other disinfectants used in this trial, both in isolates with and without the ability of in vitro expressing biolfim. Further studies are required, performed with a higher number of bacterial isolates and evaluating the influence of time of disinfectant exposure upon its performance.

Keywords biofilms; companion animals; catheters; chlorhexidine

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Effect of essential oils on the planktonic of *S.aureus* and *E.coli* cells

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The essential oils of aromatic plants and their components have a wide range of applications in ethno-medicine, preservation, food flavoring and fragrances and in the perfume industries. Some essential oils derived from plants have revealed promising antimicrobial activity against a wide range of bacteria, including antibiotic resistant species.

The aim of this study was to examine the antimicrobial effect of essential oils of *Cinnamomum zeylanicum* and *Cymbopogon martini* against planktonic *Staphylococcus aureus* and *Escherichia coli* growth.

The antimicrobial activity of the essential oils was checked by bacterial growth, at 37 ºC and 120 rpm, in the presence of increasing concentrations of each essential oil for 24 h. Essential oils were dissolved in DMSO (2.0 %) and saline water (0.85 %) with tween 80 (0.5 %) in order to obtain final concentrations of 0.06 %, 0.09 % and 0.12%, for *E. coli*, and 0.09 %, 0, 12 %, 0.36 % and 0.48 %, for *S. aureus*. Bacterial planktonic growth over time was followed by the quantification of the number of viable through cultivation of aliquots in TSA.

Data showed that *E. coli* was more sensitive to the action of both essential oils, since complete planktonic growth inhibition was attained with a concentration of 0.09 % of the essential oil of *C. zeylanicum* oil and 0.06 % of the essential oil of *C. Martini*. Conversely, *S. aureus* was less sensitive to the antimicrobial action of the essential oils. *C. zeylanicum* essential oil inhibited *S. aureus* growth only at concentrations of 0.36 % and 0.48 %, after 4 and 2 hours of growth. However, unexpectedly after 24 hours those *S. aureus* cells recovered gradually their planktonic growth.

The data pointed out that it is crucial to check the bacterial behavior in the presence of antimicrobial products in different concentrations and over time due to the possible development of bacterial tolerance towards the mechanisms of action of those products. In fact, antimicrobials may have a positive effect in the early hours of application, as demonstrated by some results of this experiment. However, for longer times, the inhibitory effect of antimicrobials can be reverted by bacteria making ineffective their use as disinfectants in food industries. Addiotionally, the continuous exposure of bacteria to antimicrobials can influence the process of microbial resistance de development and increase. These preliminary results demonstrated the possibility of using essential oils of *C. zeylanicum* and *C. martini* against two bacteria that are responsible for foodborne illnesses at low concentrations but only for slightly prolonged periods of exposure.

Keywords: natural antimicrobial agents, planktonic growth, S.aureus, E.coli, essential oils

Epidemiological study of *Acinetobacter baumannii* from the Caracas University Hospital

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Acinetobacter baumannii is one of the most important nosocomial pathogens worldwide. High-level antimicrobial resistance is a noteworthy characteristic of this specie. The presence of these bacteria in the hospital environment constitutes a risk factor especially in intensive care units (ICUs). In our country, as well as in the rest of the world, nosocomial infections are a major public health concern and studies have focused mainly on determinants of resistance to antibiotics. Molecular typing techniques have had a positive impact on environmental studies, both hospital and natural, and the role of environmental bacteria in the transmission of infectious diseases. The aim of this study was to determine the possible sources of transmission of nosocomial infections caused by *A. baumannii* isolated from the hospital environment.

During a one year study in the ICU and Neonatal Service, NICU included, at the Caracas University Hospital, samples were collected both from patients with nosocomial infections and from the environment of each unit. More than 200 Gram negative colonies were identified using an automated system. Resistance to 15 antibiotics was determined. Plasmid elements were analyzed. The presence of Class 1 integrons were determined by PCR and sequenced. ERIC-PCR and REP-PCR were used for genotyping, isolates genetically related by the two techniques were considered as such.

We isolated 74 *A. baumannii,* 14 from ICU patients and 42 from its environment, and 1 from a Neonatal patient and 17 from the environment of this service. Environmental isolates were obtained from all surface items sampled in every unit analyzed in this study; isolates from staff´s hands were also included. Overall, 44.6% of the isolates showed resistance to at least 7 antibiotics. Environmental isolates from the Neonatal Service were concomitantly more susceptible to the antibiotics tested. Through analysis of restriction patterns was shown that all bacterial isolates harbored not related high molecular weight plasmids. A total of 58% of the isolates harbored one to eight class 1 integrons, which contained variable regions of up to 3000 bp. Sequence analysis of the 3000pb class 1 integrons harbored in patient and environmental samples demonstrated a high homology to an integron from a pathogenic island previously reported in this specie, AbaR5. It was found that 73% of the isolates from patients were closely related clones, and also 28.8% of the environmental isolates were grouped in 7 indistinguishable clones groups. Among the isolates analyzed only two environmental isolates are possibly related to two patient isolates.

Our results showed that: 1) In the Caracas University Hospital *A. baumannii* is the most important nosocomial pathogen; 2) The multiple antibiotic resistance profiles demonstrate the need for antibiotic usage surveillance, 3) The presence of an integrón included in a pathogenic island, not previously reported in our country, needs to be evaluated; 4) Given the high incidence of multiresistant pathogens in the environment of these critic care units, hand wash and surface cleaning methodologies have to be evaluated, and 5) The presence of related bacterial clones and the high incidence of movable genetic elements in different patients and the environment highlights the importance of surveillance measures in the hospital.

Keywords: Nosocomial infection; *Acinetobacter baumannii*; antibiotic resistance

Evaluation of cholesterol removal in MRS and GS culture media by *Lactobacillus acidophilus* and optimization of biomass production in GS culture medium

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Cholesterol is an important compound in most of the biological reactions which the excess of it can be seen as a harmful compound of causing heart diseases. Probiotics are bacteria that are able to reduce cholesterol levels. These bacteria according to the least negative effect on the body alternative to chemical drugs are considered.

In this study, the amount of cholesterol removal by *Lactobacillus acidophilus* strain (ATCC4356) in vitro culture (MRS and GS) by spectrophotometer method was evaluated .Also optimal GS culture medium compositions for growth of this bacteria were determined. Response surface method of optimization was employed to find the optimal values of the four most important substrates from the culture medium (Glucose, yeast extract, di Potassium hydrogen phosphate, Potassium di-hydrogen phosphate).

Key words: probiotic, *Lactobacillus acidophilus*, cholesterol removal, optimization

Evaluation of the distribution of *qac* disinfectants resistance genes on strains isolated from hospital settings

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Antiseptics and disinfectants are extensively used in hospital infection control. The most widely used antiseptics include cationic quaternary ammonium compounds (QACs).The daily use of these compounds has led to the selection of resistant bacterial strains that increase the risk of acquiring nosocomial infections. This antiseptic and disinfectant resistance is usually associated with efflux pumps encoded by the gene family *qac,* contained in plasmids. The aim of this study was to evaluate the distribution of antiseptic and disinfectant resistance genes (*qacA, qacB, qacC, qacE, qacEΔ1, qacF, qacG, qacH* and *qacJ*) and its association with phenotypes of resistance in strains isolated from hospitalized patients and their surrounding environment.

We analyzed 12 strains of *Stenotrophomonas maltophilia* and 39 strains of *Acinetobacter baumannii* isolated from patients and inanimate surfaces in the environment of the Intensive Care Unit (ICU) and Neonatal Service (NS), of the Caracas University Hospital. To evaluate the resistance phenotypes tests recommended by the Association of Official Analytical Chemistry (AOAC) were used; the Quantitative Suspension test (number of viable colonies) and the Cell Death Rate Test (turbidity on liquid culture), both determinations were performed after the exposure of the microorganisms to the disinfectant. To assess the distribution of *qac* genes, the Polymerase Chain Reaction (PCR) was used.

The microorganisms tested in this study have been reported as the main agents of most nosocomial infections in this healthcare center. Our results showed that 29 out of 51 isolates exhibited the resistance phenotypes to the quaternary ammonium compound disinfectant agents evaluated, 5 strains of *S. maltophilia* (2 isolates from hospitalized patients and 3 from the ICU environment) and 24 strains of *A. baumannii* (7 isolated from patients, 6 from the NS environment and 11 from the ICU environment). Of these strains, 7% harbored the *qacA* gene, 65% the *qacB* gene, 3% the *qacC* gene, 65% the *qacEΔ1* gene, 28% the *qacF* gene and 7% the *qacH* and *qacG* genes. The *qacJ* and *qacE* genes were not detected. Positive PCR results to more than two genes simultaneously were obtained in 14 of the strains tested. *qacB*, *qacEΔ1, qacF, qacG* and *qacH* genes detected in environmental isolates of *A. baumannii* and *S. maltophilia,* NS and ICU respectively, suggest that these multiresistant strains are settling in inanimate surfaces in these hospital units, being potential genetic reservoirs for multiple disinfectant resistance genes. The high prevalence of *qacEΔ1* genes was positively correlated with the presence of class 1 integrons in each of the strains analyzed.

This is the first study in Venezuela that evaluates the presence of these genes in hospital related isolates, highlighting the high prevalence of genes *qacEΔ1 and qacB* distributed in isolates from both the environment and patients with nosocomial infections. These results represent a warning to the authorities of this health center in order to prevent the spread of these resistance determinants, given that the presence of these genes are a selective advantage that increases the survival of these multiresistant isolates in a hospital environment.

Keywords: disinfectants resistance; *qac* genes; PCR

Exploring the anti-MRSA activity of sixteen medicinal plants endemic in Khuzestan province, Iran

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Today, Methicillin Resistant *Staphylococcus Aureus* (MRSA) has emerged as an important cause of serious infection in hospitals and in the community. Medicinal plants have always been sources for new drug discovery. The main objective of this study was to explore the antibacterial activity of sixteen Iranian plant extracts including *Quercus brantii*, *Ziziphus spina-christi*, *Peganum harmala*, *Oliveira decumbens*, *Galium tricornutum*, *Vitex pseudo-negundo*, *Salvia sclarea*, *Vaccaria pyramidata*, *Teucrium polium*, *Plantago ovata*, *Cordia myxa*, *Callistemon citrinus*, *Albizia lebback*, *Malva neglecta* Wallr and *Hibiscus rosa-sinensis* against clinical MRSA strains(Table1).

The studied strains were isolated from urine, stool, blood and wound of infected patients at Shahid Iranpour hospital located in Omidiyeh city (South of Khuzestan province, Iran) from November 2007 to May 2008. Sixteen medicinal plants collected from several locations in Khuzestan province (South west of Iran) including Behbahan (South east of Khuzestan province), Izeh (North of Khuzestan province) and Ahvaz (Center of Khuzestan province). In further, the antibacterial activity of ethanolic local plant extracts were tested on clinical isolates by disc diffusion method.

A total of 9 strains were resistant to meticillin and cefixime. The highest antibacterial activity were belong to *Q. brantii*, *P. harmala*, *Z. spina-Christi* and *O. decumbens* extracts with 11-40 mm, 15-28 mm, 8-26 mm and 10-20 mm of diameters, respectively. Intermediate antibacterial activity was exhibited by *G. tricornutum* and *V. pseudo-negundo*. The *S. slarea*, *V. pyramidata* and *C. myxa*, however, showed no antibacterial activity against the studied strains. Other plants presented relatively poor anti-MRSA effect. Most plants growing in Khuzestan province have to undergo a period of the strict drying climate as the stress condition that may result in the production of some special secondary metabolite with the pharmaceutical importance. In conclusion, the *Q. brantii* and *P. harmala* seed extract can be considered as the source of natural antibiotics for treatment of infections caused by MRSA strains.

Keywords MRSA; native medicinal plant; *Quercus brantii*; *Peganum harmala*

Table 1. List of plants with a high-level of anti-MRSA activity and their families, vernacular name, used parts and collection sites.

Scientific name	Family	Vernacular name	Part used	Collection site
Oliveria decumbens	Umbellifereae	Laal Kouhestan	Aerial part	Behbahan
Peganum harmala	Nitrariaceae	Espand	Seed	Behbahan
Quercus brantii	Fagaceae	Balout	Seed	Izeh
Ziziphus spina-christi	Rhamnaceae	Sedr, Konar	Leave	Ahvaz

Frequency of isolation of *Staphylococcus lugdunensis* in nosocomial infections

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OBJECTIVE: A one year study was done to determine the frequency of nosocomial *S. lugdunensis* infections and sensitivity of the isolates to available antibiotics. *S. lugdunensis* has recently been acknowledged as an important pathogen because of the increased use of medical devices, vascular grafts, prosthetic heart valves and prosthetic joints. Many clinical laboratories do not routinely report *S.lugdunensis* and are usually ignored as contaminants in specimens such as blood, cerebrospinal fluid, urine, pus, and sputum. However, vital statistics underlining the major cause of morbidity in Pakistan indicate that *S. lugdunensis* are not just contaminants. The prime pathogenic mechanism of *S. lugdunensis* is biofilm formation. In addition, *S.lugdunensis* also cause disease by producing heat stable hemolysin, as well as Staphylococcal enterotoxins.

METHODOLOGY: A total of 56 isolates were recovered in a 1 year study from urine,pus and blood samples from hospitalized patients. *S.lugdunensis* was identified using API Staph 20 and 16s Ribosomal RNA gene sequencing. The hemolytic effect of the isolates was observed on each of human, sheep, buffalo, cow and rabbit blood agar plates. Their antibiotic susceptibility was tested using disk diffusion method. Secreted enterotoxin was determined using the Staphylococcal Enterotoxin Test-Reversed Passive Agglutination kit.

RESULT: Of the total *S. lugdunensis isolates*, urine samples yielded 12.5%, pus 37.5% and blood 50% strains. The most effective drugs among the 17 antibiotics used were Vancomycin (100%) and Linezolid (100%), followed by Teicoplanin (95%) and Amoxyclav (78%). Methicillin resistance was noted in 60% of the isolates.Enterotoxins detected were SEA and SEC.

CONCLUSION: *S. lugdunensis* has emerged as a cause of many nosocomial infections and it should be given proper attention to reduce morbidity and mortality associated with nosocomial infections. *S. lugdunensis* should decidedly be identified in labs using appropriate tests and not be ignored as a contaminant.

Keywords: S. *lugdunensis*, nosocomial infections, prosthetic, biofilm, enterotoxin, hemolysin.

Growth of *Escherichia coli* O157:H7 on packed fresh-cut lettuce treated with electrolyzed water

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Escherichia coli O157:H7 is a serious concern for fresh-cut vegetable industry in Spain and other countries since vegetables may become contaminated at field by this pathogen. The main disinfection step applied in Industry consists of treating vegetables with chlorinated water, however, results microorganisms can survive and then grow when conditions are optimum. Several decontamination methods have been proposed as alternative to chlorination such as irradiation, organic acid and electrolized water. However, the most promising results have been obtained for electrolized water treatments since bacterial reduction is similar to chlorine-based treatment and generated toxic by-products are much lower than in chlorination. The purpose of this study was to evaluate and model the growth of *E. coli* O157:H7 in packed lettuce under different storage temperatures, previously submitted to an electrolized water treatment.

A cocktail of *E. coli* O157:H7 (CECT 4267, 4076, 4782, 4783, and 5947) resistant to nalidixic acid was inoculated on fresh iceberg lettuce (~ 5 log cfu/g) and then submitted to a novel electrolyzed water treatment based on Boron-doped diamond electrodes. After packaging, lettuce bags were stored at different temperatures (4, 8, 13, 16 ºC) during 27 day-period. The pathogen growth was monitored by plating on Chromocult agar supplemented with nalidixic acid. In order to capture the biological variability, eight replicates were analyzed for each point. The primary growth model described by Baranyi and Roberts (1994) was fitted to growth raw data by means of DMFit excel Add-In (kindly provided by J. Baranyi, Institute of Food Research, Norwich, UK),

The electrolyzed water treatment resulted in a reduction of ~ 2 log cfu which was similar to those often reported for clorination. These reduction resulted in an initial count in packaged lettuce of around 3.5 log cfu/g. The growth data indicated that *E. coli* O157:H7 was able to grow at 8, 13, and 16 ºC. On the contrary, at 4 ºC, a decline of 2.5 log ufc/g was observed after 27 days. At 8 ºC, growth curves showed a lag phase, which elapsed 15 days and then, an increase of 1.5 log ufc/g was obtained during the following 12 days. For the 13 ºC, non-lag was observed and a 3-log increase was reached in 8 days giving a final population of 7 log cfu/g, approximately. Finally at 16 °C, bacteria was able to grow up to 7 log ufc/g in only 3 days. Regarding modelling, there was a lack of fit for data obtained at low temperatures which could be consequence of the stringent conditions. For instance, at 8 °C, data presented a great variability thereby producing lower R^2 (0.40) and high Standard Error (0.62). In turn, for 13 and 16 ºC, growth models showed better R^2 (0.78 and 0.62, respectively). It is worthy to note that a 3 ºC increase (from 13 to 16 ºC) resulted in a 3-fold increase of growth rate, from 0.33 log cfu/day at 13 ºC to 1.06 log cfu/day at 16 ºC. These data suggest that *E. coli* O157:H7 is able to survive on lettuce after a electrolyzed water ion treatment, and then to grow significantly at refrigeration temperatures ≥8 C. Models here developed might be applied to predict grow and to complete quantitative risk assessment studies including electrolized water treatment.

Keywords: Foodborne pathogens: *Escherichia coli* O157:H7, electrolyzed water,disinfection, minimally processed vegetables, predictive modeling.

Identification and Multiple drug resistance of bacterial isolates from effluents collected from pharmaceuticals industry, Islamabad

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The study was based on the isolation and identification of bacteria from the effluents of pharmaceutical company. The pharmaceutical effluent poses harmful effects both on health and environment. The pH of effluent was 7.12 at 25° C and its electrical conductivity was 841µS/cm at 31.8 ^{o}C. Isolation was done by serial dilution, spread plate and streak plate method. Isolates were identified by studying colony morphology, gram staining, and growth on differential media and biochemical tests finally. Biochemical tests done were triple sugar iron test, Indole test, Citrate utilization test, methyl red-vogas proskeur test, gelatin liquefaction test and starch hydrolysis. The identified strains were gram positive Bacilli, *Streptobacillus sp., Gram positive Cocci, Cocobacillus sp, Staphylococcus aureus, Staphylococcus epiderdimis, Escherichia coli, Klebsiella sp.and salmonella sp.*the identified strains were checked against different antibiotics to test their multiple resistance. The antibiotics used were sparfloxacin (SPX 10µg), kanamycin (K 30µg), norfloxacin (NOR 10µg) , sulzone (SCF 105 µg), enoxacin (ENX 30 µg), fusidic acid (FD 10 µg), Carbinicillin (30 µg), imipenum (IPM 10 µg). clarithromycin (CLA 15 µg), Erthyromycin (E 5 µg), vancomycin (30 µg), cefepime (FEP 30 µg), Gentamycin (CN 10 µg), ciprofloxacin (CIP 5 µg), cefixime (CFM 5 µg), Disc diffusion method was used to check antibiotic resistance against fifteen antibiotics respectively. *Klebsiella sp.* and *streptobacillus sp.* Showed high resistance against all antibiotics. FEP 30 µg) is no more effective against strains *E.coli sp*, *Bacilli* sp*., coccobacillus sp., staphylococcus epidermidis sp., klebsiella sp., cocci., streptobacillus sp.* The largest zone was given by imipenem (IPM 10 µg) having a diameter of 40mm against streptobacillus . least effective medicine is cefixime (CFM 5 µg) and Cefepime (FEP 30 µg) because they showed no zone against majority of bacterial strains therefore there should search for production of new antibiotics.

Key words: pharmaceuticals, antibacterial resistance, biochemical tests.

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Identification of Metabolic States and their Relation to Operational Conditions in Urokinase Production by HT1080 Cells

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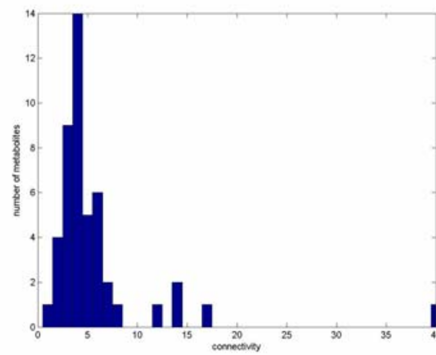
Urokinase is a serine protease that converts plasminogen into plasmin and initiates the process of fibrinolysis. In the event of stroke and myocardial infarction, urokinase is one of the preferred drugs for clinical intervention as it has no known immunological reaction and few reported incidences of hemorrhagic complications. A clinical formulation of urokinase contains the active protein of molecular weight ranging from 50 to 80 kDa. Commercially, urokinase is produced primarily by mammalian cell culture. Since mammalian cells have a slower growth rate compared to microbes, maximizing productivity while meeting quality and regulatory requirements presents a formidable challenge. This work attempts to address these issues in part by identifying metabolic states that associated with high production rates of urokinase and correlating these with operating conditions. Human fibrosarcoma HT1080 cells were employed for the production of urokinase. The reactions of the metabolic pathway were elucidated and the biosynthetic pathway was created. Flux map distributions for various metabolic states were calculated using the data obtained from bioreactor studies. The optimal flux distributions corresponding to both cell growth and urokinase synthesis were determined. Extreme pathways were identified and examined to understand amino acid metabolism during urokinase production.



Keywords Urokinase, HT1080 cells, metabolic flux analysis, flux optimization, bioreactor.

Identification of *Nocardia brasiliensis* strains isolated from actinomycetoma in Mexico using specie-specific primers

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Actinomicetoma is a chronic granulomatous disease caused by Gram-positive actinobacteria. It is endemic in tropical and subtropical regions, especially in India, Central Africa and Central and South America In Mexico the disease is considered to be one of the most frequent mycoses and *N.brasiliensis* (85%) y *Actinomadura madurae* (10%) are the predominant etiological agents. The molecular identification methods are now more frequently used and the most used is the amplification and sequencing of the 16S rRNA gene, present in all the *Nocardia* species. A method has been described for the amplification and sequencing of a 606 bp region of the 16S rRNA gene that is efficient for the identification of the different *Nocardia* species without sequencing the complete gene. These two methods have been used in several studies with positive results.

The object of this research was to bring up to date the actinomycetoma situation in the State of Mexico and to identify the causal agent.

To localize actinomycetoma cases the patient's medical records from the dermatology departments of general and private hospitals were reviewed. Pathologic strains were looked for in general hospital patients and in reference institutes in Mexico City. The clinical strains were kept in Bennett agar and later on were identified through polyphasic approaches including a parcial sequence of the 16S rDNA using specie-specific primers designed to recognize *Nocardia* species. Eleven cases of actinomycetoma and 5 species of pathological strains were identified. Most of the cases were found in the Southeast zone of the State of Mexico and *Nocardia brasiliensis* was the isolated species.

This research confirmed the presence of actinomycetoma in the State of Mexico as well as the zones of high incidence and the most frequent pathological strain.

Key words: actinomycetoma, State of Mexico, 16S rRNA identification, characterization phenotypic, *Nocardia brasiliensis*.

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Immunomodulatory molecules of the probiotic *Lactobacillus rhamnosus* GG

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Contact with resident gastrointestinal microbes, but also with harmless bacteria from the environment, is important for development of immune homeostasis and prevention of diseases such as inflammatory bowel diseases (IBD) and allergy. Based on these observations, probiotics such as *Lactobacillus rhamnosus* GG (LGG) are explored as immunomodulatory agents. Clinical studies with LGG have shown prevention and relieve of acute diarrhea in children, prevention of atopic disease and prevention of relapse in IBD, among other effects, yet without much knowledge on the mode of action.

In this study, molecular mechanisms of action of LGG are explored by applying a mutagenesis approach. Various LGG knock-out mutants that lack putative immunomodulatory surface molecules were constructed. The cytokine induction profiles in intestinal epithelial and dendritic-like cells were subsequently determined for each mutant. By comparison of the immunomodulatory capacity of these mutants with LGG wild-type, the role of these surface molecules is studied *in situ* on live bacteria. In parallel, the immunomodulatory capacity of isolated surface molecules was determined.

These analyses showed LGG has long flexible surface appendages, termed pili, which play a key role in adhesion to the gut mucosa and modulation of cytokine expression in intestinal epithelial cells. In addition, the cell surface of LGG is surrounded by an exopolysaccharide (EPS)-layer, which promotes survival in the intestine and modulates cytokine induction. The cell surface of LGG also contains D-alanylated lipoteichoic acids (LTA), which appear to have a pro-inflammatory capacity.

Thus, the cell surface of LGG contains multiple molecules with immunomodulatory capacity. Further studies are ongoing to delineate the exact immunomodulatory role of the pili, EPS, LTA and other LGG molecules. The final host response provoked by LGG will depend on the sum of the dynamic interactions of diverse surface molecules with their cognate immune receptors, of which many details remain to be unraveled. Ultimately, this molecular research should provide a molecular framework enabling the selection of the best strain (which is possibly a mutant) or molecules for each disease or application.

Keywords probiotics; immunomodulation

Impact of nutritional conditions on colony morphology variants isolated from *P. aeruginosa* and *S. aureus* biofilms

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In natural habitats, microorganisms are challenged all the time due to stress conditions imposed by the surrounding environment. To adapt to these environmental changes, bacteria alter their physiological and genetic traits. This adaptive behavior may be achieved by phenotype switching. This process consists in a reversible switch of phenotypes, as a mechanism ON/OFF, which occurs at high frequencies than spontaneous mutations. Colony morphology variation is the macroscopic feature of the phenotypic switching. Colony variation may have serious impact on bacterial virulence and antimicrobial resistance potentiating its ability to cause disease. Some colony variants are strongly associated to antibiotic resistance due to their presence in chronic infections despite antibiotic therapy. In cystic fibrosis, the switch of *P. aeruginosa* from non-mucoid to mucoid morphotype, which overproduce alginate, is a crucial stage to the establishment of this recalcitrant disease. Small colony variants (SCV) are other well-known resistant morphotype. These variants exhibited small size because its slow growth rate, pigmentation, haemolysis, reduced range of carbohydrate utilization and higher resistance to aminoglycosides antibiotics and cell-wall inhibitors.

It has been growing the number of studies related with phenotypic switching and colony morphology characterization. However, normally each study reports the use of different solid growth media which makes the comparison between studies inaccurate. In order to clarify the role of nutritional conditions on bacterial colony morphologies and on its populational diversity, *P. aeruginosa* and *S. aureus* planktonic and biofilm-growing cells were spread onto the most common solid laboratory media (TSA, MHA, LB agar, MacConkey agar and Columbia horse blood agar). Additionally, the reproducibility of each medium was also inspected.

Data showed that *P. aeruginosa* and *S. aureus* colony morphotypes are strongly influenced by the plating medium used. The main differences observed were the size, texture and form of colonies. The largest colonies were detected in TSA, MHA and LB agar. Colonies grown on MHA and LB agar were very similar possibly due to their identical nutritional composition. All the solid media tested showed reproducibility between assays except the Columbia horse blood agar which exhibited some inconsistency probably due to the presence of blood in its composition. Amongst the solid media tested, for planktonic and biofilm cultures, TSA gave rise to higher number of colony variants. Phenotype diversity seems to be more influenced by nutritional factors when bacteria derived from biofilms.

This study allows concluding that, in contrast to fungi, bacterial colony appearance is influenced by the nutritional conditions of the solid media used to spread the cells. This evidence should be taking into account when important phenomena as phenotypic switching are going to be studied. The data obtained with this preliminary work may question the classification of colony morphotypes used until now.

Keywords Phenotypic switching; colony morphology, nutritional conditions, solid growth media

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In vitro inhibitory activity of vancomycin, daptomycin, linezolid and tigecycline against methicillin resistant *Staphylococcus aureus*

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OBJETIVE

To compare the *in vitro* inhibitory activity of vancomycin, daptomycin, linezolid and tigecycline against methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical samples.

MATERIAL AND METHODS

All the MRSA isolates collected at Hospital Universitario de Canarias (Tenerife, Spain) during the period 2009-2010 were included in the study. Only one isolate per patient episode was considered. The identification and susceptibility testing were performed by VITEK2 (bioMerieux ®, France). Methicillin resistance was confirmed by using the MRSA-screen test (Denka Seiken Co., Japan) to detect the PBP2a protein. In addition, an in-house real-time PCR was carried out to detect the *mecA* gene. MICs were determined by the broth microdilution method according to CLSI recommendations using Cation-Adjusted Mueller-Hinton II Broth (Becton Dickinson, USA). For daptomycin susceptibility testing, the broth was supplemented to reach a Ca^{2+} final concentration of 50 ug/ml. Fresh medium (<15 hours) was used for tigecycline susceptibility testing. *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were included as quality control strains. Susceptibility interpretations were performed according to the CLSI (2010) criteria, when available. A tigecycline susceptibility breakpoint of 0,5 µg/mL was used in accordance with Guide to Antimicrobial Therapy (J. Mensa et al, 2010),

RESULTS

During the study period, a total of 88 MRSA isolates (including 17 from Intensive Care Unit patients) were collected from 54 exudates, 17 blood cultures, 13 respiratory samples and 6 urine specimens. Regarding patients, the male-female ratio was 55-33 and the average age was 67 years (SD = ± 17.33), ranging from 21 to 92 years. Comparative data for the inhibitory activity and interpretation of susceptibility to the antimicrobial agents are shown in the following table:

Antimicrobial agents	MIC (µg/mL)			Susceptibility (%)
	Range	50%	90%	
Vancomycin	0,125 - 2	0,5	1	100
Daptomycin	0,125 - 1	0,5	0,5	100
Linezolid	1 - 4	2	4	100
Tigecycline	<0,0016 - 0,5	0,0625	0,125	100

CONCLUSION

The data show that all the MRSA isolates included were susceptible to the four antimicrobial agents tested in vitro. However, the MIC_{90} is only 1, 1 and 2 fold dilutions lower than the susceptibility breakpoint for vancomycin, daptomycin and tigecycline, respectively. Regarding linezolid, the MIC_{90} was equal to the susceptibility breakpoint.

KEYWORDS: MRSA, vancomycin, daptomycin, linezolid, tigecycline.

In-Vitro anti fungal activities of Cinnamon extract on *candida spp* and *Aspergillus spp*

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Introduction: Several studies have shown that cinnamon has antibacterial and antifungal activity. Furthermore cinnamon has been described to have medicinal usages in some fungal infections like Candidiasis and Aspergillusis.

Methods: To determine inhibitory and fatality dose of cinnamon extract, we prepared serial dilution of the extract including 1/20, 1/40, 1/80, 1/160, 1/320 and 1/640 in 1 mL of liquid medium Sabouraudo Dextrose Broth (SDB). Given numbers of candida yeasts in 1ml were added to above dilution tubes. *Candida spp.* and *Aspergillus spp.* Cultures were incubated at $30^{°c}$ and $25^{°c}$ respectively for 24-72 hours.

Results: we observed that the concentration of 0/25 g/dL of cinnamon extract showed an inhibitory and killing effect on more than 50% of the isolates. In the agar dilution method, some changes were observed on morphological features (depends on the extract dilution) as well as quantitative effects of dilution of extract on the colonies.

Conclusion: We found that the extract of cinnamon had a prominent antifungal activity and inhibitory effect on *candida spp* and *Aspergillus spp* isolates.

Keywords: Cinnamon, *Candida spp*, *Aspergillus spp*, Inhibitory

Inactivation of *Bacillus subtilis* spores by hydrogen peroxide vapour; acquisition of statistically robust inactivation kinetics

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Hydrogen peroxide possesses many characteristics that make it an ideal disinfectant, principal amongst these being that it ultimately decomposes to oxygen and water. The use of this agent is receiving much attention in the healthcare sector as a means of reducing the incidence of healthcare associated infections (HAI) such as *C. difficile* and MRSA. For such applications, hydrogen peroxide is typically generated in the form of a vapour in order to achieve maximum efficacy in environments that are populated with a variety of surfaces in a number of complex configurations. Our ultimate aim is to design disinfection protocols that are tailored to specific environments. This approach requires the use of computational fluid dynamics (CFD) coupled with robust microbial inactivation data. As a first stage towards achieving this objective, we present inactivation kinetics for spores of *Bacillus subtilis* that were obtained using a chamber of novel design in which the concentration of hydrogen peroxide and time of exposure are carefully controlled. We present here inactivation data for spores obtained at concentrations of hydrogen peroxide between 10 and 90 ppm and exposure times ranging from minutes to several hours. A series-event model is shown to provide a good fit of the experimental data.

Keywords hydrogen peroxide; disinfection

Increased susceptibility of *Staphylococcus epidermidis* to sandalwood oil in a pulsed electromagnetic field

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The antimicrobial activity of sandalwood oil was significantly enhanced against *Staphylococcus epidermidis* in a pulsed electromagnetic field. These findings support other workers who found that electromagnetic fields enhance antimicrobial activity.

Keywords sandalwood oil; pulsed electromagnetic field; static magnetic field, antimicrobial activity.

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Investigation of the antimicrobial activity of the essential oil of *Cymbopogon martini* on *S.aureus* and *E.coli* biofilms

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Biofilms are sessile communities of microbial cells embedded in an exopolymeric secreted matrix that can adhere both to abiotic and living surfaces, serving as a permanent source of contamination. Essential oils (EOs) have different characteristics depending on the plant due to a large number of compounds (eugenol, citral, carvacrol, among others). It has been noticed that EOs have promising antibacterial activity that can be explored as an effective alternative to control biofilms. The aim of this study was to assess the antimicrobial activity of the essential oil of *Cymbopogon martini* against pre-established single biofilms developed by *Staphylococcus aureus* and *Escherichia coli*. Biofilms were developed in 96-well microtiter plates for 24 h at 37 ºC, in an orbital shaker at 120 rpm, being afterwards submitted to EOs aggression for 15, 30 e 60 minutes. The essential oil were dissolved in DMSO (2.0 %) and saline water (0.85 %) with tween 80 (0.5 %) in order to obtain final concentrations of 0,12, 0,48, 0,96 and 1,92 %. Biofilms were characterized, before and after EO treatment, by total biomass, through crystal violet (CV), and number of cultivable bacterial cells, expressed as Log CFU per cm^2.

The *C. martini* essential oil did not have any effective antimicrobial action against *S. aureus* biofilms, since there was no significant reduction of the biofilm cultivable cells and biomass. Conversely, this essential oil showed a promising antimicrobial activity against *E.coli* biofilms as it was observed a significant reduction of the cultivable biofilm-growing cells, in general, for all the concentrations tested and exposure time periods. Similarly to *S.aureus* biofilms, the *C. martinii* essential oil was not effective in reducing the biomass of *E. coli*.

From the data, it can be concluded that under the conditions tested, the *C. martinii* essential oil was more effective in the inhibition of the bacterial cells entrapped in *E. coli* biofilms than in the removal of biofilm mass. This inability to remove biofilm s from surfaces can be a drawback since the viable cells remaining within the biofilms after EOs treatment are protected by the exopolysaccharides matrix, allowing its multiplication. To overcome this situation, it would be interesting to assess the anti-biofilm potential of the *C. martinii* essential oil, as well as its synergistic activity with an antimicrobial agent with biofilm disrupting properties.

Keywords: biofilms, sanitizer, natural antimicrobial, bacteria, essential oil

Isolation and identification of antibiotic-producing actinomycetes from Moroccan biotopes

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Actinomycetes are gram positive filamentous bacteria known for their ability to produce a large number of antibiotics. These bacteria continue to be a reservoir of bioactive molecules and several laboratories are still working on the isolation of new strains of actinomycetes capable of producing new antibiotic molecules. In this communication, we present the results of the isolation, characterization and phylogenetic analysis of the actinomycetes strains producing antimicrobial substances from Moroccan habitats. The antimicrobial activity was tested against Gram positive bacteria (*Bacillus subtilis 5262, Bacillus cereus cip 14579, Staphylococcus aureus 7625, Staphylococcus epidermidis 6821*), Gram negative bacteria (*Pseudomanas aeroginosa 76110, E.coli cip 7624, Erwinia chrysenthemi), Mycobacterium smegmatis* and *Candida albicans.* The 14 strains out of 18 displayed high activity against at least one the test-strains. Our further work is focused on the structure determination of the one of the molecules with high activity against gram positive bacteria.

Key words: Actinomycetes, Streptomycetes, antibiotics, molecular identification, 16S rDNA.

Isolation, purification and partial characterization of antifungal metabolites from novel Actinomycetes spp

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Antifungal antibiotics constitute a small, but an important group of drugs existing in the market, and they play a significant role in the control of fungal diseases. Actinomycetes are well-known for their ability to produce several bioactive compounds, which may be associated with antibacterial and antifungal properties. The increased incidence of fungal infections has accentuated the need for new, safe and more effective antifungal agents.

Several actinomycetes spp were screened for possible antifungal activity using agar plate bioassays. In preliminary results, it was found that the actinomycete sp A-03-116 showed complete inhibition of various fungi such as *Mucor, Aspergillus niger, Aspergillus oryzae, Trichoderma reesei, Alternaria solani, Fusarium moniliforme, Curvularia fallax, Curvularia lunata, Claviceps purpurea, Helmintosporium*, etc. indicating that the organism has potential antifungal activity. Furthermore, the extracellular antifungal compounds were produced through batch fermentation, was extracted, purified and tested for antifungal activity.

Ethyl acetate, chloroform and dichloromethane were used for the extraction of antifungal metabolites and found that ethyl acetate extract had the maximum antifungal activity. Moreover, the culture filtrates, and the mycelia were extracted with ethyl acetate. The antifungal activity was found to be largely extracellular. The crude filtrate, and the mycelial extract were devoid of 1, 3 glucanase and chitinase activities. The residue after extraction showed antifungal activity when tested against several fungi, including plant pathogens. Thin layer chromatography showed four prominent spot, and the spot C was found to be most active. Flash chromatography was used for the purification of ethyl acetate extracts, which were further purified using HPLC. The most active fraction (fraction C) had the UV absorbance at 254 nm and a mass of 1139.79. Infrared spectroscopy confirmed the presence of hydroxyl, ester and amide group in the purified compound. The MIC of the purified compound for *Aspergillus sp.* and *Conidiobolus sp.* was determined to be 1µg/ml, which is well within the range allowed for antifungal compounds used.

Keywords: Actinomycete, antifungal activity, bioactive compounds

Leaching of tetracycline resistant bacteria from pig manure applied to two field sites

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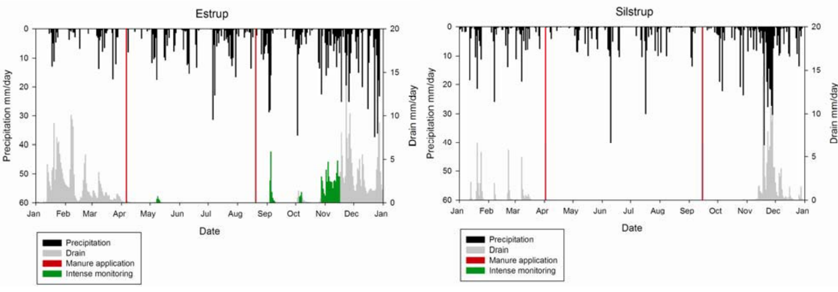
There is growing evidence of resistance to antibiotics in both commensal and pathogenic bacteria.

The spreading of manure on agricultural land is an economic and practical solution for improving soil quality. However, the large scale application of animal manure onto agricultural land release great quantities of antibiotics, resistance genes and resistant bacteria into the soil environment. Potential antibiotic resistant pathogens have been shown to survive for prolonged periods of time ranging from a few days to many months. Important factors influencing survival are initial concentration in the manure, soil water content, manure type, manure application method and temperature.

In the present study we followed the survival of natural occurring tetracycline resistant bacteria and *E. coli* in pig manure injected into two field sites. Furthermore concentrations of tetracycline resistant bacteria and *E. coli* was monitored in drainage water and groundwater based on natural precipitation.

200 of the tetracycline resistant colonies found in the water was genetic characterised by 16SrRNA gene sequencing and fingerprinting techniques.



Precipitation and drainage runoff from Estrup and Silstrup. The vertical red lines indicate manure application time and the green area symbolises intensive sampling with drainage water analysed for every 2 mm drainage runoff.

Keywords antibiotic resistance, water quality, manure, genetic characterization

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Metabolomic study in human urine samples using HPLC-TOF-MS of cranberry syrup with antibacterial activity

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Cranberry (*Vaccinium macrocarpon*) is a rich source of various polyphenols which has antimicrobial property and has been used to prevent urinary tract infection (UTI). In this sense, the aim of the present study was to evaluate whether we could identify polyphenols and their metabolites in human urine samples after comsumption of cranberry syrup used in ISRCTN16968287 clinical trial. Furthermore, a study of its antibacterial activity has been also carried out.

10 male & female volunteers (25-45 years old) were participated in the study. The subjects did low-diet of polyphenols for 2 days before the experiments. On the third day, urine samples were collected before ingestion of the cranberry syrup with dose 0.6 mL/kg and after 2 hours of ingestion. The urine samples were stored at -20^{0}C before analysis. HPLC analyses were made with an Agilent 1200 series rapid-resolution LC system (Agilent Technologies, USA) equipped with a binary pump, an autosampler and a diode array detector (DAD). Separation was carried out with a Zorbax Eclipse Plus C_{18} analytical column (150 mm x 4.6 mm, 1.8 µm particle size). TOF-MS was conducted using a microTOF^{TM} (Bruker Daltonics, Germany) equipped with an electrospray ionization (ESI) interface. Both of them were used in negative and positive ion modes.

By using the proposed extraction method, several phenolic compounds and their metabolites were detected in human urine samples by interpreting the data obtained via TOF-MS. Thus, 10 phenolic compounds in urine samples in negative ionization mode and 8 phenolic compounds in positive ionization mode were identified. In this sense, it is important to highlight that all the detected compounds were found in the urine samples and not found in the controls.

As conclusion, the combined use of HPLC separation assisted by DAD and TOF-MS has proved to be a useful tool in the identification of polyphenolic compounds and their metabolites in human urine samples. Furthermore, antibacterial activity has also been studied and it has been proved that very low concentrations of cranberry extract are able to modify the non-specific adherence properties of *E. coli* producing a reduction in the surface hydrophobicity. This study is a preliminary research for better known of the method we used to identify phenolic metabolites.

Keywords: cranberry; human study; urine; metabolites; HPLC-MS; *Escherichia coli*; adherence

Molecular analysis of community acquired methicillin resistant *Staphylococcus aureus* isolated from skin and soft tissue infections, Botucatu Medical School, Brazil

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Staphylococcus aureus methicillin resistant (MRSA) are usually isolated in nosocomial environments and are considered as agents of several infectious processes. CA-MRSA (community acquired methicillin *Staphylococcus aureus*) was first reported in 1981, infecting people addicted to intravenous drugs and did not have any risk factors. These strains harbor the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) type IV which contains the *mec*A gene codifying for methicillin resistance. CA-MRSA strains usually carry PVL (Panton-Valentine Leucocidine) a virulence factor responsible for tissue invasion, causing systemic infections and leading to serious complications. The aims of this work were to detect the *mec*A gene, SCC*mec* characterization and to detect the PVL gene of the *S. aureus* strains isolated from patients diagnosed with skin and soft tissue infections attended in Dermatological-admission Room (BMS-DAR). Among 127 collected samples 66 (51.9%) were *S. aureus* and, from that, 7 (10.6%) harbored *mec*A gene. SCC*mec* characterization showed 3 (42%) harboring cassette type IV, characteristic from CA-MRSA, 3 (42%) carrying type II and 1 (16%) type Ia. None of these samples carried the PVL gene, but it was detected in 10 (15,1%) samples of *S. aureus* methicillin sensible (MSSA). Our study suggests the *S. aureus* strains that harbor PVL gene and MRSA are present in the community as important pathogens. The presence of PVL gene among MSSA strains suggest that not only resistant strains can cause serious damages to the patient and also these strains can become source of virulence genes to the resistant and non-virulent strains. Some groups are at risk of acquiring infections caused by CA-MRSA and these results must be taken into consideration in order to help its spread, which can constitute sources of infections with some treatment difficulties and evolve to dissemination of resistant strains among commonwealth.

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Molecular characterization of *Staphylococcus aureus* from backyard dairy farms: zoonotic potential of local strains and insights into biological control

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Staphylococcus aureus is a pathogen that causes diverse pathologies in different hosts. In humans, nosocomial infections like osteomyelitis, endocarditis and septicemia have been related to Hospital-Acquired (HA) strains, while dermatitis, toxic shock syndrome are caused by Community-Acquired (CA) strains. In animals *S. aureus* causes mainly bovine mastitis and dermatitis in companion animals. The use of molecular techniques to characterize *S. aureus* has allowed tracing of this pathogen in the community to establish regional surveillance programs. MLST (Multilocus Sequence Typing) and *spa*- typing have classified *S. aureus* according to the host and the associated pathology. In this work we characterized *S. aureus* isolates related to bovine mastitis from backyard dairy farms in the municipality of Tarímbaro, in the State of Michoacán; México.

Mastitis was detected with the California Mastitis Test in 13 backyard dairy farms to determine its prevalence. Questionnaires were applied to identify risk hazards for the transmission of *S. aureus*. Samples from milk and pharyngeal swab from humans related to dairy milk production were used to isolate *S. aureus*. 16S rRNA gene sequence analysis was used to identify *S. aureus.* The isolates presented resistance to penicillin or ampicillin (50%) or lyncosamide (45%). Resistance to three or more antibiotic occurred in 73% of the isolates. Molecular analysis with MLST revealed 11 new STs, 3 of them related to the identification of new alleles and the rest containing new combinations of pre-existing alleles. Allelic profiles obtained from isolates from milk samples were grouped in Clonal Complexes CC97 (ST97, ST126, ST352, ST1476, and new STs), typically associated to bovines and CC8 (ST8) typically associated with CA, human associated strains. One isolate from human origin showed ST188 which groups in the CC97. Isolates related with CC97 present *spa*-types t224, t267, t605, t693, t1965 and t4570 commonly associated with this CC. *spa*-types t008, t189 were found in CC8-related isolates in compliance with previous reports. Several isolates were not typable with *spa*-typing because of the absence of canonical 5’ and 3’ signature sequences or the presence of new sequence repeats. Minimum spanning tree analysis revealed that one of the new STs from CC97 behaved as a founder clone for the rest of the strains. The fact that isolates with human-related genotypes found in bovine milk samples and with bovine genotypes found in human samples, suggest a high risk of zoonotic behaviour of backyard farm multiresistant *S. aureus* isolates. *spa*-typing suggests that specific allelic variations of this virulence gene are associated with bovine mastitis.

As an alternative for biological control of *S. aureus* infections, lytic bacteriophages have been isolated from waste water treatment facilities. Phages will be tested against finely genotyped isolates to select those with wide host-range.

Keywords *Staphylococcus aureus*; MLST, *spa*-typing, bovine mastitis, phage therapy

Monitoring the effect of plasmid DNA fermentation strategies on host cell physiology and plasmid stability

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As gene therapy clinical trials are entering phase IV, the potential use of plasmid DNA as biopharmaceutical is increasing. Naked/plasmid DNA is the third most used vectors worldwide, with 316 clinical trials currently using these vectors. In order to provide sufficient pharmaceutical-grade plasmid DNA material it is essential to gain a comprehensive knowledge of the bioprocesses involved; so, the development of protocols and techniques that allow a fast monitoring of process performance is a valuable tool for bioprocess design. Regarding plasmid DNA production, the metabolic stress of the host strain as well as plasmid stability have been identified as two of the key parameters that greatly influence plasmid DNA yields. These two factors are closely related, since plasmid copy number increase will lead to a higher metabolic stress whereas a higher metabolic stress (Silva *et al*., 2009) could lead to the decrease of segregational stability, hence decreasing plasmid copy number. Therefore, to improve plasmid DNA titres it is necessary to monitor these two factors throughout fermentation. Flow cytometry and real-time quantitative PCR can be used as at-line monitoring techniques that allow a fast assessment of cell physiology and plasmid copy number (Silva *et al*., 2011), enabling to change the process design to achieve a better performance. The present work describes the impact of batch and fed-batch fermentations using different C/N ratios and different feeding profiles on cell physiology and plasmid stability, investigating the potential of these two monitoring techniques as valuable tools for bioprocess development and design. In this work, all fermentation strategies caused cell filamentation and decreased viability at the end of fermentation. The results obtained in batch fermentations suggest that lower C/N ratios result in higher plasmid yields and that higher C/N eventually leads to lower plasmid copy number and overall plasmid yields. Regarding fed-batch fermentations, the strategies with exponential feeding profiles, on opposite to constant feeding, showed higher biomass (37.2 g dcw/L) and plasmid yields with higher plasmid stability. The data obtained clearly showed that a fed-batch fermentation with an exponential feeding profile resulted in the highest volumetric yields (382 mg/L) with reasonable specific yields (10 mg pDNA/g dcw) and plasmid copy number (800 copies per cell) at the end of the fermentation. In conclusion, this study allowed clarifying the bioprocess performance based on cell physiology and plasmid stability assessment, allowing the improvement of plasmid DNA yield and cell growth.

Keywords: plasmid DNA, batch, fed-batch, cell physiology, segregational stability

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Moroccan plants essential oils as potential chemosensitizers restoring the antibiotic activity in resistant Gram-negative bacteria

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Bacterial drug resistance is a worrying problem of public health. Antibiotic efflux is the major non-specific resistance mechanism used by bacteria and efflux pumps are involved in the low level susceptibility of various important Gram-negative pathogens. The use of molecules that can block bacterial pumps is an attractive strategy, but several researches report only a partial efficacy due to limits of these molecules (stability, selectivity, bioavailability, toxicity...).

Our objective is to search natural sources of molecules able to inhibit efflux pump systems of resistant Gram-negative bacteria (*Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Salmonella enterica* Typhimurium and *Pseudomonas aeruginosa*). The results indicate that the studied essential oils exhibit an interesting activity against the tested bacteria. This activity is significantly enhanced in the presence of efflux pump inhibitor such as phenylalanine arginyl β-naphthylamide. The role of lipopolysaccharide (LPS) structure in the effect of essential oils was also reported in *Salmonella* LPS deep rough mutants. In addition, essential oils of *Thymus maroccanus* and *Thymus broussonetii*, used at a low concentration (a fraction of the Mimimum Inhibitory Concentration), are able to significantly increase chloramphenicol susceptibility of several resistant isolates. These results demonstrate that these essential oils can alter the efflux pump activity, and may be attractive candidates to develop new drugs for chemosensitizing multidrug resistant strains to clinically used antibiotics.

Keywords Antibiotics; Antibiotic resistance; Chemosensitizers; Efflux pumps inhibitors; Efflux systems; Essential oils; Gram-negative bacteria; Multidrug resistance

Oligodynamic action of Cadmium, Zinc and Silver on Enteric pathogens

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High concentration of metals and other trace elements could restricted bacterial growth and modify their metabolic pattern as well. Bacteria use various types of resistance mechanisms in response to heavy metal toxicity. This study aimed to find out the influence of Zinc, Cadmium, Silver on growth of potential gram negative pathogens, *E. coli*, *Klebsiella sp* and *Pseudomonas aeuroginosa* against a series of metal ion solutions (1.0M to 0.1mM). The antibacterial activity of metals was evaluated using agar well assay and a crystal violet biofilm assay in 96 well ELISA titre plate. In both 96 well microtiter plate assay and agar well assay, all the pathogens were observed to tolerate 0.0001-0.001 molar concentration of heavy metals. However, *Ps aeuroginosa* was found to be very resistance giving a zone of 12 mm at 1M silver nitrate. The pathogens lost their ability to form biofilm at higher concentrations of metal ions. Further work is warranted to determine the minimum inhibitory concentration of heavy metal against the pathogen and to study the resistance mechanism in *Ps aeuroginosa.*

Keywords Toxicity, Heavy Metals, pathogens, biofilm

Phenotypic and genotypic approach to ciprofloxacin resistance in *Campylobacter* from human and broiler in Portugal: the contribution of an efflux pump inhibitor

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Infections with thermophilic *Campylobacter* species, namely *C. jejuni* and *C. coli,* have become one of the most common bacterial cause of human diarrhea worldwide. Being self-limiting, campylobacteriosis typically does not require treatment although for some patients antibiotic therapy may become necessary and ciprofloxacin is one of the drugs of choice. This fluoroquinolone is also often used for empiric treatment given its coverage of other bacterial pathogens. Ciprofloxacin acts by inhibiting the activity of DNAgyrase, essential for DNA replication and transcription. In *Campylobacter*, a modification at amino acid 86 of GyrA (Thr to Ile) has been described as the main mechanism of resistance to fluoroquinolones that may act synergistically with an active efflux pump. Our main purpose was to evaluate the susceptibility of *C. coli* and *C. jejuni* strains isolated from human and broiler samples (intensive, extensive indoor and organic) from different production systems to different antibiotics, gentamycin, tetracycline, erythromycin and specially ciprofloxacin and also biocides (sodium hipochloride (SH) and benzalkonium chloride (BC). The effect of efflux pump inhibitor (phe-Arg-β-naphtylamide) in *Campylobacter* resistance to ciprofloxacin was also determined. Sampling was performed for caecum, carcass (neck skin) and breast meat at a poultry slaughterhouse, considering flocks' traceability for organic, extensive indoor and intensive production system. From the total of the isolated Campylobacter strains (n=144), 31 (21,5 %) *C. coli* and 25 (17%) *C.jejuni* were selected as Multidrug resistant strains (data not shown). Human multidrug resistant strains (n=14) were selected from an initial group of 72 strains that were kindly provided by INSA and were obtained from patients with campylobacteriosis during 2008 and 2009. Detection of *Campylobacter* was performed according to EN/ISO 10272-1:2006 and isolates were identified by multiplex PCR. The minimum inhibitory concentrations of antimicrobial agents were performed according to the *CA-SFM* (2010). We found that all Campylobacter strains were susceptible to gentamycin, 81.5% were susceptible to tetracycline while 36% of all strains were resistant to erythromycin. Finally, 78.6% strains showed resistance to ciprofloxacin, such a result was corroborated by genetic study using MAMA-PCR (according to Zirnstein *et al*., 1999). 13 and 15% of all strains showed resistance patterns till 128 mg/L for SH and 4 mg/L for BC, respectively. Human and intensive production strains showed the highest levels of resistance, generally steady at 16 mg/L, to ciprofloxacin although evidence of two strains from organic and extensive indoor origin revealed the highest levels of resistance, still growing in the presence of 512 mg/L ciprofloxacin concentration. The occurrence of the efflux pump inhibitor better potentiated ciprofloxacin activity in low level resistant strains than in high level resistant strains.

Keywords: *Campylobacter* spp., Multidrug resistance, Fluoroquinolones, Efflux pump inhibitor

Prevalence of *Legionella pneumophila* antibodies in immunocompromised patients and analysis of risk factors for development of legionellosis

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Immunocompromised patients, such as dialysis patients and patients after renal transplantation, can be predisposed to Legionella infections. The risk of catching Legionnaires' disease in this group of patients is largely determined by the immunosuppressive effects of uremia, the immunosuppressive drugs used and the nature and extent of environmental exposure.

The aim of this work was to investigate the prevalence of *Legionella pneumophila* serogroups 1-7 (SG 1-7) antibodies in dialysis patients and in patients after renal transplantation, as well as to analysis of the risk factors for disease development in patients with positive results.

Serum samples were collected from 212 dialysis patients and 68 patients after renal transplantation. Serum samples were also collected from 100 healthy persons as the control group.

Two commercial ELISA kits were used for detection of serum IgG (SG 1-7, SG 1) and IgM (SG 1-7) antibodies in patients and control group.

In the studied group of patients, positive results were obtained in 20 patients (7.14%). Among the dialysis patients, IgG antibodies against *L. pneumophila* SG 1-7 were detected in 13(6,13%) subjects and IgM antibodies SG 1-7 in 5 (2,35%) subject. One patient was found to have two classes of antibodies (IgM and IgG). It must be noted that in one patients the IgG antibody against *L. pneumophila* SG 1 was detected. Positive results of antibodies IgG against *L.pneumophila* SG 1-7 were obtained in 3 subjects (4.41%) out of 68 renal transplantation patients. In the control group positive results of IgG antibodies SG 1-7 were found in 3 (3,0%) patients and IgM antibodies SG 1-7 in 6 (6%) patients. The antibodies against *L. pneumophila* SG 1 were not detected in the control group.

The medical records showed that none of the patients with positive results had symptoms of *L. pneumophila* infections in the last 2 months prior to the study. The difference between the patients and control group was not statistically significant (P=0.54).

Patients with *L. pneumophila* antibodies in both groups did not differ significantly in any of the usually evaluated risk factors of clinical infection. The reported outbreaks of Legionnaires' disease in chronic dialysis patients and in patients after renal transplantation and our results of IgG and IgM antibodies calls for further investigation of infection sources and examining the efficiency of cellular immune system response to *Legionella* in these immunocompromised patients IgG and IgM positive .

Procalcitonin and sepsis in general surgery

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In recent years, it has been studied the usefulness of procalcitonin (PCT) as a marker of severity of sepsis, connecting their values with the differentiation of septic stages, as well as a predictor of postoperative complications. The principal objectives of our study is to demonstrate that the procalcitonin is a useful marker and early diagnosis and differentiation of sepsis in patients with surgical pathology and to assess the levels of procalcitonin in the diagnosis of postoperative infectious complications.

We performed a prospective study of diagnostic test evaluation of a total of 75 patients from March to July 2009 by establishing 2 groups: first, patients with infectious disease (acute cholecystitis, acute diverticulitis, mesenteric ischemia and gastrointestinal perforations) and the second group, patients undergoing non-infectious disease that presents during postoperative infectious complication rate of urinary tract infection (UTI), catheter-associated bacteremia, pneumonia, intraabdominal abscess, surgical wound infection and anastomotic dehiscence.

The results show that not only elevated procalcitonin values to a septic process, but their values are higher in relation to increased severity of the process.

Keywords: Procalcitonin, sepsis , bacterial infection

Production the probiotic chowing gum containing LAB, An effective way to enhance biofilm production capacity of Streptococcus. Mutans

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Probiotics were defined by FAO/WHO (The Food Agricultural Organization/World Health Organization) as live microorganisms which when administered in adequate amounts (in food or as a dietary supplement) confer a health benefit on the host (improving microbiological balance in intestinal tract). These bacteria have many profitable effects such as oral cavity prevention potential. Their profits is the first cause of producing more probiotic foods. One of the best ways for this target is adding these bacteria to the foods(non dairy and non fermented, like chowing gum or biscuits). Successful addition is reached by protecting these bacteria through storage and….Between common ways, microencapsulation is more effective and profitable. Chewing gum is a good product to be the oral cavity preventor bacteria carrier. To reach the best results in this aim, we tested different material as the coating layer for these capsules, to have abilities such as long shelf life, ability to be released as fast as entered saliva in oral, protectable in different conditions through consumption, and many other profitable abilities together. At last, a kind of protein base substance accepted because of containing many desirable effects.

Key words: Probiotics, Oral cavity, Microencapsulation, Chewing gum

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Pseudomonas aeruginosa isolated from cutaneous infections in a tribal area in South India

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Pseudomonas aeruginosa is one of the most important clinically significant and opportunistic pathogens, often causing nosocomial infections. The clinical manifestation ranges from life threatening septicemia and pneumonia to generally milder skin infections. *Pseudomonas* has been implicated to cause folliculitis and other papular or vesicular lesions in the skin of otherwise healthy individuals besides pyoderma gangrenosum in neutropenic patients. In this study, *Pseudomonas* species were isolated from skin infections of patients from a tribal area in South India. The isolates were confirmed as *P. aeruginosa* by BD Crystal automatic identification system for enteric/non-fermenter (Becton Dickinson and Co, Maryland, USA) and using a PCR targeting two gene loci from 16S rRNA.

P. aeruginosa isolates exhibited resistance towards several antibiotics including broad spectrum antibiotics, cephalosporin, macrolides, sulfanilamide and other sulfonamides. However, the isolates were susceptible towards many commonly used antibiotics belonging to quinolones, β-lactams and aminoglycosides. Globally, most of *P. aeruginosa* isolates from hospitals have shown resistance towards quinolones, β-lactams and aminoglycosides. Tribal people are generally custodians of medicinal plants and avoid antibiotics and other modern medicines. Hence, all the *P. aeruginosa* isolates were found susceptible to commonly used quinolones, β-lactams and aminoglycosides in hospitals worldwide. Hence, judicious use of antibiotics is imperative for controlling spread of antibiotics resistance among bacteria.

Keywords *Pseudomonas*; cutaneous infection

Q fever among wool sorters in Belgium

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Coxiella burnetii, an obligate intracellular gram-negative bacteriumbelonging to the *Legionellales* order of gamma-proteobacteria, is the causative agent of Q fever. The disease is a worldwide zoonosis affecting mammals, birds and anthropods. The bacterium is extremely resistant to harsh environmental conditions due to spore formation, it readily becomes airborne, and it is highly infectious for humans. For these properties, it is on the list of BW agents in "Category B". Human infections arise as a result of contact with infected farm animals such as cattle, sheep and goats, although pet animals such as cats have also been implicated particularly in urban outbreaks.In most cases, the infection follows inhalation of aerosols derived from the excretions and secretions of infected animals.Ingestion of *C. burnetii*,especially by consumption of contaminated dairy products, is considered a rare alternative for acquiring infection. The acute form of Q fever is characterized as a flu-like illness or atypical pneumonia, or less frequently as granulomatous hepatitis with a significant incidence of neurological complications.Persistent infections may lead to chronic form of the disease. Q fever endocarditis is the most frequent clinical manifestation of this form of the disease.Currently, serological assayscombined with PCR are the most commonly employed methods for diagnosing *C.* burnetii/Q fever.We performed a serological research on the occurrence of Q fever in workers from Belgian scouring factory processing wool and goat hair products. Our results highlighted a high Q fever seroprevalence among the workers under study. Continuous exposure to the Q fever agent was the probable cause of some atypical antibody responses evoking sometimes a "chronic" or "relapsing" disease and stressed out the need to analyze paired serum samples and to rely on detailed clinical evaluations prior establishing a final diagnosis. Considering the continuous occupational risk to which these workers are exposed, hiring of pregnant women or persons with underlying medical conditions such as vavulopathy or immunological depression should be avoided. Moreover, annual serological testing should be conducted on all exposed individuals to detect any evolution towards the chronic form of the disease, which can be life-threatening. Though less dangerous than anthrax, Q fever is a highly prevalent occupational disease affecting those working with animal products in the respective industrial branches and environments.

Keywords *Coxiellaburnetii*; Q fever

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Quorum Quenching Quandary: Resistance to Antivirulence Compounds

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Background: Quorum sensing (QS) is the regulation of gene expression in response to the concentration of signal molecules, and its inactivation (quroum quenching) has been suggested to have great potential to attenuate microbial virulence since QS activates the expression of multiple virulence determinants and it is assumed that there should be less Darwinian selection pressure for resistance against QS inhibitors compared to antimicrobials. However, the assumption that bacteria will not evolve resistance to quorum quenching compounds is suspect because the tests utilized to show the QS inhibitors do not affect growth are usually conducted in rich medium that does not mirror the disease state. **Methods**: Using the best-characterized bacterium in regard to QS, *Pseudomonas aeruginosa,* and inhibitors of both the *N*-acyl homoserine lactone QS systems (:4-bromo-5-(bromomethylene)-2(*5H*)-furanone, C-30) and the quinoline dependant QS system, halogenated analogues of the anthranilic acid (AA), it was shown here that resistance to QS inhibitors can arise easily. To generate conditions in which QS inhibition could affect growth, we utilized growth on adenosine as the sole carbon source to select strains resistant to C-30, and growth in minimal medium with low iron to select strains resistant to the AA analogues. Growth on adenosine was used since utilization of this substrate requires QS and is related to disease in the gastrointestinal tract, and growth in low iron was used since the expression of siderophores like pyoverdine also requires QS. Multiple QS related phenotypes were studied, and a *Caenorhabditis elegans* virulence model was used to test the virulence of the C-30 resistant mutants. **Results**: In the presence of C-30, growth on adenosine in minimal medium was inhibited with the wild-type strain, and within 15 generations, both transposon mutants and spontaneous mutants were identified that overcame the inhibition by C-30. Mutations that made the cells less sensitive to the QS inhibition by C-30 were identified in *mexR* and *nalC,* which encode repressors of the *mexAB-oprM* multi-drug resistance operon; inactivation of MexR led to enhanced efflux of C-30. The *mexR* mutant was pathogenic to *C. elegans* in the presence of C-30 whereas virulence was decreased markedly for the wild-type strain with C-30. Critically, *P. aeruginosa* strains that cause chronic cystic fibrosis infections have the same efflux pump mutations and also show resistance to C-30. In the presence of AA analogues, growth was inhibited in minimal medium with low iron, and as in the case of C-30, both transposon and spontaneous mutants able to overcame the inhibition by AA analogues were obtained after a few rounds of selection. Currently the mutations responsible of the resistance are being identified, but in contrast with those mutations that lead to resistance to C-30, the mutations that yield resitance to AA are not related to the *mexAB-oprM* multi-drug resistance operon, since the *mexR* mutant is not resistant to the AA analogues. **Conclusion**: Our results demonstrate that single mutations in key loci give rise to resistance against anti-virulence compounds. Therefore, bacteria may readily evolve resistance to many new pharmaceuticals that are under development under the rationale they are impervious to resistance.

Quorum sensing inhibitors from epiphytic bacteria isolated from wild berries

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Antibiotic resistance is a reemerging problem facing health care settings globally. Among the possible routes to offset this challenge is to try and find out means of combating resistance and pathogenecity by reducing the virulence rather than growth inhibition. One of the most promising proposals is inhibiting or modifying quorum sensing systems in bacteria. This study focuses on screening epiphytic bacteria from wild berries for quorum sensing inhibitors(QSI). For this purpose 600 bacterial isolates were isolated and screened for quorum sensing substances using the Chromobacterium violaceum monitor strain system. Eleven isolates showed clear QSI activity against C.violaceum both on agar plates and when culture supernatant was tested using agar diffusion method. Most of the positive isolates were gram-positive aerobic rods.Opimization of production of the QSI materials by some isolates is underway together with partial identification of these materials.

Keywords: epiphytic bacteria, quorum sensing, Chromobacterium violaceum

Red foxes (*Vulpes vulpes*) as reservoirs of extended-spectrum beta-lactamases-producing *Escherichia coli* isolates

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Background:
The production of Extended-spectrum beta-lactamases (ESBLs) by *Escherichia coli* has been increasingly reported in the last few years**.** The purpose of our work was to analyze the faecal carriage of ESBL-producing *E. coli* isolates in red foxes (*Vulpes vulpes*) in Portugal, to identify the type of ESBLs, to detect the presence of other antimicrobial resistance markers, as well as study the phylogenetic groups and virulence factors in these bacteria.

Observations: 52 fecal samples were seeded in Levine supplemented with cefotaxime (CTX). Susceptibility to 16 antibiotics was performed by disk diffusion method. ESBL-phenotypic detection was carried out by the double-disk test and genes encoding TEM, OXA, SHV and CTX-M type ß-lactamases were studied by PCR and sequencing. Antibiotic resistance mechanisms, phylogenetic groups and virulence factors were also performed by PCR. CTX-resistant *E. coli* isolates have been isolated from 3 of 52 faecal samples (5.8%), obtaining a total of 9 *E. coli* isolates (3 per sample). ESBLs detected were the following (n of isolates): CTX-M-1 (1), CTXM-15 (2) and TEM-1b (6). *tet*(A) and/or *tet*(B) genes were detected in 8 tetracycline-resistant isolates, *aad*A gene in all streptomycin-resistant isolates, *cml*A gene in 3 of 4 chloramphenicol-resistant isolates, and *aac*(3)-II gene in the 2 gentamicin-resistant isolates. Different combinations of *sul*1, *sul*2 and *sul*3 genes were demonstrated in 6 of 9 trimethoprim–sulfamethoxazole-resistant isolates. The *fim*A and *aer* genes were detected in 77.8% and 33.3% of the isolates, respectively. All our isolates belonged to phylogenetic group A and B1 (3 and 6 isolates, respectively).

Conclusions:
Red foxes (*Vulpes vulpes*) constitue a reservoir of ESBLs-producing *E. coli* isolates and antibiotic resistance genes that could be transmitted to other pathogenic ones representing a public health problem

Keywords: ESBLs; *Escherichia coli*; virulence factors

Restriction analysis of the Orotidine Monophosphate Pyrophosphorylase (*URA5*) gene of Portuguese *Cryptococcus neoformans* isolates

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Cryptococcus neoformans is an encapsulated basidiomycetous yeast with a worldwide distribution, and frequently implicated in meningoencephalitis in HIV-positive individuals and other immunocompromised patients. Two varieties, *C. neoformans* var. *grubii* (serotype A) and *C. neoformans* var. *neoformans* (serotype D) are known, as well as hybrid strains (serotype AD). Also, the closely-related *Cryptococcus gattii* species corresponds to serotypes B and C. The restriction analysis of the PCR-amplified orotidine monophosphate pyrophosphorylase (*URA5*) gene has been used worldwide for assessing the epidemiology of these clinically relevant species(1,2). Eight major molecular types are described: VNI and VNII (both corresponding to *C. neoformans* var. *grubi* strains), VNIII (serotype AD hybrid strains), VNIV (*C. neoformans* var. *neoformans*), and VGI, VGII, VGIII and VGIV (corresponding to *C. gattii*). The aim of this work was to assess the genetic heterogeneity of clinical and environmental Portuguese isolates of *C. neoformans* and to undertake a comparative analysis of the results with those of other similar studies previously published, concerning the epidemiology of this species in Spain, South-America and other regions. For this study, 105 *C. neoformans* isolates were selected and obtained from several Portuguese hospitals and regions, having been specifically identified using conventional mycological diagnosis. The molecular type of each isolate was determined by restriction analysis of the *URA5* gene(2). Five molecular types were found among the Portuguese *C. neoformans* isolates: VNI and VNII (serotype A), VNIII (serotype AD), VNIV (serotype D) and VGIII (*C. gattii*). The VNI type was the most abundant (44.8%), followed by the VNIII (32.4%, the hybrid groups between *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans*) and the VNIV types (10.5%, including all serotype D strains). The less abundant type was the VNII (8.5%). It is noteworthy that one isolate of *C. gattii* (type VGIII) was found among the Portuguese clinical strains isolated from AIDS patients. A higher ratio of the VNI type was found in Portugal compared to previous results from similar studies in other regions of the world. Of note, we here report the identification of a significantly higher percentage of type VNIII (hybrid) isolates in Portugal compared to any other study ever documented for other regions of the world.

(1) Matsumoto *et al*. Genotyping, serotyping and determination of mating-type of *Cryptococcus neoformans* clinical isolates from São Paulo state, Brazil. Rev. Inst. Med. Trop. S. Paulo, 49: 41-47, 2007.

(2) Meyer *et al*. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerg. Infect. Dis. 9: 189-195, 2003.

Keywords *Cryptococcus neoformans*; *URA5* gene; molecular types

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Role of enzymes involved in a cholesterol metabolism in the pathogenicity of *Mycobacterium tuberculosis*

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Tuberculosis is a chronic bacterial infection that causes human million deaths each year. Sequensing of *Mycobacterium tuberculosis* (Mtb) genome revealed at least 250 genes involved in the lipid metabolism. The ChoD and KsdD genes encode cholesterol oxidase and ketosteroid dehydrogenase, respectively. Both enzymes are involved in the degradation of cholesterol. It was demonstrated that Mtb can use cholesterol as a sole carbon and energy source. However, the role of cholesterol in the pathogenesis of tuberculosis remains unclear. We generated Mtb H37Rv strains with inactivated ChoD or KsdD genes by using the technique of gene replacement based on the process of homologous recombination. We obtained the Mtb mutants: Δ*ChoD* and Δ*KsdD* lacking functional copy of gene encoding cholesterol oxidase or ketosteroid dehydrogenase.

The aim of these studies was to analyze the phagocytic and bactericidal activity of human macrophages infected with MtbH37Rv in comparison with MtbΔ*ChoD* and MtbΔ*KsdD* mutants. The monocyte-macrphages cell line (THP1) activated or not with interferon γ (IFN-γ) were infected with non-opsonized or opsonized with human serum Mtbs. The phagocytosis and intracellular killing of bacteria as well as production of nitric oxide (NO), reactive oxygen species (ROS) and tumor necrosis factor-α (TNF-α) by macrophages were tested. Phagocytic activity of activated and non-activated macrophages in the presence of all tested strains was similar. We observed similar intracellular replication of Mtb strains: H37Rv, Δ*ChoD* and Δ*KsdD* in activated macrophages at day 6 post-infection. In contrast, growth of non-opsonized, MtbΔ*ChoD* and MtbΔ*KsdD* strains was impaired in non-activated macrophages when compared to MtbsH37Rv. Control strains carrying the Δ*ChoD* or Δ*KsdD* gene complemented with intact *ChoD* or *KsdD* replicated similar to the wild type strain. Activated macrophages infected with tested Mtbs produced similar amount of NO. MtbΔ*ChoD* and MtbΔ*KsdD* but not wild type and complemented strains stimulated non-activated macrophages to produce NO. We noticed that all tested Mtbs inhibited the ROS production by activated and non-activated macrophages. However, the non-opsonized MtbΔ*ChoD* strain (but not control strain) was significantly less active in the inhibition of ROS production by non-activated macrophages than Mtb wild type. We did not notice any differences in the production of TNF-α by macrophages infected with Mtb strains.

We conclude that enzymes involved in the degradation of cholesterol can be important in the infection of human macrophages before being activated by IFN-γ. Additionally, non-opsonized *M. tuberculosis* stimulate bactericidal activity of macrophages more effectively than opsonized bacteria.

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Screening and isolation of macrolides producing actinomycetes from soil of Iran

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Macrolides are one of the most commonly used families of antibiotics and belong to polyketide type I, a large class of bioactive natural products. They inhibit RNA-dependent protein synthesis by reversibly binding to the 50S ribosomal subunits of susceptible microorganisms. The aim of this project is using molecular screening to find macrolides producing actinomycetes isolated from soils of Iran. The amounts of 50 actinomycetes were isolated from different soils of Iran. Their DNAs were extracted by modified phenol-chloroform method. By using PCR reaction and specific primers for *pks I* gene, thirty two isolates having *pks I* genes were identified. Primary identification of these isolates was done by 16S rRNA gene sequence analysis. Base blast analysis has demonstrated that similarity of *pks* gene in 10 actinomycetes with that of corresponding genes submitted in NCBI was less than 90%. Antimicrobial activities of these strains were assayed against *Pseudomonas aeruginosa, Bacillus subtilis*, methicilin resistant *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. In this group, all 10 selected isolates exhibited broad-spectrum antimicrobial activities. For confirmation of macrolides production by the selected isolates, thin chromatography layer (TLC), high performance liquid chromatography (HPLC), IR spectroscopy and NMR were applied. Five macrolide producer isolates were selected. They have maximum similarity to *Nocardia soli*, 100%; *Saccharothrix australiensis*, 97.9%; *Micromonospora tulbaghiae*, 99.7%; *Nocardia harenae,* 100% and *Actinophytocola oryzae*, 97.5%. The results obtained have shown high potential of the soils of Iran and the efficiency of molecular screening to find new strains capable to produce valuable bioactive compound.

Key word: Actinomycetes, Macrolides, Molecular screening, Polyketide syntheses.

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Screening of endophytic actinobacteria isolated from Moroccan aromatic and medicinal plants against human pathogenic microorganisms

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Since the discovery of actinomycin, actinobacteria have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances. Approximately two thirds of the thousands of naturally occurring antibiotics have been isolated from these microorganisms. In many academic as well as industrial laboratories, the search for novel products has switched in emphasis to rare genera or to well characterized species that are found in unusual environments. In fact, the list of novel actinomycetes and products found in microbiologically unexplored ecosystems around the world suggests that a careful exploration of new habitats such as endophyllosphere and endorhizosphere of aromatic and medicinal plants. In this context the aim of the present study is the screening of endophytic actinobacteria isolated from Moroccan endemic aromatic and medicinal plants for their abilities to produce bioactives compounds.

Endophytic actinobacteria were isolated from medicinal plants in high Atlas Mountains and Essaouira ecosystems. Isolation was conducted from root, stem and leafs using three isolation culture media and two methods. Obtained isolates were screened against many human pathogens for antibacterial activities and using brine Shrimp test for assessing cytotoxic activity.

Obtained results showed that 88% of isolated actinobacteria were active against at least one of tested microorganisms and two isolates have a broad spectrum with significant antimicrobial activity against all tested bacteria and a high percentage (> 80%) of mortality against brine Shrimp larvae. The amplification of 16S rDNA gene indicates that the actives isolates belong to the genus *Streptomyces*.

Keywords: endophytic actinobecteria, antimicrobial activity, cytotoxic activity, 16S rDNA.

Serotyping study the activity of antibiotics and support of some genetic resistance of 100 strains of salmonella isolated from Gallus gallus in four wilaya in central Algeria

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Serotyping study the activity of antibiotics and support of some genetic resistance of 100 strains of salmonella isolated from Gallus gallus in four wilaya in central Algeria

This work aims to:

- Serotype after logging (NF U 47-100 / 2005), 100 strains of salmonella,
- And determine:
    - The activity of antibiotics (technical CLSI)
    - And the support of the resistance plasmid (Kado and Liu technique, 1981)

These are salmonella isolated from poultry from the wilaya of Tizi-Ouzou, Bouira, Bejaia and Boumerdes, in the year 2007.

Bouira had 48% of salmonella. Thirteen serotypes were identified with 24% of S. Heidelberg.

53% of strains were resistant to 8 of 34 antibiotics tested, including 15.09% multiresistant. Quinolone resistance was dominant with 58.49%. The transfer plasmid performed on 53 strains showed that 11 were transferred resistance markers, including ampicillin, tetracycline, sulfonamides, and kanamycin. The character tetracycline was present in 72.72% of the transconjugants, those of lactams and sulfonamides in 27.27% each and that of aminoglycosides in 9.09%. The incompatibility groups of plasmids belonging to the families and F1me Com1. The molecular weight of the plasmid DNA was greater than 100 kb.

The results confirm the phenotypic and genotypic clonal spread of Salmonella in poultry transferable to humans in the study area.

Simultaneous and rapid detection of *Bacillus anthracis*, *Salmonella typhi* and *Yersinia pestis* by multiplex PCR

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Background: Rapid detection of biological agents are prime importance in countering the emerging infectious disease and bioterrorism events

Methods: We have tested *Bacillus anthracis* Vaccine strain and *Yesrinia pestis* from recombinant clone containing F1 gene for Vaccine purposes but for *salmonella typhi* we used DNA extracted directly from strain obtailed from reference laboratory that is isolated from clinical sample.

Results: We report here for the first time the development of a rapid PCR method for simultaneous detection of the *Bacillus anthracis*, *salmonella typhi* and *Yersinia pestis* with Multiplex mixture of 6 specific primer sets designed and tested specifically for these agents.

These methods may provide a rapid tool for the simultaneous detection and identification of the three category A bacterial species listed as biological threats and can be used in reference laboratories, clinical and diagnostic labs and specially for filed analysis of samples or contaminated letters in mobile labs .

Key words: Simultaneous detection. Rapid Detection, PCR. Bacillus anthracis, Yersinia pestis and Salmonella typhi

Specific detection of *Aspergillus* species in blood from patients with invasive aspergilosis

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In the last two decades, there has been an increasing incidence of invasive pulmonary aspergillosis, with extremely high morbidity and mortality rates. This situation results from the intensive use of chemotherapy and immunosuppressive agents, mainly in immunocompromised patients and, among these, in hemato-oncology patients and transplant recipients of bone marrow. These fungal infections are caused by some species of the genus *Aspergillus*, where *Aspergillus fumigatus* is the species isolated more often from human infections. However, other species like *A. flavus, A. niger, A. terreus, A. versicolor, A. nidulans* and *A. glaucus* are also responsible for potentially severe fungal infection in humans. A key factor limiting more efficient interventions against the increasingly incident invasive aspergillosis is the inability to establish an early definitive diagnosis, useful for prompt beginning of specific antifungal therapeutic, in order to ensure favorable prognosis of these patients.

The main goal of this study was to detect *Aspergillus* sp. DNA in whole blood samples of patients by nested-PCR[(1)], to compare the results with those obtained from galactomannan detection in patients sera and to evaluate the effectiveness of both methods for early diagnosis of invasive pulmonary aspergillosis.

In this work, 37 blood samples were studied, 45.9% belonging to females and 54.1% to males. Among them, 75.7% were diagnosed with clinical acute myeloid leukemia, 8.1% with chronic lymphocytic leukemia, 2.7% with tricoleukemia and 5.4% with myelodysplastic syndrome. Galactomannan antigen (GM-Ag) determination in the blood was performed with the Platelia®Aspergillus EIA kit, whilst, for molecular diagnosis, a nested-PCR reaction was carried out.

Our results showed no discordant results between the two techniques - all positive patients by nested-PCR were also positive by the GM-Ag test. From the 37 studied patient samples, 35.1% showed the presence of the GM-Ag in serum; in 32.4%, it was possible to detect fungal DNA using specific primers, through the nested-PCR reaction. Only in two cases, the GM-Ag test evidenced a little more sensitivity, while, in one case, nested-PCR was the more sensitive method.

Although, in general, the molecular method did not exhibited higher sensitivity than that of the GM-Ag test, we consider that the nested-PCR assay is a practical screening test for excluding diagnosis of invasive aspergillosis. This technique, due to its simplicity and rapidity, may become a standardized tool for the diagnosis of invasive pulmonary aspergillosis, especially if used in parallel with other methods (eg galactomannan determination and high-resolution CT or HRCT) to better assure definite and timely specific detection of *Aspergillus* infections.

(1) Skladny *et al*, Specific Detection of *Aspergillus* Species in Blood and Bronchoalveolar Lavage Samples of Immunocompromised Patients by Two-Step PCR. J. Clin. Microbiol. 37(12):3865–3871. 1999

Keywords: invasive aspergillosis; *Aspergillus*; molecular diagnosis

Staff can harbour methicillin-resistant staphylococci (MRS) on a farm when animals do not

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This work aimed to detect methicillin-resistant staphylococci (MRS) in animals and staff in a farm. Samples were obtained from dairy cattle (36), beef cattle (26), sheep (19), horses (21), pigs (23), goats (23) and humans (13), yielding a total of 161. A screening using selective media was performed and after that, the isolates were tested by PCR aiming the gene *mec*A in order to establish resistance to methicilin. Antimicrobial-resistance testing to penicillin, meropenem, ceftriaxone, cephalothin, oxacillin, levofloxacin, enrofloxacin, chloramphenicol, ciprofloxacin, gentamicin, clindamycin, erytromycin, linezolid, sulfamethoxazole/trimethoprim, tetracycline, doxycycline and vancomycin was performed on all *mec*A+ isolates. Four methicillin-resistant coagulase-negative staphylococci (MRCoNS) were isolated from human beings and none from animals, yielding a prevalence of 31% and 0% respectively. Methicillin-resistant *Staphylococcus aureus* (MRSA) was not found. The isolates of MRCoNS of this work presented different antimicrobial resistance patterns. The results show that MRCoNS may be present in humans associated with animals while not present in the animals. It is possible that selective pressure outside of the farm and/or lack of MRCoNS transmission between humans and animals may be responsible for this lack of correlation

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*The full paper related to this abstract can be found at DOI: 10.1111/j.1863-2378.2011.01413.x

Keywords farm animals, MRSA, MRS, mecA, resistance to methicillin, *Staphylococcus* spp.

Strain specificity in antibacterial activity copper and silver nanopaticles

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The antibacterial properties of copper and silver nanoparticles were investigated using *Escherichia coli* (3 strains), *Klebsiella pneumonia* (2 strains), *pseudomonas aeroginosa* (1 strain), *Entrobacter aerogenes* (1 strain), *Salmonella entritidis* (1 strain), *Staphylococcus aureus* (3 strains), *Basillus cereus* (1 strain) and *Actinomycets* (1 strain). The average of the silver and copper nanoparticles were 4nm.

The bactericidal effect of silver and copper nanoparticles were compared based on diameter of inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nanoparticles dispersed in both cultures. Bacterial sensitivity to nanoparticles was found to vary depending on the microbial species. Disk diffusion studies with all of the bacteria in this study revealed greater effectiveness of the silver nanoparticles. *E.coli* depicted the highest sensitivity to nanoparticles compared to the other strains and was more adversely affected by the copper nanoparticles. Good correlation was observed between MIC and MBC measured in liquid cultures.

For copper nanoparticles a good negative correlation was observed between the inhibition zone observed in disk diffusion test and MIC/ MBC determined based on liquid cultures with the various strains. Although strain-specific variation in MIC/MBC was negligible for *E.coli* some strain-specific variation was observed for *S.aureus*

Keywords:Antibacterial,Copper,Silver,Nanoparticles

Streptococcus intermedius can module the expression of some virulence factors in Porphyromonas gingivalis

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Introduction: Periodontal disease has been associated with a poor dental care, which promotes the accumulation of bacteria and the development of diseases of the mouth. Porphyromonas gingivalis is anaerobic Gram-negative bacteria found in the in subgingival plaque, and is largely responsible for chronic periodontal disease and Streptococcus intermedius is a Gram positive coconut, found in supragingival plaque. Our hypothesis is that when S. intermedius cell number is higher it can to trigger the expression of virulence genes in P. gingivalis.

Objective: Evaluate gene expression of P.gingivalis using a heterologus microarray of E. Coli.

Material and Methods Porpyhromonas gingivalis W50 ATCC 25611 and Streptococcus intermedius were placed in Tripticaseine sodium broth and grown in four flasks: ratio of 1:1 (1 ml of the two bacteria), and finally 1:9 (1ml S.intermedius P.gingivalis and 9) after 48 hours. (Logarithmic phase) is an RNA extraction and applies the genetic chip Ecoli_07_04 hybridizing with samples labeled with Alexa555 1:1 versus 1:9 sample labeled with Alexa647 genArise analysis was performed to find the folder Ecoli_07_04gA, the results of genes regulated UP or DOWN are obtained depending on the sample 9:1. Then there was the annotation of genes identified by the site genArise and David GO for P. gingivalis and E. Coli.

Results: The information obtained shows how different genes are expressed in native state, increase their activity with the concentration form. The Genes PG0520, PG0538 and PG1280, in 1:9 increases significantly more than in the other conditions. These data were confirmed using qPCR of each of those genes which showed greater change PG0538

Study of the antibacterial activity of acetone extracts of *Thapsia garganica* L. growing wild in Algeria

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Thapsia garganica is an endemic plant which grows spontaneously in Mediterranean basin. It is used by Algerian population for some cases of rhumatism.

In this study we tested the antibacterial activity of acetonic extracts of the leaves and roots of *Thapsia garganica* on four bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Listeria innocua, Staphylococcus aureus* for this four concentrations of the extracts were prepared 100mg/ml, 50mg/ml , 25mg/ml and 12.5mg/ml.

Results indicated that acetonic extracts of the leaves have the best antibacterial activity with inhibition zones varying from 6 to 32 mm. Extracts at 100mg/ml were better than the other dilutions. The Gram negative becteria were more sensitive than the Gram negative ones.

MIC results indicated that all the strains have a minimum inhibition concentration higher than 4mg/ml except for *Staphylococcus aureus* wich showed a MIC of 3.1mg/ml.

Key words : Thapsia garganica, acetone extracts, antibacterial, MIC.

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Study of the antimicrobial activity of four algerian marin algae species

SAIDANI karima and BEDJOU fatiha

Antibiotic resistance in bacteria is one of the emerging health related problem in the world nowadays. Medicinal plants are valuable natural sources effective against infectious agents.

In this study, methanolic extracts of four marine algae of Algeria coast were investigated for antibiotical and antifungal activities against respectively six pathogenixc bacteria (*Bacillus subtilis*, *Listeria inocua*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), and three specis of fungi (*Candida albicans*, *Mucor ramanianus*, *Aspergillus niger*)

All algae extracts showed antibacterial activity against four of the six pathogenic bacteria tested, with MIC value (minimum inhibitory concentration) ranged between 0.25-3mg/ml.

Extracts of *Rhodomela confervoïdes* exhibited highest activity against *Bacillus subtilis* (24mm), *Cystoseira tamariscifolia* exhibited the highest activity against *Listeria innocua* (19.67mm). All the extracts exhibited antifungal activity. The highest inhibiting effect was noted for *Rhodomella confervoïdes* and *Padina pavonica* againts respectively *Candida albicans* and *Mucor ramanianus* for the first one. *Aspergillus niger* showed resistance against the majority of methanolic extracts.

Key words: antimicrobial activity, marine algae, polyphenols, méthanolic extracts.

Synergism effect of citric acid, citrus oil and nisin on growth inhibition of *Escherichia coli*

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Recent outbreaks regarding to contamination of *Escherichia coli* has been associated of consumption of fresh vegetables, resulting in deaths in the European Community. The washing and sanitizing are the most important steps during the process of fresh produce to avoid contamination, and chlorine usually is the most common antimicrobial agent used. However, the use of chlorine as sanitizer is limited due to the amines formed as residual products, so alternatives to chlorine for sanitizing have been extensively investigated. Moreover, the increasing demand for natural alternatives by the consumers stimulates studies on investigating natural antimicrobial agents. In this context, the objective of this work was to evaluate the synergism effect of citric acid, lemon oil, lime oil and nisin as sanitizer to inhibit the growth of *Escherichia coli*, "in vitro", using statistical design. The statistical experiment design was a 2^3 factorial with three centre points, constituting 11 assays. The experiment was carried out, varying the concentrations of citric acid (% w/v, 0; 0.5; 1.0), lime oil (% v/v, 0; 1.0; 2.0) and nisin (IU/mL, 0; 50; 100), using nutrient broth as media. *Escherichia coli* was inoculated into 11 flasks (10^6 CFU/mL), which were incubated at 37 °C. The interaction between lime and lemon oil was also studied. The colonies were determined after a sequential of dilutions (1:10) of the samples flasks, from which 100 µL were spread onto the MacConkey agar plates and incubated at 37 °C for 24 h. The results showed that after 1h, only citric acid and lime oil had significant effects on inhibition growth of the *E. coli* as well their interaction ($p<0.05$). When the factors were combined, the reduction was 100%. At first hour, nisin showed a little reduction on the colonies formed, but after 24h the bacteria kept growing into the flask (10^9 CFU/mL). No synergism effect was observed between lime and lemon oils.

Keywords: antimicrobial activity; pathogens; microorganism; sanitizer; statistical design

Synergistic activity between coriander essential oil and conventional antibiotics against *Acinetobacter baumannii*

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Essential oils can be seen as a supply of new antimicrobial agents and have been recognized for their antimicrobial activity against several pathogenic microorganisms. This study focused on the essential oil obtained from seeds of *Coriandrum sativum L.*, which is typically used as cooking ingredient, in perfumery, cosmetics, flavouring agent in pharmaceutical preparations, as a stomachic, spasmolytic or carminative and has been reported as having a very good antibacterial activity against some pathogenic bacteria.

Owing to the rising number of multidrug-resistant pathogens it is necessary to investigate new antimicrobial agents, such as essential oils, that when used together with conventional antibiotics could potentiate the activity of the later ones.

In this work we investigated the existence of synergistic antibacterial effect between coriander essential oil and six different antibacterial drugs, against *Acinetobacter baumannii*, one microorganism that remains an important and difficult-to-treat pathogen whose resistance patterns result in significant challenges for the clinician. The antibacterial activity of coriander oil was assessed using microdilution susceptibility testing and synergistic interaction by checkerboard assays.

The results obtained highlighted the occurrence of a pronounced synergistic interaction with the association of coriander essential oil with chloramphenicol, ciprofloxacin, gentamicin and tetracycline against two reference strains of *Acinetobacter baumannii* (LMG 1025 and LMG 1041) with a FIC index ranging from 0.047 to 0.375.

When tested the involvement between coriander essential oil and piperacillin or cefoperazone, the isobolograms and FIC index showed an additive interaction. The in vitro interaction could improve the antimicrobial effectiveness of ciprofloxacin, gentamicin and tetracycline and may contribute to resensitize *A. baumannii* to the action of chloramphenicol.

So, coriander essential oil has shown a remarkable potential to be associated with some conventional antibiotics against *Acinetobacter baumanni,* that has emerged as nosocomial multi-resistant pathogen.

Keywords Coriander oil, *Acinetobacter baumannii*, antibiotics

The role of oxidative stress in the antibacterial mechanism of action of a natural clay mineral mixture

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As bacterial pathogens continue to develop antibiotic resistance, the urgency to identify novel therapeutic agents is becoming increasingly pressing. To this end, we have identified a natural clay mineral mixture, designated CB10, that exhibits antibacterial activity against a broad-spectrum of pathogens. This natural mineral mixture holds outstanding potential as a complementary or alternative antibacterial agent, serving as both a therapeutic agent and a medium for delivery. To begin investigating the mechanism of action of the CB10 minerals, we prepared mineral leachates that lack physical particles and only contain solubilized ions released from the surface of the minerals. These leachates maintained antibacterial activity, demonstrating that the mechanism of action is dependent on chemical, not physical, interactions. Inductively-coupled plasma optical emission spectroscopy (ICP-OES) analyses identified several redox-active metal ions, such as iron and copper, present in the mineral leachates. These metal ions are known to participate in Fenton, Fenton-like, and Haber-Weiss reactions, whereby the metal ions react with hydrogen peroxide to produce superoxide anion and hydroxyl radicals which can damage DNA, RNA, lipids, and proteins. Hydrogen peroxide was detected in 10% CB10 mineral suspensions and leachates at steady concentrations of 3-4 µM and 1-2 µM, respectively. Oxidation – reduction potential measurements of CB10 leachate (CB10-L) yielded a value of 321 mV, indicative of an oxidizing environment. To test the hypothesis that reactive oxygen species (ROS) contribute to the antibacterial mechanism of action, we used the ROS-sensitive fluorescent dye, dyhydrorhodamine 123 dihydrochloride to measure ROS levels in the CB10-L extracellular environment and the *Escherichia coli* intracellular environment during exposure to CB10-L. These data revealed that CB10-L alone produces 10-fold higher ROS after 24 h than the respective water control, while the intracellular ROS content in CB10-L-exposed cells is increased 2-fold over the respective water-exposed control condition. These data demonstrate that while ROS generation transpires both internally and externally, the external environment is the predominant stress location. For this reason, we hypothesize that the mechanism of action of CB10 minerals occurs at the cellular membrane. Previously, we demonstrated that the *E. coli* membrane is not permeabilized following bactericidal exposure to CB10-L. We have used a luciferase-based assay to measure ATP production, as an indicator of cellular respiration, during CB10-L exposure. During a 4 h exposure to 10% CB10, ATP levels dropped significantly compared to the minimal media-only control conditions ($p < 0.0001$). Based on these data, we have confirmed the participation of ROS in the antibacterial mechanism of action of these minerals and have demonstrated that cellular respiration is diminished during exposure to CB10 minerals.

Keywords Complementary medicine; Natural antibacterial agents; Clay minerals; Reactive oxygen species

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The tail fiber protein of ϕAB6, an *Acinetobacter baumannii* phage, may possess polysaccharide depolymerization activity

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Acinetobacter baumannii is a non-fermentative, Gram-negative bacterium. In recent years, frequency of infections caused by this organism keeps increasing and multidrug-resistant strains (MDRAB) are emerging in hospitalized patients. Therefore, as therapeutic options become limited, the potential of using phages as natural antimicrobial agents to control the infections is worthy reconsideration. We have isolated 10 virulent bacteriophages with double-stranded DNA, ϕAB1 to ϕAB9 and ϕAB11, and found that each has a narrow host range, infecting only low percentages of 253 MDRAB strains isolated in Taiwan. Electron microscopy showed that 8 of the 10 virions each consists of a small icosohedral head with short tail fibers, similar to members of the *Podoviridae* family. Genomic sequences of ϕAB1 and ϕAB6 displayed high degrees of conservation between most of their genes except *gp41* which was predicted to be the tail fiber gene, coding for polypeptides of 882 and 699 residues, respectively. Immunogold electron microscopy with antibodies raised against recombinant $Gp41^{\phi AB1}$, in which cross-reaction was observed, confirmed that Gp41 is a component of the tail apparatus of these phages. The N-termini of $Gp41^{\phi AB6}$ and $Gp41^{\phi AB1}$ share similarities with the N-terminus of phage T7 tail protein, indicating by analogy that these domains function to connect Gp41 to the virion's head. A central domain of 105 residues present in $Gp41^{\phi AB6}$, but not in $Gp41^{\phi AB1}$, is highly similar to exo-beta-1,3-glucanase from fungi, suggesting that this domain may function to depolymerize exopolysaccharide of host cell surface, as the cases in other phages, to facilitate phage entry. In addition, replacement of C-terminus of $Gp41^{\phi AB1}$ with the corresponding region of $Gp41^{\phi AB6}$ caused a change in host specificity.

Transfer of *Listeria monocytogenes* during slicing operations and its resistance to disinfecting agents

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Nowadays, supermarkets provide a wide variety of Ready-to-Eat (RTE) meat products. Examples of these commodities may include RTE sliced meat products such as iberian RTE cured loin of pork. Operations linked to food slicing may pose a human health risk because foodstuffs may come into contact with contaminated surfaces if proper hygiene practices are not applied. As these products do not require further treatment such as cooking before consumption, contamination events during processing plays a major role in foodborne human diseases. According to the European Food Safety Authority (EFSA) RTE meat products have been involved in human listeriosis. In 2009, listeriosis accounted for 1,645 confirmed human cases which were mainly caused by *Listeria monocytogenes*. In this work, experiments were performed in order to study the transfer of *Listeria monocytogenes* during slicing operations and its resistance to disinfecting agents, a cuaternary ammonium. The aim was to know (i) the transfer of *Listeria monocytogenes* from stainless steel blade to iberian RTE cured loin of pork during slicing operations with and without disinfection of the blades before slicing, and (ii) the efficacy of a disinfecting agent in two laboratory media. For the first purpose, *Listeria monocytogenes* was inoculated on two stainless steel blades (around 10^9 ufc/blade). After inoculation, the blades were left in laminar cabinets at 25ºC until inoculua were dried. Then, a commercial disinfectant was sprayed on one of the two blades and left in laminar cabinet until drying, following the application protocol detailed in the disinfectant instructions. In order to study the transfer of *Listeria monocytogenes* during slicing, the iberian RTE cured loin of pork was sliced in two stages: just after inoculation, and after 3 hours at 25ºC from inoculation; both disinfected and non-disinfected blades were used. Paralelly, the efficacy of the disinfectant agent was studied in two media matrix, i.e. broth and agar. In the first place, a series of four tubes containing 1 mL of Brain Heart Infusion (BHI) broth were inoculated with 1 mL of *Listeria monocytogenes* (10^9 cfu/mL), and then, 2 mL of the commercial disinfectant were added. In the second place, the disinfectant agent was sprayed on Plate Count Agar (PCA) plates previously inoculated with *Listeria monocytogenes*. In both cases, the media were stored at 37ºC for 24 h to allow for possible recovery of bacterial cells. Detection of *Listeria* was studied by the application of the ISO 11290-1 standard method. *Listeria monocytogenes* was transferred from non-disinfected blade to iberian RTE cured loin of pork in ≈90% of slices. However, when the disinfectant agent was applied to blade, the transfer of *Listeria monocytogenes* was lower (≈67%), but still high. The disinfection action in tubes containing BHI broth and *Listeria* cells was 100% effective, as the microorganism did not survive, and no growth was detected. Growth of *Listeria* on PCA plates after disinfection treatment was only observed in one replicate experiment out of three. These results show that *Listeria monocytogenes* is able to survive on stainless steel blades and transfer to foods during slicing with and without spray application of commercial disinfectants. However, it could be hypothesized that broth media, which allows free movement of their components (bacteria, disinfecting agent, nutrients), lets them enter into close contact, facilitating the death of, *Listeria* cells. The survival ability of *Listeria monocytogenes* on disinfected blades may be explained by the physical allocation of *Listeria* cells on the blade, which could let some cells escape from direct contact with the disinfectant agent. More research is needed to clarify the action of disinfecting agents against bacteria on food contact surfaces as well as the matrix and application protocols effects.

Keywords: *Listeria monocytogenes*; transfer; slicing; disinfecting agent.

Use of Moringa oleifera flowers to treat bacterial contamination in water

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The urbanization process around water-bodies has caused damage to the environmental balance. Increase of microorganism populations in drinking water has been responsible for waterborne infections which account for 80 % of all diseases worldwide and 90 % of all infectious diseases in developing countries. Chlorine is the main disinfectant used to remove microbial contamination from water. However, its use leads to formation of carcinogenic compounds, which justifies the search for safe and natural disinfectants. This work reports the evaluation of antibacterial activity from protein preparations of *Moringa oleifera* flowers against bacteria that may be present in contaminated water and their use to disinfect water of Cavouco Lake (Recife, Brazil). Minimal inhibitory (MIC) and bactericide (MBC) protein concentrations were determined. Aqueous extract (MoE) was obtained by homogenization of *M. oleifera* flowers with distilled water using a blender. The extract was treated with ammonium sulfate (60%) and the precipitated protein fraction (MoPF) was dialyzed against distilled water. The presence of alkaloids and trypsin inhibitor activity in MoE and MoPF was evaluated. MoE and MoPF were active against Gram-negative (*Escherichia coli*, *Proteus mirabilis* and *Salmonella enteritidis*) and Gram-positive (*Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus*) bacteria. The preparations were most active against *E. coli*, the best indicator of fecal contamination; MIC of 0.023 and 0.018 mg/mL as well as MBC of 0.366 and 0.297 mg/mL were obtained to MoE and MoPF, respectively. MoE and MoPF abolished the growth of bacteria from lake water. Alkaloids were absent in both preparations. Trypsin activity (425 mU/mL) was reduced to 125 and 212 mU/mL, in the presence of MoE and MoPF, respectively. Trypsin inhibitor may be related with antibacterial activity. In conclusion, the biodegradable flowers contain antibacterial compounds and constitute a source of water disinfectant agents.

Key words: *Moringa oleifera;* flowers; antibacterial activity; water treatment.

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Validation of specifics oligonucleotides for detection of Enteropathogenic (EPEC) and Enterotoxigenic (ETEC) *Escherichia coli* strains isolated in a Mexican region

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Escherichia coli colonize the human intestinal tract within hours of birth and it is considered a non-pathogenic member of the normal intestinal biota. However, there are six *E. coli* pathogenic groups that may produce diarrhea: enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enteroinvasive (EIEC) and diffusely adhering (DAEC) groups. The unavailability of routine diagnostic tests to differentiate commensal and pathogenic *E. coli* does not contribute to resolve the *E .coli* health problem worldwide. Epidemiological studies performed by molecular biology methods in Latin America countries, including Mexico, show that EPEC and ETEC are the most pathogenic groups which have been isolated from fecal samples during disaster events. Although there are several techniques for detecting specific virulence genes for each pathogen type, these techniques are used for researching studies only and are unavailable for most diagnosis laboratory.

We are interested in determining the incidence of these pathogens in order to design a sensible, fast and accurate test for ETEC and EPEC *E. coli* diagnosis. For this determination, we have obtained isolates from patients with gastrointestinal infections and use these colonies for simultaneous PCR assays. Oligonucleotides based in two specific genes for each pathotype were used: EPEC effacing intimin and bundle-forming pilus genes and ETEC heat-stable and heat-labile enterotoxins. A PCR assay simultaneous using the oligonucleotides selected for the reference strains E2348/69 and H-10407 was standardized. Ten copies of EPEC and one copy of ETEC were detected in DNA crude extracts of laboratory strains. The oligonucleotides were assayed with genes no recognized specifically by designed sequences (*Shigella, Salmonella, E. coli* commensal, *Klebsiella oxytoca, Proteus mirabillis, Enterobacter cloacae and Enterobacter sakasaki)* showing their specificity.

Our results show that EPEC is a strain with incidence in this region because we detected at least one pathogenic bacterium among forty-five fecal isolates collected in different clinic laboratories. Furthermore, two more pathogenic ETEC were identified in the urine of patients with urinary infections.

The results described above confirm the existence of EPEC and ETEC affecting the Mexican population in the state of Guanajuato, located at the center of the country. *E. coli* pathotypes are not subject to analysis in daily medical practice. Our results show that it is possible and feasible to perform an amplification of the virulence genes from categories of diarrheagenic *E. coli* (EPEC and ETEC) with high specificity and sensibility. On this basis, it will be possible to design an accurate technique to be applied for the etiologic diagnosis of patients with sporadic diarrhea. Furthermore, we believe that a routine test for these pathogens must to be included in regions with moderate incidence of diarrhea or high prevalence of intestinal infections.

Keywords Enteropathogenic *E. coli,* enterotoxigenic *E. coli,* PCR.

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Methods

Quantitative Models and Bioinformatics in Microbiology

Technology development

Analysis of *Saccharomyces cerevisae* protein interactions with Mnn2p using an open access database

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The transfer of mannosylphosphate (Man-P) groups into N-linked oligosaccharides in *Saccharomyces cerevisiae* has been widely studied in the last decades using different approaches. The pioneer studies by Ballou's group with the isolation and characterization of mnn mutants (Hernandez et al., 1989; reviewed in Balou, 1990) were followed by biochemical and genetic studies by Jigami's group (reviewed by Jigami and Odani, 1999). They found that at least two proteins participated in Man-P transfer: Mnn6p was the putative transferase while Mnn4p was a required regulator. Later studies by our group demonstrated that Mnn2p, an alpha 1,2-mannosyltransferase, was also involved in some step(s) of the process (Olivero et al., 2000). This finding suggested that probably more proteins might participate in the transfer, and we designed a new approach to look for them: the genome-wide search for genes which deletion affects the transfer of Man-P (Corbacho *et al.*, 2005). We found a long list of genes whose deletion resulted in a decrease in Man-P transfer. Most of them as a result of an indirect effect, but also including several candidates to directly participate in the process.

In order to go deeper in the study and try to find possible functional interactions between proteins involved in Man-P transfer, in this work we tried a different approach: we used a *in silico* protein analysis tool (Zang *et al.*, 2011), based in the Global Proteome Machine Database (GPMDB) which is the largest curated and publicly available proteomic data repository derived from tandem mass spectrometry. We have focused our study in Mnn2p because the available data for Mnn4p and Mnn6p were not enough to perform a reliable analysis.

The study starts with information of 318 data sets and after several processing steps and application of some filters (same raw data set filter, confidence of identification filter, data set size filter, ProDis filter and frequency of occurrence filter), we end up with 15 data sets. The protein list associated to each one was analyzed to get information about Mnn2p interactions. In a general manner, we found that Mnn2p was related to several proteins required for efficient transport of secretory proteins to the Golgi (Erv25p and Erv29p) and from the Golgi (Chs1p, Chs5p and Chs7p), proteins involved in MAP Kinase pathway (Bck1p, Stt4p and Pkc1p), and two transferases functionaly related to Mnn2p: Mnn1p and Mnn5p which, as Mnn2p, both are alpha-mannosyltransferases responsible for the addition of mannoses to the branches of outer chain(Mnn1p and Mnn5p) and core (Mnn1p), in the N-oligosaccharides of *S. cerevisiae*.

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Keywords: glycosylation, Mnn2p, GPMDB, protein-protein interaction, *Saccharomyces cerevisiae*

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Application of Bacterial Biosensors for Ecological Hazard Assessment of Chinese Soils

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There are significant worries about the impact of pollutants in soils during urbanisation and industrialisation in the developing countries. The routine chemical analysis of soils is used to characterise the concentration of metals translocated by point source and diffuse actions, this fails to put in context the bioavailability or potency of these analytes. Biosensors offer a novel and direct method for hazard assessment of soils impacted with anthropogenic contamination. However, few significant examples of thorough applications of biosensor in environment. This study makes use of comprehensively characterised biosensors for diagnostic applications to complex environmental matrices. Both constitutively marked and metal specific sensors were optimised, but only the constitute were responsive to a wide range of soils and chemicals of concern. As the matrix became increasingly complex, the data became more uncertain. For the metal induced sensor, the response to standard aqueous samples was predictable but as these became impacted by the presence of co-contaminants, soil associated carbon or variations in the pH value. The constitutively-marked biosensors offered much greater predictability of response and this is the reason why these are so widely adopted in soil applications. Bacterial biosensors provide a useful tool for assessing the bioavailable fraction of analytes in soils and for complementing chemical analysis. If matrix matched control samples can be collected, then this technology can be applied to a wide range of contrasting soils with a suite of contaminants to aid in the development of generic hazard evaluation of contrasting soils.

Keywords: bacterial biosensors, heavy metal bioavailability, environmental application, soil

Automated Cryobank of Microorganisms: Unique Possibilities for Long-Term Authorized Depositing of Commercial Microbial Strains

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Liconic Instruments' automated −80°C Tube Store becomes an extremely useful, simple and intuitive system for a long-term storage of microorganisms at optimum conditions without the loss of their valuable properties. This ultra low temperature store is based on the chest freezer principle and characterized by the most efficient way of running a store at −80°C. Special plates with 300 µl tubes marked with bar codes are used. Plates are loaded into storage chamber via the interface unit (-20°C). This ensures a stable temperature and dry conditions into the storage compartment. Computer passwords used by depositors during the sample load operation ensure an authorized access to commercial strains of microorganisms. STC Tube Store Sample management software contains all information about the strains deposited: description, location in the store and movements of each sample. Unique "real time" online database of microorganisms has been organized on basis of the STC Tube Store Sample management software. This database allows depositors to monitor their samples and climatic conditions in the depository during storage. Reliable functioning of the automated Tube Store is provided with a backup cooling (reserve cooler and LN2 backup system) and self-alternator as well. Capacity of this depository is 200 thousands samples. Such kind of store gives possibilities to intensify the search of new microbial cultures, keeping in mind the huge soil microbe's biodiversity and a necessity to mobilize genetic resources of microorganisms for an agricultural production.

Keywords microbial collections; long-term maintenance; authorized depositing of strains

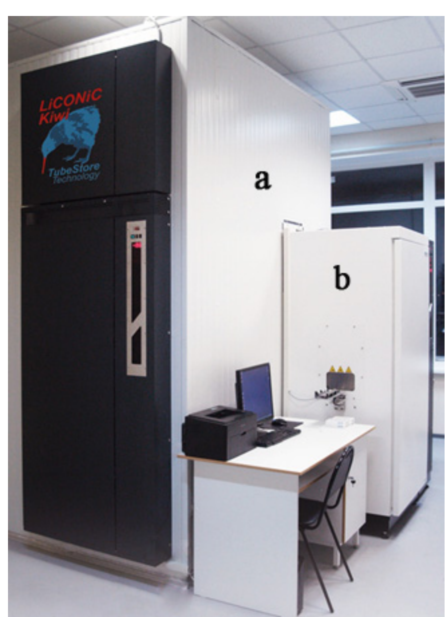

Fig.1 Appearance of the Liconic Instruments automated -80°C Tube Store.
a- Storage Chamber (-80°C)
b - Interface unit (−20°C) that is used for transportation of plates into/out of the store and selection of single samples for unloading

Comparison of nucleosome positions in promoters of orthologous genes between *Aspergillus fumigatus* and *Saccharomyces cerevisiae*

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Nucleosome consists of an octamer of histones, around which DNA is wrapped in 1.65 turns. Neighboring nucleosomes are separately by unwrapped linker DNA. Generally, the eukaryotes have more conserved nucleosome positions in gene promoters than other regions and the histone modifications in gene promoters play an important role in the gene regulation. We have already performed nucleosome maps of the filamentous ascomycete *Aspergillus fumigatus* and the ascomycetous budding yeast *Saccharomyces cerevisiae*. Most of the nucleosome positions of *A. fumigatus* are conserved in gene promoters, even after treatment with the histone deacetylase inhibitor trichostatin A (Nishida et al., 2009; Nishida et al., 2010). In addition, most of the nulceosome positions of *S. cerevisiae* are conserved in gene promoters between the control and the histone acetyltransferase gene *ELP3* deletion mutant, and between the control and the histone deacetylase gene *HOS2* deletion mutant (Matsumoto et al., 2011). The ELP3 and HOS2 have the highest and the third highest evolutionary conservation level among the fungal histone modification proteins respectively (Nishida, 2009). In the present study, I compared the nucleosome positions in promoters of orthologous genes between *A. fumigatus* and *S. cerevisiae* on the basis of the two nucleosome maps. First, I extracted 466 genes of *S. cerevisiae* with very conserved (correlation coefficient $r > 0.95$) nucleosome positions in gene promoters between the control and *ELP3* deletion mutant, and between the control and *HOS2* deletion mutant (Matsumoto et al., 2011). Among the 466 genes of *S. cerevisiae*, 300 genes had orthologus genes in *A. fumigatus*. Next, I compared the nucleosome positions in the promoters of the orthologous genes between *A. fumigatus* and *S. cerevisiae*. As a result, only 18 orthologous genes had correlation (correlation coefficient $r > 0.7$) between the two nucleosome position profiles and the others had low correlation. This result is probably related to the fact that the two fungi are evolutionarily distant (*A. fumigatus* and *S. cerevisiae* belong to the subphyla Pezizomycotina and Saccharomycotina respectively). I believe that comparison of nucleosome positions in gene promoters is a powerful tool for gene identification between evolutionarily close organisms.

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Keywords nucleosome position; gene promoter; orthologous gene; *Aspergillus fumigatus*; *Saccharomyces cerevisiae*

Detection of antibodies and DNA by multiplex microbead arrays using the VideoScan platform technology

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There is an increasing demand for multiplex techniques enabling the simultaneous assessment of multiple biomolecules in one biological sample in research and clinical diagnostics. Due to their high flexibility microbead-based multiplex detection systems close a gap between single and high-throughput detection methods.

Within the framework of the InnoProfile project "Real-time-PCR-Array" we developed a microbead-based multiplex antibody and DNA detection technology. Main pillars for this technology are A) Fluorescent microbead populations which are defined by varying ratios of two fluorophores and B) The multi-fluorescent color detection platform VideoScan with novel pattern-recognition algorithms.

A) Microbeads: Proteins for the detection of antibodies or oligonucleotide capture probes for the detection of PCR products are coupled on microbeads. Specific antibodies bind to microbead-coupled antigens and are detected by a fluorescence-labeled secondary antibody. PCR products are fluorescence-labeled during the PCR reaction and hybridize to microbead-coupled capture probes. Binding of fluorescence-labeled secondary antibodies or PCR products results in a specific fluorescence corona around a microbead. The intensity of a fluorescence signal correlates with quantities of bound antibodies or PCR products. Fluorescence corona and affiliation of one microbead to one of 18 microbead populations are automatically analyzed by the VideoScan platform. Size, surface, thermostability and degree of carboxylation of microbeads as well as photostability of fluorophores determine efficiency and robustness of the interpretation process.

B) The VideoScan platform is a flexible imaging technology with a broad spectrum of applications such as I) Protein and DNA quantification due to fluorescence signals on microbead surfaces, II) Cell- and tissue-based immunofluorescence assays, III) Bacterial adhesion and invasion assays by the detection and quantification of fluorescent bacteria. The VideoScan platform is based on automatic capturing and processing of two-dimensional images and consists of the following main components: motorized inverse microscope with three fluorescence channels, motorized scan-stage, digital grey-scale camera and computer running software for controlling and processing.

The VideoScan is a flexible and automatic multi-parametric analysis system with a great potential for further diagnostic and biochemical applications.

Development of a web-based tool for assessing and managing microbial risk in minimally processed vegetables

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Minimally processed and ready-to-eat vegetables are of growing importance in Europe. This type of foods should be among the most relevant in order to be submitted to Risk Assessment, as it is widely spreading, and its safety could be compromised, since scientific and epidemiologic evidence has shown that this type of foods can be contaminated by pathogens coming from primary production or the factory environment (cross-contamination or recontamination), and industrial processes aiming at reducing the microbial load of the product does not guarantee their complete elimination.

The aim of this project was developed a computer tool, based on Risk Assessment framework, easy to use by risk assessors and managers to estimate and mitigate microbial risk in minimally processed vegetables.

The development of the web-based tool has been based on the consecution of 6 phases: 1) hazard identification in minimally processed and ready-to-eat vegetables; 2) modelling processes along farm-to-table chain with a flexible structure by means of predictive and/or stochastic models; 3) characterization of identified hazards; 4) development of a model for risk characterization; 5) proposal and implementation of risk management measures; and 6) development of a computer tool involving the results of the previous objectives, and easy to use and interpret.

With this tool, assessors and managers are able to introduce data which are usually confidential (e.g. prevalence and concentration of pathogens in foods from official analyses), and obtain a final estimate of the risk. Beside this, it is also possible to select different management measures and to know their impact on risk mitigation. The realization of this project represents a decisive advance in the assessment and management of safety of this type of foods in Europe, demonstrating transparency with scientific basis in making decisions.

Keywords: risk assessment, predictive microbiology, vegetables, food-borne pathogens, web-based tool.

Encapsulation in monodisperse hydrogel microspheres enables fast and sensitive phenotypic analyses of bacteria, yeast and human cells using flow cytometers

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Characterization of microorganisms usually involves culture during more than 20 generations in order to achieve the formation of macrocolonies on solid media. Alternatively, microencapsulation allows the detection of microbial growth by monitoring the development of microcolonies from encapsulated individual cells. Microbial proliferation inside the microcapsules can be detected using flow cytometry, provided that the population of microparticles exhibits appropriate optical and mechanical properties and is monodisperse in size and shape.

Using a Cellena® Flow Focusing® microencapsulator (Figures 1 and 2), we managed to produce monodisperse alginate microparticles containing individual bacteria, yeast and human stem cells (Figure 3). Alginate particle sizes were reproducibly selected from less than 100 µm to over 600 µm, by replacing the disposable nozzle. Sterility was preserved during the microencapsulation procedure, preventing undesired contaminations.

Microencapsulated microorganisms were used for a variety of application: from characterizing secreted enzymes to detection of thermosensitive mutants. Proliferation inside the particles was monitored by flow cytometry without requiring fluorescent labelling.

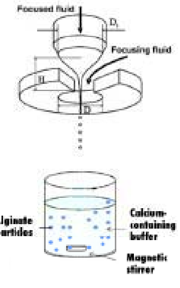
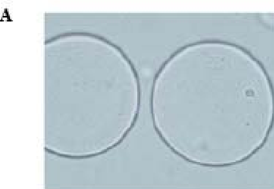


Figure 1. Flow Focusing® microencapsulation. Figure 2. Cellena® microencapsulator .

A



B

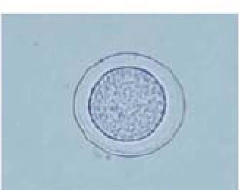


Figure 3. Encapsulated yeast cells. (A) single cell after immediately after encapsulation. (B) Microcolonie after 14 h incubation in YPD medium at 30°C.

Keywords microencapsulation, flow cytometry

General detection of microbial contamination in technical fluids by adsorption to chemically functionalized surfaces

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This work presents first results of a new approach for the detection of biological contamination using the Surface Plasmon Resonance sensor (SPR) technology. SPR sensors have been commonly employed for the detection of proteins, bacteria and chemical substances. However, only specific interactions can be detected so far that rely on specific and sensor immobilized receptor molecules. It is of importance to overcome this limitation as technical fluids like cooling lubricants, paints and varnishes often show contaminations with different strains of microorganisms. The here presented general approach will focus on a distinguished mode of action. The aim is an unspecific adsorption of bacteria and other microorganisms to heavy metal ions immobilized in a polymeric coating on the gold sensor surface. The adsorption is based on the interaction between multivalent cations and negatively charged membrane proteins in the outer membranes of the contaminating microorganisms. The processed sensor surface is characterized by means of contact angle measurements, scanning electron microscopy, and bacterial and fungal binding studies. Besides, one of the main advantages of the sensor is the usability in a multitude of liquids such as deeply contaminated emulsions and other technical fluids, providing a broad applicability of the sensor system.

Keywords surface plasmon resonance, detection, fluid, surface modification, bacterial contamination

Genome-wide nucleosome maps of the histone acetyltransferase *ELP3* and the deacetylase *HOS2* gene disruptants of *Saccharomyces cerevisiae*

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Eukaryotic genomic DNA is packaged with histone proteins to form chromatin [1], the most fundamental repeating unit of which is the nucleosome. The precise organization of this chromatin is of utmost importance for the maintenance of eukaryotic genomic DNA. Generally, nucleosomal histone proteins are post-translationally modified. The acetylation and deacetylation of the core histone tails play an important role in the regulation of transcription [2]. Although the histone proteins are so conserved among the eukaryotes, the nucleosomal DNA lengths are different among phylogenetically closed ascomycetous yeasts [3]. Our previous analyses indicated that the distribution of the nucleosomal DNA lengths of the filamentous ascomycete *Aspergillus fumigatus* showed 2 peaks at 135 nt and at 150 nt [4]. On the other hand, the distribution of the nucleosomal DNA lengths of *A. fumigatus* with the hyperacetylated histones induced by the histone deacetylase inhibitor trichostatin A shifted toward longer with a single peak at 168 nt [5], suggesting that hyperacetylation of histones induced to elongate the nucleosomal DNA length.

In order to elucidate the influence of histone acetylation upon nucleosomal DNA length and nucleosome position, we compared nucleosome maps of the following three yeast strains; strain BY4741 (control), the *ELP3* (one of histone acetyltransferase genes) deletion mutant, and the *HOS2* (one of histone deacetylase genes) deletion mutant of *S. cerevisiae*. We sequenced nucleosomal DNA fragments after treatment with micrococcal nuclease. After mapping the DNA fragments to the genome, we identified the nucleosome positions. We showed that the distributions of the nucleosomal DNA lengths of the control and the *HOS2* disruptant were similar. On the other hand, the distribution of the nucleosomal DNA lengths of the *ELP3* disruptant shifted toward shorter than that of the control. It strongly suggests that inhibition of ELP3-induced histone acetylation causes the nucleosomal DNA length reduction. Next, we compared the profiles of nucleosome mapping numbers in gene promoter regions between the control and the disruptant. We detected 283 genes with low conservation level (correlation coefficient $r < 0.5$) of nucleosome positions in promoters between the control and the *ELP3* disruptant, and 53 genes with low conservation between the control and the *HOS2* disruptant. In addition, we detected 24 genes with low conservation level between the control and the *ELP3* disruptant as well as between the control and the *HOS2* disruptant. It indicates that both ELP3-induced acetylation and HOS2-induced deacetylation influence the nucleosome positions in the promoters of those 24 genes. Interestingly, in 19 of the 24 genes, the profiles of nucleosome mapping numbers were similar between the two disruptants [6].

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Keywords nucleosome; histone-acetylation; histone-deacetylation; *Saccharomyces cerevisiae*

Identification of Bacterial Community in Thermophilic Oil Reservoir Using Restriction Fragment Length Polymorphism (RFLP) and Culture-Base Method

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The diversity of microorganism implies the characteristics of its environment. The physical and chemical parameters in an oil reservoir may limit the ability of bacteria to grow as the temperature and pressure are the main limiting factor. The aim of this study is to determine the bacterial communities from Semarang oil reservoir using molecular and live cultural approached. Restriction Fragment Length Polymorphism (RFLP) is a technique of fingerprinting with a cloning strategy that targeted the 16S rRNA of bacterial genes. This method is used to estimate the culturable and non-culturable bacterial communities. For culturable bacteria, five types of media were used for isolation - Trypticase Soy agar/broth, Luria Bertani agar/broth, Viande Levure agar/broth and Sulphate Reducing Bacteria agar/broth. The pH and salinity of the media were adjusted according to the water sample environments which were 7.5 and 1.6%, respectively. Based on the cultivated population which was done by spread plate and streaking method, about 152 isolates were obtained from water samples. These isolates were then incubated at different temperature ranging from 40 to 55°C. At 55°C, only 15 isolates could survive and most of them were *Bacillus sp.* and one *Desulfotomaculum sp.*. Meanwhile, by RFLP technique, out of 161 clones, about 104 clones showed differences in the restriction profile after digested with *Msp1* and *Taq1* enzymes. Sequencing and phylogenetic analysis showed the screened clones were dominated by *Marinobacter sp.* (43%), *Flexistipes* sp. (12.5%), *Pelobacter* sp. (1.9%), *Methanolobus* sp. (1.9%) and uncultured bacterium (11.5%). Another eight genera which showed a low percentage of similarity after aligned their sequences with BLAST were *Ralstonia* sp. (8.6%), *Rhodococcus* sp. (1.9%), *Garciella* sp. (0.96%), *Beta proteobacterium* (1.9%), *Dethiosulfatibacter* sp. (1.9%), *Burkholderiales bacterium* (0.96%), *Spirochaeta* sp. (0.96%) and *Pseudomonas* sp. (6.7%). This result showed that not all bacteria are cultivable. Critical environmental conditions such as pressure, temperature, amount of oxygen and nutrient contents may affect the growth of these bacteria.

Keywords Bacterial community, Culturable, Oil reservoir, Restriction Fragment Length Polymorphism

Identification of CD44 and CD127 cells from human dental pulp using MACS technology

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INTRODUCTION

Stem-cells have proved for years now to be the new potential for disease cures and therefore have been subject to many investigations. Due to this fact, it has become important to find the best way to identify, and successfully cultivate these cells, in order to able to work with them. (add info and references)

Magnetic Activated Cell Sorting (MACS) technology has been found useful for the correct identification and culture of numerous and varied tissues such as (examples and reference). These, later on have served a range of purposes, from (examples and reference). This is where the idea surges, that this technology could be used for stem-cell segregation, cooperating with the better identification and culture of the same. Due to the fact that stem-cell processing needs to be as time and cost efficient as possible, we find that MACS technology is a great window of possibility. The current outlook in tissue engineering is the use of stem cells isolated from tissues present in the adult, this in order to carry out a future therapeutic use of autologous manner in this patient.

Material and Methods

The dental pulp samples were collected from the intact (3rd) molar extraction of 8 patients, ages 16 -25 years of Mandibule . Facilitated by Dra. Belinda I. Beltran- Salinas from Faculty of Dentistry of the Universidad Autonoma de Nuevo Leon / Ministry of Health, they were all recollected the morning they were to be processed.

After extraction of the dental pulps, the procedure followed is as follows. Add 4.75 mL of PBs in a GentleMACS C tube and insert it in the GentleMACS instrument ensuring the sample is in touch with the tube vanes. Run either program A or B. Remove the tube and digest the pulps in a 3mg/mL collagenase I and 4mg/mL dispase solution. Incubate for 1 hr at 37°C in a water bath. Once incubated, reintroduce the tube on the GentleMACS and run programs B or C.

RESULTS

Stem cells were isolated from third molars in young adults using the technique of magnetic separation *(Miltenyi Biotec)* in which monoclonal antibodies were used CD271 and CD44, the cells were observed in inverted microscope morphologies found elongated, flattened and fibroblastic dental pulp stem cells, subsequently by flow cytometry these cells were quantified positive results in both markers CD271 *(0.6)* CD44 *(95.7 + -3.8)* analysis in 10,000 cells.

Kinetics survive of *Eschericha Coli* in Tryptic Soy Broth (TSB) under High hidrostatic pressure. Fitting a mathematical model, prediction and validation of a experimental nonlinear model

Samuel Lima Jácome; Daniela Saucedo Reyes; Mª Dolores Rodrigo Aliaga; Manuela Vaz Velho; Antonio López Martínez;

It has been determined inactivation kinetics of *E*. coli CECT 433 by high hydrostatic pressure inoculated in nutrient broth (pH 7.4 ± 0.2), for a range of pressures of 150, 175, 200 and 225 MPa with exposure times of 0 to 14 minutes, and 30ºC. The survival curves were adjusted to three mathematical models: Weibull, Baranyi and Gompertz. The best goodness of fit was obtained for the modified Gompertz equation with of values R^2_C = 0990, and *RMSE* = 0138. The validation of mathematical modeling was done through calculating accuracy and bias factor (A_f and B_f), being the best Gompertz model. Finally, it was determined the secondary kinetic parameter (Z (P)) or constant resistance to pressure, for each model, specifically the value determined by the Gompertz model resulted in 125 MPa.

Licorice root Ochratoxin A contamination detection by inverse ion mobility Spectrometry

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Licorice is one of the most commonly used crude drugs not only in the Orient but also in the Occident. It has been used for the treatment of gastric and duodenal ulcers, bronchial asthma, inflammation and other diseases. Ochratoxin A (OTA), a mycotoxin which contaminates a wide range of food commodities is one of the major licorice contaminants. OTA has been shown to possess nephrotoxic, carcinogenic, immunosuppressive and teratogenic properties and was classified as carcinogenic for humans (group 2B). This secondary metabolite is produced by filamentous fungi such as *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus westerdijkiae* and *A. steynii* as well as *Penicillium verrucosum* and *Penicillium nordicum*. In tropical zones, OTA is mainly produced in coffee beans by *A. ochraceus* and *A. westerdijkiae* (section Circumdati), which was recently dismembered from *A. ochraceus,* due to their important OTA production and occurrence

Despite the recent, successful efforts to detect mycotoxins, new methods are still required to achieve higher sensitivity, more simplicity, higher speed, and higher accuracy at lower costs. This paper describes the determination of ochratoxin A (OTA) using corona discharge ion mobility spectrometry (IMS) in the licorice root. A quick screening and measuring method is proposed to be employed after cleaning up the extracted OTA by immunoaffinity columns. The ion mobility spectrometer is used in the inverse mode to better differentiate the OTA peak from the neighboring ones. After optimization of the experimental conditions such as corona voltage, injection port temperature, and IMS cell temperature, a limit of detection (LOD) of 0.010 ng is obtained. Furthermore, the calibration curve is found to be in the range of0.01–1 ng with a correlation coefficient (R2) of 0.988. Licorice roots were analyzed for their OTA content to demonstrate the capability of the proposed method in the quantitative detection of OTA in real samples.

Key words: Licorice root, Ochratoxin A, Detection, Ion mobility spectrometry, Limit of detection

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Magnetic Activated Cell Sorting for Human Dental Pulp Stem-Cell Identification

L.M. Rodríguez-Serrano, S.L. Alva-Sandoval, C.del Angel-Mosqueda, A. Gómez-Treviño, M. E. Mercado-Morales, R. Hernández-Delgadillo, M.A. de la Garza-Ramos, A. Mendiola-Jiménez.

INTRODUCTION

Stem-cells have proved for years now to be the new potential for disease cures and therefore have been subject to many investigations. Due to this fact, it has become important to find the best way to identify, and successfully cultivate them, so that they can be worked with.

Magnetic Activated Cell Sorting (MACS) technology, has been found useful for the correct identification and culture of numerous and varied tissues and cells such as T, B, NK, epithelial, and endothelial cells, among others;even stem cells have already been separated before, but never from human dental pulp. Due to the fact that stem-cell processing needs to be as time and cost efficient as possible, we find that MACS technology is a great window of possibility.

The current outlook in tissue engineeringtoday, is the use of stem cells isolated from tissues present in adults, in order todevelop later on, the therapeutic use of autologous implants.

MATERIAL AND METHODS

The dental pulp samples were collected from the intact 3rd molar extraction of 8 patients, ages 16 -25 years. Facilitated by Dra. Belinda I. Beltran- Salinas from Faculty of Dentistry of the Universidad Autónoma de Nuevo Leon / Ministry of Health, they were all recollected the morning they were to be processed.After extraction of the dental pulps, the procedure followed is as follows. Add 4.75 mL of PBs in a GentleMACS C tube and insert it in the GentleMACS instrument ensuring the sample is in touch with the tube vanes. Run either program A or B. Remove the tube and digest the pulps in a 3mg/mL collagenase I and 4mg/mL dispase solution. Incubate for 1 hr at 37°C in a water bath. Once incubated, reintroduce the tube on the GentleMACS and run programs B or C.

After tissue was disgregated, stem cells were isolated from all other tissue cells using the magnetic separation technique *(MiltenyiBiotec),* in which CD271 and CD44 monoclonal antibodies were used.

RESULTS

The cells were observed in an inverted microscope finding elongated, flattened, and fibroblastic dental pulp stem-cell morphologies. Subsequently, by means of flow cytometry, these cells were quantified and presented positive results for both markers, CD271 *(0.6)* and CD44*(95.7 + -3.8)* in 10,000 cells.

Methodological optimization of the extraction of outer membrane subproteome in *Vibrio harveyi*

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In gram-negative bacteria, outer membrane proteins (OMPs) play a key role in the adaptation to changes in external environments (5). Thus, OMPs profile of *Escherichia coli* changes during the transition to the viable but nonculturable state (3) or, in the case of *Vibrio alginolyticus,* by effect of sodium concentrations (5). Furthermore, the development of rapid and simple techniques for the detection of specific microorganisms is sometimes based on immunological methods (4). In this sense, Kimata *et al.* (1) reported the suitability of OMPs for detection of *Pseudomonas* in seawater by these methods.

One of the most laborious steps in the proteome study is the analysis and comparison of the 2D-PAGE gels. Often poor 2-D results are obtained due to high conductivity or high levels of interfering substances. In order to improve 2-D results there are currently several commercially available kits and solutions, such as 2-D-Clean-Up kit (GE Healthcare) to remove interfering substances or DeStreak Rehydration Solution (GE Healthcare) to reduce streak between spots.

In this study, to prove the suitability of these solutions in the study of OMPs, we used stationary-phase cultures of *V. harveyi* Spanish Type Culture Collection (STCC) 525 and *E. coli* STCC 416. OMPs of these bacteria were extracted according the method proposed by Molloy *et al.* (2). After extraction, and according to manufacturer's instructions, we proved the 2-D-Clean-Up kit and the DeStreak Rehydration Solution and compared the protein profiles by SDS-PAGE.

Our results indicate that the kits clean preparations effectively and simplify the spot pattern. So, after the determination of the OM subproteome, they can be appropriate for the isolation of specific spots. However, we have observed that some spots are lost during the process and protein expression varies in some cases. Consequently, these solutions could not be appropriate for comparative studies of the subproteomes.

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Optimization and standardization of sample preparation with the Bead-beating technology in microbiology studies

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In the context of sample preparation and cell lysis, Bertin Technologies (France) has developed a technology dedicated to the homogenization and grinding of soft to hard materials. The goal is to improve the first critical step in any molecular biology process and follow the latest requirements of analysis equipments which have radically improved in terms of throughput, reproducibility, detection limits and linearity.

Following specific mechanical engineering studies of bead beating technology, a high speed figure-8 multidirectional motion gives shaking energy to the beads that grind/homogenize samples in 2mL and 7mL sealed vials. This solution plays a large part in the analyze chain of rapid method to extract and detect or quantify DNA, RNA, proteins, drugs or biomarkers. Thanks to Cryolys option, temperature inside Precellys24 tubes is maintained at an optimal level during homogenization. Cryolys technology permits temperature-sensitive molecules to keep their native state for any analysis.

Bertin and its partners have been investigating mechanical lysis with the Precellys or Minilys bead beater vs. manual, chemical or sonicator methods. Several applications on proteins, nucleic acids or biomarkers extraction, from animal, plant tissues, soil or cells illustrate the contribution of this equipment to the improvement of life's sciences analysis and particularly in microbiological studies (medical microbiology; gene expression; environmental, marine, aquatic microbiology; geo-microbiology; soil, forest microbiology, food microbiology…).

Bead beating technology was successfully evaluated in these applications and satisfied users in term of efficiency without degradation of the material, reproducibility, time and labor saving that are mains items to considering.

Keywords sample preparation, standardisation, homogenizer, bead beating, Precellys, protein, nucleic acid,

Plasmid copy number quantification by spectrofluorometry

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Fluorescence emission by green fluorescence protein (GFP) can be used to quantify the number of copies of a plasmid (Lobner-Olesen, EMBO J. 1999; 18:1712). In this work we have determined the plasmid copy number (pcn) for different plasmids carrying the construction *pBAD-GFPmut2*. We have analysed plasmids with control of replication by iterons, such as F, P1 and pSC101; and plasmids controlled by antisense RNA, such as R1 and the members of the ColE1 plasmid family p15A and pBR322. We have determined the pcn in MC1000 *Escherichia coli* strain carrying the different plasmids, in cultures exponentially growing at 30ºC, 37ºC and 41ºC. To quantify GFP fluorescence we have tested two methods: spectrofluorometry (plasmids/mass) and flow cytometry (plasmids/cell).

The pcn has been quantified as the average fluorescence intensity obtained in at least three different experiments. To obtain relative values, the copy number of F plasmid was set to 1. The pcn values obtained at 37ºC, relative to F, by spectrofluorometry were 5.53 for P1, 3.72 for pSC101, 1.62 for R1, 18.13 for p15A and 27.58 for pBR322; and by flow cytometry 7.42 for P1, 4.10 for pSC101, 2.07 for R1, 40.02 for p15A and 92.33 for pBR322.

In order to determine the more accurate method to quantify the pcn, these results have been compared to data obtained by 'Southern blot' (Lobner-Olesen, EMBO J. 1999; 18:1712). The differences found ranged from 6 to 17% when comparing spectrofluorometry to Southern, and from 8 to 291% comparing flow cytometry to Southern**.** Considering the pcn determined from cultures growing at 30ºC, 37ºC and 41ºC, this parameter seems to be dependent on culture growth rate. For every plasmid, the faster the growth rate, the higher the pcn. The linear regression analysis of the pcn at different temperatures revealed a better adjustment of spectrofluorometry data than the one from flow cytometry. These facts support that spectrofluorometry is a simpler and more reliable method than flow cytometry to quantify the pcn.

Keywords plasmid copy number; pcn; GFP expression; spectrofluorometry; flow cytometry

Prediction of type A trichothecene accumulation in wheat grain contaminated with *Fusarium sporotrichioides* using neural networks

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Fusarium species are important plant pathogens causing diseases such as crown rot, head blight, scab on cereal grains and vascular wilts on a wide range of crops with the dominant species depending on the area and the type of the crop. Various *Fusarium* species produce mycotoxins. *F. sporotrichioides, F. poae, F. langsethiae, F. equiseti, F. graminearum* and *F. culmorum* are considered the most toxic *Fusarium* species in animal feed stuffs and the increasing incidence of mycotoxins in grain, along with the highly toxic nature of these mycotoxins are of particular concern to inter-governmental trade organizations, such as the European Union.

Trichothecenes are further grouped in two main types depending on the absence (type A trichothecenes: T-2 toxin, HT-2 toxin, diacetoxyscirpenol) or presence of a keto group (type B trichothecenes: deoxynivalenol and nivalenol) at the C-8 position. Most of these mycotoxins represent a serious health threat to animal and human consumers, causing a wide range of symptoms varying in severity.

Production of T-2 and HT-2 has been well documented in two species in particular, *F. sporotrichioides* and *F. langsethiae*. These mycotoxins are commonly found in various cereal crops (wheat, maize, barley, oats, and rye) and processed grains (malt, beer and bread).The general toxicity, haematotoxicity and immunotoxicity of T-2 and HT2 toxin are considered to be the critical effects. The production of mycotoxins in crops is influenced by different factors including water activity (a_w), extent of infection, temperature, substrate, microbial interactions and fungal isolates.

The aim of the present task was the application of predictive models based on neural networks (NN) to assess the levels attained by the sum of T-2 and HT-2 toxins in wheat grain inoculated with a strain of *F. sporotrichioides*. To accomplish this purpose a factorial design was carried out involving three temperatures, three aw values and three different levels of inoculum. The cultures were further analyzed for both toxins at selected days by LC. The results were summed and a dataset containing the values of the variables linked to the environment of cultures (temperature and aw), size of the inoculum (diameter of the inoculating disk from a culture of the strain in petri dish), incubation time together the sum of the concentrations reached by both toxins in cultures was used to train, validate and test different models. They were multilayer perceptrons (MLP-NN) with 1-2 hidden layers and radial basis function networks (RBFN). Within the dataset the four independent variables were used as inputs while the sum of toxin levels was the output. Various algorithms and validation techniques were tried to train the MLPs.

The MLP-NN provided the best prediction ability on the basis of low mean square error (MSE) and high determination coefficient (R^2) between predicted and observed (target) levels of T2 plus HT2. Single and double hidden-layer perceptrons proved useful but the former were more straightforward and the MSE was slightly lower. RBFN can give similar results to the best MLP when a high number of nodes are employed in the hidden layer and computational time is relatively lower. Thus, these models can be useful to predict the concentrations of both trichothecenes in wheat contaminated with *F. sporotrichioides*.

Keywords predictive models; climate change; type A trichothecenes; wheat

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Production of Recombinant Dog Sperm Protein Izumo as an Immunogenic Antigen

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Production of purified and sufficient proteins is necessary to investigate the role and function of them. Use of the bacteria are the most efficient way to produce a large amount of proteins.

In this study, E. coli BL21 was used to express recombinant dog Izumo (dIzumo) protein to determine if it can use as a potential immunogenic antigen.

After designing specific primers and polymerase chain reaction (PCR), Izumo gene was amplified and after purification, it was sub-cloned in plasmid expression vector pqe60. Then pqe60-Izumo was transferred to E. coli BL21and Protein generation induced by IPTG. The recombinant protein was purified and its concentration was assayed by Bradford method. Western-Blot analysis was run after that.

The recombinant dIzumo was successfully produced. Rabbits inoculated with polyA-dIzumo developed a specific serum antibody and the highest antibody titer lasted at least 6 weeks. Cellular immunity was increased after this period.

Because of the increase in the level of specific antibody and number of T-cells in the injected rabbit' s blood, It can be concluded that Izumo can be immunogenic protein in dog and accordingly may promote the immune system against sperm in both male and female dogs.

Keywords Izumo; E. coli BL21; immunogenic, sperm

Rapid and sensitive detection of *Giardia* cysts and *Cryptosporidium* oocysts from glass slides

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In humans, giardiasis is a common cause of parasitic gastroenteritis and is a major health concern worldwide. The recognition of *Cryptosporidium* as an important pathogen and the global increase in immunocompromised populations have resulted in a strong demand for sensitive and reliable detection and typing systems for both clinical and environmental samples. Frequently, *Cryptospiridium* oocysts and *Giardia* cysts occur at low densities in water. The application of molecular techniques for species identification and genotyping from glass slides has the potential to provide detailed epidemiological information on the cryptosporidia in circulation in each water catchment. However, until recently, few procedures were sensitive and accurate enough to recover low numbers of oocysts (Sunnotel et al., 2006; Robertson et al., 2009; Ruecker et al. 2005; Nichols et al., 2006, Amar et al. 2001) and cysts (Amar et al. 2002) fixed to glass slides. This study reports the comparison of different published protocols for DNA extraction from oo(cysts) fixed to glass slides and subjected to PCR and real-time PCR (qPCR) for species and genotype identification.

DNA extraction from oo(cysts) positive slides: *C. parvum* oocysts (bovine, Iowa isolate) and *G. lamblia* (H3 strain) cysts were purchased from Waterborne. The cover-slip from each slide was carefully removed and retained, top-side down 25 µl aliquots of AL or ATL lysis buffers or 10-50 µl aliquots of TE lysis buffer were added, respectively to the slide wells. The wells were carefully and gently scraped using a sterile scalpel blade. The buffer and scrapings were then pipetted into a microcentrifuge tube. This process was repeated 2-4 times, collecting the buffers and scrapings into the appropriate tube for each slide. The cover-slip was replaced onto the slide which was then re-screened. A freeze-thaw protocol (Nichols *et al*., 2003) and a heat protocol (Robertson *et al.* 2009) for DNA extraction from oocysts and cysts were compared. The DNA was isolated from cysts and oocysts using the QIAamp DNA minikit. PCR assays: PCR was conducted of the *Cryptosporidium* SSU rRNA gene and *Giardia* β-giardin gene using published primers. qPCR and multiplex qPCR assays: primers and probes for *Cryptosporidium* and *Giardia* detection were adopted from a previously reported qPCR method targeting a 151-bp region of the COWP gene and 74-bp region of the β-giardin gene, respectively. Quantitative PCR and data analysis were performed with the Light-Cycler-2.0 PCR system.

The scraping method removed nearly all oocysts and cysts from the slide well surface. Our results showed that any remaining mounting medium after removed the coverslips from the enumerated slides had no noticeable inhibitory effects on the detection of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts by qPCR, as indicated by similar threshold cycle (Ct) values. The Ct values for ATL, AL and TE lysis buffers showed similar threshold cycle (Ct) values as indicated by the presence of a PCR product of the expected size with band intensity similar. Quantifying and genotyping oocysts and cysts from a single sample not only reduces overall costs but also eliminates the disparity between duplicate samples caused by the random distribution of small number of oocysts and cysts in water.

In conclusion, in this work we show that the application of the protocols assayed could be used to detect *Cryptosporidium* oocysts and *Giardia* cysts from stored glass slides in public health and water service laboratories, helping to identify and trace pathogens and their routes of infection.

Keywords *Cryptosporidium*, *Giardia*, qPCR, glass slides, PCR, multiplex qPCR

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Separation of low number of microorganisms from real samples by capillary electromigration techniques with UV detection and MALDI-TOF MS

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The identification of phenotypically similar species by the conventional laboratory methods is still time-consuming and often unsufficient for ensuring early targeted therapy. As an example may be *Candida* species which are the second most common yeast species isolated from bloodstream infections and they belong to the important nosocomial pathogens. Moreover, manifestations of candidaemia are associated with the formation of biofilm on host tissue or indwelling medical devices. The differences in the physico-chemical properties of the cell surfaces, e.g., the values of the cell surfaces charge, may result in different electromigration behavior. Capillary isoelectric focusing and capillary electrophoresis could be appropriate tools for the efficient separation of the phenotypically similar microorganisms according to their isoelectric points, p*I*'s, or mobilities and for improvement of their characterization.

Simultaneously, the analysis of low number of microorganisms from real biological samples requires sample preparation step including a concentrating of microorganisms from large sample volumes with the high and reproducible efficiency. The electromigration techniques have a great potential to include the pre-concentration, separation and detection of the whole cells and therefore they can rapidly indicate a presence of risk pathogens.

In our experiments common etiological agents of CNS and nosocomial infections, *Candida* species, *S. epidermidis*, *E. coli*, *St. aureus,* were selected as model bioparticles. All microorganisms icluding biofilm-positive and biofilm-negative strains of *Candida* species were separated according to their isoelectric points. The coupling of the filtration cartridge with the separation capillary improved the detection limit of the isoelectric focusing with the UV-detection by at least five orders of magnitude down to tens detected cells in 1 ml of the real biological sample. For better identification some results were supplemented by MALDI-TOF MS fingerprints spectra.

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Keywords capillary electromigration techniques; microorganisms

Study of indirect RABIT curves fitted with modified Gompertz equations and correlation with relevant microbial parameters

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Background – The indirect Rapid Automated Bacterial Impedance Technique (RABIT) is an easy-to-use and fast technique used to enumerate microorganisms in environmental samples and to perform inhibition tests. Microbial growth is monitored by trapping CO_2 in a KOH/agar-bridge where conductivity is measured and a negative shaped growth curve will be observed. The Time To Detection (TTD) corresponds to the point where the conductance variation rate shows a predetermined deviation from the base line. The correlation between TTD and the initial number of cells may be used for calibration. However, if a mathematical best fit is performed on the raw data, microbial parameters, i.e. total change, lag phase and slope can be calculated more accurately. Although the classic Gompertz model makes a good estimation of these parameters, it has been observed that RABIT signals with long lag phases affect the accuracy of the model.

Objectives – The first goal of this study was to propose two modified Gompertz equations, taking the linear start of the curve into account. These mathematical fits should result in more accurate and microbiologically relevant estimates, i.e. lag phase and slope. The novel fit of the RABIT curve combines a linear or exponential function with the original Gompertz equation. The second goal was to observe whether the linear start of the curves, obtained by the indirect RABIT, could be compared with curves from more classic measurements, i.e. plate count, concentration of metabolized glucose and produced ethanol.

Methods – The two alternative equations were compared with the Gompertz curve described by Zwietering *et al.* (1990) using statistical criteria such as the sum of squares (SS), adjusted coefficient of determination (R^2), Akaike Information Criterion (AIC) and Schwarz Bayesian Information Criterion (BIC). The equations were tested on data sets obtained with indirect RABIT measurements on all four statistical criteria. These data sets were acquired for a fungus (*Trichoderma*), yeast (*Saccharomyces cerevisiae)* and bacterium (*Pseudomonas putida)*. Both the statistical assay and the non-linear modelling were performed using R (version 2.12.1). To observe if indirect RABIT measurements correlated with more classic measurements, a RABIT experiment was performed with *S. cerevisiae*. At different points in time during the experiment, both the microbial growth and ethanol concentration were measured. The microbial growth was measured by plating the yeast on Potato Dextrose Agar, produced ethanol concentrations were measured using head-space GC-FID.

Results and conclusions – The statistical analysis revealed that the model described by Zwietering *et al*. (1990) is the least best fit. Based on the observed data, the approach of Zwietering *et al.* (1990) underestimates parameters (except the total change which is overestimated). It is difficult to state which of the alternative fits is preferred, because a significant difference based on statistical assays was not noticed. In case of fast growing organisms, all three models are equally good, as no significant discrimination could be made. Keeping this in mind, it can be stated that a curve with a long linear onset influences the estimation of parameters. The use of the alternative approaches can help to reduce this problem by including a linear or exponential section in the equation. The linear onset was not observed by increasing ethanol concentrations over time. The observed lag phase of the RABIT curve, due to CO_2 production, generates a faster detection time. Altogether it can be concluded that the indirect RABIT method remains a very useful bio-assay for fast microbial detection, more accurate values for lag phase and slope are obtained when the technique is used in combination with the new proposed equations.

Keywords Modelling; Impedance Technique; Microbial growth curve

The potential of calorimetry for real-time monitoring of anaerobic bioprocesses shown at the example of aceton-butanol fermentation

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In search for real-time monitoring tools for anaerobic bioprocesses we looked at the potential of calorimetry. Changes in stoichiometry and kinetic of bioprocesses are reflected by heat production rates in real-time. The combination with other monitoring tools either on-line (e.g. gas emmisions, pH etc.) or off-line (intermediates) allows the separation of stoichiometric and kinetic information using thermokinetic models. Forming the enthalpy balance of a reactor allows the determination of the metabolic heat production rate in an easy way (von Stockar and Marison, 1991). This approach was already successfully demonstrated at different scales for aerobic bioprocesses (van Kleeff et al., 1996; Voisard et al., 2002; Türker, 2003; Schubert et al., 2007; Biener et al., 2010) and it's principle should also be applicable to anaerobic digestion processes. Since with any scale-up the ratio of the heat producing volume to the heat exchanging surfaces increases and therefore the accuracy of the heat measurement improves this calorimetric principle might be particularly advantageous for anaerobic bioprocesses as they are typically performed in large tanks.

For exploring the information content of the calorimetric signal the heat production rate as well as the gas emission and pH shifts of *Clostridium acetobutylicum* was monitored in real time. This test system was chosen because it has been used for production of butanol and aceton for decades. Thus, being a well-studied bacterium some information on kinetics, stoichiometry, genetics, and proteomics are available from literature.

For this study Clostridium acetobutylicum (ATTC 824) was cultivated in a bench-scale reaction calorimeter Mettler Toledo BioRC1 at 37°C on a minimal synthetic growth medium under anaerobic conditions. Heat production rate was analyzed and compared with conventionally derived growth kinetics and product formation during acetogenic and solventogenic metabolic phases. Data for pH, redox potential and gas production were logged online. For offline analysis of substrate consumption and product formation samples were taken from fermentation broth and exhaust gases. All of the data were thermokinetically modeled. A similar experiment was done using a thermal sensor (i.e. a flow-through microcalorimeter) at line. The experimental data show the interrelation between gas emission, pH shift, metabolic pattern and heat production rate. Correlations between these parameters are currently analyzed in more detail and will be presented in time.

Keywords biotehrmodynamics; calorimetry; anerobic bioprocesses; aceton-butanol fermentation; clostridium acetobutylicum

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The QCM detection of *Bacillus atrophaeus* spores enhanced by magnetic particles

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Detection of bio-aerosols is very important especially in relation to the threat of attacks using biological warfare agents (BWA). The possibility for using anthrax spores as a BWA is quite high, as was shown in the attacks in 2001. *The anthrax spores are* serious threat for people health and security. The early detection is thus extremely relevant for the immediate warning and deployment of treatment. The detection of BWA in the form of aerosols is challenging due to the low concentration of microbes; usually, a suitable air-sampling system - cyclone is used to collect all particles from air into a small volume of liquid. The main goal of this study is improvement of limit detection by further preconcentration of target agents with using magnetic (nano)particles (MPs).

Three kinds of imunospecific MPs were conjugated with polyclonal antibodies and were compared. The MPs were used for preconcentration of diluted spore (*Bacillus atrophaeus*) suspension (model of aerosolised spores). The MPs bound to the secondary antibodies were added into the suspension of spores and magnetically separated. Utility of this procedure was examined by the quartz crystal microbalance (QCM) based immunosensor. The QCM is relatively simple and highly sensitive device. The antibodies immobilised on its surface make QCM highly sensitive to the antigen (spores in this case). The antigens interact with antibodies on the surface and increase the mass loaded on the surface which is equal to decreasing frequency of the QCM resonator. Simultaneously, MPs amplified the negative shift in QCM frequency. The responses were compared with direct measurements of the same suspension concentrations.

Keywords QCM (Quartz Crystal Microbalance); magnetic nanoparticles; *Bacillus atrophaeus*; biosensor; bio-aerosols

Microbial Physiology, Metabolism and Gene Expression

Activity of selected groups of microorganisms in aerobic granular sludge during SBR cycles

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The activity of microorganisms involved in nitrogen conversions was investigated in three constantly aerated column SBRs with granular sludge treating anaerobic sludge digester supernatant. The SBRs differed in the length of a working cycle that was 6 h, 8 h and 12 h in reactors 1, 2 and 3, respectively. Total Kjeldahl nitrogen and COD concentrations in the supernatant averaged 570 mg N/L and 1500 mg O_2/L. The biomass samples were collected during SBR cycles in 0.5-2-hour intervals in the period of stable reactor performance. Isolated RNA was reverse transcribed and the cDNA obtained was used for relative quantification Real-Time PCR. Total bacteria (16S rRNA), ammonia-oxidizing bacteria (*amoA* gene), denitrifiers' (*nosZ* gene) and Anammox bacteria (16S rRNA) activities were analyzed.

The expression of *amoA* mRNA in all reactors was high at the beginning of aeration, however, it lowered 0.3-4-times after 1 hour to gradually increase and reach a maximum after 4-6 hours of aeration. The fluctuations of expression were more pronounced in reactors with the shorter SBR cycle lengths (6 and 8 h) and the highest level of *amoA* mRNA correlated with the highest concentration of nitrites in the aeration cycle. In all reactors, the constant presence of *nosZ* mRNA was noted with the highest values 0.5 h after the beginning of the SBR cycle. This probably resulted from the introduction of wastewater rich in organic carbon used for denitrification and from a periodical lowering of dissolved oxygen concentration due to excessive biochemical conversions of introduced substrates. The level of Anammox bacteria 16S rRNA, regardless of the cycle length, was similar in all reactors and did not undergo fluctuations connected with the substrate availability. The experiments showed that the three-dimensional structure of aerobic granules enables a large number of microenvironments to evolve that, in spite of constant aeration, allow for simultaneous nitrification, denitrification and Anammox in a single reactor.

Keywords aerobic granular sludge; real-time PCR, microbial activity, RNA

Analysis of interaction between GbdR, a choline metabolism regulator, with DNA and RNA polymerase

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Many genes encoding proteins involved in the pathogenesis of *Pseudomonas aeruginosa* such as phospholipase C and phosphorylcholine phosphatase are regulated by GbdR, a protein encoded by the gene PA5380. This regulator also activates the genes of choline catabolism. In this work we studied the relationship between structure and function of *P. aeruginosa* GbdR. For this, *gbdR* was cloned, the recombinant protein was obtained and the interaction with DNA was demonstrated by gel shift assays.

GbdR is homologue with MarA, a transcriptional activator of the AraC family that activates at least 24 promoters of *E. coli*. The structure of MarA was determined by NMR in complex with a synthetic DNA and the C-terminal domain of the α subunit of RNA polymerase (α-CTD-RNAP) (PDB: 1XS9).

Using threading techniques, we have modeled *P. aeruginosa* GbdR using as template the atomic coordinates of MarA of *E. coli* (PDB:1XS9) for the C-terminal domain and, the protein Atu0886 from *A. tumefasciens* for N-terminal domain. The obtained model, evaluated using the assessment tools available in Swiss-Model workspace, indicated the presence of two DNA binding motifs helix-turn-helix (HTH), suggesting that these are the critical regions of GbdR that interact with DNA.

To understand the function of the DNA-regulator-polymerase complex, the α-CTD domain of *P. aeruginosa* RpoA was also modeled by comparative modeling in Swiss-Model workspace using atomic coordinated of the same protein of *E. coli* (PDB:1XS9). The complex α-CTD-RNAP-GbdR-DNA was obtained and we suggest that the two α-helix of GbdR that interact with DNA are: ^{254}RRQLERLFQKYL^{265} and ^{304}TPHFSKCYREYF^{315}. In addition, we also propose that the regions interacting in the protein-protein interface are: ^{19}V, ^{20}R, ^{23}N, ^{24}C, ^{49}N, and ^{84}D for RNAP; ^{219}L, ^{228}E, ^{231}A, ^{232}L, and ^{235}A for GbdR.

In conclusion, we were able to identify the residues of GbdR that might be involved in the interaction with DNA and with RNAP. So, with all these bioinformatic results we have opened a way to discover the relevance of these regions which will be tested experimentally by site-directed mutations.

Keywords: *Pseudomonas aeruginosa,* GbdR regulator, choline, structure-function.

Approach to the analysis of the biological role performed by the laccase produced by *Streptomyces cyaneus* CECT 3335

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Laccases (EC 1.10.3.2) are widely distributed in nature but most of the existing knowledge about these enzymes arises from the study of ligninolytic system of the white-rot fungi. Its role in these microorganisms seems clear enough and laccase production is a common feature among these lignin degrading organisms.

In recent years it has been shown that these enzymes are also present in a few bacteria such as Azospirillum lipoferum Marinomonas mediterranea, Bacillus subtilis, Myxococcus xanthus, Streptomyces griseus, Streptomyces cyaneus and Streptomyces ipomoea. The study of the biological function of these bacterial laccases has been done in different contexts and its function has been implicated in processes of mobility (Azospirillum), sporulation (Bacillus), copper resistance (Myxococcus) and within the Streptomyces genus, in sporulation and morphogenesis processes and in the case of laccase S. cyaneus in lignin degradation.

This work deals on the study of the possible biological role of the laccase produced by the *Streptomyces cyaneus* CECT 3335, both in lignin degradation and/or its cycle of differentiation.

To this end, a mutant strain for laccase activity was obtained (*S. cyaneus* SclA$^-$) and a comparative study of the morphological features along the time course of growth between wild-type strain and mutant strain was carried out. On the other hand, the ability to degrade a commercial lignin (AT Indulin) was also tested for both strains.

Mutagenesis of the gene that encodes for the laccse of *S. cyaneus* is carried out using the technique of gene disruption. Thus, a fragment of the gene was amplified and cloned in the vector pOJ260. The final construction (pOJ260-SclA) was used to combine spores of S. *cyaneus* wild-type with *Escherichia coli* ET12567 (pUB307). The mutants were selected for their resistance to the antibiotic Apramicin, conferred by the plasmid pOJ260. The absence of laccase activity was verified through the cultivation of the mutant SclA$^-$ in Manitol-Soy medium supplemented with Apramicin and measuring the activity daily against 2, 2´-azinobis [3-etilbenzotiazolin-6-sulfonic] (ABTS). As expected, no laccase activity was detected neither in solid nor liquid MS medium in *S. cyaneus* SclA$^-$ mutant. In addition, to check the presence of the mutation at a molecular level, a Southern Blot was perform using chromosomal DNA of both strains and as probe, the pOJ260-SclA construction used in the experiments of conjugation. Thus, in the chromosomal DNA, the *sclA* gene could be detected in a hybridation band of 2.3 kb in *S. cyaneus* wild type; while in the mutant strain was detected in a band of approximately 8 kb, indicating the inclusion of the construction in the chromosomal DNA by single crossover.

Once obtained the mutant strain, a morphological study of the cycle of development of both strains was carried out through the observation under optical and the scanning electron microscopes along the time course of growth. No significant differences in mycelial development or in the process of sporulation were detected but the lack of color in the spores of the mutant strain.

Finally, both strains were assayed to show up their lignin degradation ability using a commercial kraft lignin (indulin AT) in a minimal salt medium supplemented with glycerol and ammonium sulphate. A higher degradation degree of the polymeric lignin was found in the cultures of wild-type strain, although a significant reduction in the lignin content was also detected in the laccase-lacking strain compared with the control. Although these results can be considered as preliminary, they point to lacasa produced by *S. cyaneus*, to have an important role in lignin degradation.

Keywords *Streptomyces*; laccase; biological function;

Auxotrophic markers enhance the growth deficiencies of *Saccharomyces cerevisiae* BY4741*Δmnn9* in standard YPD medium

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Saccharomyces cerevisiae strains with different combinations of auxotrophic markers are commonly used in genetic, molecular biology, and physiological studies as well as in biotechnological applications. Some researchers have warned of the risks of habitual use of auxotrophic strains of *S. cerevisiae* (reviewed by Pronk 2002). These genetic modifications can often cause side effects, so that the results obtained with such strains growing in minimal media, supplemented with the complementing compounds, may not be comparable to those obtained with their prototrophic counterparts. However, conventional complex media have routinely been used to grow auxotrophic strains, it being assumed that its components provide adequate quantities of the relevant nutrients to mask the nutritional deficiencies of auxotrophic strains. In a previous work (Corbacho *et al.,*2011) we compared the growth parameters of *Saccharomyces cerevisiae* (S288C) and its derived auxotrophic strains FY1679-14C and BY4741 in standard YPD medium, and we found that the biomass production of both auxotrophic strains was markedly lower than that of the prototrophic one.

In this work we analyze how the presence of auxotrophic markers can affect the growth of *S. cerevisiae Δmnn9* mutant strain, growing in YPD, compared to its wild-type isogenic auxotrophic strain BY4741 (both from EUROSCARF) and their wild-type protrotophic strain S288C (ATCC). We choose this mutant strain because the mnn9 mutation makes a truncated outer chain in the N-oligosaccharides, and affects cell wall building and growth. The strains were grown in aerobic bath cultures in YPD, and growth parameters were determined. The maximum specific growth rate of BY4741 (*Δmnn9*) fell until 50 or 44% when compared to BY4741 or S288C respectively. With respect to the biomass production, the BY4741 (*Δmnn9*) biomass level at the stationary phase, decreased only 20% compared to BY4741, but the decrease was 55% when compared to its prototrophic counterpart. This data indicate that the poor growth exhibited by BY4741*(Δmnn9)* seems not to be attributable only to the mnn9 mutation. The confirmation of this suggestion was obtained by comparing the growth parameters of another *S.cerevisiae mnn9* mutant strain derived from X2180 (Rachke *et al*., 1973) with those of its parental wild-type strain. Both strains are prototrophic and were grown in YPD in the same conditions than the aforementioned strains. The difference between the growth parameters of both strains were 10% for the maximum specific growth rate and almost 30% for biomass production. Both differences were notably lower than those displayed by the auxotrophic mnn9Δ strain. These results seem to confirm the negative effect that auxotrophic markers exert on the auxotrophic strains growth potential, and how this effect is more evident when the auxotrophic strain carry the mnn9 mutation.

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Keywords: *Saccharomyces cerevisiae*, auxotrophic strains, growth deficiencies, growth rate; biomass production.

Biochemical and morphological studies of yeast *Rhodotorula rubra*. Sensitivity to cyanide, antimycin A, salicylhydroxamic acid, and to antibiotics

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Rhodotorula rubra (*Rhodotorula mucilaginosa*), a cryptococcacae, was considered for a long time as a common contaminant without human health risk factor. However since 1960 it had been reported as pathogen for human and related to increase in infections in immunodeficiency syndrome and in cancer treated by immunosuppressant chemotherapy [1]. Many other cases of infection associated with *R. rubra* have been reported, such as catheters-related, endocarditis, peritonitis, meningitis and endophthalmitis. In this study we show that *R. rubra* exhibits two electron transfer pathways, the cytochrome c oxidase pathway and the alternative cyanide and antimycin A insensitive oxidase (AOX) pathway. Furthermore, not only do cyanide and antimycin A fail to inhibit cell respiration in *R. rubra*, they also enhance it.

R. rubra was cultured in salt synthetic medium with glucose as substrate. Samples were removed at various intervals (4 to 48 h) during culture, for biochemical and electron microscopy studies. The dithionite reduced-minus-air oxidized difference absorption spectrum of cultured *R. rubra* showed the characteristics of a typical eukaryotic respiratory chain spectrum. Absorption bands due to, respectively, cytochrome c oxidase at 601 nm, two cytochromes b with maxima at 560 and 564 nm, cytochrome c1 at 554 nm and cytochrome c at 548 nm, were observed. The difference absorption spectrum of *R. rubra* treated with antimycin A did not exhibit the absorption bands due to cytochromes b while the absorption bands due to cytochromes c and c1 and cytochrome c oxidase were fully present. Polarographic studies showed that addition of KCN (1 to 3 mM) to *R. rubra* induced a variable enhancement of cell respiration which could reach 200% of the control value. Antimycin A (8 to 25 μM) also produced a progressive enhancement of respiration with the highest value of 140%. The enhancement of respiration with cyanide and antimycin A was inhibited by 8.3 mM salicylhydroxamic acid.

The sensitivity of *R. rubra* towards 9 antibiotics was tested by culturing the cells in Petri dishes, on agar medium containing various concentrations of the antibiotics. Colony growth was totally inhibited only in the presence of 100 μg/ml cycloheximide, 50 μg/ml amphotericin B, or 3 μg/ml nystatin.

Thin section electron microscopy showed that *R. rubra* cells were oval (2.1 ± 0.2 x 2.7 ± 0.4 μm diameter) or round (2.3 ± 0.2 μm diameter), dividing by budding. The nucleus was generally round (1.15 ± 0.15 μm diameter), occasionally polyhedric, and surrounded by a nuclear membrane. In the cytoplasm, 1-3 mitochondria (0.3 ± 0.04 x 0.42 ± 0.03 μm diameter) with abundant cristae were seen. The glycogen granules distribution in the cytoplasm was variable. In some cell sections, few glycogen particles were observed, whereas in other cells they were abundant. Roughly two granule populations, one of small granules (0.25 ± 0.1 μm diameter) and one of larger granules (0.8 ± 0.08 μm diameter) with electron dense material, presumably due to carotenoid pigments, were also observed. Peroxisomes were rarely seen. The cytoplasm was surrounded by a plasma membrane and covered with a cell wall.

The AOX is present in the mitochondria of plants and of some fungi and protozoa [2]. Moreover, cyanide-resistant respiration is considered to be a very frequent metabolic pathway in yeast [3]. The electron transport through AOX is not associated with energy conservation, the change in redox potential being lost as heat [4]. A quinol oxidizing protein containing a binuclear iron center, reduces oxygen to water [4]. Our results showed that in the presence of cyanide and antimycin A, cell respiration was significantly enhanced. This may suggest that, in *R. Rubra*, AOX biosynthesis was enhanced whenever necessary to meet the cell's metabolic requirements.

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Keywords mitochondria; cyanide; antimycin A; alternative respiratory chain; electron microscopy; *Rhodotorula rubra*

Carotenogenesis induction with hydrogen peroxide in *Xanthophyllomyces dendrorhous* colored mutants

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Xanthophyllomyces dendrorhous is a dimorphic yeast that synthesizes the oxygenated carotenoid astaxanthin as the most representative xanthophyll derivative. This compound displays antioxidant properties and its production has been induced and stimulated via reactive oxygen species (ROS), as it has been observed in several microorganisms.

The biosynthesis of carotenoids in this yeast occurs through condensation of two geranylgeranyl pyrophosphate (GGPP) molecules to form phytoene. Introduction of four double bonds in phytoene molecule forms lycopene, followed by cyclization of acyclic ends to give β-carotene, whose oxygenation of the β-rings yields astaxanthin. Given that detailed mechanisms for carotenogenesis in *X. dendrorhous* have not been elucidated, modifications of carotenoid content in mutants with different metabolic profiles allow identification of important biosynthetic steps in this microorganism.

Oxidative stress was induced by H_2O_2 in four mutants of *X. dendrorhous*: R4 (red, astaxanthin hyperproducer); W10 (white, accumulates phytoene); Y21 (yellow, accumulates β-carotene) and P26 (pink, accumulates monocyclic carotenoids). Concentrations of 5 or 10 mM H_2O_2 were added to YM culture medium at the beginning of growth of *X. dendrorhous*. Optical density at 660 nm was measured for yeast growth determination, while total carotenoid content was estimated by the method developed by Sedmak *et al*., (1990).

Addition of both H_2O_2concentrations prolonged the lag phase in all strains and this growth delay increased with H_2O_2 concentrations. P26, W10, and Y21 strains reached a similar growth at the stationary phase as that observed for control, while the R4 strain displayed a moderate reduction in growth, which was H_2O_2 concentration dependant (Table 1). The prolonged lag phase in the four strains may occur because oxidative stress could redirect ergosterol biosynthesis to carotenoid precursors.

H_2O_2 addition increased carotenoid production in R4 and Y21 strains, while in P26 and W10 this parameter remained constant. In *Chlorococcum* sp. it was observed that ROS induces β-carotene hydroxylase activity, which converts cantaxanthin into astaxanthin. The results reported herein, particularly those obtained with the R4 and Y21 strains, suggest that carotenogenesis induction by H_2O_2 may occur even before the β-carotene step formation. Also, astaxanthin could act as a feedback inhibitor of growth in the R4 strain of *X. dendrorhous*.

Table 1. Effect of H_2O_2 addition on growth and carotenoids production.

	Growth (OD)			Carotenoids (mg/OD)		
	H_2O_2 treatment			H_2O_2 treatment		
	0 mM	5 mM	10 mM	0 mM	5 mM	10 mM
R4	0.94	0.75	0.48	2.17	2.56	2.49
W10	0.79	0.70	0.89	8.71	7.03	7.54
Y21	0.90	0.91	0.95	0.69	0.89	0.66
P26	0.86	0.85	0.90	0.22	0.19	0.21

Keywords: astaxanthin, carotenoids, oxidative stress.

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Characterization of a new *Saccharomyces cerevisiae* dsRNA virus encoding a killer toxin with broad antifungal activity

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The new *Saccharomyces cerevisiae* killer toxin (K*lus*) killed all the previously known *S. cerevisiae* killer strains, in addition to other yeast species. The K*lus* phenotype is encoded by a medium-size dsRNA virus, ScV-M*lus,* whose genome size ranged from 2.1 to 2.3 kb. Its genome structure is similar to those of M1, M2, or M28 dsRNAs with a 5' terminal coding region followed by two internal A-rich sequences and a 3' terminal region without coding capacity. M*lus* positive strands carry *cis* acting signals at their 5' and 3' termini for transcription and replication similar to those of killer viruses. The ORF at the 5' portion codes for a putative preprotoxin with an N-terminal secretion signal, potential Kex2p/Kexlp processing sites and N-glycosylation sites. No sequence homology was found either between the M*lus* dsRNA and M1, M2, or M28 dsRNAs, or between K*lus* and K1, K2, or K28 toxins. The K*lus* amino acid sequence showed a significant degree of conservation with the host chromosomally-encoded ORF YFR020W of unknown function.

Keywords yeast; *Saccharomyces*; killer; dsRNA; toxins

Comparison of physiological profiles of halophilic microbial communities from different hypersaline area in Turkey

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The Biolog technique is one of the methods which rely on measurements of utilizing different carbon substrates by microorganisms. Measurements of substrate use enables qualifying microbial metabolic capabilities and hence functional diversity of a microbial community. The Biolog method has undoubted advantages but also many weaknesses.

In this preliminary study, three different hypersaline area in Turkey (Tuz Lake, Meke Lake and Çorum/Sungurlu Saltern) were chosen for the sampling. Brine samples were directly applied to the GN (Gram negative) and AN (Anaerobic) plates of the Biolog system. Plates were incubated 10 days at 37 ^{o}C. Measurements were performed every day during the incubation time. These three different hypersaline environments has similar results especially in GN plates but their anaerobic plates results were not similar. Culture-dependent studies has also been supporting these results.

Copper tolerance in *Marinobacter hydrocarbonoclasticus* - proteomic analysis of the periplasm

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While copper is essential in the cells as a cofactor of numerous metabolic and respiratory proteins, elevated levels are highly toxic. Copper has been related with oxidative stress, production of free radicals by Fenton reactions, binding to proteins, nucleic acids, polysaccharides and lipids, causing the displacement of native metal ions, as well as, changes in the structures and/or functions of the biomolecules and inhibition of metabolic pathways. The cells are equipped with regulatory systems that allow them to control copper concentrations. These systems protect cells from high concentrations of copper but are also able to maintain the levels required for cell development [1,2].

The study of copper homeostasis and tolerance mechanisms has gained importance due to the widespread use of copper compounds as bactericides in agriculture or as disinfectants in the food industry. Furthermore, it aids in the study of copper transport related diseases, such as the Menkens and Wilson diseases [1]. In this context, we have been investigating the molecular mechanism of copper resistance in a bacterial system, *Marinobacter hydrocarbonoclasticus*. A unique operon, *copSRXAB*, has been identified, suggesting different mechanism of copper resistance than the ones encountered in other bacteria [3].

The copper induced stress affects the cell morphology of *M. hydrocarbonoclasticus*: the cells are round-shaped instead of their characteristic rod-shaped aspect and cell wall rigidity appears to be lost. The aim of this work is the identification of the global changes on protein expression of *M. hydrocarbonoclasticus* induced by copper. For this purpose we are following a proteomic approach based on high-resolution 2D electrophoresis combined with highly sensitive mass spectrometry techniques. The bacterium cells were grown aerobically in synthetic sea water medium with different copper content (trace [1 μM] and 1 mM) and harvested at the stationary phase of the growth. The differences on protein patterns are being assessed through cross-comparison of the 2D maps of both samples using ImageMaster 7.0 software (GE Healthcare). Accordingly, 457 proteins were identified in the periplasmic proteome of *M. hydrocarbonoclasticus* cells and 415 in the copper stressed ones, on a pH 4-7 range. A statistical analysis showed that 25 proteins are up-regulated in the copper condition while 68 are down-regulated. Among the up-regulated proteins CopX was identified by the peptide mass fingerprint approach. In this way, the cellular strategies used by *M. hydrocarbonoclasticus* for copper resistance, as well as, an overview of the effects of copper stress can be attained.

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Dependence of the composition of methanogenic *Archaea* on the operational parameters of anaerobic dairy wastewater treatment

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The impact of temperature, organic loading rate (OLR) and method of heating on the methanogenic *Archaea* community during anaerobic dairy wastewater treatment was determined using DGGE and FISH techniques. The influent contained 1000 mg COD/L, heat supply into the reactors included a conventional heating method (CH) and microwave radiation (MR).

A predominance of non-methanogens in anaerobic sludge was observed since the *Bacteria:Archaea* ratio equaled 3.3-11.6. The increase in OLR from 1 kg/(m^3·d) to 2 kg/(m^3·d) resulted in the rise in the number of *Archaea* from 9.7 to 17.7% in CH reactors and from 7.5 to 10% in MR reactors. Independently of temperature and OLR, the participation of microorganisms from the family *Methanosarcinaceae* in the total number of *Archaea* was 1.1-2.2-times higher in MR reactors. Under conditions of thermophilic fermentation (55ºC) the abundance of *Methanosarcinaceae* was lower than in the mesophilic process (35ºC), however, the methane percentage in biogas did not decrease despite an almost 7-fold drop of biogas production at 55ºC. Operational parameters applied resulted in the total decline of methanogenic *Archaea* from the genus *Methanosaeta* that were present in the inoculum. This decline was probably connected with the acetate affinity of the microorganisms tested. Sequencing of the DGGE amplicons showed that *Archaea* belonged to the orders *Methanosarcinales* and *Methanomicrobiales.* The species composition in the reactors was strongly affected by the operational parameters of the treatment, with some amplicons occurring only at the specific OLR or temperature. During the mesophilic fermentation, significantly higher species diversity was noted in reactors heated by MR in comparison with CH reactors. Amplicons that grouped with the sequence of *Methanosarcina barkeri*, regardless of the influent composition, occurred only in MR reactors. The lowest species evenness characterized *Archaea* communities from the thermophilic reactors, suggesting that these communities underwent a strong selection pressure resulting in a domination of a few well adapted species.

Keywords methanogenic *Archaea*; biodiversity; microwave radiation

Ecological distribution of *Saccharomyces* killer yeasts in south-western Spain

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Among the four types of *Saccharomyces* killer yeasts already described (K1, K2, K28, and Klus), we found K2 and Klus killer yeasts in south-western Spain. The K2 yeasts were found in all the wine producing sub-areas during all the vintages analyzed, while the Klus yeasts were found in the warmest locations and mostly in the warmest vintages. The killer yeasts were present in most spontaneous fermentations. Most were K2 with Klus being the minority. The proportion of killer yeasts increased during fermentation, while the proportion of sensitive yeasts decreased. The fermentation speed, malic acid, ethanol yield, and wine organoleptic quality decreased in those fermentations where the killer yeasts replaced at least 15% of a dominant population of sensitive yeasts.

Keywords yeast; *Saccharomyces*; killer; dsRNA; toxins

Evaluation of Fermentative Performance of *Candida guilliermondii* Grown in Sugarcane Bagasse Hydrolysate Detoxified With Activated Charcoal or Vegetal Polymer

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The increasing search for ethanol fuel in order to reduce the dependence and to promote the substitution of fossil fuels will contribute to higher accumulation of sugarcane bagasse in the environment. This biomass that in Brazil is a by-product of the sugar-alcohol mills, although it has been used for the generation of energy in the sugar and alcohol production, can also be used as alternative for obtainment of xylitol, contributing to bring economical advantages for sugar-alcohol mills. In this sense, researches has been performed for the biotechnological use of sugarcane bagasse for the production of xylitol, a polyol with peculiar characteristics like its sweetener power similar to that of saccharose, non-cariogenic and indicated for diabetics and obese people, as well in the treatment of respiratory diseases and in the osteoporosis prevention. Its commercial production occurs by chemical catalysis of the xylose from the rich-xylan lignocellulosic materials, which has high cost. For the biotechnological xylitol production from these materials, initially the polymeric matrix deconstruction is necessary for separation of their main fractions: the cellulose, hemicellulose and lignin. In the case of xylitol, the fraction of interest is the hemicellulose due to be constituted mainly of pentose xylose, substrate for this bioprocess. The diluted acid hydrolysis has been commonly used in the researches for the obtainment of rich-xylose hemicellulosic hydrolysates. However, in this bioprocess there is also the release/formation of toxic compounds to the microorganisms, inhibitors of enzymatic activities like phenolics, organic acids, furfural, hidroxymethilfurfural, besides metallic ions. In this work it was evaluated the fermentative performance of *C. guilliermondii* FTI 20037 grown in hemicellulosic sugarcane bagasse hydrolysate (xylose: 77.23g/L; arabinose: 6.52g/L; glucose: 8.89g/L, total phenol: 10.73g/L) detoxified with activated charcoal or vegetal polymer. The detoxification two methodologies were: increase of pH to 7.0 with calcium oxide, followed by the decrease to 2.5 with phosphoric acid combined with the activated charcoal adsorption (1.0% w/v, 100rpm, 30min, 60°C); and vegetal polymer flocculation (15% w/v, 200rpm, 15min, 25ºC). Duplicate fermentation runs were carried out in Erlenmeyer flasks (125 mL) containing 50 mL of the fermentation media, and the yeast *Candida guilliermondii* FTI 20037 cultivated in detoxified hydrolysate (initial pH 5.5), supplemented with nutrients: $(NH_4)_2SO_4$ and $CaCl_2.2H_2O$. The flasks were incubated on a rotary shaker at 200 rpm, 30°C, for 96h. The hydrolysate detoxification with activated charcoal resulted in phenolics concentration 2.5 fold lower than that observed employing vegetal polymer. The lowest concentration of toxic compounds in the hydrolysate detoxified with activated charcoal resulted in lower biomass formation and the higher xylitol formation (13.54g/L) resulting a maximum values of yield (0.75g/g) and productivity (0.48g/L.h), representing a conversion efficiency of xylose to xylitol of 81.7%, and this value was around 60% higher than that observed for the detoxified hydrolysate with vegetal polymer.

Financial support: CAPES, CNPq

Keywords *Candida guilliermondii*; sugarcane bagasse hemicellulosic hydrolysate; activated charcoal; vegetal polymer

Expression analyses of oxidative stress *katA*, *katG* and *oxyR* genes in *Erwinia amylovora* in the viable but nonculturable state

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The enterobacterial plant pathogen *Erwinia amylovora* is responsible of fire blight disease, causing serious losses in apple and pear fruit-growing areas around the world. The economic importance of this pathogen is increasing since it is spreading into new geographical areas. Difficulties in control fire blight have been related to the survival abilities of this pathogen in nature under different environmental conditions. One such survival strategy is the adoption of the viable but nonculturable (VBNC) state, where the cells remain viable but unable to grow on routine solid media. Under diverse stress conditions, the loss of culturability by VBNC cells has been related to the loss of catalase activity in some bacteria. Initial studies in our laboratory indicate a similar behaviour in the fire blight pathogen, but the molecular bases of this response are still unknown. According to the annotated *E. amylovora* genomes, this bacterium produces two catalases, codified by *katA* and *katG* genes, respectively, which catalyze hydrogen peroxide degradation. This pathogen also possesses the *oxyR* gene, which in *E. coli* activates the expression of a regulon of hydrogen peroxide-inducible genes, including *katG*. Consequently, the purpose of this work has been to study the expression of these oxidative stress genes in *E. amylovora* cells present in the VBNC state. Putative changes in the expression of *katA*, *katG,* and *oxyR* genes were determined by reverse transcriptase polymerase chain reaction (RT-PCR), before and after entry of the pathogen into the VBNC state induced by incubation in oligotrophic conditions at suboptimal temperatures. Enumeration of both viable and culturable cells, and 16S rRNA gene expression, were used for comparison. Viability and culturability of this bacterium was followed during the entry into the VBNC state by performing total, viable and culturable cell counts at various times. Initial results have shown repression of *katA*, *katG* and *oxyR* genes during entry into the VBNC state. However, 16S rRNA genes were constitutively expressed in these same VBNC cells, providing evidence that the *E. amylovora* VBNC cells were alive, as reported previously for other pathogenic bacteria. Further studies are required to determine the role of *katA*, *katG,* and *oxyR* genes as well as other stress-related genes in the VBNC response by this pathogen.

Keywords plant pathogenic bacterium; fire blight; VBNC; oxidative stress; gene expression; *katA; katG; oxyR*

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Functional Analysis of an Important Gene Related to Biohydrogen Production in *Escherichia coli*

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Biohydrogen production has become pivotal subject being discussed recently due to their eco-friendly character. Its combustion only produces H_2O and it is not one of the green house gases. Essentially, biohydrogen is able to be produced by various means chemical, physical and biological approaches. Biohydrogen from fermentable biomass appears an interesting idea due to low cost substrate availability. *Escherichia coli* is a robust bacterium for developmental research based on genetic engineering because its whole genome sequence is available and its metabolic pathways are relatively well-established. To date, we conducted an exhaustive search of all *E. coli* pathways for their impact on hydrogen production through screening the entire Keio mutant library (3985 isogenic mutants) using chemochromic membranes (GVD Corp., Cambridge, MA) formed by a thin film of WO_3 covered with a catalytic layer of palladium, to detect biohydrogen gas, by a colorimetric response. Then, several uncharacterized genes related to bacterial hydrogen production were identified. In this study, ten mutants chosen from the screening process were used to analyze the relationship to biohydrogen production under anaerobic conditions. Chosen strains were used in biohydrogen assay using modified complex medium supplemented with 100 mM glucose or formate and biohydrogen amount generated in headspace was analyzed using gas chromatography (Agilent Technologies Inc., Santa Clara, CA) after one, two, four, and 24 hours. From the chosen strains, six mutants had found gave significant response to biohydrogen membrane and biohydrogen assay so called mutants *yhfX, ypdJ, yieL, yhbP, yqiG* and *phnN*. It yields were 0.53, 0.55, 0.01, 1.07, 3.38 and 3.90 µmol H_2/mg protein respectively. The yield obtained significantly lower compared to wild type with 48.21 µmol H_2/mg protein. These genes have been expended for further investigation using complex formate media. Two mutants (*yqiG* and *phnN*) have showed significant deviation from glucose whereas it consumed formate and produce hydrogen with the yield 130.05 and 80.64 µmol H_2/mg protein respectively. These mutants seem to be not affecting the FHL complex that is responsible to formate degradation. From the organic acids analysis using HPLC, all strains showed accumulation of formic acid at various concentrations from 2 mM up to 7mM compared to wild type with no formic acid detected. The selective strains will be elected to undergo molecular level investigation using qRT-PCR in order to elucidate expression level of the gene in response to hydrogenase operon.

Keywords biohydrogen; metabolic engineering; fermentation; biomass

Functional analysis of *n*-alkane degradation by *Dietzia* spp.

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High-G+C Gram-positive actinomycetes play a major role in the biodegradation of aliphatic hydrocarbons. Numerous n-alkane-degrading strains belonging to the *Dietzia* genus were recently isolated from different hydrocarbon-contaminated ecosystems. In spite of their relevance, efficiency and widespread occurrence, no experimental evidence can be found in the literature concerning the class of genes responsible for *n*-alkane degradation in *Dietzia* spp. In order to investigate the genetic background of *n*-alkane degradation in detail, a set of genetic tools, *e.g.* the first applicable vectors and a simple electroporation protocol enabling the manipulation of several *Dietzia* spp. was developed. Our newly isolated strain, *Dietzia* sp. E1, proved to have an excellent ability to degrade n-C_{12} to n-C_{38} alkane components of crude oil. The preferred substrate was the very long-chain alkane *n*-eicosane with an optimal temperature of 37 ºC and an optimal pH of 8 under aerobic conditions. Gas chromatographic/mass spectrometric analysis revealed that intracellular substrate mineralization occurred through the conversion of *n*-alkane to the corresponding *n*-alkanal. The monoterminal oxidation pathway was initiated by AlkB and CYP153 terminal alkane hydroxylases, both of their partial coding sequences were successfully detected in the genome of strain E1. A suicide vector carrying a 518-bp *alkB* fragment was site-specifically integrated into the E1 chromosome, and the full *alkB*, as well as its chromosomal environment was sequenced after plasmid rescue experiments. Real-time quantitative PCR experiments revealed that four out of nine putative genes were strongly induced by long-chain *n*-alkanes in wild type E1. ORF4 encoded a natural fusion protein consisting of an integral membrane alkane hydroxylase and a rubredoxin domain. The significance of the *alkB-rub* gene in *n*-alkane degradation was investigated in phenotypic tests, and the disruption mutant strain exhibited severely impaired growth on n-C_{20} alkane carbon source. The mutation was successfully complemented with the expression of five different intact *Dietzia* AlkB-Rub proteins, the full-length forms of which were detected by simultaneous immunoblotting. The presented data furnish the first experimental evidence of the *in vivo* existence of an AlkB-Rub natural fusion protein, which plays a major role in long-chain *n*-alkane degradation.

Keywords *n*-alkane; *Dietzia*; manipulation tools; alkane-hydroxylase; rubredoxin; fusion protein; CYP153

Gene expression by *Erwinia amylovora* during starvation in natural water

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Erwinia amylovora is the bacterium that causes fire blight, one of the most important and difficult to control diseases of pome fruit trees worldwide. This harmful organism is a serious threat for susceptible cultivars, being subject to phytosanitary legislation in the European Union. Like other pathogenic bacteria*, E. amylovora* must be able to survive in non-host environments until contact with a susceptible host plant. Nutrient limitation is a common stressing factor in natural environments affecting bacterial survival most of the time. This stress frequently stimulates a starvation-survival response in non-differentiating bacteria, allowing their subsistence in a non-growing but culturable state. A starvation response has been described to allow *E. amylovora* survival in oligotrophic conditions, maintaining pathogenicity, but the molecular mechanisms underlying this response are relatively unknown. The aim of this study was to investigate the expression of some starvation-related genes (*rpoS*, *cstA* and *dps*) as well as pathogenicity genes (*hrpL, rcsB, rlsA* and *dfoA*) in *E. amylovora* under starvation conditions in natural water microcosms. Gene expression was analyzed periodically in cells exposed to oligotrophic water, by amplifying mRNA by reverse transcription (RT)-PCR, and using the 16S rRNA gene as an internal control. Additionally, total and viable cell counts (staining with the Invitrogen Baclight viability kit and epifluorescence microscopy), and culturable cell counts on KB agar plates, were performed. Initial results have shown that all studied genes modulated their expression through the first 24 h after exposure to nutrient-limiting conditions, reaching expression levels that remained nearly constant until the final sampling point at time 7 days. The expression of *rpoS*, *cstA* and *dps* starvation-related genes agrees with the population dynamics of the pathogen during starvation and with previous studies in other bacteria under similar conditions. Interestingly, the *hrpL, rcsB, rlsA* and *dfoA* virulence genes were also expressed under nutrient-limiting conditions during the assayed time. These results appear to confirm that starved *E. amylovora* cells retain their pathogenic potential.

Keywords plant pathogenic bacterium*;* fire blight; starvation response; gene expression; starvation-related genes; virulence genes.

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Glycogen and Trehalose Acumulation in *Candida albicans* and *Candida rugosa*

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Trehalose and glycogen are common carbohydrates in many organisms, including bacteria, yeasts, plants, and invertebrates. Especially in yeasts, trehalose has distinct functions as stress protectant metabolite. It also has a regulatory role in the control of glycolytic flux. Orginally, trehalose and glycogen were identified as reserve carbohydrates, however, later studies showed that trehalose and glycogen accumulate in response to various environmental stress conditions to protect cellular components

In this study, we have investitaged trehalose and glycogen contents of *Candida rugosa*, and *Candida albians* in response to different stress condition such as heat shock, oxidative stress, and metal stress. There was a similarities and differences between these two yeast species in response to stress conditions regarding their trehalose and glycogen contents. We have found that the heat shock results in a significant level of glycogen and trehalose accumulation in these yeasts. Trehalose synthesis is also activated by metal stresses such as iron and cobalt in *C. albicans* and *C. rugosa*. Metal stress activates glycogen accumulation in *C. rugosa* but not in *C. albicans*. Oxidative stress did not led to trehalose accumulation in *C. albicans* and *C. rugosa*. However, high levels of glycogen accumulation were detected in *Candida rugosa* in response to oxidative stress. We have used standart *S. cerevisiae* strain (FY2) in our research as a control yeast since trehalose and glycogen metabolism is known well in this microorganism. Our results indicated that trehalose and glycogen contents are much more higher in *C. albicans* and *C. rugosa* than in *S. cerevisiae* when subjected to heat and oxidative stresses. Based on our results, it can be concluded that the trehalose and glycogen metabolism has differences and similarities between *S. cerevisiae* and *Candida* strains used in this study.

Keywords: Trehalose, Glycogen, Stress, *C. albicans*, *C. rugosa*

Growth assessment methods for *Helicobacter pylori* in liquid medium

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Helicobacter pylori is known to be associated with chronic gastritis, peptic ulcers and gastric cancer. The lack of physiological data has hampered the uncover of mechanisms associated with *H. pylori* infection and consequently, many aspects related with the appearance of diseases remain unclear.

It is well known that *H. pylori* can change cell morphology from spiral to coccoid form when exposed to adverse conditions. Some authors have reported the existence of a viable but nonculturable state of this bacterium. The development of robust methods to grow this bacterium and reliable methods for the assessment of growth are needed for a better characterization of its physiology. As such, the purpose of this work was to study *H. pylori* growth in a chemically defined medium, compare different methods to assess the growth and observe the changes of morphology.

Cultures were grown at 37ºC under controlled conditions in Ham´s F-12 medium supplemented with fetal bovine serum. Samples were collected until 72 hours. For growth assessment, the following methods were used and compared: optical density, cultivable cell counts, total cell counts using DAPI staining, evaluation of viability with the Live/Dead viability kit and a PNA FISH probe which evaluates the content of stable rRNA. Cell counts and analysis of cell morphology were assessed using an epifluorescence microscope.

Under the conditions of atmospheric oxygen 6.5%, pH 7, and shaking speed 110 rpm, *H. pylori* was in exponential growth from 0 to 4 hours. In comparison to total counts, PNA FISH displayed, in general, lower counts, particularly after cells have reached the stationary phase. Changes in morphology and viability were observed. After 60 hours of culture cells were mainly coccoid and nonviable.

Keywords: *Helicobacter pylori*; growth; morphology

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Growth conditions influence *E. coli* persisters formation during stationary phase of growth

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"Persister" cells are transiently antibiotic-tolerant cells and usually constitute a small part of bacterial populations. These bacteria remain dormant and are able to regrow after antibiotic treatment. For that reason, persisters can be the most common cause of chronic infections. Persister cells can be detected in biofilms as well as in planktonic, especially stationary cultures. The exact mechanisms underlying persisters formation are not well-known. In this study we investigated the influence of growth conditions on *E. coli* persisters formation under stationary phase. We found that the availability of oxygen and glucose in a medium affected the level of persisters. Under aerobic conditions the number of persister cells was diminished in the presence of glucose. Conversely, under anaerobic conditions, we observed a marked increase in the amount of persister cells in the culture supplemented with glucose. Our previous experiments demonstrated that under the same conditions (aerobic stationary cultures without glucose and anaerobic cultures with glucose) *E. coli* cells accumulated aggregates of misfolded proteins. These proteins are normally involved in a variety of cellular processes including translation, metabolism, cell architecture and stress responses. The above results suggested that the aggregation of proteins can be correlated with persisters formation under stationary phase. Further studies confirmed this assumption. We revealed that in MOPS-buffered stationary cultures, in which protein aggregation was inhibited, the production of persisters was significantly decreased.

Keywords: antibiotics, *E. coli*, persisters, protein aggregation, stationary phase

How do they affect different conditions of culture to the cellular stress in biofilms?

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Introduction: Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) have been extensively studied in planktonic bacterial physiology; however, the precise role of cellular stress in biofilm development is unclear. Oxidative stress is caused by an imbalance between the production of oxidants and the levels of antioxidants presents in the biological system. In this situation, the overproduction of reactive oxygen species (ROS) can lead to the damage of cellular components. If this damage is not repaired, mutagenesis and cellular death can occur.It has been documented that at least 65% of all microbial infections are related to formation of biofilms, and participate in the pathogenesis of different diseases such as chronic rhinosinusitis (chronic otitis, chronic tonsillitis, cholesteatomas and other inflammatory and infectious disorders), associated with *Staphylococcus aureus*; and illnesses associated with *Escherichia coli* infections such as hemolytic uremic syndrome and pyelonephritis. Both of them have been associated with the presence of biofilms.

Objective: The aim of this work was to characterize the cellular stress, the production of ROS, the liberation of nitric oxide (NO) and the enzymatic activity of superoxide dismutase (SOD), under different culture conditions.

Materials and methods: In this study, the biofilm formation of the reference strain of *E.coli* EDL 933 and three clinical isolates (associated with hemolytic uremic syndrome) were tested. To be able to realize a comparative study between a Gram negative and Gram positive specie, three pathogenic *S. aureus* clinical strains (associated with different indwelling medical devices) and an ATCC 29213 strain (a biofilm control) were used.

We studied the influence of different range of culture conditions (pH, sugars, osmotic stress, and reduction conditions) by quantitative methods of biofilm detection. The biofilms-forming ability was measured by determination of the adhesion to 96-well polypropylene microtiter plate to form mature biofilms, which is based on the ability of bacteria to form biofilms on solid surfaces and uses CV to stain biofilms.

The extracellular production of ROS was detected by the reduction of nitro blue tetrazolium to nitroblue diformazan. The supernatant was separated by measuring the extracellular production of ROS (ROS). The reaction was proportional to the ROS generated in biofilm and was measured by optical density (OD) at 540 nm. The NO production was evaluated as nitrite by a microplate assay method using the Griess reagent. Absorbance was measured at 540 nm in a microplate reader and results were expressed as μM. Total SOD activity was assayed photochemically based on the inhibition of nitro blue tetrazolium (NBT) reduction.

Results: We observed that ROS, RNI and its downstream derivatives played an important role in biofilm development. This suggests that cellular stress occurred inside microcolonies, affecting to the biofilms grown under different conditions (high pH and osmotic stress), with ROS and RNI production being intimately correlated with differentiation and dispersal events in situ in biofilms. On the other hand, the SOD activity of biofilms was lower than that found in culture controls. In favorable conditions cultures (sugars and reduction conditions) for biofilm formation, the high SOD activity was found. In this way, this antioxidant power could be the reason for which the production of ROS and NO was not detectable.

Conclusions: The decrease of the extracellular matrix under unfavorable conditions for increasing the cellular stress caused radical oxidizers to accumulate in an extracellular medium and thereby affect the matrix. In favorable conditions cultures for biofilm formation, the polysaccharide matrix was increased because the sessile cells were present under lower cellular stress.

In conclusion, we suggest that biofilm formations in a variety of environmental conditions are influenced by cellular stress. Improved knowledge of ROS, RNI and enzymatic pathway regulation, may help in clarifying the relevance of biofilm formation in the pathogenesis of infections associated with medical devices, and new advances in this aspect could also be of great value in the development of better preventive and therapeutic measures.

Keywords: biofilms; stress; reactive oxygen species; nitric oxide; superoxide dismutase

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Influence of hydrogen on the growth of hyperthermophilic organotrophic archaea of *Crenarchaeota* phylum

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Organotrophic hyperthermophilic archaea of the *Crenarchaeota* phylum grow on a wide range of substrates: proteins, peptides, polysaccharides, mono- and disaccharides. The growth of representatives of *Desulfurococcus* and *Fervidicoccus* on peptone is significantly stimulated by elemental sulfur, which is reduced to H_2S. In the absence of sulfur, H_2 is the reduced fermentation product formed by these organisms.

We studied the influence of different concentrations of hydrogen added to the gas phase on the growth of three thermophilic archaea isolated from Uzon Caldera, Kamchatka. The final cell yield of *Desulfurococcus kamchatkensis* (1) in peptone medium without elemental sulfur and exogenous H_2 was $5.7*10^7$, and the yield of *Desulfurococcus fermentans* (2) and *Fervidicoccus fontis* (3) was $3.5*10^7$ cells ml^{-1}. The final concentration of H_2 formed by the microorganisms on this medium was 4-4.5%. It was found that the increase of the H_2 concentration added to the gas phase slowed down the growth of these organisms on peptone in the absence of sulfur and the final cell yield decreased, but the response of these organisms was different. *F. fontis* was more sensitive than the desulfurococci to low concentrations of H_2 (5% H_2 decreased the final cell yield to 33%), but at high concentrations of H_2 (20 and 100%) *F. fontis* was still able to grow (the cell yield was 11% of the control). The growth of representatives of the genus *Desulfurococcus* was more stable at low concentrations of H_2 but was completely inhibited at high concentrations of H_2.

Complete genomes of *D. kamchatkensis* and *F.fontis* were analyzed, and it was found that they both contain genes encoding ferredoxin-dependent membrane-bound energy-converting hydrogenase (4,5). However, *F. fontis* also has a cytoplasmic NADPH-dependent hydrogenase (5). Production of hydrogen from NADPH allows less energy to be conserved and therefore should remain thermodynamically possible at higher hydrogen concentrations. Thus, the metabolism of *F. fontis* is more flexible, giving it an additional advantage in the competition for substrates in microbial communities of terrestrial hot springs (6).

Keywords: hyperthermophilic Crenarchaeota; hydrogenases

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Mesorhizobium type strains show distinct tolerances to several environmental stresses

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One of the most widespread problems facing agriculture is the degradation of soil quality due to desiccation, salinity and acidity. Furthermore, the symbiosis between rhizobia and legumes is also affected by these different environmental conditions. Hence, the stress tolerance of rhizobia is valuable for strain improvement in agriculture or bioremediation of soils at elevated temperatures, salinity and extreme pH values, since it is more practical and less expensive to select naturally occurring tolerant rhizobia strains, than to construct genetically modified tolerant strains. The main aim of this study was to evaluate stress tolerance of *Mesorhizobium* species and to investigate species-specific stress response mechanisms. Our specific aims were to evaluate the tolerance of type strains of *Mesorhizobium* species to temperature, salt and pH stress and to investigate changes induced by stress in total protein profiles as well as in the expression levels of the chaperone gene *groEL*. Tolerance evaluation revealed a high diversity of tolerance phenotypes among *Mesorhizobium* type strains in response to several abiotic stress conditions, namely temperature, salinity and pH stress. *M. plurifarium* showed higher growth at 37ºC. *M. thiogangeticum* showed highest growth with 1.5% NaCl and *M. ciceri* at pH5 and thus may be considered as moderately halophilic and acidophilic, respectively. No correlation was depicted between tolerance to an environmental stress and strain origin or host plant. However, correspondence analysis pointed out individual associations between strain origin and growth under different stresses. SDS-PAGE analysis revealed changes in the protein profiles, namely the overexpression of a 60kDa protein, following heat stress. Under salt stress, five overexpressed proteins were identified in *M. plurifarium* and *M. thiogangeticum*. The overproduced proteins (40–85 kDa) detected in the tolerant strains *M. huakuii*, *M. plurifarium* and *M. thiogangeticum*, grown with 1.5 and 3% NaCl, may be involved in saline stress tolerance. Under salt stress a protein with approximately 15 kDa was consistently detected in tolerant and sensitive strains. In conclusion, several proteins were overproduced in different strains and may be involved in stress tolerance. Northern analysis revealed that *Mesorhizobium* strains show different *groESL* transcriptional responses to heat and salt shock: upon heat shock the *groESL* mRNA levels increased, while a slight decrease was observed upon salt shock. Northern analysis revealed an increase in *groESL* expression in *M. huakuii* and *M. septentrionale* after heat shock; by contrast, a decrease was detected in *M. albiziae* and *M. thiogangeticum*, upon salt shock. To our knowledge, this is the first study focusing tolerance to temperature, salt and pH stress in *Mesorhizobium* type strains. We have shown that *groESL* genes have distinct transcriptional responses to environmental changes, namely temperature and saline shocks, but also that there are differences in transcriptional levels between tolerant and sensitive strains. Further studies are in course to elucidate the function of overexpressed genes in order to clarify their role in temperature, salt and pH stress tolerance of mesorhizobia, as well as their contribution to symbiotic effectiveness.

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Keywords *Mesorhizobium*; stress; temperature, salt; pH; *groEL*

Monitoring of the inorganic polyphosphate accumulation and acid and alkaline phosphatase activity in *Cunninghamella elegans* strains using fatorial design

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Phosphate metabolism is a great important factor to cell development of live organism, the main way to phosphate disponibilyzation happen through of phosphates compounds hydrolysis, which is carried out by phosphates enzymes. Inorganic polyphosphate is a polymer constituted by repeat units of orthophosphates residues linked by high-energy phosphoanhydride bonds, which is ubiquitous in nature and have been considered a alternative energetic source. In this work was investigated the enzymatic activity of acid and alkaline phosphatases in ten strains of *Cunninghamella elegans*, a filamentous fungi belong to Zigomycetes. Assays were performed in agreement to a factorial arrangement with two levels (2^2) without central point, where glucose concentration in the medium and growth temperature were the independent variables and enzymatic activity was the variable response. Obtained results showed enzymatic activities for all tested samples. Highest activity for two enzymes was demonstrated by *C. elegans* (UCP 542), which were 0,59 U.I/g of biomass to acid phosphatase and 0,34 U.I to alkaline. PolyP accumulation hasn't influenced by phosphatases activity. Best results were obtained with the independent variables in the maximum level (+1) and the variable temperature showed effect most significant to variable response.

Key-words: enzymes, polyphosphate, Zygomycetes.

Perspectives of Applied Microbiology with Purple Bacteria, driven by Systems Biology

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Anoxygenic photosynthetic purple bacteria are amongst the most versatile life forms and offer highly attractive opportunities for industrial applications. Potential products derived from intracytoplasmic photosynthetic membranes (ICM) are amongst others, photosynthetic pigments (porphyrines, carotenoids), coenzymes (Q_{10}), biohydrogen, biopolymers and recombinant membrane proteins. Since high levels of ICM are formed at low-light intensities at anaerobic conditions, most attempts to exploit purple bacteria for producing high value compounds were so far conducted phototrophically, using light as energy source. However, mass cultivation of phototrophic bacteria is generally inefficient due to the inevitable limitation of light-supply when cell densities become very high. It is thus interesting that *Rhodospirillumrubrum* produces high-levels of ICM completely separated of light, when grown semi-aerobically in the dark with a two-carbon substrate growth medium. On the basis of this cultivation process, we applied a systems approach using a combination of bioreactor cultivations, metabolomics, as well as mathematical modelling to develop *R. rubrum* for biotechnological applications. These efforts include the optimization of fed-batch processes to yield high cell densities to levels commonly employed in industry. For improving the understanding of underlying metabolic and regulatory events, stoichiometric and dynamic computational models of metabolism and electron transfer chains were developed and applied. Furthermore, a metabolomic profile was obtained with different carbons sources which indicate additional flexibility in acetate and carbon dioxide assimilation pathways. This work is intended to open a new perspective for utilizing photosynthetic bacteria as producers in biotechnology.

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Proteomic analyses of the white-rot fungus *Trametes hirsuta* grown on different substrates

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Woody biomass is a complex mixture of cellulose, hemicellulose and lignin. Lignin is a three-dimensional polymer of phenylpropanoid units which is highly resistant towards chemical and biological degradation. White-rot fungi have the apparently unique ability to degrade lignin due to production of extracellular oxidative enzymes (oxidoreductases) such as lignin peroxidases, manganese peroxidases and laccases.

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are copper-containing phenol oxidases that catalyze the oxidation of a broad range of aromatic substrates including o,p-diphenols, aminophenols, polyphenols, polyamines and model lignin structures, also some inorganic compounds. Fungal laccases have been extensively exploited for industrial purposes and there is a wealth of information available concerning their reaction mechanism, biological role and several molecular aspects, including cloning, heterologous expression and transcriptional analyses.

Synthesis and secretion of laccases are strictly influenced by nutrients' levels, culture conditions, and developmental stage as well as by presence of different inducers. For a number of white-rot fungi the induction of laccase secretion by Cu(I) and/or Cu(II) ions has been shown. Nevertheless there are only few studies on laccase biosynthesis and its regulation in Basidiomycetes. There is also lack of knowledge about the protein factors involved in cellular copper homeostasis and copper incorporation in laccase.

As these factors appear to play an important role in the biosynthesis of the copper containing proteins including laccases, the present study is focused on comparative analysis of proteome of Basidiomycetes *Trametes hirsuta* – an efficient producer of high redox potential laccase under submerged cultivation on glucose-peptone medium (G-P) and medium containing lignocellulose materials (glucose-peptone medium with oat straw, G-P+S). Additionally, the experiments were carried out using both cultivation media (G-P and G-P+S) supplied with $CuSO_4$ as inducer of laccase biosynthesis. The intracellular proteins of *Trametes hirsuta* grown on different media with and without inducer were separated using 2D electrophoresis. The identification of intracellular proteins was carried out by means of MALDI-TOF mass-spectrometry.

The laccase production reached the maximal level of 88 $U \cdot ml^{-1}$ on the sixth day of cultivation under submerged cultivation on G-P medium, containing 10.0 g glucose per liter and 0.25 g $CuSO_4$. However, laccase production on G-P+S medium (with 10 g of oat straw per liter) comprised 82 $U \cdot ml^{-1}$ only after 69 days of cultivation. It should be mentioned that the levels of laccase activity under cultivation without $CuSO_4$ on G-P and G-P+S were significantly lower comprising 10 $U \cdot ml^{-1}$ and 44 $U \cdot ml^{-1}$ after 6 and 69 days of growth respectively.

The comparative analysis of 2-DE maps of proteome were performed using fungal mycelium collected when the highest laccase activity in the cultural broth was measured during the fungi growth on G-P and G-P+S media. The significant differences were observed in total number and distribution pattern of protein spots on 2-DE maps. The 10 protein spots corresponding to proteins with molecular weights in the range of 40-60 kDa and pI - 8.0-9.0 were revealed in proteome of *T. hirsuta* grown on G-P medium. The amount of these proteins was significantly increased when $CuSO_4$ was added as inducer. Two proteins were identified as the β-subunits of ATP synthase. The other one shows rather high level of homology with chaperone activator Sti1 which is a potent effector for the ATPase, the latter is known can serve as Cu transporter within Golgi apparatus, where laccase folding and copper incorporation is taken place. The identification of other proteins is under progress. The analysis of proteins produced after 69 days of fungal growth on G-P+S with and without copper revealed the presence of nearly 15 well reserved protein spots corresponding to the proteins with molecular weight in range of 15-20 kDa and pI – 9.0-9.5 which have not been observed in proteomes of fungi grown both on G-P with copper and G-P without copper. The data obtained can be explained by difference of laccase biosynthesis regulation mechanisms on G-P and G-P+S media.

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Keywords white-rot fungi, proteome, lignin degradation, laccase

Quorum Sensing systems of *Serratia proteamaculans* 94

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Quorum Sensing (QS) regulation is a specific type of the gene expression regulation depending on the density of bacterial populations. QS systems include low molecular weight signal molecules (autoinducers) that can easily diffuse across the cell wall, and receptor proteins interacting with autoinducers. The best studied QS systems are those employing

N-acyl homoserine lactones (AHLs) in Gramnegative bacteria. QS systems play a key role in the regulation of many bacterial cell processes.

The present work is devoted to studying of two types of *Serratia proteamaculans* QS systems and their roles in the control of bacterial metabolism. The *S. proteamaculans* strain 94 was isolated from rotten meat kept in an industrial fridge. This strain is able to hydrolyze the collagen at low temperature and to produce a number of extracellular proteases.

Using different AHL-biosensors, TLC chromatography and LC-ESI-LTQ-FTIC-MS analysis we have shown that *S. proteamaculans* 94 produced two main types of AHL signal molecules, N-(3-oxo-hexanoyl)-l-homoserine lactone and N-(3-hydroxyhexanoyl)-l-homoserine lactone. We identified, cloned and sequenced genes of the first QS system: *sprI* gene encoding the AHL-synthase and *sprR* gene encoding the receptor protein. Both of these genes were transcribed convergently and their reading frames partly overlapped. Lux-box overlapped with the sequence of -10 site was detected before *sprR* gene. In addition, we identified the second type QS signal molecule autoinducer AI-2 in *S. proteamaculans* strain 94. *luxS* gene coding for AI-2 synthase was cloned and sequenced.

S. proteamaculans 94 mutants with inactivated genes of the AHL synthase, the receptor protein and the AI-2 synthase were constructed. To define roles of the QS-systems in regulation of *S. proteamaculans* 94 processes of metabolism we compared activities of different enzymes from mutant and wild type cells. Cells *S. proteamaculans* 94 was shown to display extracellular proteolytic, lipase and chitinase activities which were the same in mutant cells with the inactivated receptor protein gene *sprR*. Mutants deficient in AHL synthase and AI-2 synthase were shown to drastically decrease extracellular proteolytic and chitinase activities. All three mutants were shown to have decreased capacity to form biofilms.

Keywords N-acyl homoserine lactone; *Serratia proteamaculans*; Quorum Sensing

Regulatory roles of alternative sigma factors in transcription of genes in Corynebacterium *glutamicum*

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Bacterial core RNA polymerase associates with a sigma subunit (factor) to recognize promoter sequences. *Corynebacterium glutamicum* encodes seven sigma factors of RNA polymerase: the principle sigma SigA, the principle-like SigB and five alternative sigma factors (SigC, SigD, SigE, SigH and SigM). Each sigma factor is responsible for recognizing promoters of genes belonging to a sigmulon, which is involved in specific functions of the cell. Most promoters of *C. glutamicum* housekeeping genes are recognized by RNAP associated with SigA, whereas SigB is involved in transcription of a large group of genes active during the transition phase between the exponential and stationary growth phases. The SigH sigmulon consists of the genes involved in heat shock response and oxidative stress response including genes coding for regulators and other sigma factors. This suggests that SigH occupies a central position in the cross-regulated network of sigma factors and controls their concerted response to various stress conditions in *C. glutamicum*. The SigM factor was found to regulate genes responding to oxidative stress. The main role of SigE is to activate genes involved in response to a cell surface stress. Presence of 2 to 4 promoters upstream of several *C. glutamicum* genes was proved. We mapped such multiple promoters particularly within the upstream regions of several stress-response genes (*dnaK*, *dnaJ2*, *clpC*, *arnA*) and the genes coding for the sigma factors themselves (*sigH* and *sigE*). In most cases, both SigA-dependent and SigH-dependent promoters were localized by transcriptional start determinations. Transcription of the *sigH* gene and *sigE* gene was driven from 3 and 4 SigA-dependent promoters, respectively. In both cases, presence of still another (SigH-dependent) promoter is supposed. To confirm the recognition specificity of particular *C. glutamicum* sigma factors, the *in vitro* transcription system based on the isolated *C. glutamicum* RNA polymerase and chosen sigma factors was developed. We confirmed that SigH is involved in transcription of *dnaK* by this *in vitro* transcription system. The *sigM* gene and the *rshA* gene encoding anti-SigH factor were found to be transcribed from single SigH-dependent promoters. Further analyzed genes were chosen from the group of genes whose expression was upregulated by deletion of the gene for anti-sigma factor RshA: *pup*, *uvrA* and *mca*. A complex regulation of gene expression involving multiple promoters recognized by different sigma factors and control by various DNA-binding proteins integrates the effects of external stimuli and tunes the expression profiles of the genes as required by growth conditions. Based on the results of mapping the promoters of sigma factor genes, analysis of the functions of anti-sigma factors CseE (anti-SigE) and RshA (anti-sigH) and other published data, the first model of sigma regulatory network has been proposed.

This work was supported by grant 204/09/J015 from the Scientific Council of the Czech Republic

Keywords *Corynebacterium glutamicum*, sigma factors, regulation of transcription

Saccharomyces cerevisiae plasma membrane dicarboxylate transporter is a probable sensor of extracellular pH

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Yeast *S. cerevisiae* sometimes inhabit in the environment, acidity of which is connected with significant L-malate concentrations. For example, such situation develops during manufacture of dry grape wines. Low-active transporter-receptor of inorganic phosphate with pH optimum in alkaline area was characterized recently in plasma membrane of this yeast. Earlier we have found out low-active (13.8 ± 0.4 nmol/min/1 mg of dry weight) plasma membrane dicarboxylate transporter sensitive to 2-undecyl malonate. It has been difficult to study because of its low activity and special properties. This *S. cerevisiae* transporter has alkaline pH-optimum and transports succinate more effectively, than citrate. After 15-18h of aerobic preincubation at 0°C of yeast cells, external L- and D-malate stimulates respiration and 2-undecyl malonate inhibits oxidation of both substrates. However the malate oxidation becomes negligible after 24-26h of such preincubation. These stereoisomers competitively inhibits the succinate oxidation and transplasmalemmal transport of this dicarboxylate into cells limits respiration at that. Change of the incubation medium pH value from 5.5 to 6.5 causes growth of the ratio value of IC_{50} (one of the parameters of inhibition) of D-malate and IC_{50} of L-malate from 1.0 to 6.4. At the same time succinate dianion affinity varies a little bit. Such selective regulation of transplasmalemmal dicarboxylate transporter affinity may indicate that this carrier is a malate receptor and a probable sensor of extracellular pH.

Keywords *S. cerevisiae*; transporter; malate; succinate

Stringent response is critical for survival of *Escherichia coli* upon treatment with inhibitors of aminoacyl-tRNA-synthetases, microcin C and albomycin

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Aminoacyl-tRNA-synthetases (aaRSs) are essential enzymes which charge their cognate tRNAs with the corresponding amino acids. During the last years, bacterial aaRSs are regarded as attractive targets for developing new antimicrobial drugs. Microcin C (McC) and albomycin (Amc) are natural antibiotics-inhibitors of aaRSs (AspRS and SerRS, respectively), which use the “Trojan horse” strategy to attack the susceptible bacterial species. They rapidly penetrate through cellular envelope using dedicated transport systems, and once in the cytoplasm they are processed by cellular peptidases with formation of proper inhibitors, mimetics of aminoacyl-adenylates, capable of blocking tRNA aminoacylation and consequently protein synthesis and bacterial growth. Theoretically, treatment with McC or Amc should mimic what happens when the bacterial cell starves for Asp or Ser, leading to accumulation of uncharged tRNAs which in turn triggers the stringent response.

When the stringent response is triggered by the presence of uncharged tRNAs, the signaling nucleotides pppGpp and ppGpp (further referred as ppGpp) are synthesized on the ribosome by RelA protein from ATP and GTP. Accumulation of ppGpp causes dramatic alterations in gene expression, reducing transcription from promoters serving for growth (genes for rRNAs, tRNAs, ribosomal proteins etc.) in favor of promoters responsible for maintenance and survival (genes for amino acid biosynthesis, stress factors etc.). In this study, we evaluated the McC and Amc capabilities of causing the stringent response in *E. coli* by comparing changes in transcription efficiency for a set of well-characterized promoters (*rrnB* P1, *fis*, *thrL*) as well as for promoters of ribosomal protein operons (*rpsB, rpsA P1, rpsO, rpsT P1*) by using RT-PCR. The results clearly show that both McC and Amc cause classical stringent response in wild-type *E. coli* strains, that was further corroborated by using mutants unable to produce ppGpp (ppGpp^0) or its cofactor DksA. These mutants exhibited relaxed phenotype, i.e. transcription from promoters of ribosomal genes did not react any more to the presence of the inhibitors. To evaluate the viability of cells, aliquots of treated and untreated cell cultures were diluted into fresh medium, and serial 10-fold dilutions were dropped on agar surface. Remarkably, though both McC and Amc cause abrupt cessation of cell growth, the cells able to produce ppGpp (including *dksA* mutant!) retained 100% survival after treatment, while the ppGpp^0 strains lost their viability, indicating the bactericidal effect in the absence of ppGpp. When mutations in the *rpoD* gene, which decrease the ability of sigma 70 to compete for core RNA-polymerase with alternative sigma factors, were transfered into ppGpp^0-background, full recovery of the viability was observed. These and other data allow us to conclude that the main cause of cell death upon treatment with McC or Amc in the absence of ppGpp is the inability of the cell to switch the transcription to utilizing the alternative sigma factor E, the only alternative sigma factor essential for *E. coli*.

This work was supported by RFBR grant 09-04-01014a.

Keywords stringent response, ppGpp, microcin C, albomycin.

Taxonomy of sterol-degrading species of the actinomycetal genus, *Rhodococcu*s

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Several actinomycetal strains were isolated from soil for their capability to catabolize plant sterols as sole carbon and energy sources. These were identified according to their morphology, physiology and wall chemotype to belong to the genus *Rhodococcus*. Amongst the isolates, three strains: *Rhodococcus* sp. CIP 105335 (strain GK1), strain GK3 and strain GK12 were found to possess a high capability for sterol degradation. This catabolic capability can meet biotechnological applications. Therefore, investigation of these strains is important at the fundamental knowledge and also at the application level. The nucleotide sequence of the 16S rRNA gene was determined for the strains GK1 and GK12. According to all the obtained data, strain GK1 might be a new species of the genus, and the isolate GK12 is a strain of *Rhodococcus erythropolis*. Besides, rhodococci have been distinguishable amongst bacteria that catabolize sterol by the location of cholesterol oxidase, the first enzyme of the sterol catabolism. This oxidase is either released in strain growth medium and/or located in the cell surface layer. This location was demonstrated for the three strains and confirmed their appurtenance to the specified genus.

Keywords: *Rhodococcus species*, sterol degradation, taxonomy

The haloacid operon of *Burkholderia sp.* MBA4 is catabolically repressed

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Degradation of haloacids such as monochloroacetate (MCA) in *Burkholderia sp.* MBA4 is mediated by the expression of a haloacid operon producing a dehalogenase (Deh4a) and an associated permease. When MBA4 was cultivated in a defined medium containing succinate and MCA a biphasic growth pattern was demonstrated. The release of chloride was witnessed apparently after succinate was depleted (Fig. 1).

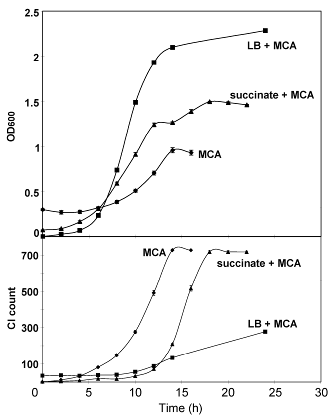

Fig. 1. Growth and release of chloride from chloroacetate by *Burkholderia sp.* MBA4. Growth was monitored by a spectrophotometer and chloride amount was determined by a chloride counter. MCA, chloroacetate, LB, Luria-Bertani broth.

Quantitative reverse-transcriptase PCR was used to quantify the expression levels of the dehalogenase gene (*deh4a*). When MCA was the only carbon source and the expression level of *deh4a* was treated as 100, then the relative expressions were 16, 42, 40 and 23%, respectively, when Luria-Bertani broth without sodium chloride (LB), succinate, pyruvate and glucose, were supplemented in the medium (Fig. 2). This showed that the haloacid operon was catabolically repressed.

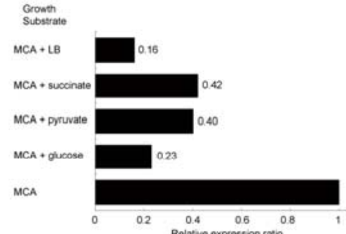

Fig. 2. Relative expression levels of dehalogenase *deh4a* in various substrates. Quantitative RT-PCR was used to determine the transcript levels. The level in cells grown in MCA was used as 1.

A transposon carrying plasmid, pOT182, was conjugated from *E. coli* to MBA4 to generate a library with randomly disrupted genes. Transconjugants were isolated for relieved degradation of MCA in LB^- by a colorimetric method. A transconjugant, 131M04, was found to express *deh4a* to 42% of the MCA-induced level. The DNA sequence of the disrupted gene was determined and was found to encode for a putative branched-chain amino acid (BCA) transporter of 638 residues with two bacterial-protein-dependent-transport-2 domains. Cloning and expression of this gene in *E. coli*, strain B7634, defective in BCA transport systems, helped the cell to grow in medium with low isoleucine concentration. When leucine, isoleucine and valine were supplemented to defined medium containing MCA, the expression of *deh4a* in MBA4 was repressed 50%. Under similar growth conditions, the expression level of mutant 131M04 was relieved to 80% of the induced level. These results indicated that the expression of the haloacid operon was catabolically repressed and BCA is one of the inhibitors.

Keywords Burkholderia; catabolite repression; amino acid

The highest synthesis of GbdR, an essential regulator of genes induced by choline in *Pseudomonas aeruginosa*, depends on the use of choline as an alternative nitrogen source

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Choline, a quaternary ammonium compound, is present in high concentrations in different tissues, such us lung, corneal and urinary epithelium, where *P. aeruginosa* causes infections. It is also found as phosphatidylcholine, phosphorylcholine, and acetylcholine or as choline-free. In this way, choline, can be used as a source of carbon (C) and nitrogen (N) and favors the colonization of this pathogen. Its metabolism in bacteria has been studied and in *P. aeruginosa* it was recently described that GbdR, a regulator that belongs to AraC/XylS family, is essential for the used of choline as a nutrient for the microorganism. PA*5380* is the gen that encodes for GbdR, and is in a context in *P. aeruginosa* genome of genes responsible for the synthesis of many proteins involved in the catabolism of choline. The two components system regulators NtrBC and CbrAB which are responsible for the intracellular C: N balance in Pseudomonas, are also involved in the use of choline as nutrient. The aim of this work was to know the transcriptional regulation of *gbdR* gene and the relationship between its expression and the use of choline as an alternative C and N source. We also strove to find out if the *gbdR* expression depends on the presence of RpoN factor and hence of the global NtrC and CbrB regulators. Previously, the +1 start site was determined by 5´RACE, and the highest expression of *gbdR* promoter (P*gbdR*) was obtained with the construction comprising 700pb, that included 80pb downstream and 620 upstream from the ATG. To carry out the present work, transcriptional fusions to *lacZ* were performed (P*gbdR::lacZ*) and inserted into the chromosome of *P. aeruginosa* WT strain. To assess whether RpoN, NtrC or CbrB are required for the activation of *gbdR*, the P*gbdR*::*lacZ* construct was introduced into the bacterial chromosome of Δ*rpoN*, Δ*ntrC* and Δ*cbrB* strains, respectively. β-galactosidase, as reporter activity, was determined to measure the promoter expression. The upstream region of *gbd*R gene was analyzed using the Promscan and Prodoric programs. Bioinformatic analyses revealed that this P*gbdR* region contains: two hypothetical overlapped −12/−24 promoter elements, a consensus sequence for the integration host factor, two overlapped palindromic sequences resembling the binding sites for potential enhancers binding proteins: NtrC and CbrB. Molecular and physiological experiments allowed us to conclude that: *i)* the use of choline as the only substrate for bacterial growth, activated the P*gbdR* in a similar level (3-fold) as the cells grown on betaine, dimethylglycine or histidine, compared with succinate/ammonium grown cells; *ii)* the highest expression (12-fold) was found when choline was used as sole N source and succinate as preferential C source; *iii)* the highest expression did not occur with the addition of other nitrogen (betaine, dimethylglycine or histidine) or carbon sources (glucose or mannitol) used for the bacterial growth; *iv)* a choline concentration as low as 1 mM was sufficient to trigger the activating effect on *gbdR* expression; *v)* β-galactosidase activity was highly reduced (≅90%) in the Δ*rpoN* and Δ*ntrC* mutant strains compared to the to the WT strain growing on succinate/choline; *vi)* an increase of ≅20% in the expression of P*gbdR* in Δ*cbrB* strain with respect to WT strain grown in succinate/choline showed that both enhancers could compete for the same potential binding site. With all these data we felt able to propose that the use of choline as N source is sufficient to promote the *gbdR* expression, and that NtrC is the enhancer of this system. Therefore, we suggest another pathway for choline, which depends on intracellular imbalance in the C: N ratio caused by an excess of C given by succinate.

Keywords: *Pseudomonas aeruginosa*, choline, GbdR regulator. transcriptional regulation

The phosphatidylserine synthase is required for motility of *Vibrio parahaemolyticus*

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Phosphatidylethanolamine (PE) is an abundant phospholipid in bacterial cell membrane. In bacteria, the biosynthesis of PE proceeds via CDP-DAG pathway. On the pathway, PS is generated by a reaction between CDP-diacylglycerol (CDP-DAG) and serine, which is catalyzed by the single identified PS synthase (PSS). The PS produced is decarboxylated to form the major membrane phospholipid PE. This shows that PS plays a central role in phospholipids biosynthesis pathway. The *pssA* mutants of *E. coli* were conditional lethal and temperature-sensitive. Since PS is a precursor of PE through the action of phosphatidylserine decarboxylase (PSD), the level of PE of these mutants declines dramatically. It is interesting that the *pssA* mutants of *E. coli* lack PE can be suppressed by the addition of sucrose and divalent cations, such as Mg^{2+}, Ca^{2+}. Apparently, the properties of cardiolipin change can substitute for PE in the presence of divalent cations. In this study, we constructed the *pssA* deletion mutant by using gene replacement method in a clinical strain *V. parahaemolyticus* No. 93, which lacking of *tdh* and *trh*. We examined the physiological role of PE biosynthesis gene *pssA* of *Vibrio parahaemolyticus*. The growth rate of *pssA* deletion mutant (Δ*pssA*) was severely impaired no matter in the medium of TSB3 and APSB3, but finding the addition of $MgCl_2$ could help their growth. The Δ*pssA* was decreased in swimming motility when compared with that of wild-type in all test medium. The differential expression of the identified flagella genes in Δ*pssA* was further investigated by QPCR. The results showed that the transcription level of *flaD* and *fliS* were markedly lower by 2.9 and 4.8 folds in Δ*pssA*, respectively. It indicated that the *pssA* gene is required for the motility of *V. parahaemolyticus*.

Keywords *Vibrio parahaemolyticus*; phosphatidylserine synthase; *pssA*, motility

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The regulation of alkaline serine protease, PrtA, of *Vibrio parahaemolyticus* is involved in LuxO-OpaR system

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Vibrio parahaemolyticus is a gram-negative bacterium which grows in the marine environment and the estuary. It is an important food-borne pathogen causative of gastroenteritis and diarrhea in Taiwan, Asia and coast area. The outbreaks often associated with the consumption of raw or undercooked seafood. One clinical strain isolated from Taiwan was TDH and TRH negative and was designated as *V. parahaemolyticus* no.93. Previously, we have demonstrated that the alkaline serine protease (PrtA, VPA0227) from this strain has the cytotoxic effects on CHO, HeLa and CaCO2 cells. It was reported that LuxR-like transcriptional regulator might be a positive regulator of the protease gene as well as involved in negative regulation of the cytolysin gene in *V. cholerae*. The LuxR homologue in *V. alginolyticus* caused decrease in the transcript levels of extracellular alkaline serine protease A (*proA*), which was an important virulent factor of *V. alginolyticus*. However, the LuxO-OpaR system exhibits highly homologous in the *Vibrio* spp.. There was a conserved sequence for the LuxR-like protein binding on the upstream of *prtA* promoter. Therefore, we hypothesized that the extracellular proteases in *V. parahaemolyticus* are regulated by LuxR homolog, OpaR. A precondition assay was performed in our laboratory to determine this point of view to be present. Continuously, the bacterial mutants were costructed by using homologous recombination gene replacement approach. The Δ*opaR* was observed to enhance the swarming activity, but was opposite in that of Δ*luxO*. However, no obvious change was occurred in colony transparency. In addtion, the QPCR results showed that *prtA* and *vppC* were expressed strongest at 3 and 6 hr, respevctively. In the contrast, the expression of *prtV* was sustained steadily. The results showed that the expression of *prtA* was increased in Δ*luxO* but *vppC* was decreased in Δ*opaR*. It indicated that *prtA* was positively regulated by OpaR. However, *prtV* and *vppC* was not obviously regulated in these strains. The same results of PrtA expression are exhibited by Western blot. We concluded that PrtA was regulated by LuxO-OpaR system positively. Due to the literature reports are currently absent about the collagenases and the regulated mechanism for the toxicity in *V. parahaemolyticus*. We will further observe the role of LuxO-OpaR system in the virulent mechanism of *V. parahaemolyticus* in the near future.

Keywords *Vibrio parahaemolyticus*; alkaline serine protease; quorum sensing

Unbalanced hunger response of glucose-growing *Pseudomonas putida* results in cell lysis

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Growth of bacteria is often completely restricted due to the lack of suitable carbon sources in the environment. There are also habitats, for example rhizosphere, where carbon source is present, but the amount of nutrients is still relatively low prohibiting the maximum speed of proliferation. Bacteria can grow in such environments of nutrient-limitation, but they are still in the state that is defined as "hunger".

Limitation of carbon source and competition with other inhabitants of the niche requires specific changes from gene expression to the cell membrane. We have investigated the hunger response of glucose grown *Pseudomonas putida*. Rearrangements for better acquisition of glucose are complex and require functioning of two-component signal transduction system ColRS.

We have discovered that *P. putida* deficient in ColRS signalling experiences serious glucose-specific stress that leads to the lysis of bacteria growing on solid medium. Analysis of cells by flow cytometry has revealed population heterogeneity: in addition to the wild-type-like population, there is a subpopulation of cells with damaged membrane. More specific investigation revealed that the subpopulation prone to lysis is locating in the periphery of bacterial culture growing on solid medium and the lysis is depending on certain glucose concentration, which most probably provides the hunger signal for bacteria. Analysis of membrane protein pattern revealed several hunger-induced changes in bacterial outer membrane: the amount of OprB1 porin is significantly increased at glucose limitation, whereas the amount of OprE porin is decreased. Therefore, it is not surprising that the essentiality of ColRS in hunger response can be bypassed by reducing the amount of certain outer membrane proteins. We were able to demonstrate that the depletion of OprB1, down-regulation of OprF porin and hindering the SecB-dependent protein secretion can suppress the cell lysis of bacteria deficient in ColRS signalling.

Altogether, our studies have shown that *P. putida* growing on solid glucose medium adapts to glucose limitation through up-regulation of sugar porin OprB1, which allows enhanced acquisition of a limiting nutrient. However, bacteria need ColRS signalling to survive this adjustment. Thus, ColRS system can be considered a safety factor of hunger response ensuring the welfare of cell membrane during the increased expression of certain membrane proteins.

Keywords: *Pseudomonas*; hunger; cell lysis; cell membrane; glucose; ColRS; OprB1

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Use of the red fluorescent protein mCherry as a reporter gene in *Pseudomonas putida* to achieve higher levels of expression in the XylS/*Pm* regulator/promoter system

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The XylS/*Pm* expression cassette consists of the regulator XylS of the AraC-XylS family of positively regulated transcription factors and its cognate promoter *Pm*. Originating from the *Pseudomonas putida* TOL plasmid, which encodes a pathway for the degradation of toluene and xylenes, the system is inducible with a range of different benzoate derivatives. Expression levels have earlier been greatly improved in *Escherichia coli* by introducing mutations in the *Pm* promoter and its 5'-untranslated (UTR) DNA region (Bakke et al., 2009 and Berg et al., 2009). However, attempts to use the same variants for improved expression in *P. putida* and *Pseudomonas fluorescens* did not yield the desired results, indicating that specific mutants may be needed for each host of interest. Using the red fluorescent protein mCherry as a reporter, we have now been able to screen a library of *Pm* and UTR mutant sequences in *P. putida*. The results show that expression can be strongly improved by selecting *Pm* and UTR mutants displaying enhanced activity in this specific host.

Keywords XylS/*Pm* regulator/promoter system, *Pseudomonas putida*, mCherry

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Volatile organic compounds of *Pseudomonas* and *Serratia* and their action on phytopathogenic fungi and bacteria

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The bacteria are able to produce volatile organic compounds (VOCs) that suppress growth of other microorganisms. A number of volatiles released by bacteria are also known as a plant growth stimulation factors. Volatiles are considered to be able to play the significant role in antagonistic interactions between microorganisms occupying the same ecological niches. The production of these compounds can be important for biological control of plant diseases by plant-associated bacteria.

In the present work it was shown that VOCs produced by *Pseudomonas* and *Serratia* strains suppressed the growth of *Agrobacterium tumefaciens*, *Erwinia carotovora*, cyanobacteria *Synechococcus* and *Anabaena* and phytopathogenic fungi *Rhizoctonia solani* and *Helminthosporium sativum.* Besides, they caused changes in the morphology of the fungi grown on plates and mycelium degradation visible under electronic microscopy of *Verticillium dahliae, Helminthosporium sativum*, *Sclerotinia sclerotiorum, Fusarium oxysporum, Pythium aphanidermatum* and *R. solani*. To investigate the mechanisms of the regulation of the VOCs production we studied the influence of mutations inactivated genes responsible for the global regulation of bacterial genes expression on VOCs synthesis. It was found that mutations in genes *rpoS*, *vfr*, *phzA*, *phzB* of *P. chlororaphis* did not affect the VOCs production. Mutant of strain *S. proteamaculans* 94 with inactivated gene *sprI* (N-acyl homoserine lactones synthase gene) didn't produce volatile compounds suppressing phytopathogenic fungi growth. Some decrease of volatiles effect is observed in case of strains with mutations in genes of global systems *gacA/gacS* and *grrA/grrS* in strains *P. chlororaphis* and *S. plymuthica*, respectively.

VOCs emitted by strain *P. chlororaphis* 449 were identified with using the gas chromatography–mass spectrometry analysis. The antimicrobial effects of individual compounds are currently under investigation.

Keywords antagonistic interaction; bacteria; phytopathogenic fungi; volatile organic compound

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Biodegradation and Bioremediation

A case study of the bioremediation of a methyl *tert*-butyl ether-polluted Hungarian aquifer

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A bioremediation field study has been recently performed at an abandoned Hungarian gasoline storage tank facility. The site assessment activities indicated that the groundwater beneath the surface was heavily polluted with methyl tert-butyl ether (MTBE), BTEX components and aliphatic hydrocarbons. The contamination plume was sampled and the biodegradation rates for each pollutant were evaluated. Laboratory results revealed that the addition of a proper mixture of mineral salts stimulated the endemic bacterial flora. The degradation of both aromatic and aliphatic contaminants could be intensified, but MTBE was found to be recalcitrant during the tests under aerobic conditions. Therefore, *Methylibium* sp. T29, a novel bacterial isolate capable of the efficient mineralization of MTBE was cultured and injected along with solutions of ammonium sulphate, potassium phosphates and trace elements. In order to increase the concentration of dissolved oxygen in groundwater, magnesium peroxide suspensions were also injected into four upstream wells. During the field study, groundwater samples were collected from 12 wells regularly and analyzed for organic substances and geochemical indicators including pH and concentrations of dissolved oxygen, ammonium, orthophosphate and sulphate ions. Culture-independent and culture-based microbiological methods were also applied to monitor the multiplication of *Methylibium* sp. T29 and the indigenous bacteria, respectively, throughout the *on-site* test. Data suggested that spontaneous breakdown of magnesium peroxide resulted in the slow and stable release of molecular oxygen (up to 7.2 mg/L), which enhanced the aerobic microbial degrading processes and enabled the significant depletion of different hydrocarbons. In line with the decrease of the concentrations of BTEX and other TPH compounds, MTBE was also decomposed rapidly. Analytical and denaturing gradient gel electrophoretic (DGGE) results confirmed that the loss of MTBE coexisted with the spreading and proliferation of microbes belonging to the *Methylibium* genus. Based on these observations, the presented biotechnological method appears to be suitable for the effective cleanup of aquifers contaminated with MTBE and various petroleum hydrocarbons.

Keywords bioremediation; MTBE; *Methylibium*; magnesium peroxide; DGGE

Ampelomyces quisqualis Ces. ex Schlecht. as an alternative measure of protection

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To date, the Republic of Serbia has registered no fungicides for suppression of pathogens in the forest ecosystems. In order to introduce proper use of new disease-fighting agents into the country, certain relevant principles, requirements and criteria prescribed by the Forest Stewardship Council (FSC) must be observed, primarily with respect to measures of assessment and mitigation of risks, the list of dangerous and highly dangerous pesticides with the possibility of alternative protection. One of the main goals of the research was adjustment of the protective measures to the FSC policy through selection of eco-toxicologically favourable fungicides, given the fact that only preparations named on the list of permitted active matters are approved for use in certified forests.

The occurrence of mass dieback in oak forests is another consequence of the presence of powdery mildew, which is caused by the pathogenic fungi *Microsphaera alphitoides* Griff. et Maubl. (1910) and which affects in particular new, young foliage susceptible to infections. Oak powdery mildew is a serious problem on seedlings in nurseries as well as on naturally and artificially introduced progeny.

We study alternative protection on the occurrence of mass dieback in oak forests seedlings in Central Serbia (caused by *M. alphitoides*) with various dosages of AQ-10 biofungicide, which is a pelleted formulation of conidia of *Ampelomyces quisqualis* Ces. ex Schlechtend.. Simultaneous testing was conducted on the efficiency of a chemical sulphur-based preparation (used in this area for many years as a measure of suppression of powdery mildews, without the possibility of developing resistance of the pathogen to the active matter). Examinations were performed by standard EPPO methods. The intensity of the infection was followed according to methods PP1/69 (2): 100-102 and PP 1/152 (2): 37-51 (1997a and 1997b), while the method PP 1/135 (2): 31-36 (1997c) was applied in the case of phytotoxicity. In data processing, statistical methods were used – the intensity of infection according to Towsend-Heuberger, and the efficiency according to Abott and method PP/181 (2): 52-58 (EPPO 1997d). The differences in the intensity of infection were determined through the variance analysis and LSD test.

The results of the research have demonstrated that AQ-10 biofungicide can be used as a part of integrated disease management programmes as an alternative. The best results in suppression of oak powdery mildew were attained through use of sulphur SC in the concentration of 0.5%, while very satisfactory results were obtained by use of AQ-10 biofungicide in the highest dosage of application (70 g/ha). The number of treatments was proven to have no significant impact on increased efficiency of the bio-preparation, or in other words, that besides the application dosage, the high efficiency of the bio-preparation depends primarily on proper timing of the application.

Key words: pedunculate oak, alternative measures of protection, efficacy

An investigation of mixed microbial populations for use in the treatment of waste fats, oils and greases (FOGs)

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The removal of fats, oils and greases (FOGs) from wastewater is critically important to ensure that wastewater is disposed of efficiently and economically avoiding blockages of sewers and problems in municipal wastewater treatment plants. FOGs may be intercepted at source using grease traps and treated biologically *in situ* using bioaugmentation. In Ireland, bioaugmentation products used in grease traps must contain only bacteria.

Batch studies were carried out in the laboratory to evaluate the ability of mixed microbial populations to degrade various fats, oils and greases (butter, highly refined olive oil and Greek extra virgin olive oil) in 1% w/v concentration. The inoculum was added (10^6org/ml) to 250 ml Erlenmeyer flasks containing 100 ml of sterile culture medium to which the carbon source was added. Two culture media were investigated, a minimal medium and an enriched nutrient medium. The flasks were incubated aerobically at 30° C and agitated at 150 rpm for 7 to 14 days.

The microbial populations investigated comprised mainly bacteria of the genera *Bacillus* and *Pseudomonas*. Optimal results were obtained when a mixture of *Bacillus spp*. and a *Pseudomonas putida* was used. This mixture was capable of degrading both hard fats and oils and also displayed an interesting aggregative growth behaviour – desirable for retention in a grease trap when designing bioaugmentation products.

Keywords Fats, Oils and Greases (FOGs), greasetrap, bioaugmentation, *Bacillus*, *Pseudomonas*

Application of Flotation and Bioremediation to Eliminate Persistent Organic Pollutants in the Influent Stream of Cerny Prikop (Czech Republic)

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The paper deals with decontamination of sediments polluted by persistent organic pollutants in the influent stream of Cerny prikop in Ostrava, the Czech Republic. Flotation is used as the first stage treatment method when the individual contaminants accumulate in the flotation concentrate. Consequently, bacterial leaching applying the bacteria of genus *Rhodococcus* sp. is used as the second treatment method. The results imply that the combination of both the methods is very effective and leads to a removal of up to 70% C_{10}-C_{40}, 60% PAHs, 70% PCBs.

Keywords flotation; bioremediation; *Rhodococcus* sp.; persistent organic pollutants

Application of polymeric biosurfactant produced by *Candida glabrata* for bioremediation of soil contaminated by hydrophobic pollutant

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The impacts on the environment promoted by organic compounds of petroleum origin are from accidental spills and deliberate discharge of oily waste in soil or water. These accidents have become numerous and have caused many ecological and social problems. An alternative to the removal the contaminants can be accomplished through the use of biosurfactants. Biosurfactant are molecules that have hydrophilic and hydrophobic groups acting on the interface between fluid phases, produced by biotransformation of renewable raw materials, with major advantages over chemically synthesized surfactants. The application of biosurfactant-type polymer produced by *C. glabrata* was carried out in sandy soil contaminated with burned oil engine to increase the interaction of surface water / oil and promote this way, treatment of soil by bioremediation. The treatment process for this soil was used 10 ml of burned motor oil adsorbed in 20 g of sandy soil, coming from the beach of Boa Viagem (Recife-PE, Brazil). The treatment was carried out using the net metabolic cell-free, containing the biosurfactant and distilled water as control. The experiments were submitted to shaker at 150 rpm during 48 hours at 28 ° C. The polymeric biosurfactant showed excellent result and removed 95.77% of the hydrophobic pollutant from sandy soil. The results showed higher biotechnological eficiency of the biosurfactant produced by *C. glabrata* strain.

Keywords: Bioremediation; *Candida glabrata*; Biosurfactant; hidrophobic pollutant.

Bioaugmentation of Microbial Consortia and Supplementation of Bulking Agentsin Removal of Crude Oil from Soil

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Currently, with the increasing attention towards the environment conservation, bioremediation has become a valuable alternative to chemical treatments. Bioremediation is an ecologically acceptable technology that uses microorganisms to efficiently degrade pollutants such as oil and oil products in the environment to non-toxic products.Biodegradation experiment was carried out to evaluate the effectsof addition of microbial consortium and supplementation of bulking agents on biodegradation of crude oil in soil. The soil with indigenous microbes was spiked with crude oilat 50 000 mg/kgand microbial consortia in the ratio 1:1:1:1 (v/w) which consisted of *Pseudomonas* sp. UKMP 14-T, *Acinetobacter*sp. UKMP 12-T and two fungi isolates of *Trichoderma*sp. (TriUKMP-1M and TriUKMP-2M). Bulking agents (sugarcane bagasse (SB) and empty fruit bunch (EFB) from oil palm) at 15% and 20% (w/w), respectivelywere mixed with the soil thoroughly. The pH of the soil was maintained at6.5 using 0.05%(v/w)calcium carbonate (Ca_2CO_3)and the moisture content was maintained at40% VWC. The degradation of crude oil from the soil was analyzed using gas chromatography-flame ionization detector (GC-FID) and the growth of bacteria was estimated using spread plate method. Degradation of crude oil was expressed as Total Petroleum Hydrocarbon (TPH). The result showed degradation of crude oil by microbial consortia with addition of SB produced 100% TPH degradation compared to91% with EFBafter 30 daysof incubation. The control plot which containedonly indigenous microbes showed 62% degradationfor the same period of incubation. This experiment indicated that the types of bulking agent may influence the intake of the nutrient source by microbial consortia, hence influence the percentage of the TPH.This system is inexpensive, efficientand environmentally compatible and may offer a viable choice for crude oil-contaminated soil bioremediation.

Keywords: Biostimulation, bulking agent, crude oil, microbial consortia

Biodegradation of atrazine and terbutryne by a mixed microbial community in a packed bed biofilm reactor

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Atrazine and terbutryne are triazine herbicides widely used for broad leaved weed control. Those herbicides have potential deleterious effects on aquatic systems. Their specific interference with photosynthetic electron transport and toxicity to primary producers in the trophic chain is well known. Due to their extended use in agriculture and to their persistence, atrazine and terbutryne have to be considered a potential risk for water life as well as for drinking water quality.

For the aerobic biodegradation of those herbicides, an internal airlift bioreactor (ALR) was constructed. The reactor's riser was packed with a porous support of volcanic stone fragments.

A bacterial community isolated from agricultural soils of Central Mexico formerly treated with triazine herbicides was used along this work. Selection process was carried out by the technique of microbial enrichment by successive culture transfer into mineral salt medium (MS) containing terbutryne and atrazine (21 mg L^{-1} and 24 mg L^{-1}, respectively) as sole carbon and nitrogen source. The herbicide (Atermix), a commercial presentation used in Mexico, was diluted to obtain proper concentrations. The cultivable bacterial strains constituting the community were identified by sequence comparison of 16S rDNA amplicons. They were *Acinetobacter* sp. (NC014259.1; 96%), *Pseudomonas fluorescens* (NC007492.2; 96%), *Acinetobacter baumannii* (NC010611.1; 94%) and *Arthobacter* sp. (NC008541.1; 90%). In parentheses, the NCBI accession numbers and % of similitude are indicated.

The mixed bacteria culture was immobilized in the PB-ALR and operated by repeated batch and continuous culture and fed with MS medium containing terbutryne and atrazine (21 mg L^{-1} and 24 mg L^{-1}, respectively) as carbon and nitrogen sources. The overall removal efficiency obtained with this community was in a range of 80 to 100%, determined by HPLC and by the decrease of the chemical oxygen demand. In addition, as an important byproduct, the cyanuric acid content was evaluated. In this case, the removal efficiency was in a range of 70 to 80%.

Keywords: Biodegradation; atrazine; terbutryne; biofilm reactor.

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Biodegradation of fluoroquinolones by a bacterial consortium

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The present concern in pharmaceuticals in the environment is well known and research studies in this area have been regularly reported. Pharmaceuticals reach the environment by several ways but mostly due to their incorrect disposal and the incomplete elimination during the treatment processes in Wastewater Treatment Plants (WWTP). These residues continuously enter aquatic environments and many of them are resistant to degradation, being so called as pseudo-persistent pollutants. In aquatic compartments, pharmaceutical residues reach concentrations in the ng L^{-1} to µg L^{-1} range.

This work describes the biodegradation of four fluoroquinolones, Ofloxacin (OFL), Norfloxacin (NOR), Ciprofloxacin (CPF) and Moxifloxacin (MOX), by a bacterial consortium constituted by three bacterial species isolated in our labs, namely F11, FP1 and S2, known to degrade different aromoatic fluorinated compounds. The experiments were conducted in batch mode using a mineral medium supplemented with acetate and 10 mg l^{-1} of each compound or 10 mg l^{-1} of a mix of the compounds. The bacterial consortium was capable of aerobic biodegradation of OFL, NOR and CPF during successive feedings of the compounds to the medium, as measured by monitoring removal of the compounds by HPLC-FD and fluoride release by potenciometry. Degradation profile of the fluoroquinolones used in this study indicated that intermediate metabolites were accumulated. Two of the initial constituting strains, F11, belonging to the α-proteobacteria group, and S2, belonging to the Actinobacteria group, were recovered from the medium, F11 predominating in cultures fed with moxifloxacin whereas S2 was mostly found in the remaining cultures. Degradation by single bacteria is under evaluation.

Keywords Biodegradation; Pharmaceuticals; Fluoroquinolones

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Biodegradation potential and molecular detection of the cathechol 1, 2-dioxygenase gene of actinobacteria isolated from wastewater treatment plants in Spain

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Actinomycetes are diverse and nutritionally versatile bacteria that have been widely studied due to the production of secondary metabolites and also because of their ability to degrade environmental toxins. Species of the genera *Gordonia, Rhodococcus* and *Mycobacterium* are known as degraders of recalcitrant pollutants. These species have been suggested as tools for biotechnological applications such as bioremediation. At the same time several species are known as opportunistic human pathogens. Therefore, the accurate identification of isolates with potential for bioremediations applications is important. The benzene ring structure is the most widely spread chemical in nature after the glycosides residues, so it is not surprising that organisms have evolved metabolic pathways that enable them to mineralize these compounds. The toxic effect of hydrocarbons aromatics such as phenol and naphthalene has been described in the literature. This study focuses on the effectiveness of biodegradation by different actinobacteria isolated from wastewater treatment plants (WWTPs) with foaming and toxicity problems.

Actinomycetes strains were isolated in Czapeck medium and identified by polyphasic taxonomy procedures. Diaminopimelic acid, whole cell sugars and mycolic acids were detected by standard procedures. PCR amplification of the 16S rDNA was carried out using universal primers. Multiple sequences alignments were performed using CLUSTAL X software and phylogenetic analysis was done by the neighbour-joining method. Three different mineral media, supplemented with 0.1% w/v phenol and naphthalene as sole carbon source, were used to evaluate biodegradation potential. Previously, strains were subjected to an adaptation culture in the same media supplemented with 0.1% w/v of glucose. Results were scored using the growth with glucose as positive control. The *catA* gene encodes for catechol 1, 2-dioxygenase, which is a key enzyme involved in the first step catalysis of monoaromatic pollutants such as benzene ring and derivates. Presence of these catabolic genes could determine the biodegradation potential of the microorganisms. C12OF and C12OR primers were used to amplify the *catA* gene from the different genera.

We have isolated 156 strains from wastewater treatment plants. Chemotaxonomic test and analysis of 16S rDNA showed that the isolated strains belonged to the genera *Corynebacterium* (2 strains), *Dietzia* (2 strains), *Gordonia* (97 strains), *Microbacterium* (1 strains), *Mycobacterium* (24 strains), *Pseudonocardia* (4 strains), *Rhodococcus* (9 strains), *Tsukamurella* (16 strains) and *Williamsia* (1 strains). The biodegradation assays showed that 56 strains were capable of degrading at least one toxic product. The best degraders' strains are *Pseudonocardia* and *Mycobacterium* (degrading naphthalene), *Gordonia* (degrading naphthalene and phenol) and *Rhodococcus* (degrading phenol). The *catA* gen was amplified in 38 strains.

In conclusion, in this work we show that wastewater treatment plants can be a good source of microorganisms with many potential applications, such as bioremediation and biodegradation.

Keywords biodegradation potential, actinobacteria, phenol, naphthalene, cathechol 1, 2-dioxygenase gen (*catA* gene)

Bioremediation of direct dyes in simulated textile effluents by paramorphogenic form of *Aspergillus oryzae*

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Azo dyes are extensively used for coloring textiles, paper, food, leather, drink, pharmaceutical products, cosmetics and inks . The textile industry consumes the largest amount of azo dyes, and it is estimated that approximately 10 – 15% of dyes used for coloring textiles might be lost in waste streams. Almost all azo dyes are synthetic and resist biodegradation, however, they can be readly reduced by a number of chemical and biological reducing systems. Biological treatment is advantageous over physical and chemical method as result of its low cost and little disturbance to the environment.

This research focuses on the utilization of *Aspergillus oryzae,* to remove some kinds of azo dyes from aqueous solutions. The fungi, physically induced in its paramorphogenic form (called ,"pellets"),were used in the dyes biosorption studies with both non autoclave and autoclaved hyphas, at differents pH values . Thus the goals are the removal of dyes by biosorption and the decrease of its toxicity.

The utilized dyes were the Direct Red 23 and Direct Violet 51. Their spectral stabilty (325-700nm) was analised at different pH values (2.50,4.50 and 6.50).The best biosorptive pH value and the toxicity limit(which is given by the lethal concentration (LC^{100}) were then determined. Each dye showed the same spectrum at different pH values. The best biosorptive pH was 2.50, for non autoclaved and autoclaved hyphas of *Aspergillus oryzae*. The toxicity level of the dyes was determinated using Trimmed Spearman – Karber`s Method, with *Daphnia similis* in all bioassays . The Direct Violet 51 (LC^{100} 400 $\mu g.mL^{-1}$)was found to be the most toxic dye, followed by the Direct Red 23 (LC^{100} 900 $\mu g.mL^{-1}$).

The toxicity bioassays for each dye have showed that it is possible to decrease the toxicity level to zero adding a smal quantity of biomass from *Aspergillus oryzae* in its paramorphogenic form. The autoclaved biomass have showed a higher biosorptive capacity with the dye than the non autoclaved ones.

The results have showed that the bioremediation does occur on the *Aspergillus oryzae* in its paramorphogenic form, and it provides the remarkable condition that it can be used as biosorptive substract for industrial waste water treament containing azo dyes.

Keywords bioremediation, biosorption, *Aspergillus oryzae*, *Daphnia similis,* paramorphogenic form, "pellets", azo dye, toxicity.

Bioremediation of PAH-contaminated soil in semi-arid conditions: effect of autochthonous bioaugmentation strategies

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Autochthonous bioaugmentation (ABA) is defined as a bioaugmentation technology that uses microorganisms isolated from the contaminated sites to be remediated, which should be the much better adapted to the historically or artificially contaminated environments.

The aim of this work was to investigate the effects of the ABA strategy in artificially phenanthrene-contaminated Patagonian soil microcosms maintained under semi-arid conditions, on phenanthrene elimination rate and soil microbial community. The phenanthrene-degrading strain used as inoculum, which showed a phylogenetic relationship with the *Sphingomonadaceae* family, was previously isolated from a PAH-contaminated soil sample collected in semiarid Patagonia, and selected by its resistance to drying conditions.

Two treatments were carried out in triplicate trays: control (C) and inoculated (IN) and the microcosms were incubated sequentially under three different abiotic conditions. The microcosms were contaminated with 2000 mg Kg^{-1} of phenanthrene. The IN microcosms were inoculated with $1.4x10^{8}$ cfu/g of dry soil at 0; 86 and 150 days of treatment. At the beginning of the experiment the microcosms were maintained to 10% (w/w) of water content and keeping the original C/N/P rate of the soil (100/2/0.3); after of 86 days the incubation the microcosms were fertilized taking the relation C/N/P to 100/5/2; and finally after 150 days of incubation the water content was increased to 15%.

The concentration of the remaining phenanthrene concentration (HPLC), the number of cultivable heterotrophic (R2A) and PAH-degrading (NMP) bacteria, the total soil microbial activity (dehydrogenase activity) and the genetic diversity of the bacteria soil community (PCR-DGGE of 16S rDNA) were determined at different time intervals.

During the first 150 days of treatment the concentration of phenanthrene did not shows significant changes in the C microcosms but neither in the inoculated microcosms. However the IN microcosms showed highest PAH-degrading bacteria counts and the DGGE profile of samples from the IN microcosm during the entire investigation period were strikingly different and less than 20% similar to the profiles of samples from C microcosms, with a clear predominance of a band corresponding to the inoculum in the DGGE profiles of IN microcosms.

The change of the C/N/P ratio done on the 86th day of treatment caused a small increase of the microbial activity in C and IN microcosms, with a drastic change in the DGGE profile of C microcosms. However the elimination of phenanthrene was not observed. The increase in the water content done after 150 days of treatment produced, in C and IN microcosms, an increment of the heterotrophic bacteria counts, the dehydrogenase values and the genetic diversity (DGGE), with a concomitant phenanthrene elimination. After 20 days that the water content was increased the residual concentration of phenanthrene was significantly lower in the IN microcosms than in the C microcosms.

The results of the present study showed that the semi-arid conditions strongly limit the phenanthrene degradation, in spite of the presence not only of the indigenous phenanthrene-degrading bacteria but also the long-term establishment of the inoculum; and that clearly during the entire experiment the phenanthrene was bioavailable. Although ABA strategy produced strong changes in the soil microbial community, a stimulatory effect on phenanthrene degradation principally occurred when the water content was increased.

Keywords: Bioaugmentation; Soil; Semi-arid conditions; Phenanthrene

Bioremediation of Sites Contaminated with Textile Effluents: Role of Designer Bacterial Consortium and Plasmids in the Decolorization of Various Textile Azo Dyes

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The decolorization of textile colorants (textile azo dyes) is one of the vital concerned environmental issues which still await successful field remedy. Biodegradation of azo dyes by bacteria is one of the important areas of research to provide solid remedy to the problem.

Approach: This work investigated the primary isolation of 78 bacteria from various samples. Twenty four organisms were screened on the basis of their decolorization potential during secondary screening. Biochemical characterization of these isolates was performed. Decolorization study of 16 textile dyes was carried out employing a designer consortium consisting seven bacterial isolates. Isolation of plasmids from the isolates used to design consortium and their curing was carried out to confirm the role of plasmid in decolorization of textile dyes .

Results: More dye decolorization activity was achieved in presence of designer consortium compared to individual organisms. Curing of plasmids significantly decreases the decolorization activity indicating involvement of plasmid in the decolorization activity of bacteria.

Key words: Decolorization, Designer Bacterial Consortium, Plasmid

Biosurfactant production by *Rhodotorula glutinis* UCP 1555 using industrial wastes

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The biosurfactants are molecules that have hydrophilic and hydrophobic group acting on the interface between fluid phases. The formation of a molecular film between the interfaces, you can reduce the surface and interfacial tensions. These properties make biosurfactants suitable for a wide range of industrial applications such as low toxicity, biodegradability, production from renewable substrates and stability at extreme pH values and temperature.

The main of this work was to evaluate the biosurfactant production by *Rhodotorula glutinis* using submerged fermentation in medium composed of industrial waste [cassava wastewater , corn steep liquor and ice cream waste], in order to achieve the best ratio of nutrients. The concentrations of waste were established by Central Composite Rotational Design (DCCR) of 2^3 and the variable response was the reduction of surface tension and emulsifier index as biosurfactant production. The fermentation in Erlenmeyer's flasks was carried during 72 hours, at 28° C under orbital agitation at 150 rpm. The liquid metabolic was centrifuged and filtered to obtained the biomass was measured gravimetrically. The liquid metabolic free cells was subjected to determination of surface and interfacial tensions, emulsification index, stability against different temperatures, concentrations of sodium chloride and pH. The kinetics of the growth of *R. glutinis* and biosurfactant production was evaluated during 152 hours. The results showed the lower surface tension was 28mN/m and was obtained in the assay containing higher levels of cassava wastewater (30%), corn steep liquor (9%), and lower residue of ice cream (10%). Significant results were obtained for the interfacial tension (5.1 mN/m), and the emulsification index (93%) using hydrocarbons as motor oil. The biosurfactant in the assay selected showed thermal stability in a wide temperature range (up to 100 ° C) determined by surface tension, and pH stability in 4, 8, 10 and 12 determined by the rate of emulsification. The biosurfactant produced by *R. glutinis* becomes economically viable using industrial wastes, and the biotechnological process offered economic production and a great potential of applications.

Keywords: *Rhodotorula glutinis*; Biosurfactant; Industrial Waste; Stable; Surface Tension

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Biosurfactant production from agro-industrial residue by *Pseudomonas aeruginosa* LBI

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Biosurfactants are secondary metabolites that exhibit surface activity and are synthesized by a variety of microorganisms. The advantages of these compounds compared to synthetic surfactants are based on structural diversity, low toxicity and high biodegradability. Besides, they can be produced from renewable and low cost sources such as agro-industrial residues. These biosurfactants contribute to environmental pollution reduction and they allow aggregate market value to agro-industrial residues. Bacteria as *Pseudomonas* genus are noted for biosurfactant synthesis known as rhamnolipids, which are structurally glycolipids with fatty acid molecules linked to rhamnose.

This work aimed for rhamnolipids (biosurfactant) production by *Pseudomonas* from an agro-industrual residue.

The microorganism used was *Pseudomonas aeruginosa* LBI isolated from petroleum-contaminated site and soybean soapstock was used as carbon source. The culture was maintained on nutrient agar at -4 ºC and it was reactivated in 50 mL nutrient broth during 24 h at 30 °C – 150 rpm by a rotary shaker for bacterial growth. A 1.0 mL aliquot of the bacterial growth was added to 250 mL Enlermeyer flask cointaining 50 mL mineral salts medium with 2.0% w/v of soybean oil as carbon source (inoculum). After 24 h at 30 ºC – 200 rpm, 1.0 mL of the inoculum was added to 125 mL Erlenmeyer flasks with 25 mL of production medium composed by mineral salts and 2.0% w/v of soybean soapstock. Initial pH of production medium was adjusted to 6.8–6.9 and the incubation was 120 h at 30 ºC - 200 rpm . Experiments were conducted in three independent replicates. Rhamnolipids were quantified from the cell free broth as rhamnose. The rhamnose concentration was determined by the reaction with H_2SO_4 and thioglycolic acid and absorbance measure at 400-430 nm. Hence, rhamnolipid content was determined by multiplying rhamnose values by 3.

It was observed that *Pseudomonas aeruginosa* LBI was able to grow in mineral salts medium with soybean carbon source (oil and soapstock). The biosurfactant production from soybean soapstock was demonstrated using the rhamnose concentration analysis. Initially, there was 0,165 g L^{-1} of rhamnolipids at mineral salts medium with 2.0% w/v of soybean soapstock. After 120 h incubated, *Pseudomonas aerugiona* LBI produced 3,823 g L^{-1} of biosurfactant (rhamnolipids).

Therefore, the use of agro-industrial residues such for rhamnolipid production by *Pseudomonas* is a viable alternative to reduce biosurfactant costs. Also, renewable sources as soybean soapstock contributes to a sustainable and ecological development.

Keywords rhamnolipids; soapstock, glycolipid, soybean oil

Characterization of Alkylphenol Degradation and Alkylphenol Degradation Gene Cluster of *Sphingobium fuliginis* strain TIK-1

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Alkylphenols (APs) are ubiquitous pollutants in urban aquatic environments and show estrogenic activities and other toxic effects on aquatic organisms and humans. We have recently isolated *Sphingobium fuliginis* strain TIK-1 that is capable of utilizing 4-*tert*-butylphenol (4-*tert*-BP) as a sole carbon and energy source via a *meta*-cleavage pathway (Applied and Environmental Microbiology, 2010, 76:6733-3740). To investigate biodegradation mechanisms of APs, we examined here (i) characterization of AP degradation by strain TIK-1 and (ii) characterization of AP degradation gene cluster of strain TIK-1.

Twenty APs with various alkyl chains and bisphenol A (BPA) were used for growth experiments with strain TIK-1 and for degradation experiments using whole cells of strain TIK-1 grown on 4-*tert*-BP or glucose. Strain TIK-1 grown on 4-*tert*-BP or glucose could degrade 4-APs with various length and branched side chains (ethyl, *n*-propyl, isopropyl, *n*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *tert*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *tert*-octyl, *n*-nonyl, and branched nonyl) and BPA via a *meta*-cleavage pathway but not 2- or 3-APs. Strain TIK-1 is potentially useful for removal of wide range of 4-APs and BPA.

Table Utilization and degradability of various APs by *S. fuliginis* strain TIK-1

Substrate	Growth	Transformation ratio (%)	Main degradation product
4-Ethylphenol	−	100	Not detected
2-*n*-Propylphenol	−	0	Not detected
2-Isopropylphenol	−	0	Not detected
3-Isopropylphenol	−	0	Not detected
4-*n*-Propylphenol	−	100	2-Pentanone
4-Isopropylphenol	+	100	3-Methyl-2-butanone
2-*sec*-Butylphenol	−	0	Not detected
2-*tert*-Butylphenol	−	9.7	Not detected
3-*tert*-Butylphenol	−	0	Not detected
4-*n*-Butylphenol	−	100	2-Hexanone
4-*sec*-Butylphenol	+	100	3-Methyl-2-pentanone
4-*tert*-Butylphenol	+	100	3,3-Dimethyl-2-butanone
4-*n*-Pentylphenol	−	100	2-Heptanone
4-*tert*-Pentylphenol	+	100	3,3-Dimethyl-2-pentanone
4-*n*-Hexylphenol	−	100	2-Octanone
4-*n*-Heptylphenol	−	100	2-Nonanone
4-*n*-Octylphenol	−	100	2-Decanone
4-*tert*-Octylphenol	−	98.0	3,3,5,5-Tetramethyl-2-hexanone
4-*n*-Nonylphenol	−	100	2-Undecanone
Technical nonylphenol	−	64.0	Methyl branched nonyl ketones
Bisphenol A	−	100	Under analysis

Complete genome sequence of strain TIK-1 was determined by Roche Genome Sequencer FLX (GS FLX) System. The sequences of about 831,000 reads were assembled by Rhoche GS *De Novo* Assembler. The prediction and annotation of open reading frames (ORFs) were performed using NCBI BLAST and the In Silico Molecular Cloning software suite. The genome of strain TIK-1 contains a circular 4,347,408-bp chromosome with 64.2% G+C content. We found 14,402 ORFs and then identified AP degradation gene cluster which encodes the enzymes of AP metabolic pathway from strain TIK-1. This gene cluster consisted of 18 genes. Among the genes, we identified 6 genes encoding a multicomponent phenol hydroxylase and one gene encoding catechol 2,3-dioxygenase (aromatic ring *meta*-cleavage enzyme). Our results indicate that this AP degrading gene cluster is involved in various 4-AP and BPA degradation in strain TIK-1.

Keywords alkylphenol; degradation; *Sphingobium fuliginis*

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Color removal of Algodoais river water by chitosan obtained from *Absidia corymbifera*

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The filamentous Zygomycetes fungi class has been considered an attractive chitosan source, considering the many biotechnological applications and bioremediation process. Investigations were carried out using microbiological chitosan by adsorption process applied to residuary water treatment. In this context the main objective was the chitosan production by Absidia *corymbifera* using a 2^3 factorial design, applying as independent variables corn steep liquor, glycerin and urea, and as variable response chitosan production. The fungus was grown at 37°C on PDA (Potato Dextrose) medium, and was used as inoculums to fermentation according the factorial design, and incubated in an orbital shaker at 150rpm during 96h. The chitosan was obtained from biomass of *A. corymbifera*, and it was characterized by spectroscopy by infrared to calculate the deacetylation degree and X- ray diffraction to determinate the cristalinity index (IC). The best results showed the deacetylation chitosan degree (GD) variated to 90.3% and 78.6% commercial chitosan (CC). Besides that the microbiologycal chitosan showed 21% of IC and 56,64% of the commercial one. The best condition of deacetylation degree was applied to the discoloration process of the Algodoias River (Suape, Pernambuco, Brazil). The investigation was used 2^1 factorial design, using as independent variable the microbiological and CC concentration, respectively, and the variable response the color removal (RC) from river water. The results indicated higher efficiency of RC (93%) using 5g/L concentration of the microbiological chitosan in static condition. The results obtained suggest higher biotechnological potential of *A. corymbifera* to produced chitosan with the best deacetylation degree, considering the ability to RC of river water.

Keywords: A. *corymbifera*, Chitosan, Color removal, Algodoais River.

Comparison of hydrocarbon-degradation by isolates of *Pseudomonas fluorescens* Chao, *P. putida* P13 and *Pantoea agglomerans* P5 in presence of Gas oil, Toluene and Phenanthrene

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Soil and water contamination by oil is one of great environmental concerns. Bioremediation is one of principal strategies for remediation. Bioremediation is the use of microorganism to remove pollutants and can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Among bacteria Pseudomonas has been well known as one of bacteria with high potentiality to remediation of different types of hydrocarbons. According to the positive role of Pseudomonas genus in bioremediation, in this study the potential of two Pseudomonas bacteria, including P. fluorescens Chao, P. putida P13 and a non-Pseudomonad Pantoea agglomerans as hydrocarbon degraders were estimated in presence of different compounds including gas oil, toluene and phenanthrene.

Pure cultures of three bacteria were cultured by enrichment technique in Carbon Free Minimal Medium containing 1% Gas oil. The grown cells on a rotary shaker were recovered by centrifugation after 1 or 2 days of growth, washed and re-suspended in CFMM. An inoculum of bacteria containing 108 cfu/ml was used in plate and liquid assays, which were performed in factorial experiment based on completely randomized design with 3 replications. Detection of hydrocarbon-degrading activity was first performed using the CFMM plate assay containing 1% toluene and 0.05% phenanthrene, which was measured based on the diameter of bacterial colonies. Utilization of hydrocarbon sources were again detected in CFMM broth supplemented with 2% gas oil, 1% toluene and 0.05% phenanthrene, where the mean viable cell count recovered from hydrocarbon-supplemented broth cultures were measured at different times of 0, 72, 144, 216h.

Mean comparison by Duncan's method showed that in CFMM plate assay there weren't any significant difference between bacterial isolates and also toluene and phenanthrene even though P. fluorescens Chao had higher means in presence phenanthrene. In broth assay P. putida P13 and P. fluorescence Chao had similar behavior in presence of Gas oil, toluene and phenathrene and trend of bacterial growth change at different times was the same, while with P. agglomerans P5 lowest number of bacteria was achieved. Among three compounds highest degradability in bioremediation was related to gas oil, and it's followed by phenanthrene and toluene, respectively. The ability of bacterial isolates for degradation of oil-compounds increased by increasing time of incubation.

To conclude, this study suggests the potential use of P. putida strain P13 isolated from alkaline soil of Iran and P. fluorescence Chao for bioremediation of hydrocarbon-contaminated environments especially Gas oil.

Keywords Pseudomonas, Bioremediation, Gas oil.

Development of a clean and environmental-friendly process for the treatment of industrial effluents containing heavy metals

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Electroplating industries are one of the biggest contributors of heavy metal bearing effluents. Conventional chemical (precipitation) or physical treatments (ion exchange, membrane technologies) appear to be inadequate for the treatment of electroplating effluents due to its reduced efficiency at low metal concentrations, high investment and running costs. Biological treatment has been shown a cheaper and effective alternative on the removal of metals from bearing effluents. Even though, the metal pollution problem requires bigger research efforts, not only to remove metals efficiently from the effluents, but also for its selective recovering and recycling in the productive processes.

The strategy of this work passes to the development of a new technology, a multi-stage process, combining the use of yeast cells of *S. cerevisiae* with chemical/electrochemical methodologies, for an effective removal of the metals (Cr, Cu, Ni and Zn) from real electroplating effluents and its subsequent selective recovery.

For this purpose, two real effluents containing multi-metal (Cu, Ni and Zn or Cr, Cu, Ni and Zn) were treated combining chemical precipitation at pH 6.0 with a subsequent biotechnological-based process (using heat-inactivated brewing yeast cells of *S. cerevisiae*). After the third batch of biomass, metal concentrations were lowered to values below the legal limits of discharge of the U.S.-Environmental Protection Agency and Portuguese law; ≥ 89, 91, 92 and 94% Ni, Cu, Cr and Zn were removed, respectively. In the case of effluents containing Cr, two strategies were considered: reduction of Cr^{6+} to Cr^{3+}, pH adjustment to 6.0 and subsequent treatment with a serial batch of yeast cells. Alternatively, Cr^{6+} was selectively removed (98%) by yeast biomass at pH 2.3; subsequently, pH of the effluent was raised up to 6.0 and treated with a serial batch of yeast cells.

Heavy metals can be subsequently recovered, from the contaminated yeast cells, with a high yield and purity. For this purpose, biomass was incinerated, the ashes were acid digested and heavy metals selectively recovered by combining electrochemical and chemical processes.

In conclusion, this multidisciplinary approach allowed the development of a closed process for removing and recovering selectively Cr, Cu, Ni and Zn from electroplating wastewaters, suitable for future industrial applications.

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Keywords: heavy metals removal, effluent bioremediation, metal selective recovery, *Saccharomyces cerevisiae*

Direct Violet 51 dye Biosorption at different pH values using *Phanerochaete chrysosporium* and *Aspergillus oryzae* autoclaved pellets

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Textile industry uses large amounts of synthetic dyes in dyeing processes. Azo dyes are among the most widely used dyes, characterized by the presence of one or more azo bonds (-N = N-) in its chemical structure. The complexity attached to these molecules makes them highly stable, difficult to be degraded, and persistent in the environment. Therefore, dye presence dificults textile effluents treatment which becomes a source of aquatic contamination. An alternative these processes is microbiological treatment where fungi are widely studied. Fungi can be applied in the degradation or adsorption of these molecules. Autoclaved fungi act only as an adsorbent, since there is no enzyme release that could break dye molecule bonds, which could contribuite to potentially toxic byproducts formation. Recently,biological materials such as peat, chitosan, yeast, fungi and bacteria are used as biosorbents to concentrate and remove dyes. Seeking new ways to properly treat textile effluents, this study aimed to investigate biosorption potential of dye Direct Violet 51 (DV51) solution in different pH values for *Phanerochaete chrysosporium* and *Aspergillus oryzae* autoclaved pellets. Test solutions were prepared with 1 mL of *P. chrysosporium* and *A. oryzae* pellets, pH adjusted (2.50, 4.50 and 6.50) distilled water with a DV51 stock dye solution addition (obtaining final dye concentrations of 100, 80, 60, 40 and 20 mg / mL). Each biomass mL corresponds to 10.33 mg/ ml dry weight for P.chrysosporium and 11 mg / ml for A. oryzae. Control solution was prepared only with 1 ml stock dye solution and 9ml of pH 2.50, 4.50 and 6.50 distilled water resulting in a final concentration of 100 micrograms per milliliter. Test solutions were incubated at 30°C for 120 minutes. Then, they were centrifuged at 4000 rpm for 20 minutes and scaned in a UV-VIS spectrophotometer at 544 nm. Experiment results expressed as discoloration percentage were obtained from the formula: (Absorbance of control - Absorbance of sample / absorbance of control) x 100. Discoloration percentage was determined for all fungi solutions tested. The best results were obtained at a 20 mg / mL *A. oryzae* solution, and 95.04% at pH 2.5, 69.72% at pH 4.5 and pH 6.5 in 77.12, and 40 mg / mL for P. chrysosporium, in which dye discoloration was 99.09% at pH 2.5, 92.87% at pH 4.5 and 90.44% at pH 6.5. P. chrysosporium was more efficient in dye removal as it is shown in the *P. chrysosporium* and *A. oryzae* biosorption comparison tested in three pH values solutions with initial dye concentration of 100 mg / mL (Figure 1). Also, both fungi have a greater biosorptive capacity at pH 2.50. Hence, both fungi have a great potential in textile effluent dye removal, but *P. chrysosporium* has a higher DV51dye biosorptive capacity in a pH 2.50 acid solution.

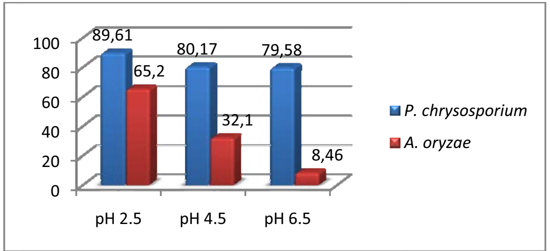

Figure 1: *P. chrysosporiumand A. Oryzae* comparative Direct Violet 51 dye biosorption analysis in pH2.50, 4.50 and 6.50 from a 100 mg/mL initial dye solution.

Keywords: Biosorption; pellets; dye, *Phanerochaete crhysosporium*; *Arspergillus oryzae*

Effect of acetate concentration-inoculum ratio on methanogenesis, using UASB granular sludge as inoculum

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Introduction. Anaerobic digestion is a complex biological process regulated by microbial consortia comprised of different trophical groups. In addition, this technology helps to reduce emission of greenhouse gases, meet the growing demad for fuels due to the production of methane, an alternative form of energy [1]. Although, acetate is the major carbon source for the methanogenic archaeas in anaerobic digesters [2], its availability in non-ionized form inhibits the methanogenesis. The presence of non-ionized form of acids depends not only on pH, but also on its own concentration. There are limited studies on the effect of acetate concentrations on its bioavailability form and the relation between acetate concentration/ inoculum ratio on methanogenesis [3]. With this aim the present study was carried out in order to identify the optimum acetate concentration/ inoculum ratio to achieve an efficient metanogenesis in anaerobic reactors.

Methods. Assays were carried out in 250 mL batch reactors with a working volume of 100 mL, containing Barker's medium [4]. Different acetate concentrations tested were 50, 100, 200, 250, 300, 350, 400, 500 mM. Three different level of inoculum (5, 10 and 20%) was added to each reactor in order to have different acetate/inoculum ratio. Initial pH of the reactors was at 7.5 and was not adjusted. Granular sludge obtained from a functioning UASB reactot treating brewery wastewater was used as inoculum. All reactors were incubated at 37°C and were maintained in duplicate. Methane was analyzed by gas chromatograph (PerkinElmer, Clarus600) equipped with a flame ionization detector and capillary column (Elite-WAX, 50mts x 0.32mm). pH, Chemical Oxygen Demand (COD), Total Solids (TS) and Volatile Solids (VS), were determined according to standard methods [5]. All data obtained were analyzed by multifactorial ANOVA using STATGRAPHICS Centurion XV 15.2.06.

Results and discussion. It was observed that there was significant difference in methane yield in relation to the acetate concentrations. Higher methane yield was obtained at 250 mM acetate concentration with 10 and 20% inoculum, followed by 200 mM, which was almost on par to the former. On the contrary, at 5% inoculum level, 200 mM recorded higher methane production than 250 mM, which was not significantly different. It was earlier reported that concentrations more than 100mM of acetate inhibited pure culture methanogens [7]. In the case of the different proportions of inoculum, 20% recorded significantly different methane yield than other levels. Further it was observed that the lag phase was higher with 5 and 10% inoculum than with 20% inocuum. In terms of acetate concentration/ inoculum ratio, the maximum methane yield was obtained for a ratio of 25:1, which did not record a lag phase too. The same ratio recorded higher COD removal of 88.15%.

Conclusion. The inhibitory effect of higher acetate concentration could be minimized with increase in inoculum proportion. Of the various ratio of acetate concentration/ inoculum level tested, 25 recorded the higher methane yield and a corresponding high COD removal.

Keywords anaerobic digestion; substrate-inoculum ratio; granular sludge; biogas; methane

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eMBR: Bioinformatics Resources for Bioremediation Research

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Contamination of ecosystems by xenobiotic substances has led to significant negative impacts on affected ecologies and on the health and economic livelihood of affected peoples. Bioremediation, particularly microbial bioremediation has proven to be a safe, low-cost and environmentally friendly method for remediation of such areas but a lack of complete understanding of the metabolic, enzymatic and cellular processes involved has made it difficult to model and predict field processes thereby hampering its development. A global survey was undertaken to highlight priorities, practices and needs of the bioremediation sector following which a web portal containing relevant bioinformatics resources was developed.

This article highlights the results of the survey and describes the electronic Microbial BioRemediation (*eMBR*) web portal which serves to promote synergy and improved collaboration in the bioremediation research community. It also describes the three bioinformatics resources developed and deployed via the portal. *eMBRLitMine* addresses the problem of identifying which microorganisms would be suitable for remediating sites contaminated by named compounds. It combines named entity recognition algorithms, graph visualization capabilities of GraphViz, a mySQL database and relevant Perl scripts to create, from the vast information available within published literature a statistical co-occurrence matrix which it uses to infer possible associations between microorganisms and particular contaminants. This provides valuable insights into bacteria/contaminant relationships and highlights possible bacterial species that could be used in remediation of named contaminants. Stored in eXtensible Mark-up Language, *eMBRCaseCatalogue* is a moderated and searchable database cataloguing bioremediation case studies, thereby providing a source of background knowledge necessary for planning and execution of bioremediation activities. Following the construction of a comprehensive metabolic biodegradation network, *eMBRHelper* enables the elucidation of possible biodegradation pathways for named contaminants. By integrating relevant chemical, enzymatic and genomics information, the resource attempts to model the interplay between contaminants, enzymes, degradation pathways and microorganisms, which enables researchers to make informed decisions for improved outcomes in remediation exercises involving bioaugmentation.

The article introduces future perspectives and highlights avenues for further informatics support for the bioremediation research sector.

Keywords bioremediation, literature-mining, web portal, case-studies, decision-support

Exploitation of olive mill wastewater and selected *Azotobacter chroococcum* strains for composting of agricultural wastes

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Olive mill wastewater (OMW) is a by-product of the industrial olive oil extraction process; it contains high amounts of organic compounds and minerals, such as potassium, phosphorus, calcium and magnesium, with key roles in soil fertility. Although OMW is substantially free of risk factors, such as microbial pathogens, viruses or heavy metals, it is characterized by a high content in polyphenolic compounds, which have strong antimicrobial action and limited biodegradability. Several adverse effects of the agricultural use of fresh OMW, such as soil salinization, phytotoxicity, and ground water quality degradation, are documented. Based on these premises, physico-chemical or biological treatment of OMW is crucial for the agricultural exploitation of this bioresource. Composting is one of the most promising biological technologies for the transformation of OMW into a valuable fertilizer and soil amendment. In the present study, we have examined the co-composting process of OMWs from different olive oil extraction systems and various agricultural wastes (straw and olive husk). The effect of the preliminary feed-batch fermentation of OMWs by selected cultures of *A. chroococcum* on the content in water-soluble phenols of the mature compost was also investigated. The choice to use these microorganisms was prompted by the acknowledged capacity of azotobacters for metabolizing polyphenolic compounds; fixing atmospheric nitrogen; and producing exopolysaccharides and phyto-stimulating hormones (auxins and gibberellins). OMWs samples were analyzed for: moisture, pH, and concentration of reducing sugars and soluble phenols (as gallic acid). Forty-one nitrogen-fixing bacteria were isolated from soil samples withdrawn from farmlands sporadically irrigated with fresh OMW. All the isolates were assayed in Erlenmeyer flasks for the ability to reduce the content of OMWs in total polyphenols by using the Folin-Ciocalteu reagent. The three most promising strains were molecularly identified by Amplified Ribosomal DNA Restriction Analysis (ARDRA) and 16S rRNA gene sequencing as *Azotobacter chroococcum* and mixed in a consortium for fermentation of OMWs used in the further composting assays.

The agricultural waste mixtures were added with either fresh or fermented OMWs, placed in open boxes and turned twice a week during the bio-oxidative phase of the composting period. To activate the process, ammonium sulphate and glucose (both at 0.5% w/v) were added as nitrogen and carbon sources, respectively. Aliquots of OMW were applied gradually each time the compost temperature dropped below 30°C and the moisture content was less than 50%. Compost samples were regularly taken and analysed for: pH, oxygen consumption rate, concentration of water-soluble phenols.

The main results of the study are briefly summarized. Three out of 41 bacterial isolates were able to notably reduce the OMWs content in polyphenolic compounds. The suitability of OMW for composting of agricultural waste materials was demonstrated and a significantly lower content in water-soluble phenols was found in the mature compost repeatedly added with fermented OMWs.

Keywords bioremediation; olive oil extraction industry; free-living nitrogen-fixing bacteria

Functional diversity of microbial communities in a bioreactor fed with bio-remediated metal-working fluid

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The membrane biorreactor (MBR) is an innovative technology which combines biodegradation together with membrane separation in a single process, thereby separating microorganisms and suspended solids from the treated water by membrane filtration. (Chang *et al.*, 2010).

In the process of manufacturing metal pieces, water-oil emulsions are used to lubricate and to cool the contact surface between the cutting tool and the manufactured piece. Water-based cutting fluids and coolants used in machining operations typically contain ethanolamines, polyglycols, chlorinated or sulfonated paraffins, mineral oil and similar molecules (Backer *et al.*, 2010). When physicochemical properties are lost, the emulsion is pretreated by a physical process and this is a waste solution that cannot be released in this form and needs further processing. This metal-working fluid is the target material to be biorremediated in this work.

The aim of this study is to know the MBR functional diversity. To this aim, 5 samples of the MBR microbial community (culturable and non-culturable) were taken. Biodiversity (CHAO and SHANNON) and metabolic diversity by Biolog® (Gram -, gram + and anaerobia) plates were studied.

The biodiversity study has shown a significant reduction between the first sample and the others. It could be due to an increase in chemical oxygen demand (COD) in the influent wastewater. Temporal fluctuations in influent wastewater characteristics are generally thought to affect microbial community structure in WWTPs (Wan *et al*., 2010). However, the Biolog® study showed that the first sample had the lowest metabolic diversity. This could indicate that the COD increase did not increase the toxic effect in the environment, but stimulated the microbial community production. Other studies have demonstrated that the addition of contaminants (like retardants or fertilisers) cause an increase of the microbial activity (García-Villaraco Velasco et al., 2009; Basanta et al. 2002; Adams and Attiwil, 1991; Dunn et al., 1985). The Biolog® study also revealed that the aromatic compounds and the polymers are the preferred carbon sources consumed by anaerobic organisms. However, the Biolog® gram + and gram – have shown that these compounds are the less consumed by non-anaerobic organisms.

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Keywords R.B.M, metal-working fluid, biorremediation, Biolog®.

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Hexavalent chromium reduction by bacteria from tannery efluent

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Chromium is generated from several industrial processes. It occurs in different oxidation states but Cr (III) and Cr (VI) are the most significant. Cr (VI) is a toxic, soluble environmental contaminant. Bacteria can reduce **hexavalent chromium** to the insoluble and less toxic Cr (III), and thus **chromate** bio**remediation** is of considerable interest. An indigenous chromium reducing bacterial strain, Rb-2 isolated from the tannery water sample, were identified as *Ochrobacterum intermedium,* on the basis of 16S rRNA gene sequencing. This strain can tolerate up to 40 mg K_2CrO_4 ml $^{-1}$on L- agar, 25 mg ml $^{-1}$ in L- broth, and up to 10 mg ml $^{-1}$ in acetate-minimal media. Chromium reduction potential was determined in L – broth and acetate minimal broth, after 24 hours. Influence of different incubation temperature (28, 37 and 42ºC) was checked to estimate the Cr (VI) reduction potential of the bacteria. The effect of different carbon sources on Cr (VI) reducing ability of bacteria was studied. It was observed that mechanism of resistance to metal was not due to the change in the permeability barrier of the cell membrane and the enzyme activity was found to be constitutive. Scanning electron microscopy revealed chromium precipitates on bacterial cell surfaces of both strains while Transmission electron microscopy showed the inner distribution of Cr (VI). This bacterial strain might be useful for Cr (VI) detoxification under a wide range of environmental conditions.

Key words: Cr (VI) reduction, *Ochrobacterum*, Bioremediation

Interactions between fungi and bacteria associated with degradation of PAHs

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It has been proved that bacteria played dominant roles in microbial bioremediation even there are a large number of other microbes existed in the polluted site, e.g. fungi, algae, protozoa. On the other hand, the fungal capability to degrade xenobiotics has attracted attention due to their predominance in contaminant site and multiplex metabolic pathways in nowadays. Diverse ligninolytic fungi had been confirmed as an effective strategy to remove pollutants from environment, e.g. white rot fungi, mycorrhizal fungi. It has been demonstrated that bacterial remediation was much more difficult to remove high-molecular-weight (HMW) organic pollutants since which are thermodynamically stable, hydrophobic and being easily absorbed to solid particles. Therefore, we are searching for a new biodegradation strategy which could use the cooperation between fungi and bacteria to degrade HMW pollutants. This study investigated the interactions between fungi and bacteria which are associated with degradation of polycyclic aromatic hydrocarbons (PAHs).

Through combination the methods of enrichment culture, quantification of PAHs degradation and analysis of PCR-RFLP, 4 soil bacteria and 7 soil fungi were obtained for their ability to remove phenanthrene, fluoranthene and pyrene, respectively. All strains obtained in this study were identified through 16S rRNA gene (for bacteria) and 28S rRNA gene (for fungi) sequence analysis.

Maximal pyrene degradation rate (60%, 28d) was obtained after co-culture of multi-fungi-bacteria at soil condition, as compared to a degradation rate of 28% for multi-fungi group and 47% in multi-bacteria group, respectively. The denaturing gradient gel electrophoresis (DGGE) profile showed a dynamic change in population and variety during the co-cultural procedure. As we supposed the outstanding PAHs degradation in fungi-bacteria co-culture was due to the middle metabolic products which were catalyzed by fungi will be more achievable for bacterial metabolism. To confirm this hypothesis, we conducted a metabolites analysis of cells exposed to pyrene using one strain of fungi combining with one strain of bacteria. Gas chromatography-mass spectrometry (GC-MS) was used to analyze pyrene-metabolites which are related to the synergistic function between fungi and bacteria. 1-hydroxypyrene and 1-methoxypyrene were detected as main metabolic products from fungal strains of *Fusarium* sp. TKF4, *Penicillium* sp. TKF5, *Trichoderma* sp. TKF7. Furthermore, bacterial strains of *Pseudomonas* sp. TKB1, *Labrys* sp. TKB2 were detected to be capable of removing 1-hydroxypyrene effectively when using as sole source of carbon and energy (above 60%, 21d). On the other hand, 1-hydroxypyrene showed metabolic toxicity to *Penicillium* sp. TKF5 from which spores could not be detected after culturing. At present, further analysis is under processing.

Keywords PAHs; fungi; bacteria; DGGE; degradation

Kerosene biodegradation and biosurfactant production in sea water by the haloalkalitolerant yeast *Candida lipolytica* UCP 0988

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Little is known about biodegradation of kerosene in sea water by haloalkalitolerant yeasts. In this work, the kerosene degradation and bioemulsifiers/biosurfactants (BE/BS) production by *Candida lipolytica* UCP 0988 in acid or alkaline seawater with low oxygenation, supplemented with nitrogen and phosphorus sources, was investigated. A 2^4 full factorial design, with four central points, was carried out to evaluate the effects and interactions of the pH and concentrations of kerosene, ammonium sulphate and potassium dihydrogen phosphate on the biomass concentration, the emulsification activity and the surface tension of 120 h cell-free culture filtrates. The experiments were carried out at 28° C and 200 rpm in Erlemeyer flasks of 1000 mL with a working volume of 750 mL. Kerosene was added to the flasks after yeast inoculation creating a limited-oxygen environment. The yeast was able to use kerosene (10, 20 or 30% v/v) in seawater with initial pH 6, 10 e 14 and produce emulsifier/biosurfactant. The ability of *C.lipolytica* UCP 0988 to tolerate salt concentrations was studied over a initial salinity range of 35–70 ‰. The biomass growth and the extent of biodegradation were affected by media constituents and pH. The increase of pH from 6 to 14 was the factor that more influenced the increase of biomass and consequently the biodegradation of kerosene, the only carbon source of the experiment. Biomass concentration was used as an indirect, fast, simples and low cost indicator for the kerosene biodegradation. The yeast showed features of extreme alkaliphile, growing better in the media with initial pH 14. The three highest biomass concentrations (22,58 g/L, 22,16 and 17,96 g/l), the three highest emulsification activities (6 UAE) and the lowest surface tension (25,04 g/l) were obtained in experiments with initial pH 14 and final values of pH around 12. The results show the potential of the yeast *C.lipolytica* and of its BE/BS for bioremediation of marine environments contaminated with kerosene and others petroleum commercial products.

Keywords biodegradation, kerosene, biosurfactant, seawater, *Candida lipolytica*, alkaliphile

Microbial Activity Studies in Phytoremediation of VOCs-contaminated Soil at Pak Chong, Thailand

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The phytoremediation of VOCs contaminated-soil at Pak Chong, Thailand was performed by the Shimizu Corporation and contractors. The clean-up area was treated with Vetiver grass, Horse tamarind, Acacia mangium, Guinea grass, Napier grass, Sunflower, and Physic nut for 272 days. Soil samples were collected from five top soil locations in each treatment at different times of remediation and tested to determine the total count of microorganisms and the amount of benzene/toluene dioxygenase gene containing bacteria. The 330 bp PCR product of benzene/toluene dioxygenase gene from *Comamonas acidovorans* T5/10 was amplified by AF/AR primers, cloned into pDrive and then transformed into *Escherichia coli* JM109 by commercial kits. The plasmid pDriveTBdi was used to prepare a standard curve measured by Real-Time PCR using SYBR Green I. The microbial populations in Soil Extract Agar and Peptone-Meat Extract-Soil Extract Agar were high with a range of 8-10 log CFU/ml and in Rose Bengal Streptomycin Medium Agar with a range of 4-6 log CFU/ml in all treatments. The results from statistical analysis showed that there were a lot more microorganisms, especially bacteria, in all phytoremediation treatments than in the control ($p<0.05$). The microbial populations were no significantly different among plant treatments. This indicated that all plant species stimulated microbial activities and both played important roles in remediation of VOCs. Bacteria may play a more important role than fungi. The Real-Time PCR technique with specific primer AF/AR can be used to monitor benzene/toluene dioxygenase containing bacteria in phytoremediation of VOCs. The benzene/toluene dioxygenase containing bacteria could be induced and were found to be highly in the early phase of remediation and decreased at the end, corresponding with the profile of VOCs as substrates and CO_2 from microbial activities. This showed that benzene/toluene dioxygenase containing bacteria may also play an important role in this remediation.

Keywords: ; phytoremediation, VOCs, Real-time PCR, benzene/toluene dioxygenase gene

Microbial electricity generation enhances PBDEs degradation by stimulating microbial viability

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As a result of the widespread use, Polybrominated diphenyl ethers (PBDEs) residues are found in a wide variety of environmental samples and the concentrations have increased exponentially. Recent works have shown that microbially mediated debromination appears to be one of the most important routes for environmental PBDEs transformation. However, it is very slow for these highly persistent compounds to be transformed under the normally anaerobic sediments conditions. In order to develop new strategies for stimulating the anaerobic PBDEs biodebromination process, two dual-chamber microbial fuel cells (MFCs) were constructed to investigate the possibility of PBDEs debromination by inoculating an anaerobic PBDEs debrominating culture which has been enriched in 150 ml defined PBDE-degrading medium under anaerobic condition for more than 90 days after collected from the PBDEs contaminated river sediment. And the cultures in the normal anaerobic glass serum bottles were treated as control. After 70 days performance, higher debromination rate and markedly different PBDEs congener compositions were observed in the MFC treatments. Integrated pyrosequencing-based and GeoChip-based metagenomic approaches were used to investigate the responses of PBDE-degrading microbial community to the MFC performance. It was found that the functional genes number detected from the MFC treatments were ten time higher than those from control. The microbial community diversity in MFC treatment is also significantly higher than control. In addition, dramatic differences were observed in the microbial community structures between the biofilm and the plankton in the anodic chamber of MFC, although the genus numbers detected are close. Within the 52 genera detected, *Geobacter* was ranked as the most abundant organisms in the biofilm, while *Pseudomonas* was ranked as the most abundant organisms in the plankton. High microbial viabilities could be maintained during electricity generation. These results suggested that the viabilities of debrominating microbes could be significantly enhanced and accelerated PBDE degradation during microbial electricity generation, which will be greatly useful for developing *in situ* PBDEs bioremediation technologies.

Keywords: microbial fuel cells (MFCs), Polybrominated diphenyl ethers (PBDEs), microbial viability, biodegradation

Migration and biodegradation of mineral oil in tropical soil

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Abstract - Petroleum hydrocarbons (PHC) are some of the most problematic pollutants found in soil and aquifers throughout the world, despite their inherent biodegradability in the environment. Therefore, the application of remediation techniques requires a good understanding of transport and degradation mechanisms. However, those processes are still insufficiently understood when tropical and residual soils are concerned. This study evaluated mineral oil migration in inert artificial soil and in natural residual soil from Southeast Brazil.

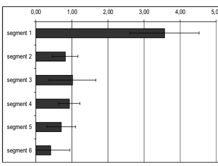

Figure 1 - Oil amounts in inert soil after 24 hours (PPM)

Oil was introduced at the top of soil columns and its downward migration was monitored for five months. Gravimetric determination of total PHC assessed contaminant amounts in the columns segments, while measurements of microbial activity (FDA hydrolysis by soil enzymes) also monitored degrading activities in the natural soil.

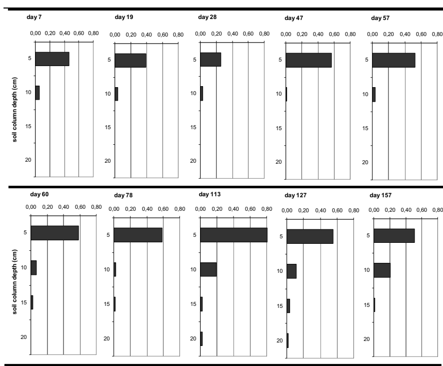

Figure 2 – Oil infiltration in soil columns during the 157-day assay (PPM)

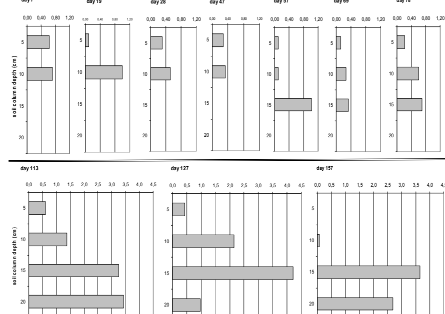

Figure 3 - Microbial activity during the assay (μg hydrolysed FDA x min^{-1} x g^{-1} soil)

Results showed that mineral oil migrated easily in inert quartz sand (40cm in 24h - fig.1), as expected given its even grain size (0.15 to 0.19 mm) and the absence of organic matter, providing no physical-chemical interaction between soil and the contaminant. Its movement was much slower in the structured soil, reaching a 20cm depth in 113 days (fig.2), revealing the role of soil composition and structure.

Besides, the onset of biodegradation processes hindered oil movement deeper into the soil column and contamination receded, as on the 157th day no detectable oil was found below 15cm deep, showing its attenuation by the action of soil microorganisms. This is corroborated by rates of microbial activity, much higher in the deeper layers than in the surface (fig.3), where microbial activity is impacted by the great amounts of contaminant. Biodegradability tests confirm the biodegrading capability of the autochthonous microbiota: 20.1% degraded oil observed after 10-day incubation showed oil intrinsic degradability; and 29.7% degradation after 30 days indicated the potential of final degradation by this soil microbiota.

Hence, these results indicate that, should a spill of this oil happen in this type of soil, its migration would initially be slowed down by soil chemical-physical characteristics and, in a second moment, by biodegradation processes. These residual soils are ubiquitous in Brazil, and natural attenuation is always the more friendly and cost-effective approach to address environmental contamination issues.

Keywords mineral oil contamination; soil microbiota

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Modulating Bacterial Deposition in Saturated Porous Media with Biostimulants

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Bacterial movement through saturated porous media may be restricted by several factors, including increased path lengths (tortuosity), bacterial adhesion to surfaces, and geometrical restrictions due to pores of small diameter and dead ends. We propose bacterial tactic response as a mechanism that could change the swimming behavior and hence improve bacterial transport in saturated porous media. We characterized the tactic response of naphthalene-degrading *Pseudomonas putida G7* to additives used in bioremediation (humic acids, sunflower root exudates and a oleophilic fertilizer), by capillary assays and by computer-assisted motion analysis. Then, in well-controlled column systems, we assessed the influence of these biostimulants on bacterial strain deposition in porous media (sand). We suggest that chemotactic sensing combined with changed swimming modes could be the reason for the enhanced bacterial transport observed with plant exudates. The compounds present in the exudates (aminoacids and organic acids) changed the bacterial motility pattern and this could have a relevant effect on bacterial deposition. We analyzed some motility parameters like speed, rate of changes of direction and net-gross-displacement ratio to determine the effect of exudates on swimming trajectories. Humic acids and the oleophilic fertilizer also promoted bacterial transport, but this effect was caused by other mechanism different to chemotaxis, and possibly involved physical interactions between the cell surface, the porous material and the biostimulant.

Keywords: motility pattern, biostimulants, deposition, transport

Motility pattern analysis and its effect on bacterial dispersal

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Phytoremediation is one of the most useful techniques to decontaminate PAH-polluted sites due to its low cost and high effectiveness. We propose sunflower root exudates as a powerful chemoeffector to improve rhizo- and phytoremediation techniqes. Bacterial chemotaxis towards root exudates could enhance bacterial transport through saturated porous media. Chemotactic bacteria can therefore constitute a useful vector for relevant catabolic activities and/or nutrients in bioremediation projects.

We studied the chemotactic response of naphtalene-degrading *Pseudomonas putida G7* towards root exudates of *Helianthus annuus* and its individual components (organic acids, aminoacids and sugars) by capillary assays. We analyzed the motility behavior concerning parameters like speed, rate of change of direction or net-gross-displacement ratio by computer-assisted motion analyses (Cell Track). In well-controlled column systems, we studied the effect of these compounds on bacterial transport in porous media.

Our data suggest that *Helianthus annuus* exudates are a powerful chemoattractant for *Pseudomonas putida G7* and it could be used in bioremediation, improving bacterial dispersal.

Keywords: chemoeffector, root exudate, bacterial dispersal

New dissimilatory arsenate reducers – isolation, characteristic and potential application in biometallurgy

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Arsenic that occurs in the form of minerals such as: enargit (Cu_3AsS_4) or tenantit ($Cu_{12}As_4S_{13}$), very often accompanies copper ores containing minerals such as pyrite (FeS_2), covelite (CuS), chalcocite (Cu_2S) or digenit (Cu_9S_5). The occurrence of arsenic in copper sulphide ores hinders smelting processes and utilization of SO_2. Moreover, arsenic removal in pyrometallurgical processes causes the emission of gaseous pollutants. Several physical and chemical methods were proposed to avoid the production of toxic arsenic compounds, but they are very often inefficient and expensive. An alternative to current technologies are biological methods, in which microorganisms are used for selective leaching of arsenic.

This paper provides physiological and functional characteristic of seven new dissimilatory arsenate reducing bacteria (DARB), that could be used in selective arsenic removal from copper concentrates and flotation tailings.

The source of isolates were arsenic-rich bottom sediments sampled from the ancient Zloty Stok (SW Poland) gold mine. Analysis of the 16S rRNA gene sequence classified isolated bacteria to the following genera: *Shewanella* sp. (strain OM1), *Pseudomonas* sp. (strain: OM2, OM3 and OM22), *Aeromnas* sp. (strain: OM4 and OM5) and *Serratia* sp. (strain: OM17). All isolates showed extreme tolerance to arsenite (up to 16mM) and arsenate (up to 400 mM) and were able to grow in the absence of oxygen, by arsenic respiration coupled with lactate oxidation. They used sodium arsenate as well as arsenic containing minerals as a terminal electron acceptor. The presence of dissimilatory arsenate reductase gene (*arrA*) was confirmed in all strains and interestingly, in the genome of three strains (OM2, OM3 and OM5) arsenite oxidase gene (*aoxB*) was also identified. Besides the presence of *aoxB* gene, none of isolated bacteria could utilize arsenite as an electron donor in chemolithoautotrophic processes as well as in heterotrophic oxidation. In addition to arsenic redox transformation properties, all isolates were tested for ability of copper concentrates dissolution and flotation tailing containing arsenic. Arsenic mobilization experiments showed that all seven strains promoted selective and effective mobilization of arsenic from copper minerals only in the absence of oxygen. In anaerobic conditions, the mean concentrations of released arsenic were at 25% and 20% of primary concentration in copper concentrates and flotation tailings, respectively. In aerobic conditions, isolated bacteria were unable to grow with copper minerals as the only one source of energy, and thus were not able to mobilize arsenic from tested minerals.

The presented studies demonstrates that isolated bacteria could be used in biometallurgy as a specific (selective) tools for removing arsenic from copper ores. They can be utilized as a supplement to commercial microbial consortia used for bioleaching of polymetallic ores.

Keywords: dissimilatory arsenate reducers, copper concentrates, mining wastes, arsenic removal

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New procedure for evaluation of diffuse environmental pollution with aromatic organic compounds based on real-time PCR quantification of degradation potential in microbial communities

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Diffuse pollution is understood as a long term contamination of the environment by very low concentration of pollutants due to variety of anthropogenic activities, thus leading to negative environmental impact. Main task of our study was a complex evaluation of microbial degradation potential linked with threshold concentrations of aromatic pollutants in soil, sediments, groundwater and surface water. A criterion for potential environmental risk was the presumption that increased *in-situ* degradation potential indicates the presence of diffuse pollution. Main pollutants of interest were BTEX, PAHs and PCBs.

A real-time polymerase chain reaction method was used for quantification of diverse group of catabolic genes encoding the catalytic α-subunits of the Rieske non-heme iron oxygenases, which are members of the toluene/biphenyl oxygenase subfamily of aromatic ring-hydroxylating dioxygenases (RHDO). The biodegradation potential was expressed as the number of enumerated RHDO genes, reported as gene copies per ng DNA. The sensitivity of this methodology allowed detection of as much as 100 catabolic genes in ng of environmental DNA. The biodegradation potential was correlated with data from chemical, microbial cultivation analyses, and hydrochemical (groundwater, surface water) or granulometric (soil, sediments) characterization of the samples. Examples for application on multiple localities with different types of diffuse pollution will be presented. Even a negligible increase in the concentration of organic matter triggered the expansion of microbial density and catabolic gene abundance. This new approach is also relevant for practices where the intensity of the biodegradation is of main importance for a cost efficient full-scale bioremediation.

This work was supported by the Czech Ministry of Education research program 1M06011.

Keywords: diffuse pollution, aromatic organic compounds, qPCR, degradation potential

Petroleum products biodegradation profile similarity evaluated by F test comparison of weekly CO_2 production

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Bioremediation exploits the ability of some microorganisms to degrade organic contaminants and has been established as an efficient, cost-effective, and environmentally friendly treatment. Microbial biotransformation is considered a major environmental technique in treating hydrocarbon pollution in both terrestrial and aquatic ecosystems. Hence, feasibility studies are a prerequisite for any planned strategy in bioremediation contaminated environments in order to identify limitations towards biodegradation and to predict remediation performance and thereby rule out technologies that may be inappropriate for the clean-up of the substances of concern. Additionally, the environmental behavior and chemical-physical properties of oils are the basis for new fluid developments and for ecological treatment strategies. The isolation, characterization and profile of petrol derived oils biodegradation capacity studies are important when deciding the correct bioremediation strategy.

Respirometry applied to bioremediation offers a series of advantages for obtaining CO_2 production data when compared to other procedures. In this study, a water adapted respirometry methodology was applied. It was possible to determinate information regarding the atmosphere's CO_2 concentration inside the respirometer with this method. The kinetics of the whole process can be related with the biodegradation rate. Thus, by measuring CO_2 produced by microorganism, it was possible to plot the biodegradation profile of each studied substance. Seven assays with the following substances were evaluated: water control (A0), petroleum (A1), motor oil (A2), weathered motor oil (A3), diesel oil (A4), gasoline (A5), kerosene (A6), phenol (A7).

Afterwards, the CO_2 data obtained underwent an in depth statistical analysis by F test. The F test is applied to determine the degrees of difference between a data group plotted in a curve. Through this test, it was determined how much different substances biodegradation profile varied among them (Figure 1) in a 0.05 significance level. This statistical procedure returns values from a 0 to 1 range which indicates the odds of a data group to be equal to another. The higher the value, the more similar is the biodegradation profile.

	A0	A1	A2	A3	A4	A5	A6	A7
A0	1	0.002717	1.22E-06	0.000285	0.022317	0.000144	0.000000	0.045326
A1	0.002717	1	0.028719	0.472493	0.438167	0.357136	0.007759	0.283721
A2	1.22E-06	0.028719	1	0.134266	0.003676	0.194184	0.609834	0.00151
A3	0.000285	0.472493	0.134266	1	0.137955	0.838272	0.046677	0.076132
A4	0.022317	0.438167	0.003676	0.137955	1	0.092707	0.000784	0.764308
A5	0.000144	0.357136	0.194184	0.838272	0.092707	1	0.072816	0.048953
A6	0.000000	0.007759	0.609834	0.046677	0.000784	0.072816	1	0.000297
A7	0.045326	0.283721	0.00151	0.076132	0.764308	0.048953	0.000297	1

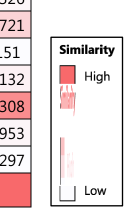

Figure 1: Biodegradation profile similarity from various petroleum products

According to Figure 1, there are some noticeable biodegradation similarity among some substances, such as weathered motor oil (A3) and gasoline (A5), presenting a 0.832 F test value. Phenol (A7) and diesel oil (A4) biodegradation profile, as well as kerosene (A6) and motor oil (A2) present a similar biodegradation profile, with 0.764 and 0.609 F test values respectively. The diagonal line of 1 value in the center of the table is due to comparison of each data group with themselves, which certainly returns a full similarity as they are equal. The biodegradation profiling proposed in this study is a complex achievement; however, the biodegradation behavior of substances must be determined. Substances are not subject to isolated environmental biodegradation. The comparison provided a more accurate knowledge of the biodegradation process on all the different types of oils by using simple respirometry techniques. Such data provides reliability when revealing important information about how the biodegradation processes happens in those residual oils, thus describing how the microorganism may react to different petrol derived contained in polluted industrial wastewater.

Keywords F test; bioremediation, respirometry

Possibility of use of the symbiotic couple *Retama sphaerocarpa-Bradyrhizobium* in the bioremediation of the degraded and affected soils

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Shrubby legumes of the *Retama* genus are endemic to the Mediterranean Basin and distributed in the various Mediterranean climates (from humid to arid) and Ecosystems including coastal dunes, maquis, and also deserts. *Retama* shrubs are tolerant to extreme drought conditions. Three species, *Retama monosperma*, *Retama raetam*, and *Retama sphaerocarpa*, were recognized within the *Retama* genus.

The symbiotic power of rhizobia associated to *Retama* opens the possibility of use in projects of revegetation of the soils. Certain works showed that *R. sphaerocarpa* allows, on one hand, the installation and the development of new vegetable species and on the other hand prevents the erosion and the desertification in the semi-arid zones. Several works recommended *R. sphaerocarpa* as effective legume to reconstitute the characteristic biodiversity as well as the physical and biological properties of soil. However this plant requires specific rhizobia and this fact certain authors recommend to inoculate it with its own native rhizobia before the culture.

In Algeria, the species of the genus *Retama* push on vast areas sand dunes of the wet coast until those of the dry areas, so expressing a presence on a big pédoclimatique contrast. They can thus establish a model indicated for the research for efficient symbiotic couples for the rehabilitation of the ecosystems degraded or the fixation of the dunes of desert sands which do not stop advancing at high speed.

11 strains of symbiotic bacteria were isolated by *Retama* of Algeria and identified as belonging to the genus *Bradyrhizobium* (Boulila and *al*., 2009).

Seeds result from plants at the origin of the isolation of *Bradyrhizobium*. Our work has for objective the study of the couple *R. sphaerocarpa-Bradyrhizobium* native in the bioremediation of soils degraded by salt or affected by metals.

Keywords: Retama , Bradyrhizobium, revegetation, bioremediation, soil

Rehabilitation of hydrocarbon contaminated soil in a gas depot using bioremedial microbes

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The study investigated the feasibility of bioremediation as a treatment option for chronically diesel-oil-polluted soil at petroleum and gas depot of OILCO (a company that is a division of Shell-S.A.) situated at the east side of Empangeni which is in the Northern KwaZulu-Natal province, South Africa. To examine the efficiency of bioremediation, one part of the contaminated site was treated with microbes, (previously isolated from the hydrocarbon-contaminated soil) to a depth of ±1,2 meters, ±5 meters wide and 2 meters in length, plus the woodshavings as a bulking agent and the other part was treated with a commercial product from ECO-SOL™. The effect of bioremediation was observed over a period of 11 weeks and samples were taken at 15 day intervals. The changes in hydrocarbon concentrations were monitored in the soil, soil leachate and the accompanying changes in the soil microbial counts and activity.

Prior to GCMS analysis, the soil texture was analysed using the Particle Size Determination Method and the soil was observed to be sandy-loam (Day, 1995). Results of the analysis of the soil microbial counts and activity indicated the presence of the following groups of microbes; *Aerobic Total Counts, Nitrofyers*, *Nitrosofyers*, and *Free-living nitrogen fixing* bacteria (Chan*, et.al*., 1993). These four groups of microbial counts were used as a biological parameter, and there was a correlation between each other as well as with the residual hydrocarbon concentration, indicating the importance of biodegradation. The effect of biostimulation of the indigenous soil microorganisms declined with time during the study.

Keywords: bioremediation, hydrocarbon, Nitrofyers, Nitrosofyers, Free-living nitrogen fixing bacteria

Structural diversity of microbial communities in a bioreactor fed with bio-remediated metal-working fluid

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The membrane biorreactor (MBR) is an innovative technology which combines biodegradation together with membrane separation in a single process, thereby separating microorganisms and suspended solids from the treated water by membrane filtration. (Chang *et al.*, 2010).

In the process of manufacturing metal pieces, water-oil emulsions are used to lubricate and to cool the contact surface between the cutting tool and the manufactured piece. Water-based cutting fluids and coolants used in machining operations typically contain ethanolamines, polyglycols, chlorinated or sulfonated paraffins, mineral oil and similar molecules (Backer *et al.*, 2010). When physicochemical properties are lost, the emulsion is pretreated by a physical process and this is a waste solution that cannot be released in this form and needs further processing. This metal-working fluid is the target material to be biorremediated in this work.

The aim of this study is to determine MBR`s structural diversity of spontaneous microbial communities. To achieve this purpose, culturable microorganisms were isolated and cloned in a library. 16S rRNA gene was amplified and the cloned plasmids were isolated using the Speedtools Plasmid DNA purification Kit (Biotools). Ninety nine culturable and 303 non-culturable clones were sequenced at Macrogen Europe Service. Sequence alignement was done on Mega 5.0. to generate a phylogenetic tree using the neighbor-joining method.

Isolates identified as *Pseudomonas sp.* appeared to dominate the bioreactor culturable population. This domination may be due to its extreme nutritional versatility (Stanier *et al*., 1966). Also, this genus has been found in cutting fluids by other investigators (Bennet, E.O., 1974; Pivnick, H., 1954) and in other MBR feeded with metal-working fluids (Backer *et al.*, 1983).

The *Caulobacteriaceae* order also appeared in the reactor, and has been reported before in other studies (Backer *et al.* (2010), but this is the first time in metal-working fluids.

In the MBR`s structural diversity (culturable and non-culturable organisms) the most abundant orders were -proteobacteria, -proteobacteria and -proteobacteria. According to others MBR studies (Wan *et al.*, 2010; Wang *et al.*, 2010) one of the most common OTUs in this systems belonged to the *Proteobacteria*.

None of the predominant bacteria from the reactor belonged to a strictly pathogenic group. Strikingly, genera with representatives of opportunistics pathogens like *Acinetobacter and Pseudomonas* were found

Acknowledgments: John Deere Ibérica S.A. (Getafe-Spain) for the metal-working fluids supplied, Gaiker Foundation (Vizcaya – España) for use of the MBR, FPU Programme from the Science and Innovation Ministry (Spain).

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Keywords R.B.M, biorremediation, Metal-working fluids.

Taxonomic analysis, degradation kinetics and soil microcosm studies of an abamectin-degrading *Burkholderia diffusa* GB-01 strain

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Abamectin is a commercial preparation of avermecrtin B_{1a}, which has wide applications in veterinary against acto/endo parasites as well as an effective insecticide/ miticide in agriculture. Recent studies have illustrated that the persistence of abamectin in the environment can pose potential risk to non-target soil and dung invertebrates leading to the instability of an ecosystem. Bacterial strain GB-01 was isolated from abamectin contaminated soils by continuous enrichment culture technique. The strain GB-01 was subjected to taxonomic analysis through a polyphasic approach, in which phenotypical, genotypical, and phylogenetical information were gathered to achieve a consensus classification. API 20 NE, ID 32 GN, API 50 CH, Biolog GN2 MicroPlating, whole cell fatty acid profile analysis, G+C mol %, DNA-DNA hybridization and phylogenetic analysis of 16S rRNA as well as *recA* genes suggested that strain GB-01 is an atypical strain of *Burkhoderia diffusa* species. The bacterium was able to degrade 80 mg L^{-1} abamectin within 36 hours in liquid cultures, while biodegradation activity was reduced to 21% upon increasing the initial concentration of abamectin to 110 mg L^{-1}. A linear relationship between initial concentration of abamectin and time required for biodegradation was observed. Hence, the Haldane equation could be used to predict the specific degradation rate (SDR) ($R^2 > 0.99$) as a function of initial concentration of abamectin. Microcosms studies performed with varying concentrations of abamectin (2-160 mg Kg^{-1}) spiked soils showed that strain GB-01 could effectively degrade abamectin over the range of 2-40 mg Kg^{-1} dry weight of soil. The doses used were higher than the recommended dose for an agricultural application of abamectin, taking in account the over-use or spill situations. A cell density of approximately 10^8 viable cells g^{-1} dry weight of soil was found to be suitable for biodegradation over a temperature range of 30-35 ^{o}C and soil pH 7.5-8.5. Our results indicated that strain GB-01 might be used as an effective bioremediation tool for abamectin contaminated sites.

Key words: Abamectin; *Burkholderia* sp. GB-01; Polyphasic taxonomy; Degradation kinetics; Soil microcosm Bioremediation

Taxonomically diverse chlorpyrifos degrading microorganisms from a chlorphyrifos contaminated tropical rice paddy

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Introduction of xenobiotic chemicals into the soil environment may have lasting effects on soil microbial dynamics – both structural and function. Chlorpyrifos is an agricultural insecticide that has been widely used for pest management in rice paddy and caused environmental contamination by virtue of their persistence. Isolation of chlorpyrifos degrading bacteria will be of immense importance for cleaning up the polluted environment. Here we report isolation of six bacterial strains that can not only hydrolyze chlorpyrifos leading to its decontamination but also use the xenobiotic molecule as a source of carbon and energy. The bacteria were isolated from CRRI farm soil planted to rice under flooded conditions and treated with chlorpyrifos for insect control. The bacterial strains were isolated and characterized using a polyphasic approach consisting of genotypic, chemotaxonomic, and phenotypic methods for determining taxonomic status. Six strains were characterized based on their 16S rRNA gene sequence analysis and assigned to diverse genera such as *Achromobacter*, *Xanthobacter*, *Stenotrophomonas*, *Inquilinus*, *Methylobacterium* and *Sphingobium*. These bacteria were tolerant to high concentration of chlorpyrifos as per recommended dosages. All the isolates were active-degraders and completely removed chlorpyrifos from liquid medium within 10 days accumulating 3,5,6 trichloro 2 pyridinol (TCP) as the metabolic by-product. Chemotaxonomic characters such as fatty acid methyl esters (FAME) and substrate-utilization (Biolog®) were analyzed for identification. A number of bacteria have been used in the development of microcosm for testing their bioremediation potential. The results highlight the potential of these bacteria for use in the clean-up of contaminated environment.

Keywords: Chlorpyrifos, degradation, bioremediation, diversity

The effect of environmental condition on phenol biodegradation by isolated bacteria from coal plant wastewater

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Phenolic wastewater is major environmental pollutants from industrial processes, such as oil refineries, cooking plants, industrial resin manufacturing and petroleum-based processing plants. Biodegradation has been extensively studied as an alternative approach due to the low costs associated with this option, as well as the possibility of complete mineralization of the xenobiotic. This study is undertaken to evaluate, the biodegradation of phenol by native bacteria strains isolated from phenolic wastewater of coal plant. Phenol degrading potential of all the strains was evaluated initially. One of the strains was found to be highly effective for the removal of phenol, which was used as sole carbon and energy source, from an initial concentration of 400 mg /L to 2000 mg /L. The probable identification of the strain was done by colony morphology, gram staining and biochemical tests. Then, the effect of temperature ranging from 25 to 45 °C, pH ranging from 6 to 8, the rate of shaking ranging from 100 to 200 rpm and glucose concentration ranging from 0 to 4 g/L on the rate of phenol degradation by that particular strain were studied. According to the results, the particular strain was found to be gram-negative, rod-shaped and probably related to the genus of *Pseudomonas*. It is revealed that the optimum temperature of phenol biodegradation was at 35 °C (85.4 % removal), optimum pH of 7 (71 % removal), optimum shaking rate of 200 rpm (71.7%) and glucose concentration of 4g/L (97.4%). Therefore it can be concluded that the efficiency of phenol biodegradation increase by the utilization of native bacterial strains from the waste water in optimum conditions.

Key word: Biodegradation; Environmental condition; Phenol; *Pseudomonas*; Wastewater

The efficiency of glyphosate Biodegradation by *Pseudomonas (aeruginosa)*

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The intensive use of glyphosate [N-(phosphonomethyl) – glycine] to control weeds in agricultural areas all over the world requires special attention due to its toxicity to non-target organisms. The use of microorganisms in the degradation and detoxification of glyphosate polluted sites in the environment is an efficient tool and bioremediation is a very useful option to conventional cleanup methods. The objective of this study was to isolate the best bacterial strain with the ability of degrading high concentrations of glyphosate, a common herbicide used in the world. Then, the ability of the isolate to degrade glyphosate under varying nutrient was evaluated. The glyphosate utilization of the bacterium was screened using mineral medium containing glyphosate as sole C, N, P or C, N, P and glyphosate along additional C, N, P sources. Of all isolated bacteria, *Pseudomonas (aeruginosa)* showed the ability to utilize glyphosate efficiently and was therefore used for further biodegradation studies. The glyphosate biodegradation by *Pseudomonas (aeroginosa)* showed significant differences among 5 nutrient medium. The comparative effects of glyphosate on the growth of the isolates showed that there was significant ($P < 0.05$) growth in the medium containing glyphosate as an additional source of C/N/P). No inhibition of growth was observed at higher concentrations. But the percentage of degradation in the above medium (58.9%) was significantly ($P <0.05$) less than media containing glyphosate as sole sources of P (90.4%) or N (71.3%) and the medium containing glyphosate as source of P, N and C (72.8%). According to the results revealed that the bacterium exhibited a high capacity to efficiently degrade glyphosate as phosphorus source. It was also observed that it could degrade 21.25 g/lit glyphosate after 96 h incubation completely. It can be concluded that application of such isolated bacteria with the potential of degrading pesticides from contaminated site, can be used to remediate soil contaminated with pesticide.

Key words: Bacteria, Biodegradation, Glyphosate, Nutrient sources, *Pseudomonas*.

The use of plant growth-promoting actinomycetes to improve phytoremediation of oil-polluted soils in the United Arab Emirates

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The ability of streptomycete actinomycetes to promote the growth of Bermuda grass in sandy soils contaminated with crude oil through the production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase was evaluated under gnotobiotic and greenhouse conditions. To achieve this, 80 isolates of *Streptomyces* spp. obtained from the grass rhizosphere in the United Arab Emirates (UAE) were initially selected for their ability to produce ACC deaminase as well as their abilities to produce plant growth regulators inclduing auxins, cytokinins, gibberellins and polyamines. Out of the 80 isolates, only 44 isolates (55%) were able to produce ACC deaminase as detected by their growth on Dworkin and Foster (DF) agar medium amended with ACC the immediate biosynthetic precursors of the hormone ethylene. The rest of the isolates grew well only on DF agar medium supplemented with $(NH_4)_2SO_4$ and failed to grow on DF-ACC agar indicating that they did not have ACC deaminase activity. Under gnotobiotic and greenhouse conditions, the application of the ACC deaminase-producing actinomycetes isolates increased plant growth in oil-contaminated soils. The application of a mixture of five different actinomycetes under greenhouse conditions significantly ($P<0.05$), reduced the endogenous levels of ACC in the roots and shoots, and significantly ($P<0.05$) increased the levels of *in planta* endogenous plant growth regulators (PGRs) including indole-3-acetic acid (IAA), indole-3-pyruvic acid (IPYA), putrescine (Put), spermidine (Spd) and spermine (Spm) in roots and shoots compared with control plants grown in oil-contaminated soils without the application of actinomycetes. The application of actinomycetes in the contaminated soils significantly ($P < 0.05$) enhanced the growth and development of Bermuda grass in the greenhouse experiment. In these treatments there were significant ($P<0.05$) increases in the fresh weights of roots and shoots, and root and shoot lengths, compared to the treatments which included only the crude oil in the soil without the application of the mixture of actinomycetes. This is the first published report for the production of ACC deaminase by actinomycetes and also the first to demonstrate the potential of actinomycetes to promote plant growth in soils contaminated with crude oil.

Keywords ACC deaminase, actinomycetes, biological fertilizers, environmental pollution, ethylene, oil pollution, plant growth-promoting rhizobacteria, phytoremediation, rhizosphere.

Treatment additives reduced cadmium and arsenic bioavailability and increased 1,2-dichloroethane biodegradation and microbial activities in co-contaminated soil

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Bioremediation of co-contaminated environments are considered difficult because of the mixed nature of the contaminants and the fact that the two components often must be treated differently. This study investigated the potential application of inorganic treatment additives namely; calcium carbonate ($CaCO_3$), gypsum ($CaSO_4.2H_2O$) and disodium phosphate plus sodium chloride (Na_2HPO_4 + NaCl) for accelerated bioremediation of soil co-contaminated with 1,2-dichloroethane (1,2-DCA) and heavy metals. Both arsenic and cadmium were inhibitory to the indigenous soil microorganisms involved in 1,2-DCA degradation at a concentration of 150 mg/kg and 170 mg/kg, respectively, leading to a significant ($p < 0.05$) decrease in 1,2-DCA degradation. Addition of treatment additives effectively resulted in an increase in 1,2-DCA degradation by up to 15.84% and 9.14% in the As^{3+} and Cd^{2+} co-contaminated soil, respectively, within the first 5 days. Furthermore, addition of $CaCO_3$ resulted in the doubling of 1,2-DCA degradation rate constant in both the As^{3+} and Cd^{2+} co-contaminated soil matrices. Overall, all treatment additives had more pronounced positive effects in the As^{3+} co-contaminated soil, compared to the Cd^{2+} co-contaminated soil, resulting in approximately 12%, 9% and 6% increase in 1,2-DCA degradation in the presence of $CaCO_3$, Na_2HPO_4 + NaCl and $CaSO_4.2H_2O$, respectively. 1,2-DCA degrading bacterial population ranged from 3.19 to 34.47 ($\times 10^6$ cfu/ml) and 2.48 to 11.63 ($\times 10^6$ cfu/ml) in the treated As^{3+} and Cd^{2+} co-contaminated soil, respectively, after 10 days, with the highest cell density observed in co-contaminated soil treated with Na_2HPO_4 + NaCl. The growth appeared biphasic with an initial decrease in bacterial density in the presence of heavy metals, followed by an increase in treated soils over time, except for the As^{3+} co-contaminated soil amended with Na_2HPO_4 + NaCl. Overall, soil dehydrogenase and urease activities were lower in the heavy metal co-contaminated sample compared to the treated soil. The alleviation of the inhibitory effect was more pronounced in As^{3+} co-contaminated soil for both $CaCO_3$ and Na_2HPO_4 + NaCl with up to 7.92% increase in dehydrogenase activity observed compared to soil co-contaminated with Cd^{2+}. Thus, addition of treatment additives to co-contaminated soil can be used as a means of reducing bioavailable fractions of the heavy metals present, thereby limiting its toxicity to organic-degrading microorganisms and ultimately leading to increased degradation of organic compound.

Keywords: Co-contamination, heavy metals, bioavailability, 1,2-dichloroethane, treatment additives

Biotechnologically Relevant Enzymes and Proteins

Adsorption of Xylanase II from *Trichoderma reesei* QM 9414 on several polymers

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The most abundant polysaccharide after cellulose in nature is xylan and the filamentous fungus *Trichoderma reesei* (anamorph of *Hipocrea jecorina*) produces four extracellular β-1,4-D-xylan xylanohydrolases (EC 3.2.1.8) to degrade xylan. Among these, Xylanase II (XYN II) is one of the major xylanases of the fungus. The polyvalent employ of xylanases in the prebleaching of kraft pulp, in textile industry and in the food industry by improving the dough properties. Disregarding the employ of xylanases and previously to the xylan hydrolysis, the first step of the process is their adsorption on xylan. As xylan is commonly accompanied by cellulose and lignin in nature in lignocellulosic materials, adsorption onto polysaccharides which are not substrates of xylanase or other polymers must be checked.

During past years, we have purified and studied several cellulases produced by the fungus *T. reesei* QM 9414 grown on wheat straw. We have also studied their adsorption on wheat straw when they coexist in the extracellular medium of the fungus cultureraw but we had no information relative to the adsorption of cellulases or hemicellulases once purified. The present study deals with the adsorption of purified xylanase II from *T. reesei* on different adsorbents isothermally at 4 ºC. We have tested oat spelt and beechwood xylan, microcrystalline cellulose, alkali-lignin wished with hot water or unwashed and wheat straw subjected to NaOH treatment and/or milling and sieving as chemical and/or physical pretreatment respectively. The experimental results were fitted to the Langmuir isotherm and the adsorption parameters were determined. The better and worse adsorbents were the sieved wheat straw and the oat spelt xylan respectively, according with the maximum amount of enzyme that can be adsorbed (Ead_{max}). The affinity that xylanase II showed to different adsorbents has been determined as the Kes value. The minor Kes, thus the maximal affinity was found with lignin and the major Kes was found with cellulose as adsorbent. The overall results are discussed in terms of the content in cellulose, hemicellulose and lignin of the straw subjected to pretreatments, the substitution in the main chain of xylan and the convenience of washing lignin to eliminate soluble phenols which potentially decrease the xylanase adsorption on lignin.

Artificial chaperones: Gold nanoparticles assisted refolding of human basic Fibroblast Growth Factor

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Despite several early reports on the refolding enhancement effect of gold nanoparticles, the exact mechanism by which gold nanoparticle interact with protein molecules to improve refolding remains not well-understood. This study provides clear elucidation of the mode of involvement of gold nanoparticles in facilitating the refolding of human basic fibroblast growth factor.

Gold nanoparticles were synthesized and found to effectively assist refolding of chemically denatured human basic Fibroblast Growth Factor.

Gold nanoparticles enhanced the refolding yield of hbFGF to 85.35% at 0.5 mg/ml refolding protein concentration. The refolded protein regained nearly 100% activity and its CD spectra were similar to corresponding CD spectra of its native form.

Keywords: Gold nanoparticles; refolding; protein; human basic fibroblast growth factor

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Bioconversion of wheat bran into polygalacturonase using *Aspergillus sojae* in solid-state fermentation

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At the beginning of this study, 10 kinds of agro-industrial residues (dried orange peel, wheat bran, ground soybean meat, coffee husk, peanut husk, corn cob, sugar beet bagasse and orange, carrot, apple pomaces) were screened in shake flasks for their polygalacturonase production potentials using solid-state fermentation process. Among these residues wheat bran was found to be the most suitable polygalacturonase producing substrate with the maximum polygalacturonase activity of 23.3 U/g substrate. Therefore, wheat bran -the major by-product of the wheat flour producing industry- was utilized as the substrate in the polygalacturonase production from *Aspergillus sojae* by solid-state fermentation. According to the statistics of United States Department of Agriculture (USDA), a total of 648,140,000 tones of wheat were produced in the period of 2010 – 2011 by the major wheat producer countries of the world. Since, bran has a 0.11-0.18 specific waste index (mass of accumulated waste/mass of saleable product) in the wheat flour production process; wheat bran can be accepted as a sustainable by-product for the microbial production of industrially important enzymes by solid-state fermentation.

The fermentation conditions such as inoculum size (10^7 spore/g substrate), incubation time (4 days), temperature (37ºC), initial moisture content (150%) and initial pH (neutral) of the substrate were optimized in the shake flasks in order to achieve the maximum polygalacturonase enzyme activity (250.8 U/g substrate). This optimization process increased the production of the polygalacturonase enzyme 10.8 times more than the screening part of the study. Moreover, the maximum polygalacturonase activity obtained in this study was nearly 2.5 and 7.1 times higher than the ones reported in the literature on fermented defatted rice bran and dried sunflower head, respectively. Other important indicators of the SSF such as total carbohydrate content, specific activity and final pH of the medium, spore count and biomass were also measured at each optimization step to figure out the evolution of the process. In contrast to other studies in the literature, high polygalacturonase activity was achieved using wheat bran without the addition of any supplementary nutrients such as salts, vitamins or additional carbon sources that increases the cost of the production process. Also, the neutral nature of the fermentation medium throughout the process is advantageous for the operational safety and waste management. Furthermore, characterization of the wheat bran was performed for its important properties such as water holding capacity (128.6%), protein content (%15.7), ash content (3.24%) etc. Utilization of the wheat bran in the production of polygalacturonase not only converts a low-cost residue into a value-added product that is widely used in many areas of the food industry, but also helps to solve the pollution problem of wheat flour producers.

Keywords: wheat bran, polygalacturonase, solid-state fermentation, *Aspergillus sojae*

Biodegradation of native feather keratin by the recombinant strain of *Bacillus subtilis dnaC 30* temperature sensitive mutant

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Environmental wastes are found in large quantities in many countries, and many of them are rich in proteins and various carbon compounds, little attention is given to utilize or recycle these wastes. Several products are produced from the biodegradation of these wastes through genetically modified *B. subtilis* strains. These include extracellular enzymes (mainly proteases), soluble proteins, peptides, amino acids and other compounds [(1)]. Utilization of chicken feather material has been maximized recently using genetically engineered microorganisms. These microorganisms were constructed to utilize the above material in a more efficient way than the native or the wild type strains.

The ability of the mutant *B. subtilis dnaC30*, which was previously transformed with an early expressed alkaline protease (*aprE*) carrying plasmid p5.2, to utilize chicken feather waste was investigated in this study. Proteolytic and keratinolytic activity was monitored throughout the growth of the recombinant *B. subtilis dnaC30* (p5.2) cells that harbor the *aprE* gene, on basal medium II, supplemented with 1 % chicken feather and kanamycin (5 µg per ml medium). Proteolytic activity, using the sensitive substrate was increased and as the incubation time was increased reached its maximum at day 1.

The increase of keratinolytic activity, as measured by the level of free amino groups due to the action of *B. subtilis dnaC30* (p5.2), was seen after two days. It reached its maximum of 80.23 µmole / ml.

Most of the feather was considerably degraded when *B. subtilis dnaC30* (p5.2) cells were grown at 37°C for 7 hours followed by growth at 48°C for 24 hours for total period of one day and half. The level of soluble proteins, that were released during the bioconversion of chicken feather by the above strain, was also determined.

Furthermore, the physical appearance of chicken feather in cultures grown at 37°C and 48°C was observed at 48°C and compared to that of 37°C. Normally, the degradation of 1 % chicken feather takes about 2 days when *B. subtilis dnaC30* (pS1) cells were grown at 37°C for 10 hours followed by growth at 48°C .

Data presented above indicated that the *aprE* gene carried by plasmid (p5.2) was highly expressed and stable in the host, consequently an enhancement of the proteolytic and keratinolytic activity in this strain was observed. Furthermore, data would indicate that the dnaC30 mutant system is efficiently able to enhance both expression and stability of the *aprE* gene. As a result, this system could represent an alternative way to improve the nutritional value of feather and would promote some industries based cheap raw materials.

Bioprocess development for the production of alkaline protease by a local archaea *Natrialba wudunaoensis* AW34 isolated from Natron Soda Lake using Response Surface Methodology

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An optimization strategy, based on statistical experimental design, is employed to enhance the production of alkaline protease enzyme by a halo-alkaliphilic archaea local isolate. The isolate which was isolated from Natron Soda Lake and identified according to 16S rDNA sequencing as *Natrialba wudunaoensis* AW34. Sequential optimization strategy, based on statistical experimental designs, was employed to enhance the production of alkaline protease by isolate AW34. The crude enzyme showed interesting characteristics for industrial applications.

Significant parameters influencing activity in submerged fermentation (Soluble starch, Beef extract, $(NH_4)_2SO_4$, $NaNO_3$, and $FeSO_4$) were identified in preliminary experiments. Response surface methodology (RSM) was adopted to acquire the best process conditions among the selected variables. In this respect, the three-level Box–Behnken design was employed to create a polynomial quadratic model correlating the relationship between the five variables and activity. The optimal combination of the major constituents of media for alkaline protease production evaluated from the non-linear optimization algorithm of EXCEL-*Solver* was as follows(%): Soluble starch, 0.54; Beef extract, 1; $(NH_4)_2SO_4$, 0.1; $NaNO_3$, 0.2; and $FeSO_4$, 0.006. The predicted optimum alkaline protease activity reached to 487.5$Uml^{-1}min^{-1}$.

Biotechnological Production and Application of Environmentally Friendly Industrial Enzymes in Bangladesh

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Microbial enzymes unlike chemical catalysts are biodegradable, having high specific catalytic activity under mild temperature and pressure conditions rendering it to be environmentally friendly. Biotechnological production of such enzymes (Alkaline protease, keratinase, cellulose-free xylanases) locally could save a considerable amount in foreign exchange besides replacing/reducing the usages of harsh environmental chemicals. Many leather manufacturers still use chemicals (lime-sulphide) for hide processing resulting in inferior leather quality and pollution problems contrary to protease and its other bio-remediation applications. In conjunction, keratinase functions in deharing of hairs from the skins and solubilize feathers to feed. Hence, this investigation aims at biotechnologically producing the enzymes alkaline protease and keratinase for technical applications employing the *Bacillus* bacteria. Three strains of *B. licheniformis* (strain # MZK-3 MZK-4, MZK-5) identified following conventional and 16SrDNA gene sequence analysis, have been successfully isolated from effluent of tanneries and poultry firms that demonstrated both proteases and keratinase activities at varying levels on a basal medium containing feather meal as C and N sources at 37°C in bioreactor and shake cultures. They also demonstrated varying properties of pH optimum and stability, temperature tolerances and protease types i.e. serine, metallo- or aspartate. The crude enzymes, viz. protease or keratinase mix and single, from *B. licheniformis* MZK-5 were found to be very effective in soaking, unhairing and bating processes of hides improving the quality of the leather while decreasing the usage of harsh chemicals by 50 %. Furthermore, the keratinase from *B. licheniformis* strains solubilized native chicken feathers completely within 8 to 12 hrs. Also the serine protease produce was stabilized with Ca and Mn ions in the complex ingredients of commercial detergents demonstrating its usage as cleansing aid in detergent formulation. The results on the cloning of the *ker*A gene in *E. coli* will also be included in the presentation.

Key words: *keratinase, dehairing, feather digestion and cleansing aid*

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Cellulases: ambigious nonhomologous enzymes in a genomic perspective

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The key material for bioethanol production is cellulose, one of the main components of the plant cell wall. Enzymatic depolymerization of cellulose, an essential step in bioethanol production, can be accomplished by fungal and bacterial cellulases. Most of the biochemically characterized bacterial cellulases come from only handful of cellulose degrading bacteria thus limiting our knowledge of a range of cellulolytic activities that exist in nature. Recent explosion of genomic data offers a unique opportunity to search for novel cellulolytic activities; however, the absence of clear understanding of structural and functional features that are important for reliable computational identification of cellulases precludes their exploration in the genomic datasets. Here we explore the diversity of cellulases and propose a genomic approach to overcome this bottleneck.

Characterization of Chitin-Degrading Bacterium Isolated from Pruning Tree Compost: Part 1

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Chitin is an insoluble polysaccharide consisting of β-(1-4)-linked N-acetyl-D-glucosamine units and a major structural component in outer shell of crustaceans, exoskeleton of arthropods, fungal cell walls, etc. It is an abundantly produced polymer on Earth. Degradation of chitin is essentially catalyzed by chitinases. Chitinases are found in a wide range of organisms such as bacteria and higher plants. Plant chitinases involve in defense mechanism against infection by phytopathogenic fungi. Bacterial chitinases are considered primarily to digest and utilize chitin as a carbon and nitrogen nutrient.

Previously, we isolated a thermophilic chitin-degrading bacterium from pruning tree compost. The isolate formed clear zones around colonies on modified Brock's basal salts (MBS) agar plate containing colloidal chitin. In the present study, we characterized the isolate phylogenetically and phenotypically. Moreover, we investigated thermostability of the chitinase from the isolate.

Genomic DNA of the isolate was extracted and used as the template for 16S rDNA amplification by PCR using the bacterial universal primers B27F and U1492RM. The PCR product was sequenced and compared with available 16S rDNA data using the BLAST. The phylogenetic tree was constructed by neighbor-joining method.

The isolate was cultured in MBS medium containing 0.1% colloidal chitin and 0.1% yeast extract at 50°C. The secreted enzyme in the culture medium was precipitated with 80% saturation of ammonium sulfate and then re-suspended in phosphate buffer. The chitinase activity in the crude enzyme solution was measured by modified Schales' method with colloidal chitin as a substrate. Thermostability was investigated by incubation of crude enzyme at 50, 60, 70 and 80°C without the substrate. Aliquots were taken after 0.5, 1.0, 1.5, 2.0 and 12 hour-incubation and cooled on ice, and residual activity was determined.

The cells of the isolate were Gram-positive, straight-rod-shaped and motile. Ellipsoidal spores were formed in swollen sporangia. The temperature and pH ranges for growth were 25-58°C and pH 6-9, with optimal growth at 50-55°C and pH 7-8. 16S rDNA sequence analysis indicated that the isolate mostly related to some *Paenibacillus* species, and that there is no 16S rDNA sequence showing over 94% to the isolate in the DNA data base. These results suggested that the isolate is novel species of the genus *Paenibacillus*.

The chitinase activity of the crude enzyme retained about 80% of the initial activity after 12 hour-preincubation at 60°C, indicating that chitinase from the isolate is thermostable. Further characterizations of the chitinase such as chitinolytic zymography assay are now in progress.

The effect of temperature and pH on chitinase activity, and thin-layer chromatography of hydrolytic products from various N-acetylchitooligosaccharides are reported in another poster, "Characterization of Chitin-Degrading Bacterium Isolated from Pruning Tree Compost: Part 2".

Keywords chitin; chitinase; thermophilic bacterium

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Characterization of Chitin-Degrading Bacterium Isolated from Pruning Tree Compost: Part 2

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Chitin is the second most abundant polymer in nature after cellulose and consists of β-(1-4)-linked units of N-acetyl-D-glucosamine (GlcNAc). Chitin degrading enzyme called chitinase is found in plants, insects, animals and bacteria. On the other hand, a vast amount of chitin waste is released from food industry, and is expected to be degrade by chitinase. In this paper, we report characterization of the chitinase which was produced by *Paenibacillus* sp. reported in the parallel presentation "Characterization of Chitin-Degrading Bacterium Isolated from Pruning Tree Compost: part 1".

Paenibacillus sp. was cultured in modified Brock's basal salts (MBS) containing 0.1% colloidal chitin and 0.1% yeast extract at 50°C, 150 rpm. After incubation, cells were collected by centrifugation and secreted proteins in culture medium were precipitated with 80% saturation of ammonium sulfate. The precipitated proteins were resuspended in phosphate buffer and resulting solution was used as crude enzyme.

The effects of temperature and pH for *Paenibacillus* sp. chitinase were examined. Chitinase activitiy was measured by modified Schales' method. The enzyme showed highest activity at 70°C, pH 7. The mode of action was also analyzed by thin layer chromatography using chito-oligosaccharides $(GlcNAc)_2$ - $(GlcNAc)_6$ as substrates. When the enzyme was incubated with $(GlcNAc)_2$ or $(GlcNAc)_3$, no product was detected. The hydrolysis of $(GlcNAc)_6$ initially yielded $(GlcNAc)_2$, $(GlcNAc)_3$ and $(GlcNAc)_4$, and then, $(GlcNAc)_4$ was hydrolyzed $(GlcNAc)_2$. These observation indicated that the existence of endochitinase activity in this chitinase.

Keywords chitin; chitinase; *Paenibacillus* sp.

Characterization of free and dried extracellular invertase produced by filamentous fungus *Fusarium graminearum* under Solid State Fermentation (SSF)

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Currently, biotechnology is accompanied by studies on the function, structure and utilization, of enzymes obtained from microorganisms, which have raised interest in research that might improve and optimize the production of these proteins. Thus, manufacturers are increasing the use of free enzymes isolated from microorganisms to obtain different products. Among these enzymes, the β-D-fructofuranosidase (EC 3.2.1.26) , also known as invertase, catalyzes the hydrolysis of the glycosidic bond of the molecule of sucrose to obtain an equimolar mixture of monosaccharides (D-glucose and D-fructose) that is named inverted sugar, which are sweeter and do not crystallize when used at high concentrations. These enzymes, also can be used in the production of fructooligosaccharides (FOS), which are sugars not metabolized by the human organism and have no caloric value. When ingested, these prebiotics promote the reduction of serum cholesterol and prevent some cancers. The aim of this work was to study the production of invertase by *Fusarium graminearum* under Solid State Fermantation (SSF), purifying and characterizing the invertase produced and then, to dry the crude extract contain the enzyme using a Spray Dryer. *F. graminearum* produced high of extracellular enzyme (110.3 U/g substrate) under SSF moistened with tap water (1:1, w/v), at 30°C and 60% relative humidity for 7 days, with wheat bran as substrate/carbon source. The invertase was purified 8.4 times using three steps, ethanol precipitation (1:1, v/v), DEAE-Cellulose and Sephacryl S200 columns, with 14% yield. The native molecular mass was estimated as 159 kDa with subunits of 66 kDa and 94 kDa determined by 12% SDS-PAGE. The *F. graminearum* invertase showed optimum temperature of activity between 45°C and 50°C and optimum pH at 4.5. The enzyme was stable at 50°C with T_{50} of 20 minutes at 60°C. When exposed to different pH, the residual activity was above 80% from 3.0 to 8.0 for 30 minutes. The invertase activity was enhanced 127% and 75% by Mn^{2+} and K^{+}, respectively. However, the enzyme was completely inhibited by Hg^{2+}. The crude extract containing *F. graminearum* invertase was subjected to Spray Dryer with a drying chamber of 215 mm x 500 mm, inlet and outlet temperatures of 100oC and 68oC respectively. For the enzymatic protection, during the drying process it was added to the crude extract different carbohydrates (starch capsul, microcrystalline cellulose, trehalose, lactose and β-cyclodextrin). The addition of starch (10%, w/v) showed the best yield (63.6%) and provided good catalytic activity (53.12 U/mL). The extract with starch has 3.9% umidity, particle diameter of 7.8 µm and 0.21 water activity. The thermostability was also investigated, and the extracts with differents carbohydrates showed better results at 70°C if compared to the soluble enzyme. In this case, the dried cellulose microcristalline enzyme was stable with residual activity above 50% for 20 minutes. In conclusion, *F. graminearum* is a good producer of invertase in SSF with a biotechnological potencial. It can be a new source to produce this enzyme using low cost substrate that is very attractive for industries purpose. Also, this invertase could be dried, what is a nice property that makes possible the commercial application of this invertase. Support: CNPq and FAPESP

Key words: enzyme production; SSF; fructofuranosidase; Spray Dryer;

Cloning and characterization of a new phosphatase gene from *Pseudomonas putida* strain P13

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The ability of phosphate solubilizing bacteria to convert insoluble phosphate to an accessible form, like orthophosphate, is an important trait which is being controled by production of phosphatase enzymes or organic acids. With respect to the important industrial and agricultural applications of phosphatases, isolation of relevant genes has been of great interest. Several phosphatase-encoding genes and also phytases genes from different bacterial sources have been isolated and cloned. Using a screening procedure developed for detection of phosphatase and phytate hydrolysing encoding gene, a new gene was cloned from *Pseudomonas putida* p13 isolated from Iran soils via genomic library construction. Screening of the genomic library was performed on solid Sperber medium supplemented with 0.025 g/l of BCIP and 100 µg/ml of ampicillin. The presence of phosphatase activity in clones was judged by the intensity of the blue color in medium. In order to gain the full lenghth of phosphatase gene, subcloning was carried out in pGEM-T easy vector. Enzyme extraction and enzyme assay was performed by a positive transformant *Escherichia coli* DH5α harbouring pGEM plus Insert grown in LB medium supplemented with ampicillin. The purified recombinant protein displayed maximum specific activity of 466 U mg^{-1} against glucose-6-phosphate. Purified enzyme was also active against fructose-6-phosphate, p-nitrophenyl phosphate and phytate. The enzyme encoded by new isolated gene showed that its optimal pH and temperature are 4.5 and 60 °C respectively. Sequence analysis revealed no similarity between novel gene and representative of known phosphatases and phytases until now. Sequence analysis revealed that this gene carrying 1386bp and consists of a phosphatase domain of 462 residues with a calculated molecular mass of 50 kDa.

We report in this paper, the isolation, purification and characterization of a new phosphatase gene from *Pseudomonas putida* strain P13 that exhibits a marked ability to dephosphorylate glucose-6-phosphate.This gene has the ability to degrade phytate as well.

Keywords Genomic library, Phosphatase, *Pseudomonas putida*.

Comparison of extracted orange peel and whole orange peel in terms of polygalacturonase production using *Aspergillus sojae* mutant strain

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Fungal pectinases are one of the most important industrial enzymes with potential applications in textile processing, degumming of plant fibers, pectic wastewater treatment, papermaking, and food industry (coffee and tea fermentations, fruit juice extraction, oil extraction, improvement of chromaticity and stability of red wines). Among pectinases, polygalacturonases (PG) are the enzymes which attract the most attention. These catalyze the hydrolytic cleavage of the polygalacturonic acid chain by introducing water across the oxygen bridge.

The worldwide orange production in 2009 was reported as 68.5 million tonnes by FAO. The disposal of the fresh orange peels is becoming a problem in many factories. Orange peel contains considerable amount of pectin which is an inducer for the production of pectinolytic enzymes by microorganisms. It is also rich in carbohydrates, proteins and contains fat in small amounts. Thus orange peel can be a suitable substrate for the production of polygalacturonase from fungal species. In the current study dry orange peel is used as substrate for polygalacturonase production.

This study was performed in order to determine the effect of substrate state on biomass, PG activity and morphology in submerged fermentation using *Aspergillus sojae* M5/6 mutant strain. With this respect fermentations with the extracted orange peel and non extracted dry orange peel was performed at different orange peel concentrations (5, 10, 15, 20, 40, 60 g/l). Besides the given concentrations of orange peel, 2.75 g/l ammonium sulfate was added to the medium. These were inoculated at 2.8*106 spore/l inoculation rate and incubated at 30°C, 350 rpm for 7 days in a shaker.

As a result PG activity increased as the substrate concentration increased and the PG values obtained with whole orange peel (227.473 U/ml) was higher than extracted orange peel (153.067 U/ml). Biomass and the difference between the biomass of whole substrate and extracted substrate increased linearly with the substrate concentration. Morphologically , extracted orange peel resulted into clearer fermentation broth with smooth and spherical pellets. However, more viscous and turbid fermentation broth with small and irregular pellets were obtained when whole orange peel was used as substrate. Additionally, SDS-PAGE profiling of the crude enzymes produced by both of the substrate types at 60 g/l concentration, producing the highest PG activity was performed in order to see if they produce the same enzymes. For the two types of substrates similar enzymes with the same molecular weight was produced. Two bands were seen at approximately 32 kDa and 42 kDa molecular weight fragments.

It can be concluded that the PG activity, biomass and morphology was found to be effected by the state of orange peel , where extracted orange peel can be advantageous due to the ease of downstream processing with clearer and non-viscous broth.

Keywords orange peel; pectinase; *Aspergillus sojae*

Effect of soluble additives on inactivation and refolding of immobilized amylase

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Amylase was very mildly immobilized on CNBr-activated Sepharose. Activity and stability of this derivative are almost identical to ones of soluble enzyme. Thermal inactivation of the immobilized amylase was performed in the presence of different additives: e.g., a 30% of polyethylene glycol 1500, glycerol and trehalose. Trehalose promoted the highest stabilizing effect. In the presence of trehalose the immobilized amylase was 10 – fold more stable than in the absence of additives.

On the other hand, experiment of unfolding – refolding of the immobilized derivative were also performed. Unfolding was promoted by the presence of 8 M guanidine and refolding was performed in the absence or presence of trehalose, the most stabilizing agent. In addition to the presence of trehalose, temperature, buffer and pH were very relevant to improve the recovery of activity after refolding. The best conditions were: 10 mM Tris buffer pH 6.0, containing 30% of trehalose at 25 ºC for 5 hours. Under these conditions a 62 % of activity was recovered after complete unfolding by guanidine. This percentage of activity was recovered after several cycles of unfolding-refolding.

The stabilizing effect of additives is interesting to improve storage and transportation of immobilized derivatives. On the other hand, unfolding-refolding strategies are also interesting to improve the operational stability of immobilized enzyme derivatives

Keywords immobilized, additives, amylase

Enzymatic degradation of Congo red by turnip (*Brassica napus)* peroxidase

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Azo dyes are recalcitrant carcinogenic compounds and might have dermal and immunological effects on human beings. Conventional methods are not effective in the treatment of azo dyes. The enzyme peroxidase is known for its capacity to remove phenolic compounds and aromatic amines from aqueous solutions and also to decolorize textile effluents. This study evaluates the potential of the enzyme turnip **(*Brassica napus)*** peroxidase (TP) in the decolourization of textile azo dyes and effluents. An azo dye, Congo Red (CR), was used as model pollutant to be treated by the enzymatic process. The effects of different operating conditions like, pH, temperature, initial dye concentration, contact time, and the amount of H_2O_2 and the enzyme were evaluated in order to determine the optimum conditions for the enzyme performance for the decolourization of Congo red solutions. The results indicated that the optimum conditions for maximum color removal was at pH 2.0, temperature 40 °C, with hydrogen peroxide (H_2O_2) concentration 50mM, CR dye concentration 50 mg/L and TP activity of 0.45 U/ml within 10 min of incubation time. Kinetic constants (K_m and V_{max}) have been also determined. Analysis of enzymatic treatment by-products by UV-vis and IR spectroscopy showed no residual compounds in the aqueous phase and a polymeric precipitated solid with no toxic effect.

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Enzyme production for animal and poultry feed by some biofilm forming *Bacillus subtilis* strains

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Many cereals have a proportion of their energy in the form of nonstarch polysaccharides (NSPs) which cannot be fully digested and utilized by animals .However, the addition of selected external enzymes (microbial enzymes) can partially degrade this NSP, lowering viscosity in the intestine and improving feed utilisation. In this study, five strains *B. subtilis* BS3-2J, B.S.35, B.S.46 (ioslated from rice fields), b.s.y12 and B.S.13H (isolated from almond phyllosphere) were surveyed for enzyme production. Determination of enzyme profile including phytase, xylanase, cellulase, α-amylase,β-gluconase and protease was routinely done using biochemical methods. Although the strains was evaluated for biofilm formation as qualitative (on Casein-Manitol medium) and quantitative methods (microtiter plate assay). Identification of the strains was carried out based on the biochemical features and molecular method including 16S rDNA amplification and sequencing. According to the results, strain BS3-2J exhibited the most phytase, xylanase and amylase activity.Although the assesment of cellulase and protease activity revealed that strain B.S.46 and B.S.13H had the most activity comparing with other strains. All examained strains exception B.S.35 had similar β-gluconase activitiy and showed dendritic growth and formed surface raised-twisted biofilm on CM agar plates. The microtiter plate assays showed that the ring of adhered bacterial cells on microtiter plate wells was different and ranged from 0.84(B.S.35) to 3.2(B.S.13H). Biochemical and molecular (16SrDNA) characterization of the bacterial strains revealed that they belong to *Bacillus subtilis* species*.* Regarding to the different enzyme patterns and biofilm formation potential,we suggest using two or three strains simultaneously in a biofilm bioreactor for enzyme production.

Keywords : Enzyme profile; *Bacillus subtilis*; Biofilm;16SrDNA; animal feed

First crystal structure of thermostable L-lysine 6-dehydrogenase as an NAD-dependent amine dehydrogenase

Toshihisa Ohshima; Kazunari Yoneda; Haruhiko Sakuraba

L-Lysine dehydrogenase catalyzes the oxidative deamination of L-lysine in the presence of NAD, This enzyme may be potentially useful as the element for biosensor and bioreactors. A gene encoding an L-lysine dehydrogenase was identified in the hyperthermophilic archaeon *Pyrococcus horikoshii*. The gene was overexpressed in Escherichia coli, and its product was purified and characterized. The expressed enzyme is the most thermostable L-lysine dehydrogenase yet described, with a half-life of 180 min at 100 degrees C. The product of the enzyme's catalytic activity is Delta(1)-piperideine-6-carboxylate, which makes this enzyme an L-lysine 6-dehydrogenase (EC 1.4.1.18) that catalyzes the reductive deamination of the epsilon- amino group and a type of NAD-dependent amine dehydrogenase. The three-dimensional structure of the enzyme was determined using the mercury-based multiple-wavelength anomalous dispersion method at a resolution of 2.44 A in the presence of NAD and sulfate ion. The asymmetric unit consisted of two subunits, and a crystallographic 2-fold axis generated the functional dimer. Each monomer consisted of a Rossmann fold domain and a C-terminal catalytic domain, and the fold of the catalytic domain showed similarity to that of saccharopine reductase. Notably, the structures of subunits A and B differed significantly. In subunit A, the active site contained a sulfate ion that was not seen in subunit B. Consequently, subunit A adopted a closed conformation, whereas subunit B adopted an open one. In each subunit, one NAD molecule was bound to the active site in an anti-conformation, indicating that the enzyme makes use of pro-R-specific hydride transfer between the two hydrides at C-4 of NADH (type A specificity). This is the first description of the three-dimensional structure of l-lysine 6-dehydrogenase as an NAD-dependent amine dehydrogenase.

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Group I introns within the chloroplast *psbA* gene encode putative HNH- and GIY-YIG homing endonucleases in lichen-forming algae

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Homing endonucleases (HEs) catalyze their duplication into recipient alleles of host genes lacking such a sequence generating site-specific recombination events. Recently, engineered homing endonucleases are being used to generate modifications in target sequences. The available complete chloroplast genomes of green algae belonging to Chlorophyta revealed the presence of a number of group I introns encoding for putative homing endonucleases, particularly within the LSU rDNA and *psbA* genes. Several studies have indicated that lichen-forming *Trebouxia* algae have a high diversity of LAGLIDADG homing endonucleases in the chloroplast LSU rDNA. However, the *psbA* gene has rarely been studied in this group. Herein we explored the presence of new group I introns in the *psbA* gene of lichen-forming algae of different genera. Our data revealed the presence of a lower number of introns when compared with the LSU rDNA in the same specimens. Most of these introns had ORFs encoding putative HEs, which belonged either to the HNH or the GIY-YIG families and were similar to those previously found in other Chlorophyta. This work has been supported by CAM - Universidad de Alcalá (CCG10-UAH/GEN-5904), MICINN (CGL2009-13429-C0200) and GVA (Prometeo 174/2008).

Keywords: Homing endonuclease; group I intron; lichen; HNH family; chloroplast; *psbA*

High redox-potential laccases from *Pycnoporus*: blue laccases for white and red biotechnology

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The genus *Pycnoporus* forms a cosmopolitan group of four species which belongs to the polyporoid white-rot fungi, the most representative group of homobasidiomycetes causing wood decay. *Pycnoporus* fungi are listed as food- and cosmetic-grade microorganisms, and emerged, at the early 90's, as a new genus whose biochemistry, biodegradation and biotechnological properties have been progressively disclosed in details. Firstly highlighted for the existence of original metabolic pathways involved in the functionalization of plant cell-wall aromatic compounds into high valuable molecules (aroma, antioxidants), *Pycnoporus* species were then explored for their potential to produce various enzymes of industrial interest such as hydrolases and oxidases. However, the most relevant characteristic of the genus *Pycnoporus* is its ability to overproduce high redox-potential laccase – a multi-copper extracellular phenoloxidase – as the predominant ligninolytic enzyme. One of the most important aspects of the *Pycnoporus* fungi is related to the use of their laccases for a variety of applications such as the bioconversion of agricultural by-products and raw plant materials into valuable products, the biopulping and biobleaching of paper pulp, as well as the biodegradation of organopollutants, xenobiotics and industrial contaminants. The biotechnological potential of three novel high redox-potential laccases from tropical *P. coccineus* and *P. sanguineus* strains was especially assessed through the degradation of various polyphenolic dyes, and oxidation of non-phenolic lignin model compounds (veratryl alcohol and adlerol). Moreover, a recent study has been carried out by using these laccases as catalyst to develop new potential natural active ingredients from rutin (quercetin-3-rutinoside, one of the best-known naturally-occurring flavonoid glycosides) for cosmetic applications. Rutin bioconversion reached about 67% for *Pycnoporus* laccases after 24 h incubation. New flavonoids oligomers were synthesized such as dimers and trimers of rutin with no, one or two *ortho*-quinone moieties. These innovative oligorutins, suitable for cosmetic applications, provided some protection against oxidative and inflammatory damage (superoxide radical scavenging activity, inhibitory effects on the cyclooxygenase COX-2 and the human matrix metalloproteinase 3 MMP-3).

Keywords laccase, *Pycnoporus*, biotechnology

Immobilization of *Aspergillus oryzae* β-galactosidase in ionic and heterofunctional support for galacto-oligosaccharides synthesis

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Galacto-oligosaccharides (GOS) are non-digestible oligosaccharides, classified as prebiotics for their selective stimulation of growth of bifidobacteria and lactobacilli in the lower intestine. GOS are produced from lactose with β-galactosidases from different microorganisms such as *Aspergillus oryzae* and *Bacillus circulans*. The present work refers to the immobilization of *A. oryzae* β-galactosidase in polyethylenimine-agarose (PEI-agarose) and amine-glyoxyl agarose to develop robust biocatalysts for their application in GOS synthesis. β-galactosidase from *A. oryzae* was immobilized on PEI-agarose via ionic adsorption on a flexible polymer with a very high density of cation moieties, allowing a reversible but very strong protein immobilization through its surface carboxylic amino acid residues. On the other hand, enzyme immobilization in the heterofunctional support amino-glyoxyl agarose support resulted in covalent multipoint attachment, where the immobilization zone is determined by the enzyme surface area with more negatively charged amino acid residues previously linked by ionic adsorption. Enzyme immobilization yields expressed in terms of hydrolytic activity were 95.1% and 52.3% for PEI-agarose and amino-glyoxyl agarose, respectively. Thermal stability at 60ºC under non reactive conditions was evaluated in terms of the stability factor (SF), defined as the ratio of the half-life of the obtained biocatalyst to the one of the soluble β-galactosidase, being 3.9 and 2.6, respectively. As can be seen, β-galactosidase immobilized in PEI-agarose was more stable than the enzyme covalently bound to amino-glyoxy agarose. This result may be explained by the strong interaction between the cationic polymer and the protein surface and for a mild covalent multipoint attachment in the amino-glyoxyl agarose biocatalyst. Biocatalysts were assessed in the synthesis of GOS at 60ºC, 50% w/w lactose solution in 0.1 M citrate-phosphate buffer pH 4.5, being the maximum conversion of lactose into GOS practically the same for both (less than 5% difference), corresponding to approximately 28% w/w at 55% lactose total conversion, which is close to the value reported for the soluble enzyme [1]. GOS specific productivity, expressed in g GOS/h/IU, were 2.3, 3.9 and 6.3 for the free enzyme, PEI-agarose and amino-glyoxyl agarose biocatalysts respectively. One international unit of β-galactosidase activity (IU) was defined as the amount of enzyme producing 1 μmol of *o*-nitro phenol (ONP) per minute from a 10 mM *o*-nitro phenyl-galactoside (ONPG) solution in citrate-phosphate buffer pH 4.5 at 25°C. It is concluded that two different immobilization techniques resulted in the development of attractive β-galactosidase biocatalysts for GOS productions, improving stability and GOS specific productivity with respect to the soluble enzyme, without reduction in the maximum conversion of lactose into GOS.

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Impact of agitation on metabolic heat in Real Time Calorimeter (RTCal) and product formation of *Aspergillus tamarii* by submerged fermentation

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Morphology of fungal pellets has a significant influence on mass transfer and turnover processes in submerged cultures. High-viscosity fermentation broth can lead to mixing and oxygen mass transfer limitation, obvious solution for this problem is to increase agitation intensity. In some processes, this will damage mycelia, affect morphology, and decrease product expression. However, in other processes increased agitation shows no effect on productivity. There are many reports in literature that biomass is not distributed homogeneously over the pellet radius, yet quantitative data is rare. Product formation of mycelial organism, like *Aspergillus tamarii*, is intimately connected with their morphology. Therefore, it is important to reveal the influence of the hydrodynamic conditions on the morphological development. In the present study, pellet morphology and protease production were studied under different agitation intensities of *A. tamarii* ie coupling of hydrodynamics and bioreaction, highlights the complex relationship between energy dissipation, substrates uptake rate and cell physiology. The present work shows that simple equations based on Monod-kinetics can describe growth and product formation with a substrate affinity of 1.3mg/mL for agricultural product which not only gave high yields of enzyme but also facilitated improvement in process economics. Measurement of metabolic heat has been attempted using continuous, dynamic heat balance, real time calorimeter. The contributions of individual heat sources influencing the temperature of the broth were evaluated. The paper also discusses the correlations between oxygen uptake rate (OUR) and metabolic heat with heat yield under dynamic conditions for different agitation rates (viz 250, 350 and 450 rpm).

Keywords: *Aspergillus tamarii*, Morphology, Protease production, Shear stress, Pellet Formation, Real Time Calorimeter(RTCal).

Improved biodelignification of lignocellulose: impact on lignocellulose structure

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Lignocellulosic biomass contains polymers of cellulose, hemicellulose, and lignin, bound together in a complex structure. Modern biorefinery concept integrates biomass conversion processes to produce fuels, electrical power and chemicals from biomass. Up to now research has been mainly focused on the production of liquid biofuels such as ethanol via fermentation of sugars derived from cellulose and hemicellulose within lignocellulosic materials. However there are additionally an enormous range of possibilities that the complex structure of lignocellulose may offer for application in different fields.

In this work we attempted a biological treatment of lignocellulose with ligninolytic fungi aimed at decreasing lignin content (biodelignify) and modifying lignocellulose surface in such a way that it could be useful for targeted applications. Lignin is the most recalcitrant polymer in lignocellulose, it cannot be easily separated into readily utilizable components. Thus, by decreasing lignin content, further processes such as anaerobic digestion for methane production or enzymatic treatment for sugars release from polysaccharides could be facilitated. Moreover, modifying lignocellulose surface may render improved raw biomass materials for the production of novel lignocellulose-containing composites.

Lignocellulose materials were inoculated with four selected ligninolytic microorganisms in two sets of experiments. In the first, the microorganisms were inoculated into wood fibres without any other nutrient source in order to oxidize lignin. In the second, coupling of amines to lignocellulose structure was the main target and it was performed by adding a certain amine to the fungal culture on lignocellulosic substrate. A final experiment was also developed to improve fungal ligninolytic activity on lignocellulose by selected fungus.

The experiments performed demonstrated that wood fibre can be modified by fungi either by oxidizing lignin, by degrading lignocellulose polymers or by coupling a secondary amine when this compound is added to the substrate. *Phanerochaete flavido-alba* (Pfa) was the most suitable microorganism for wood fibres modification. This microorganism increased carboxylic acid content, decreased more than 30% lignin and was able to mediate secondary amine coupling to lignocellulose when hydroxytyramine was added to the culture media. These wood modifying activities were improved by using rich media and inducers of ligninase activity during the inoculum production.

A method for biological modification of wood and biodelignification with live fungi is proposed in which *P. flavido-alba* grown in a medium with enzyme inducers is inoculated to wood and incubated for 21 days at 30ºC. This method may have applications as a pretreatment step in the field of biorefinery.

This work is supported by European Community grant FORBIOPLAST No.KBBE-212239.

Keywords ligninolytic; *Phanerochaete flavido- alba*; coupling

Molecular Characterization of Xylanase II from *Trichoderma reesei* QM 9414

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Xylanase II is one of the most important endoxylanases (EC 3.2.1.8) from *Trichoderma reesei* to degrade xylan in nature. The industrial employ of xylanase in processes such as the bleaching of kraft paper pulp or the improving of cattle food makes the enzyme very attractive, and studies on its thermal stability are relevant because of the high temperatures required in those industrial processes. The state of glycosylation of proteins is relevant when temperature studies are carried out since it affects their thermal stability, increasing it most of the times. The glycosylation state of xylanase II from *Trichoderma reesei* QM 9414 grown on wheat straw has been stated by carbohydrate staining of the electrophoresis gel after SDS-PAGE obtaining an apparent molecular mass for the glycosylated enzyme of 23.8 kDa which is higher than its theoretical mass (20.842 kDa). Glycosylation of xylanase II was assessed by Mass Spectrometry (MS) and peaks differing in 203 Da (an N-acetylglucosamine residue) were observed. The peak corresponding to the glycosylated xylanase disappeared in the mass spectrogram after treatment with a deglycosylating enzyme (PNGase F) followed by MS. Xylanase II from *T. reesei* holds two potential glycosylation sites determined by homology but the exact position the N-acetylglucosamine is bound is unknown. In order to assess the position of the sugar residue, tryptic digestion of xylanase II followed by mass spectrometry was carried out. The identification of tryptic fragments allowed us to discard one of the two potential glycosylation sites. A published xylanase non covalent modification related to rising temperature is the existence of xylanase dimers above 50 ºC. To detect the existence of dimers in our enzyme system, analytical ultracentrifugation was carried out at 25 ºC after heating the enzyme at 50 ºC. The major peak obtained (94.5 % of mass) showed a sedimentation coefficient only compatible with a xylanase monomer. Moreover, the existence of dimers could not been proved through glutaraldehyde crosslinking at 50ºC.

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Newly identified LAGLIDADG homing endonucleases in the chloroplast LSU rDNA of *Coccomyxa* algae

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Homing endonucleases (HEs) are enzymes that invade specific insertion sites by generating double-strand breaks on the targeted gene. This insertion process results in their duplication into recipient alleles of host genes lacking such a sequence. This enzymatic activity involves site-specific recombination events that can be exploited for the correction of DNA sequences in biotechnological and medical applications. The chloroplast-encoded large-subunit ribosomal RNA gene of lichen-forming algae belonging to the *Trebouxia* genus has diverse group I introns, which encode for LAGLIDADG-HEs. To search for new HEs, we explored the presence of group I introns in the same gene in both lichen-forming and free living algae belonging to the *Coccomyxa* genus. Preliminary results indicated the presence of a relatively high number of group I introns in the analyzed specimens. Most of these introns had ORFs encoding putative LAGLIDADGhoming endonucleases.A number of these HEs seemed to be homodimers since they had a single LAGLIDADG motif, whereas others seemed to be monomers since they had two of these motifs. A number of these introns were very similar to those previously found in *Trebouxia* algae, but some of them seemed to be very different even from those found in other organisms. These findings reveal that lichen-forming algae are potential sources of new endonucleases that can be engineered to modify specific targets of interest.This work has been supported by CAM - Universidad deAlcalá (CCG10-UAH/GEN-5904), MICINN (CGL2009-13429-C0200) and GVA (Prometeo 174/2008).

Keywords:Homing endonuclease; group I intron; lichen; LAGLIDADG; chloroplast; LSU rDNA

Nuclear targeting of a bacterial integrase which mediates site-specific recombination between bacterial and human target sequences

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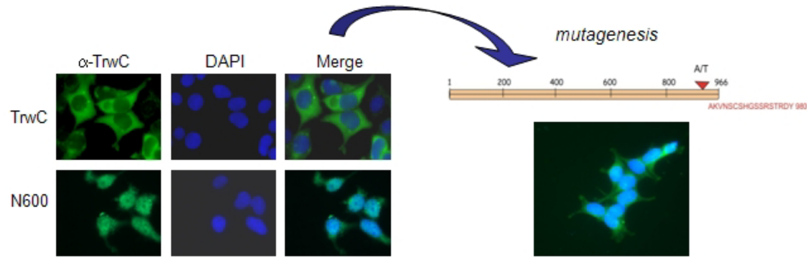
TrwC is a bacterial protein involved in conjugative transfer of plasmid R388. It is transferred together with the DNA strand into the recipient bacterial cell, where it can integrate the conjugatively transferred DNA strand into its target sequence present in the recipient cell (Draper et al., 2005). Considering that bacterial conjugation can occur between bacteria and eukaryotic cells, this protein has great biotechnological potential as a site-specific integrase (Llosa and de la Cruz, 2005).

We have determined the cellular localization of TrwC and derivatives on human cells by immunofluorescence, and also an indirect yeast-based assay to detect both nucler import and export signals. The results indicate that the recombinase domain of TrwC (N600) has nuclear localization, but full-length TrwC locates in the cytoplasm, apparently due to the presence of a nuclear export signal in its C-terminal domain. The recombinase domain of TrwC can be transported to recipient cells by conjugation in the presence of the helicase domain of TrwC, but with very low efficiency. We addressed a mutagenesis analysis of TrwC selecting for mutants with nuclear localization. We obtained one such mutant with a point A904T mutation and an extra peptide at its C-terminus, which maintained its functionality in conjugation and recombination (Agúndez et al., 2011). This TrwC mutant could be useful for future TrwC-mediated site-specific integration assays in mammalian cells.



We have searched for possible TrwC target sequences in the human genome. Recombination assays showed that TrwC efficiently catalyzes recombination between its natural target sequence and a discrete number of sequences, located in non-coding sites of the human genome, which resemble this target. TrwC also catalyzes site-specific integration of any incoming DNA into these human sequences present in the recipient cell. The integration product is stable, since reversal of the reaction requires an accessory protein from the R388 plasmid.

Altogether, these results imply that TrwC is a site-specific integrase which can be introduced in vivo into prokaryotic and eukaryotic cells covalently linked to a DNA molecule of any origin and length; once in the recipient cell, it can be targeted to the nucleus, where it could catalyze the site-specific integration of the incoming DNA into natural target sequences.

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Optimization of medium components in solid state fermentation for *Aspergillus niger* naringinase production

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Naringinase has become biotechnologically important due to its role in debittering citrus fruit juices, enhancement of wine aroma, manufacture of rhamnose, preparation of prunin and biotransformation of antibiotics. This work aimed naringinase production of *Aspergillus niger* 426 utilizing agricultural substrates (yeast extract, cane molasses and rice husk) in solid state fermentation. A full factorial design 2^3 which included three replications at the center point was used. The variables chosen were concentration (g/L) of naringin (0.4; 1.0; 1.6), yeast extract (5; 10; 15) e cane molasses (2; 4; 6). Rice husk (5g) was placed on 125mL Erlenmeyer and moistened with 12mL mineral solution (g/L): $(NH_4)H_2PO_4$ 5.0; K_2HPO_4 1.5; $MgSO_4.7H_2O$ 0.5; KCl 0.5; $FeSO_4.7H_2O$ 0.008; $MmSO_4.7H_2O$ 0.0015; $ZnSO_4.7H_2O$ 0.00008; $Na_2MoO_4.2H_2O$ 0.0008; $CuSO_4.5H_2O$ 0.0004; $NaB_4O_7.10H_2O$ 0.0004. The initial pH value was adjusted to 4.5. The inoculum was 1.2x109spores/mL. Cultivation flasks were incubated at 28°C without agitation. Samples were taken at 144 hours. One unity of naringinase activity was defined as the amount of enzyme that could hydrolyze 1 µmol of naringin. The results were analyzed by Statistica 9.0. The analysis showed that the factors significantly (at 5% level) affecting naringinase production were naringin and yeast extract. The highest naringinase activity of 2.4U/mL was obtained when naringin (0.4g/L), yeast extract (5g/L) and cane molasses (2g/L) were used. The determination coefficient (R^2) was 0.84 for naringinase activity, indicating an adequate degree of reliability in the model. The present study utilizing agroindustrial substrates could be potential cost effective production method of this important biotechnological enzyme.

Keywords: Naringinase, Agroindustrial residues, Response surface methodology.

Optimizing some factors affecting acid protease production by *Rhizopus stolonifer*: purification and characterization

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Production of extracellular acide protease by a locally isolated fungal species, *Rhizopus stolonifer* under solid state fermentation were investigated. Different cultural conditions such as rate of fermentation, effect of incubation temperature, effect of pH and medium moisture, and finally effect of carbon and nitrogen sources were optimized.

The maximum enzyme synthesis was found at 120 US/ml, after 96 h of fermentation at a temperature of 28°C. The optimum pH and medium moisture for protease synthesis were found to be 6.0 and 50.6%. The maximum protease production was found with galactose and peptone respectively.

Fermentation was then carried out under optimized conditions. The crude extract obtained was partially purified by gel filtration and the properties of purified extract were also studied.

The proteolytic activity was purified 19 fold with a yield of 9.25% and attaining a specific activity of 2851U/mg.

The extracellular acid protease was found to be stable in the temperature range of 30-55°C after 60 min heating and acts optimally at 50°C. Her optimum pH was 5.5 and was found to be stable in the citrate buffer after incubation for 24 h at 4°C.

The enhancing of milk clotting activity is found to be correlated with the increase of the concentrations of calcium chloride and enzymes respectively**.** The effect of storage on milk clotting activity of purified protease showed that it retained 100% of initial activity at -18°C for six months.

Key words: *Rhizopus stolonifer*, Milk clotting activity, Solid state fermentation, Gel filtration, Protease characterization.

Production of an extracellular lipase from *Pseudozyma aphidis* and its activity and stability in organic solvents

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The production of lipase from *Pseudozyma aphidis* (DSM 70725), microorganism closely related to the well known producer of lipases employed in various fields of biotechnology, *Pseudozyma* (*Candida) antarctica*, was monitored in media with different carbon and nitrogen sources. Since lipase production was the highest in medium with glucose as the sole carbon source and yeast extract and sodium nitrate as nitrogen sources, time course studies were conducted in order to determine the growth and lipase production characteristics of *Pseudozyma aphidis* in the optimal medium. The results reveald that the highest lipase production was achieved at the end of the log phase of growth, reaching the value of 35.0 U cm^{-3} in the fifth day of cultivation, which was over four times higher value than in previous reports.

Activity and stability of crude lipase from *Pseudozyma aphidis* was examined in various polar, water-miscible organic solvents from five organic groups (ketone, nitril, ether, alcohol, sulfoxide). The hydrolytic activity of crude lipase towards *para* nitrophenyl palmitate (*p*NPP) in aqueous media and in organic solvents was determined, using the same spectrophotometric assay in both aqueous and organic media. The crude lipase preparation exhibited activity towards *p*NPP only in acetone and acetonitrile, while lipase was stable only in acetone, with 23% of residual activity after 24 h of incubation. The stability and activity of crude *Pseudozyma aphidis* lipase in acetone, justifies the search for potential application of the enzyme in biocatalysis in such medium.

Keywords *Pseudozyma aphidis*; lipase; organic solvents

Production of Thermo-alkaliphilic keratin degrading enzyme by Egyptian local isolate *Laceyella sacchari* AM30: Numerical modelling bioprocess optimization, enzyme purification and characterization study

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Process optimization of the production of Thermo-alkaliphilic keratin degrading enzyme by the local isolate *Laceyella sac*chari AM30 was carried out through the implementation of statistical experimental factorial design. The crude enzyme showed interesting characteristics for industrial and biotechnological applications in respect to its potency to degrade bird feather at high temperature and pH (60°C and pH10). Feather degradation micrograph was SEM demonstrated.

Significant parameters influencing activity in submerged cultivation (NH_4Cl, Yeast extract and $NaNO_3$) were identified in preliminary experiments. Response surface methodology (RSM) was adopted to acquire the best process conditions among the selected variables. In this respect, the three-level Box–Behnken design was employed to create a polynomial quadratic model correlating the relationship between the three variables and activity. The optimal combination of the major constituents of media for keratinase production evaluated from the non-linear optimization algorithm of EXCEL-*Solver* was as follows (%): NH_4Cl, 0.08; Yeast extract, 0.04; and $NaNO_3$, 0.016. The predicted optimum keratinase activity increased 10 folds than the basal conditions. The produced keratinase enzyme was purified using Fast Protein Liquid Chromatography and consequently the purified enzyme was characterized.

Protease production by *Bacillus licheniformis* in the presence of industrial waste

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Enzymes are biocatalysts of great industrial interest. The enzyme application has increased by technological and economic reasons. Scientific research has been conducted for the production of metabolites of commercial value by microorganism in the presence of industrial wastes as nutrient sources. Brazil has potential to produce different microbial metabolites due to a large quantity and variety of renewable raw materials. In addition, the technology development for reuse of waste is essential for sustainable development to preserve the environment.

The proteases have great structural diversity and different mechanisms of action. These biocatalysts are used in pharmaceutical, textile, detergent and food industries; in the processing of animal feed and leather. Microbial alkaline and neutral proteases are produced mainly by bacteria of the genus *Bacillus*. The aim of this work was to determine proteolytic activity by *Bacillus licheniformis* in the presence of industrial waste.

A culture of *B. licheniformis* was grown in Nutrient Agar. After 24 h of incubation at 37ºC, the cells were innoculated in Erlenmeyer flask with 50 mL of Nutrient Broth under agitation at 250 rpm for 3 h. The inoculum with 10^8 CFU/mL was added at 10% v/v in Nutrient Broth and a industrial waste (0.5%). The experiments were performed in triplicate. The pH was determined in potenciometer. The proteolytic activity was determined by spectrophotometry at 440 nm in the presence of azocaseína 0.2% in Tris-HCl 0.1 M at pH 7.2 by the modified method of Leighton et al. (1973).

The table 1 presents the results of proteolytic activity by *B. licheniformis* in the presence of industrial wastes. The proteases production increased during the cultivation in the presence of manipueira, whey and molasses of sugar cane. The differences between the values of proteolytic activities were insignificant in the presence of glycerin and corn steep liquor for 8 and 24 h of submerged culture. The behavior of the bacillus in the presence of soybean meal was different: the highest proteolytic activity was determined for 8 h of cultivation. The maximum average production of proteases by *B. licheniformis* was 175 U/mL in the presence of glycerin and whey, respectively, the waste product of biodiesel production and the byproduct of dairy industry.

Table 1 Proteolytic activity of *B. licheniformis* in the presence of industrial wastes

Industrial waste	Proteolytic activity (8 h)	Proteolytic activity (24 h)
Manipueira	33 U/mL	64 U/mL
Whey	46 U/mL	174 U/mL
Molasses of sugar cane	32 U/mL	152 U/mL
Glycerin	176 U/mL	159 U/mL
Soybean meal	69 U/mL	18 U/mL
Corn steep liquor	135 U/mL	100 U/mL

The pH of the medium (initial pH 7.0) remained around neutrality during the first hours of cultivation. After 24 h, this physicochemical parameter decreased gradually to pH 5.7 in the presence of glycerin and it increased to pH around 8.0 in the presence of whey, soybean meal and manipueira. The behavior of pH in the presence of molasses was different: it reached pH 5.5 for 8 h and pH 6.2 for 24 h of cultivation.

Industrial wastes can be used as nutrient sources in the production of proteases by *B. licheniformis*.

Keywords: proteases; *Bacillus licheniformis*; submerged culture; industrial waste

Purification and Characterization of lipase enzyme produced by *Bacillus stearothermophilus* HU1

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An extracellular lipase enzyme from *Bacillus stearothermophilus* HU1 was purified by ammonium sulphate precipitation by bringing it to 40% saturation, followed by DEAE-cellulose ion exchange chromatography. This purification resulted in a 1.6 fold purification of lipase enzyme with 0.42% recovery yield. The molecular mass of the purified lipase enzyme was estimated to have an approximate value of 50-75 kDa by Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE). Enzyme kinetics study has shown that the lipase enzyme has a k_m and v_{max} values of 0.2353 mM and 161.2 nmol.min^{-1}.mL^{-1}, respectively.

Keywords Lipase enzyme, production, *Bacillus stearothermophilus*, enzyme purification.

Purification and Characterization of Novel Alkali-tolerant Cold-adapted α-amylase from *Microbacterium foliorum* GA2

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A psychro-tolerant bacteria, identified as *Microbacterium foliorum* GA2 was isolated from soil of Gangotri glacier, Western Himalaya, India. The α-amylase production was found maximum at 20°C and pH 9 after 120 h incubation in presence of lactose and magnesium ion. Abundant agricultural wastes may also be utilized for the production of useful products by enzymatic action as these are cheap and renewable. Result of optimization by Plackett-Burman design with SSF showed that 25% of Bagasse with 0.002M lactose at 20°C and pH 6.0 for 5.75 days of incubation was optimum for maximum amylase production. The enzyme was purified to homogeneity with 21.58-fold purification with specific activity of 161.91 U/mg. SDS-PAGE and zymogram activity staining of purified amylase showed a single band equal to a molecular mass of about 66 kDa. The optimal pH and temperature for enzyme activity was 8.0 and 22°C, respectively; however, the purified amylase was stable over a broad pH range of 6.0 to 10. The Km and Vmax of purified α-amylase was 0.37 mg/mL and 2222 Units/ml respectively. Hg^{+2}, Zn^{+2} and Ba^{+2} stimulated enzyme activity significantly; however Fe^{+2}, Cu^{+2}, Co^{+2}, EDTA, H_2O_2, $CuSO_4$, SDS and Urea inhibited the activity. Ca^{2+} showed no significant effect on enzyme activity. This research suggests that properties of cold-stable α-amylase found a good potential in starch-processing industries and its alkali tolerant behavior in detergent industry.

Key words: Cold-active enzymes, *M. foliorum*, α-amylases, Gangotri glacier, Fermentation.

Response Surface Optimization for Production Yield of Kefiran, a Newly Derived Exopolysaccharide from Kefir Grains

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Kefiran is finding increasing use in the food industry as a texturing and gelling agent. It is a water-soluble polysaccharide containing approximately equal amounts of glucose and galactose residues in a chain sequence.

The structure of the repeating unit of this EPS has been elucidated mainly by methylation analysis and NMR data. However, no studies of the structure of the kefiran obtained from kefir grains are available in the literature. Moreover, little is known about kefiran production in cheese whey. In this study, in order to better exploit EPS from kefir grains, we optimized the culture conditions for EPS production by RSM and further characterized its structure. First, central composite design (CCD) was used to determine the optimum conditions for maximum EPS production. Then, the polysacharaide's complete structure was elucidated to identify its potential application and provide a fundamental guide for correlating the intrinsic characteristics of kefiran with its putative health benefits.

Whey lactose concentration (20–100 g/l), yeast extract concentration (0–24 g/l), pH (3.5–7.5) and temperature (15–35°C) were the factors investigated. Experiments were designed according to Central Composite Design with these four factors, including central and axial points. A second-order polynomial model was developed using multiple linear regression analysis. The optimum extraction conditions were found to be: whey lactose concentration of 66.63 g/l; yeast extract concentration of 12.62 g/l; pH of 5.7 and extraction temperature of 24.13°C. Under these conditions, the experimental value was 663±25 which is well in good agreement with value predicted by the model.

Keywords: Central composite design; Cheese whey; Correlation; Kefiran polysaccharide.

Site-specific recombination and integration reactions catalyzed by conjugative relaxases: a mutagenesis approach to improve recombination activity

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Bacterial conjugation is a specialized mechanism of horizontal DNA tranfer from a donor to a recipient cell. Under laboratory conditions, DNA transfer has been described to occur from bacteria to *Saccharomyces cerevisiae*, plants, and even mammalian cells. DNA processing is driven by a nucleoprotein complex called relaxosome where the relaxase is the key protein. They are site-and strand-specific endonucleases which recognize and cleave one strand of their cognate origin of transfer (*oriT*), remain covalently bound to the 5‘ end of the DNA strand to be trasferred, and are transferred with the DNA to the recipient cell (Llosa *et al*., 2002).

TrwC is the relaxase of the conjugative system of plasmid R388. TrwC is also a site-specific recombinase capable of promoting efficient recombination between two cognate *oriT*s (César *et al*., 2006). Moreover, it can integrate the conjugatively transferred DNA strand into its target sequence present in the recipient cell (Draper *et al*., 2005). It is able to catalyze recombination between its natural target sequence and a discrete numbrer of sequences, located in noncoding sites of the human genome (Agúndez *et al*., 2011). These abilities confer TrwC a high potential as a biotechnological tool for site-specific genomic modification.

TraI is the relaxase of the F plasmid, highly related to TrwC, although it has been reported that TraI does not have recombinase activity (César *et al*., 2006). Using a TraI-mediated site-specific recombination assay we have demonstrated that TraI can catalyze recombination between two F-*oriT* copies, although the frequency of recombination mediated by F-TraI is very low compared to that of TrwC under similar assay conditions. This allows future search for TraI mutants with increased recombination activity, which will act on different target sequences.

We have used random mutagenic PCR to mutagenize the *trwC* and *traI* genes, selecting for mutants with enhanced recombination activity. We have obtained a TrwC mutant with increased recombination activity, which is completely functional in conjugation. This mutant could be used for genomic engineering with better efficiency than wild-type TrwC.

These and other results show that bacterial conjugative relaxases could be used in the future as site-specific integrases in human cells, leading the way for the development of a novel system for genomic modification which could have applications in biotechnology and biomedicine.

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Keywords: Bacterial conjugation, relaxases, TrwC, TraI, mutagenesis, genomic engineering.

Studies on cellulase from a newly isolated *Brevibacillus sp*. strain ST15c10 and molecular characterization of the bacterium

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A carboxymethyl cellulose (CMC)-degrading bacterium, ST15c10, was isolated from municipal solid bio-wastes initially by enrichment culture and identified as *Brevibacillus sp*. based on morphological, physiological and biochemical tests with 16S rDNA sequence analysis **(GenBank Acc. no HM446043)**. This rod-shaped, Gram reaction positive, endospore forming bacterium is resistant to several antibiotics and grew in media containing CMC and gelatin supplemented with potassium and magnesium. The optimum growth conditions were T_m 40°C and pH 8-9 for this organism which showed negative results in citrate, amylase and positive in gelatinase and catalase test. The 16S rRNA gene sequence data reveal that this mesophilic strain belongs to genus Brevibacillus and family Paenibacillaceae. The phylogenetic tree, constructed by MEGA4 and nucleotide homology data unveiled that strain ST15c10 has the highest sequence similarity of 98.9% with *Brevibacillus agri* isolates KZ17, *Brevibacillus formosus* strain DSM 9885T, *Brevibacillus formosus* strain LMG 16101 and *Brevibacillus brevis*. Further investigation reveal that this bacterium carry a large plasmid (~50 kb) which could attribute to its drug resistance property. The crude extra-cellular CMCase activity of this facultative cellulolytic bacterium was optimized at pH 5.6, T_m 60°C with CMC 0.5 % (w/v) as substrate. This moderately thermostable enzyme also showed significant catalytic activity towards alkali treated bagasse> alkali treated rice straw> cotton> filter paper. The present results have strong implications in bio-conversion of cellulosic agricultural and forestry wastes and in the production of essential commodity materials like sugars and biofuels.

Synthesis of *p*-hydroxybenzyl-alcohol glucoside catalyzed by α glucosidase from *Saccharomyces cerevisiae* and determination of its antioxidative properties

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p-hydroxybenzyl alcohol (*p*-HBA), one of the phenolic constituents widely distributed in various kinds of plants, including Gastridua elata Blame (Orchidaceae) (Tian ma in Chinese), has been shown to exhibit a variety of therapeutic properties including antioxidant, anti-excitotoxic, anti-inflammatory, and anti-apoptosis. Recently, *p*-HBA was found to be more effective than well known potent antioxidant melatonin in reducing hydrogen peroxide or ferrous ion-induced lipid peroxidation.

Glycosilation of phenolic compounds can improve their pharmacokinetic parameters or sometimes, glycosilation is crucial for compounds activity. Thus, it is of considerable interest to examine the antioxidant effect of *p*-HBA and its corresponding glucoside. Glycosides can be synthesized by chemical or enzymatic synthesis. Chemical syntheses of the glycosidic moieties are mainly based on time-consuming protection and deprotection strategies, activation or metal catalysis, and are often accompanied by formation of unwanted diastereomers and low yields. These difficulties can be overcome by application of enzymatic syntheses. The enzymatic synthesis provides convenience because of the synthesis of a regio- and stereoselective product commonly in one-step reaction, without environmental injuries. Transglycosylation reactions catalyzed by enzymes are well known and widely used methods for glucoside syntheses. Glycosidases, responsible for catalytic hydrolysis of the glycosidic linkage, are increasingly being used in carbohydrate synthesis. α-1,4-glucosidase (maltase) is one of the most abundant glucosyl hydrolases present in baker's yeast (*Saccharomyces cerevisiae*) and has been used for the synthesis of various glucosydes.

In our work, we synthesized glycoside of *p*-HBA, in high yield, using maltase from baker's yeast as catalyst. The effects of four reaction parameters (temperature, pH, maltose and *p*-HBA concentration) on the degree of transglucosylation were evaluated, and the optimal reaction conditions were proposed. The product was isolated, and its structure was determined by spectroscopic methods (^{1}H and ^{13}C NMR, HRMS, optical rotation). Antioxidant properties of pHBA and its glucoside were determined using DPPH, ABTS and lipid peroxidation assay, and it was shown that *p*-HBA glucoside exhibit good antioxidant properties.

Keywords *p*-HBA; glucoside; maltase; Saccharomyces cerevisiae; transglucosylation; antioxidative activity

The antifungal activity of *Streptomyces albidoflavus*, purification and characterization of its antifungal components

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Chitinases (EC 3.2.1.14) belong to glycosyl hydrolase families 18 and 19 that catalyze the hydrolytic degradation of chitin polymer to its oligo and monomeric components. It is suggested that chitinolytic microorganisms or chitinolytic enzymes have potential applications in the biocontrol of plant pathogenic fungi and insects, as a target for biopesticides, and in many other biotechnological areas. Microorganisms isolated from soils collected at different locations in Central Poland were screened on agar plates containing colloidal chitin. Among the bacteria showing the higest clear zone, strain was selected for the production and characterization of chitinase. Its identification was based on the analysis of the intergenic region ITS 16S-23S rDNA. The chitinase released to the substrate by these bacteria was purified by applying the two-step procedure: salting out with ammonium sulphate and affinity chromatography. The purified enzyme preparation was subjected to mass spectrometry analysis (IBB PAN). The protein concentration in the sample was 2 μg/ml. A protein sample previously digested using trypsin was separated on a nanoAcquity UPLC (Ultra Performance LC) system and analyzed with an Orbitrap-based mass spectrometer.

An analysis using the NCBI database revealed that the sequence obtained by test strain can be attributed to *Streptomyces albidoflavus*. The obtained results indicated the affinity of the studied enzyme with the family of 19 glycoside hydrolases. Degraded starters for PCR were designed based on the fragments of amino acid sequences of chitinase released by the studied bacteria. The nucleotide sequence of an amplified gene fragment also revealed the similarity with sequences of genes encoding the chitinases from the family of 19 glycoside hydrolases. The colloidal chitin was the best substrate for the production of chitinases. The temperature optimum of the purified chitinase was 40°C. The enzyme was characterised by thermostability at the temperature of 30°C and 40°C during 180 min preincubation. The activity of the enzyme was strongly inhibited in the presence of Hg^{2+} and Mn^{2+}, SDS. The activity of the enzyme was stabilized by the ions Ca^{2+} and Mg^{2+}. Purified and crude chitinase from *Streptomyces albidoflavus* inhibited to a different extent the development of phytopathogens and potential phytopathogens. The purified chitinase inhibited the growth of *Alternaria alternata*, *Botrytis cirenea* and *Penicillium* sp. and the crude chitinase − additionally the growth of *Penicillium purpurogenum*.

Keywords: *Streptomyces*, chitinase, biocontrol

The molecular biology of *Penicillium echinulatum* cellulolytic system: gene cloning, heterologous expression and transcription regulation studies

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Penicillium echinulatum 9A02S1 mutant strain produces and secretes large amounts of enzymes capable of saccharifying cellulose into glucose units. This fungus enzymatic properties over agricultural residues, such as sugar-cane bagasse and wheat straw, point it out as a promising agent for bioconvertion processes at the industrial level. *P. echinulatum* celulases have been successfully employed in textiles biostoning and biopolishing pilot studies. Our research group is pioneer in investigating *P. echinulatum* molecular genetics. The first described cDNA sequence, encoding endoglucanase 1 (*egl*1), was isolated and cloned in *Pichia pastoris* under the control of the AOX promoter. Recombinant enzyme (rEGL1) - presenting 387 amino acids residues, predicted molecular mass of 41 KDa and isoeletric point of 4.99 - was efficiently secreted and displayed an optimum CMCase activity over a broad pH range (5.0-9.0) and at the temperature of 60°C. rEGL1 retained 84% of its residual activity after 1 h of pre-incubation at 70°C. Calcium addition to the enzyme assay led to a 364% increase in enzyme activity. Altogether, these properties are particularly interesting to the textile industry.

Recently, we have constructed a *P. echinulatum* subtractive cDNA library from RNA molecules obtained under repressing (glucose) and induction (sugar-cane bagasse) conditions for cellulases production, in order to obtain a cDNA repertoire enriched for glycoside hydrolases-encoding sequences. In a transcriptomic approach, randomly selected cDNA clones have been sequenced. Up to now, 192 distinct clones were sequenced. Approximately 70% of them presented the sequence reading quality which allows similarity analyses (PHRED $\geq$ 20; size $\geq$ nucleotides). 18 ESTs were gathered into 8 *contigs*. 115 *singlets* were also obtained. Amongst these, we highlight cellobiohydrolase, beta-glucosidade, family 7 endoglucanase, endo-beta-galactase, calmodulin, hydrophobin and MFS multidrug transporter-encoding sequences. At the moment, we are employing a 5´/3´-RACE approach to obtain the full-length cDNA sequences corresponding to the glycoside hydrolases genes. These data are relevant to the understanding of *P. echinulatum* genome structure, since no other scientific group investigate this fungus molecular biology. Furthermore, cDNAs cloning into the *P. pastoris* expression system will generate recombinant enzymes which can be employed at agricultural residues bioconvertion into useful products at the industrial level.

The protein patterns of *Streptococcus mutans* strains in caries free and caries susceptible subjects

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The mutans Streptococci are generally considered to be the principal etiological agent of dental caries. Among them, *Streptococcus mutans* is the putative cariogenic organism more routinely associated to active lesions. However, they are widely distributed not only in populations with moderate or high caries prevalence but also in populations having no or low caries experience. So, in this study the protein patterns of *S. mutans* strains isolated from caries-susceptible and caries-free subjects were analyzed. Protein extracts of twelve *S. mutans* isolates, representing caries-susceptible and caries-free subjects were analyzed by SDS-PAGE. Results showed that, protein profiles of caries-susceptible subjects were very different from those of caries-free subjects. The SDS-PAGE of protein extracts of isolates showed between 6 to 18 bands with molecular masses in the range of 15-200 kDa. The major protein bands of SDS-PAGE analysis were observed 26-100 kDa and 45-57 kDa in caries-susceptible and caries-free subjects, respectively. The major protein bands of SDS-PAGE analysis of standard strain *S.mutans* ATCC35668 were between 35-100kDa. Major significant differences between protein patterns of two subject groups and interestingly, non-significant differences between strains of each group were also found. The significant differences between the protein bands number of standard strain and caries-free isolates were also observed while there were no significant differences between standard strain and caries-susceptible subject isolates.

It can be concluded that, the less protein bands and diversity in caries-free rather than caries-susceptible isolates may be, the less cariogenicity it may cause. It is also revealed that the protein pattern of analysis S. mutans strains can be the suitable way for differentiation of them.

Keywords: *Streptococcus mutans*, protein pattern, SDS-Page, Caries free.

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Use of *Jatropha curcas* cake as substrate for *Penicillium simplicissimum* growth: optimization of lipase production

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Lipases are glycerol ester hydrolases (EC 3.1.1.3) defined as enzymes that catalyze the hydrolysis of carboxylic ester bonds of triacylglycerols, releasing diacylglycerols, monoacylglycerols, glycerol and fatty acids. However, these enzymes are also able to catalyze synthesis reactions such as esterification, acidolysis, alcoholysis, interesterification and aminolysis in non-aqueous environments. Since the lipases are a product of industrial interest, their production must be combined with the cost reduction, which can be achieved through the use of low cost culture media (residues) from agro industry. In some applications of this enzyme, e.g. in wastewater treatment and production of biodiesel, the production cost of the biocatalyst is a limiting factor in the viability of these industrial processes.The use of agroindustrial residues in solid-state fermentation (SSF) is a very interesting alternative for obtaining enzymes at low cost. Optimization of enzymes production is another way that helps to reduce the production cost of the processes, reaching high enzymatic activity. The use of experimental design techniques allows to determine the optimum conditions to reach high activities and to verify possible interaction effect between the studied variables. Therefore, in this work, it was used *Jatropha curcas* cake as culture medium for fungal growth and lipase production. The fungus *Penicillium simplicissimum*, an excellent lipase producer, was able to grow and produce the enzyme in this residue. In order to maximize the enzyme production, two sequential designs – Plackett-Burman (variable screening) followed by central composite rotatable design (CCRD) – were carried out attaining a considerable increase in the lipase production. In the Plackett-Burman design (PB12) we evaluated, as response, the maximum lipase production in function of the variables particle size (PS: 425-1180μm), initial moisture (IM: 34%-42%),inoculum concentration (IC: 10^7 – 10^8 spores.g^{-1}) and molasses concentration (MC: 0%-10%). Using a p-value less than 0.1, the analysis indicates that initial moisture, molasses and inoculum concentrations were significant. Only the particle size was not considered significant. Thus, we can employ the particle size whole range, which results in a better waste utilization. Based on the PB12 results, a CCRD was carried out with molasses concentration and particle size values fixed in 0% (since the negative effect reached in PB12) and 425-1180 μm, respectively. The analysis of variance (ANOVA) indicated that the first-order model generated for maximum lipase activity (A) was statistically significant and showed satisfactory determination coefficient (R^2 = 0.72) for this process kind. The model shows all significant terms (p-value lower than 0.1) and marginal terms (p-value about 0.1) and it was used to construct response surface plot, showing the predicted values for lipase activity in 72h as response (Figure 1), reaching a maximum experimental activity of 175.0 U.g^{-1}. The use of experimental design strategy was efficient, resulting in 9-fold increase in the lipase production.

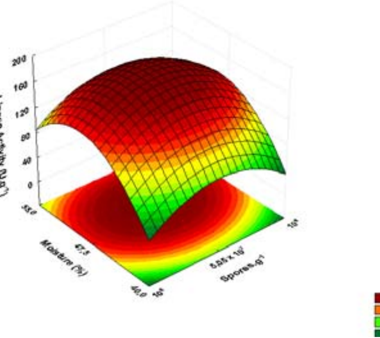

Figure 1: Response surfaces for lipase activity after 72h in function of moisture and inoculum concentration variables

Keywords: lipase production; *Jatropha curcas*, solid-state fermentation; optimization, *Penicillium simplicissimum*

Biofilms

"In vitro" antibacterial activity of farnesol against *Staphylococcus epidermidis biofilms*

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Background:
Currently, osteoarticular prosthetic infections constitute an important problem due to the difficulty of their eradication. When bacteria come in contact with the prosthetic material, a competition occurs between them and the host cells to colonize the implant. If the bacteria win, they begin to form a *biofilm* that protects them from external factors such as host immune system or antibiotic treatment. *Biofilm* formation is regulated by a bacterial communication system called *quorum sensing*, through which bacteria decide what genes to express to better adapt to their environment or, in the case of an infection, ensure the success of it.

Our study has focused on a molecule called farnesol, a sesquiterpene produced by *Candida albicans*; and which is involved in the *quorum sensing* system of this fungus. It has been shown that farnesol also acts preventing *biofilm* development in staphylococci.

Material and methods:
Two strains of *biofilm*-producing *Staphylococcus epidermidis* from the ATCC (American Type Culture Collection) (*S. epidermidis* ATCC 35983 and *S. epidermidis* ATCC 35984) were used. To complete the study, seven strains of *S. epidermidis* isolated in the Hospital Infanta Cristina of Badajoz were included. The following were determined in these strains:

The effect of sub-MICs (Minimum Inhibitory Concentration) of farnesol on *biofilm* formation by calculating the *Slime* Index, which relates the growth of a bacterial strain and its ability to form *biofilms* by measuring growth and *biofilm* formed in a spectrophotometer at 492 nm.

The activity of concentrations 10 (1500 µM) and 100 (15000 µM) times the MIC of farnesol on *biofilms* of 24 hours of *S. epidermidis* ATCC 35983 and *S. epidermidis* ATCC 35984. To these *biofilms* were added different concentrations of farnesol and remained 24 hours in contact with them. After this time, the following was determined:

1. Biomass of *biofilms* in a spectrophotometer at 492 nm after crystal violet staining.
2. Viability of bacteria included in *biofilms* and of the bacteria in the supernatant, using the ATP bioluminescence method.

Results:
The tendency of the strains is to lose the ability to form *biofilm* when the concentration of farnesol is close the MIC.

At a concentration of MICx10 of farnesol, only disintegration of the *biofilm* of *S. epidermidis* ATCC 35983 was obtained, and a concentration of MICx100 was necessary to achieve disintegration in the *biofilm* of *S. epidermidis* ATCC 35984, because it forms a denser *biofilm*.

According to the results obtained by the ATP bioluminescence method, of concentrations of 100 times the MIC were necessary for reductions in viability of about 50% in the *biofilm* in the two strains.

Conclusions:
The results obtained with sub-MICs of farnesol showed that it would be an interesting molecule for the prevention of infections associated with prostheses, because it has an inhibitory effect on *biofilm* formation. However, it does not present a good effect on mature *biofilms*, high concentrations being necessary to disintegrate it and remove the bacteria that are part of it.

Keywords: *biofilm*, *Staphylococcus epidermidis*, farnesol, prosthesis.

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Acinetobacter baumannii biofilms in hospital settings: Search for novel adhesion inhibitors

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The microorganisms are commonly attached to abiotic and biotic surfaces, including those of indwelling medical devices, organized in structured communities which are encased in an extracellular polymeric slime (EPS) matrix. *Acinetobacter baumannii* is a Gram negative bacillus (GNB) responsible of nosocomials infections especially in intensive care unit (ICU) acquired patients with impaired host defenses. The adaptation of this opportunist pathogen, in the hospital environment, is certainly due to its ability to attach to surfaces and to form biofilms structures resistant to antibacterial. In fact, this raises severe problems for public health. The challenge will be to develop a new therapeutic strategies and means of fight against the unwanted bacterial adhesion.

This preliminary study has for objective to study the adhesion of a multidrug-resistant strain of *A. baumannii*, isolated from a medical device, to various inert supports including catheter material and to estimate the kinetic of adhered biomass during a week. Besides, a study was realized with the purpose to determine the effect of the α-tocopherol on *A. baumannii* adhesion.

The obtained results showed that this bacterium adheres to the various studied materials but with different behavior towards these supports. It was also observed that the addition of the α-tocopherol with some concentrations, in culture media, revealed a significant reducing effect on *A. baumannii* adhesion after 24 hours of incubation. Also, the assay results showed that the tested molecule had no effect on the planktonic growth of bacteria. These data indicated that this antioxidant agent seem to has an effective inhibitory effect on bacterial adherence and could be consequently incorporated during the elaboration of a new anti-biofilm surfaces to prevent and control bacterial colonization and biofilms formation.

Keywords biofilm; *Acinetobacter baumannii*; inert supports; adhesion; α-tocopherol; inhibition.

An overall study of the microbial ecosystem of pigs liquid feeding systems

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In pig industry, fermented liquid feed (FLF) has gained a great interest in the last years as part of the search for feeding strategies that reduce both the incidence of Salmonella and the use of antibiotics in the herds.

When feeding pigs with FLF, it is essential to ensure the feed has properly fermented and high level of lactic acid producing bacteria (LAB). In addition, pH level should be low, i.e. 4 or less, to limit the growth of enterobacteria such as coliforms.

The microbial species that dominate in the feed may vary depending on the ingredients being fermented and the environmental conditions. Microorganisms inside biofilms that may line mixing tank and/or pipeline delivery network of the feeding system must be also considered as they can interact with the planktonic community. However, there is scarce knowledge on this subject.

This work was then devoted to improve the nutritional quality and the safety of FLF by setting up a directed microbial ecology approach in a specially designed pilot liquid feed machine (fig.1).

The optimal range of fermentation parameters (temperature, amount of residual liquid feed) favoring the development of LAB and inhibiting the growth of undesirable and pathogenic bacteria in the feed was researched. The efficiency of a selected strain (*Pedicococcus acidilactici*, Bactocell®) as culture starter and barrier flora was also investigated. In parallel, the community composition and cell density in the biofilms developed mainly on the pipeline surfaces were recorded. It was found that it closely reflect the microbiology of the feed thus validating the followed approach.

Key-words : pig nutrition, fermented liquid feed, microbial ecosystem, biofilms.

Figure 1 : The liquid feeding system used (ACEMO, France). Fermentation tanks have a working volume of 70 liters and pipelines are 25 meters long.

Analysis of the effect on the composition and the relationship established by *Pseudomonas* biofilms with the Cr(VI) in batch cultures

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Cr (VI) is soluble in water in most of their compounds, this condition increases their bioavailability and also influences so can easily pass through biological membranes. Therefore, is considered by EPA like a pollutant of priority 1 due to its genotoxic effects and high toxicity. In Guanajuato, México, there are several industries that generate waste with high contents of chromates such as tanning, a generator of 64,320 tons of waste/year (until 2006), which are released to the "Rio Turbio" without previously treatment, and "Química Central de México" (QCM) which until 2010 had accumulated about 300 000 tones of wastes of Cr(VI).

As an alternative for the treatment of sites contaminated with high concentration of heavy metals, recently has been used the abilities of native microorganisms to reduce or eliminate this compounds, looking for that this alternative be economically and environmentally sustainable.

One of the bacterial genres with high potential for bioremediation, due to the metabolic characteristics and its ability to form biofilms is *Pseudomonas*. In previous works we described and analyzed the capacity for build biofilms into several supports, and the efficiency to remove Cr(VI) in batch cultures of several *Pseudomonas* strains which were isolated of a polluted place with chromates.

In these studies we have established that biofilms formed by the *Pseudomonas* strains were better in a volcanic rock (tezontle) and in batch cultures have a removal efficiency of 300 ppm Cr(VI) in 72 h. Under these conditions, we initiated the analysis of the chromium effects about the composition and structuration of *Pseudomonas* biofilms. In biofilms exposed to Cr(VI) in one or more occasions, data analysis by SEM-EDX showed that this heavy metal has effects about the morphology and composition of this structures. This observations also indicated that the mechanism of removal Cr(VI) by *Pseudomonas* biofilms is a chemical reduction.

Keywords Chromium VI; *Pseudomonas*; Biofilms.

Anti-infective ophthalmic biomaterial surfaces based on surface-localised sensitisers

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Bacterial adherence to implanted intraocular lenses (IOLs) is recognised as a key initial stage in the development of postoperative infectious endophthalmitis (IE). The consequence of IE following cataract removal is serious, frequently resulting in blindness. To date, no strategies for prevention of IE have been successful.

Recently, we described a series of anti-infective sensitiser-incorporated hydrogels which incorporate the tetrakis(4-*N*-methylpyridyl)porphyrin (TMPyP)[1]. Excitation of this sensitiser with light equivalent to ambient lighting results in generation of highly reactive singlet oxygen ($^1O_2$), which indiscriminately initiates oxidative reactions with bacterial cell components, thereby reducing bacterial adherence to the material surface. These materials, however, suffer from relatively poor optical transparency. Herein we present a method to achieve high surface localisation of sensitisers, such that optical transparency is conserved, and which demonstrate strong anti-infective behaviour. Singlet oxygen has a short (10-50ms) lifetime, which limits the effective distance of its operation; by locating sensitiser at the surface of the biomaterial, only adherent pathogens are killed and the risk of host tissue damage is obviated.

Two series of random copolymer films based on copolymers of 2-(hydroxyethyl)methacrylate (HEMA) with varying quantities of methyl methacrylate (MMA) or methacrylic acid (MAA), were impregnated with TMPyP using an aqueous immersion method impregnation. Physiochemical properties were characterised and materials were challenged with *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

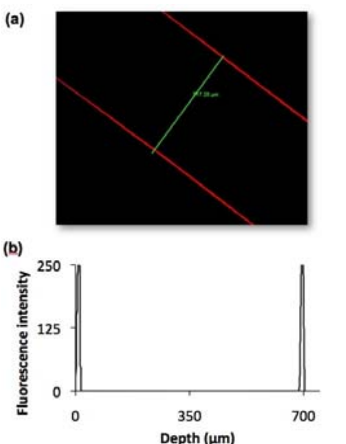

Fig.1. Confocal fluorescence microscopy image of cross-section of polymer (10%MMA) (a), and corresponding depth profile (b)

Increasing MMA content allowed a greater uptake of TMPyP, but without in a much thinner (18.24±2.18µm) sensitiser layer (Fig. 1), relative to our previous materials. This is in contrast to the MAA series where a comparatively lower loading and degree of surface localisation was seen. The results are attributable to a reduced rate of passive diffusion of sensitiser during incorporation into the more hydrophobic MMA-containing materials, in contrast to MAA-containing materials, which are significantly more porous, despite having increased numbers of electrostatic binding sites available to the oppositely-charged sensitiser.

Reductions in numbers of adherent organisms of 2-3 log cycles relative to controls were observed, which is sufficient to eradicate typical pathogen loads observed clinically.

Overall, the MMA series of materials exhibited more favourable porphyrin loading, surface localisation, mechanical properties, and reduction in bacterial adherence on light exposure. This therefore demonstrates a simple method of obtaining a high, localised concentration of photosensitiser at the surface of polymeric materials.

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Keywords anti-infective surfaces; hydrogel; sensitiser; light

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Antibacterial activity of zinc containing clinoptilolite in different water media

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Natural zeolitized tuffs (NZ) rich in clinoptilolite are good adsorbents of heavy metals due to their high cation exchange capacity. The NZ containing 70 wt. % of clinoptilolite was obtained from the sedimentary deposit Zlatokop, Serbia. The particle size of the sample was in the range 0.063-0.1 mm. The NZ was firstly converted into the Na-form by treating the NZ with 2M of NaCl solution, in order to improve the clinoptilolite exchange capacity without affecting the crystallinity of NZ. The experiments on Zn^{2+} removal from water were performed at 30°C during 24h using the batch method and $ZnCl_2$ solution in concentration of 400 mg Zn/L with mass concentration of NZ of 1g/100mL. On this way NZ containing Zn^{2+} ions (named $ClZn^{2+}$) was obtained. The $ClZn^{2+}$ was than completely dehydrated at 550ºC under air. The dehydration led to a generation of ZnO nano-particles (about 5 nm) widespread over the surface of NZ. This material was assigned as ClZnO. The leaching test of $ClZn^{2+}$ and ClZnO was performed using the 0.05M NaCl solution of pH 7.0 at 37°C during 24h. The resulting leached concentration of Zn^{2+} in water was 0.67 mg/L for $ClZn^{2+}$ whereas no leached Zn^{2+} was found for ClZnO.

After the use of clinoptilolite for zinc removal from wastewater, we looked for future application of the used material, one of which can be removal of pathogenic bacteria in the tertiary stage of wastewater treatment. The antibacterial activity of $ClZn^{2+}$ and ClZnO was tested against representative pathogenic Gram-negative (*Escherichia coli*, DSM 498) and Gram-positive (*Staphylococcus aureus*, clinical isolate at Institute of Public Health, Zagreb) bacteria. The antibacterial assay was carried out whit mass concentration of material of 1g/100mL in three different water media: Luria Bertani or LB medium, synthetic wastewater and real effluent water from the secondary stage of the biological wastewater treatment. The initial pH of used media was 7.0 and the chemical composition was as follows (in mg/L): LB medium (bacto-tryptone 10,000; bacto-yeast extract 5,000; NaCl 10,000; COD 14,000), synthetic wastewater (Na-propionate 1,000; peptone 100; $MgSO_4$ 10; $CaCl_2$ 6; KCl 30; yeast extract 10; KH_2PO_4 20; COD 1,206), effluent water (T-N 28.3; T-P 2.27; COD 31.4). All media and materials were autoclaved before the experiments were to commence. The experiments were carried out at 37°C. The CFUs of bacteria were measured on LB agar at the start and after 24h of experiment. The antibacterial activity of material was expressed as percentage of log CFU reduction when compared to control where no material was added (Table 1). The percentage of inhibition of bacteria in the presence of $ClZn^{2+}$ and ClZnO showed significantly negative correlation with the COD of water media used in assay (R = -0.987 and -0.949, respectively). The $ClZn^{2+}$ showed better antibacterial activity than ClZnO, which is ascribed to the difference in leaching of Zn^{2+} ions from materials. The *E. coli* seems to be more resistant to Zn^{2+} ions than *S. aureus*. The difference in final pH among control and experimental reactors was not higher than 0.59 units. No significant antibacterial activity of materials was observed after 1h of contact with bacteria.

Table 1. Antibacterial activity of $ClZn^{2+}$ and ClZnO against *E. coli* and *S. aureus* after 24h of incubation in different water media when compared to control. c_0 *E. coli* (10^6 CFU/mL) = 3.85±0.89; c_0 *S. aureus* (10^7 CFU/mL) = 1.42±0.24; significantly different when compared to ^{A}-LB medium, ^{B}-synthetic wastewater, ^{C}-$ClZn^{2+}$.

	***E. coli* reduction (%)**	***S. aureus* reduction (%)**	***E. coli* reduction (%)**	***S. aureus* reduction (%)**
	$ClZn^{2+}$		ClZnO	
LB medium	5.05	12.89	2.32 ^{C}	2.95 ^{C}
Synthetic wastewater	93.92 ^{A}	82.35 ^{A}	34.34 ^{A,C}	44.32 ^{A,C}
Effluent	95.07 ^{A,B}	82.06 ^{A}	53.72 ^{A,B,C}	61.35 ^{A,B,C}

The antibacterial activity of zinc containing clinoptilolite (mass concentration 1g/100mL, 24h of contact) was depended on the following factors in decreasing order: COD of the water medium, type of material and species of bacteria. The antibacterial activity of certain compound tested in the synthetic medium can be significantly different from those in real water medium.

Keywords *Escherichia coli*, Staphylococcus *aureus*, toxicity, zeolite, zinc.

Antibacterial biofilms based on calcium caseinate incorporated with carvacrol

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Biodegradable polymers have been investigated during the last few years as alternatives to non-degradable polymers currently used in films production for food packaging. It is well known that oxygen plays a very important role in the degradation of food, because it is involved in many reactions such as growth of microorganisms, enzymatic browning, vitamin loss and lipid oxidation [1]. These effects make the reduction in oxygen permeability essential to improve food preservation. On the other hand, biodegradable films based on caseinates were recently studied as an attractive solution to reduce environmental impacts in packaging industry [2]. In addition to the inherent natural origin and biodegradable character, these films show good barrier properties to oxygen, carbon dioxide and aromas [1]. Another interesting feature of caseinates is their ability to be carriers of antimicrobial substances, offering the possibility to be used in active packaging systems to control the microbial growing and proliferation in food, resulting in clear improvement of microbiological quality and shelf-life of packaged food [3]. It has been reported that some essential oils obtained from plants, such as carvacrol, a major component of the oregano essential oil, present antimicrobial activity [4]. Therefore, it is assumed that it could be incorporated to polymer matrices in order to give them antimicrobial activity. Carvacrol is also totally harmless to human health [5].

In this study, biofilms obtained from calcium caseinate plasticized with three different concentrations of glycerol (15 wt%, 25 wt% and 35 wt%), containing carvacrol as antimicrobial agent (10 wt%), were prepared. Solvent casting conditions were optimized and a complete characterization of these biofilms was carried out. In particular, oxygen transmission rate (OTR) was measured in order to evaluate the barrier properties. Antimicrobial effectiveness against *Escherichia coli* and *Staphylococcus aureus* was also evaluated for these potentially active films by using the agar diffusion method. Biodegradation of these films was also tested in compostage conditions. Calcium caseinate biofilms showed excellent oxygen barrier properties in all cases. Neither glycerol nor carvacrol affected significantly the OTR results. Biofilms incorporated with carvacrol showed clear inhibitory effects to *Escherichia coli* and *Staphylococcus aureus*. Finally, all samples showed a rapid degradation under composting conditions. In conclusion, calcium caseinate based biofilms showed excellent potential to be used in active packaging systems with good barrier properties and reduction of microbial activities, leading to the double effect to reduce oxygen chemical degradation of food and to decrease the action of pathogens.

Keywords: biofilm; calcium caseinate; carvacol; *E. coli, S. aureus.*

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Antimicrobial Activity of *Pimpinella anisum* Seed Extract

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Medical plants synthesize a vast array of secondary metabolites that are important for human life. For medical purpose, antimicrobial activity of substances derived from plant extracts has been recognized since antique years. Antibacterial activity of seed extracts of *Pimpinella anisum* (Aniseeds) was evaluated against some pathogenic bacterial strains (*Streptococcus pyogene, Psedomonas aeruginosa, Klebseilla pneumoniae and Proteus vulgaris)* on 6 different concentrations which are 1, 5, 10, 20, 50 and 100 mg/ml for methanol and aqueous extract. Aqueous extract of seed was found to be more sensitive and showed highest antibacterial activity against tested bacteria. It is exhibited varying level of antibacterial activity (20 – 100 mg/ml) on *Streptococcus pyogene, Psedomonas aeruginosa, Klebseilla pneumoniae and Proteus vulgaris*. Methanol extract showed a resistant to *Klebseilla pneumonia* and *Proteus vulgaris*. Both extract of *Pimpinella anisum* showed significant antibacterial activity against *Streptococcus pyogenes* and *Pseudomonas aeruginosa* with a greater inhibition zone (≥15mm) on 20, 50 and 100mg/ml. Mann-Whitney analysis showed there are no significant differences ($p \geq 0.05$) between methanol and aqueous extract. Based on Kruskal Wallis analysis, both aqueous and methanol extract of aniseed showed a significantly differences ($p \leq 0.01$) inhibition on bacteria gram positive (*Streptococcus pyogenes*) compared to bacteria gram negative (*Psedomonas aeruginosa, Klebseilla pneumoniae and Proteus vulgaris*). The sensitivity of test bacteria varied with the species, strains and concentration of extract applied. Spearman's analysis was done and it showed that there is positive correlation between concentrations and diameter of inhibition zone of bacteria. The higher concentration used will increased the diameter of inhibition zone.

Keywords: *Pimpinella anisum*, Antimicrobial activity, pathogenic bacteria, disk diffusion method, methanol extract

Antimicrobial resistance and biofilm formation by staphylococci subclinical mastitis isolates

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Staphylococci are recognised mastitis pathogens, often responsible for subclinical infections and chronic bovine mastitis. These infections, accountable for significant economic losses, are difficult to eradicate, given the absence of clinical signs and bacterial persistence which hamper the diagnosis and treatment. Only an appropriate management can minimize this problem, which involves, among others, the knowledge of the antimicrobial susceptibility profile of more frequent etiologic agents. Biofilm formation has been extensively described in staphylococci and may play a role in the infection outcome, contributing to bacterial persistence in the udder, evasion of immunological defences and resistance to antimicrobial action.

This study aimed at evaluating antimicrobial resistance traits and biofilm forming ability in *Staphylococcus epidermidis* (n=50), *S. chromogenes* (n=16), *S. simulans* (n=14) and *S. aureus* (n=5) isolated from subclinical bovine mastitis. Antimicrobial resistance was assessed using the disk diffusion method, according to the Clinical Laboratory Standards Institute (2008). The active ingredients tested were: amoxillin clavulanate (AMC); ampicillin (AMP); cefoperazone (CFP); cefazolin (KZ); colistin (CT); streptomycin (S); neomycin (N); gentamicin (CN); oxacillin (OX); penicillin G (P); lincomycin (MY); sulphametoxazole/trimethoprim 19:1 (SXT). After incubation for 24h, biofilm formation was evaluated using a Fluorescent *In Situ* Hybridisation (FISH) protocol, and observed under fluorescence microscopy in the x1000 amplification (Objective HCX PLAN APD) in a Leica DMR microscope. The relation between antimicrobial resistance and biofilm results was estimated using the Wilcoxon Signed Ranks Test.

S. epidermidis was the species showing the highest levels of resistance. Only one isolate of *S. chromogenes* was susceptible to all compounds tested. All isolates were susceptible to KZ. The percentages of resistance observed were: AMC 1.2%; AMP 62.4%; CFP 2.4%; KZ 0%; CT 88.2%; S 55.3%; N 30.6%; CN 35.3%; OX 4.7%; P 58.8%; MY 45.9%; SXT 54.1%. It was also found that most isolates were multiresistant (resistance to 3 or more antimicrobials with different action mechanisms). It was possible to detect biofilm formation in isolates from the species under study. The percentage of biofilm-positive isolates at 24h was higher for S. aureus (60%), followed by *S. simulans* (57%), *S. epidermidis* (48%) and *S. chromogenes* (44%). A significant relation ($p<0.05$) was found between biofilm production at 24h and antimicrobial resistance to half of the drugs tested, namely: AMC, CFP, CT, KZ, N and OX.

The antimicrobial resistance observed in *S. epidermidis*, in conjunction with the frequency of their isolation suggest that coagulase-negative staphylococci may require attention in farms from which major mastitis pathogens have been excluded. The characterization of their antimicrobial resistance profile is essential for adequate therapeutic decisions, since the mastitis is still the most frequent reason for the administration of antibiotics to dairy cattle, and treatment often begins before laboratory results are available. Recommendations for first choice drug selection have been issued by several national authorities, but they need to be assessed with data from locally obtained isolates. Also, data on antimicrobial resistance is generated bu *in vitro* tests, using bacteria in planktonic stage of growth, which may be misleading for already established infections with biofilm-producing strains. Further studies are required, aiming at better simulating *in vivo* conditions, thus providing a better model for biofilm formation in the udder.

Keywords antimicrobial resistance; biofilm; staphylococci; subclinical mastitis

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Application of silver coumarin complexes as an antibacterial substitute and their effectiveness as an antibiofilm surface coating agent

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The colonization of clinical and industrial surfaces with microorganisms, including antibacterial-resistant strains, has promoted increased research into the development of effective antibacterial and antifouling coatings. Such a coating has been designed to release antimicrobial agents over extended time periods. Sol gel coatings can be used as a delivery system due to their attractive properties such as chemical inertness, stability and homogeneity. In this present preparation metal complex (Ag-coumarin) doped methyltriethoxysilane (MTEOS) microtiter well coatings and the rapid assessment of their antibacterial activity and biofilm resistance are described. Silver coumarin complex doped sol gel colloidal solutions were prepared by the hydrolysis and condensation of an MTEOS precursor in acidic conditions (0.04 M HNO_3), by addition different concentrations (0.3, 0.5 and 0.7 %) of Ag-coumarin complexes (coumarin-3-carboxylatosilver(I) [Ag(Cca)], 6-hydroxycoumarin-3-carboxylatosilver(I) [Ag(6OHCca)], 7-hydroxycoumarin-3-carboxylatosilver(I) [Ag(7OHCca)] and 8-hydroxycoumarin-3-carboxylatosilver(I) [Ag(8OHCca)]. The wells of polystyrene flat bottom microtiter plates were coated using prepared sol gels and cured under controlled conditions. The coated wells were challenged with cultures of Gram +ve and Gram -ve bacteria including antibiotic resistant organisms (meticillin resistant MRSA ATCC 43300, multidrug resistance (ampicillin, gentamicin, ceftazidine) *Enterobacter* WT6) and the antibacterial activity was analyzed by measuring minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and kinetic growth curve study. Bio films (*Staphylococcus epidermidis* CSF 41498) were measured spectroscopically (Dye method) and microscopically (SEM) in the presence of Ag coumarin complexes with respect to a control.

The comparative antibacterial activity of Ag and Ag- coumarin complexes doped sol gel microtiter well coating against MRSA and *Enterobacter* WT6 were studied. MBC values of Ag / Ag-coumarin complexes were similar, whereas at the critical concentrations of some Ag-coumarin complexes doped sol gel coating showed higher activity than Ag doped sol gel coatings. Among all Ag-coumarin complexes [Ag (8OHCca)] coated surfaces has the most prolonged lag phase with higher antibacterial activity followed by [Ag (7OHCca)], [Ag (6OHcca)] and [Ag (Cca)]. SEM confirmed that at a critical concentration of [Ag (8OHCca)], the coated glass slide surfaces were capable of preventing bio film formation.

Assessing the development of biofouling on ultrafiltration membranes by confocal laser scanning microscopy

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Biofouling is known as a major reason for flux decline in the performance of membrane based water and wastewater treatment plants. The relevance of biofilm extracellular polymeric substances (EPS) in terms of fouling on membranes has been indicated in several studies. Therefore, a profound knowledge of the composition of biofouling is important for the development of new countermeasures in enhancing membrane permeability.

The objective of this investigation was the characterization of microbial aggregates and EPS components in biofilms that contribute to biofouling of ultrafiltration membranes using confocal laser microscopy (CLMS).

Biofouling tests were conducted using an experimental setup, where a hollow-fiber ultrafiltration (UF) membrane module made of polyethylene was fed with natural water. Dead end filtration was carried out continuously by using a constant pressure of 20mbar and an initial membrane permeability of $390 Lh^{-1}m^{-1}bar^{-1}$. One operation cycle consisted of 20min of filtration and a backwash of 20sec. Samples of fouled membranes were investigated after one, three and six cycles of filtration. The biofouling was analyzed by confocal laser scanning microscopy (CLSM) after simultaneous staining. The bacteria in the fouling were stained with DAPI specific to nucleic acids and different fluorescent labeled lectins specific to polysaccharides of the EPS.

Confocal laser microscopy showed that biofouling on the membrane was a composition of heterogeneous colonization of bacteria and extra cellular polymeric substances (EPS) containing, N-acetylglucosamine, N-acetylgalactoseamine and L fucose. The detection of the bacteria and the location of the polysaccharides could be related to the biofouling accumulation. Our investigations assume, that at first polysaccharides of the influent adsorbed to the membrane surface and serve as layer for the development of a conditioning film. Backwashing was able to remove cells from the membrane, but was unable to remove adsorbed substances of the conditioning film.

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Bacterial cellulose nanofibers for dye-affinity adsorption of recombinant human interferon-α

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The bacterial cellulose (BC) nanofibers were produced by *Acetobacter xylinum* in the Hestrin-Schramm medium in a static condition for 14 days. Then, Cibacron Blue F3GA (CB) was covalently attached onto the BC nanofibers for affinity adsorption of recombinant interferon-α (rHuIFN-α). The CB content of the BC nanofibers was 178 μmol/g. The specific surface area of the BC nanofibers was determined to be 914 m^2/g. rHuIFN-α adsorption studies were performed by batch system. The non-specific adsorption of rHuIFN-α on the BC nanofibers was very low (2.1 mg/g polymer). CB attachment onto the BC nanofibers significantly increased the rHuIFN-α adsorption (1620 mg/g). The maximum rHuIFN-α adsorption was observed at pH 6.0. The rHuIFN-α adsorption capacity decreased drastically with an increase of the aqueous phase concentration of sodium chloride. The elution studies were performed by adding 1 M NaCl to the rHuIFN-α solutions in which adsorption equilibria had been reached. The eluted rHuIFN-α had a specific activity in the range of of 3.32-3.45 x 10^8 IU/mg as inhibition of the cytopathic effect of MDBK cells. In order to determine the effects of adsorption conditions on possible conformational changes of rHuIFN-α structure, fluorescence spectrophotometry was employed. We resulted that the BC-CB nanofibers can be applied for rHuIFN-α adsorption without causing any significant conformational changes. The elution results showed that the binding of rHuIFN-α to the BC-CB nanofibers was reversible. This study demonstrated that the BC nanofibers has a potential for affinity carrier applications in protein adsorption systems

Keywords ; bacterial cellulose (BC), nanofibers, recombinant interferon-α, *Acetobacter xylinum,* Cibacron Blue F3GA

Bioactive bacterial exopolysaccharides: modification, characterization and effects on bone remodeling

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In recent years, there has been a growing interest in the isolation and identification of new microbial polysaccharides that might have new uses in many industries. They compete with polysaccharides from other sources such as seaweeds, crustaceans, animals or plants. Interest in mass culture of microorganisms from the marine environment has increased considerably, representing an innovative approach to the biotechnological use of under-exploited resources. When sulphated, carbohydrates may be glycosaminoglycan-like components that exhibit many interesting properties with medical applications.

Marine bacteria associated with deep-sea hydrothermal conditions have demonstrated their ability to produce in an aerobic carbohydrate-based medium, unusual extracellular polymers. They present original structural features that can be modified to design bioactive compounds and improve their specificity. In particular, with the aim of promoting biological activities, chemical modifications (depolymerization and substitution reactions) of one exopolysaccharide (EPS) produced by *Alteromonas infernus* have been undertaken [1]. The structure of the native EPS has been described [2]. A low molecular weight oversulphated derivative (OS-EPS) has been isolated after chemical modifications of this native EPS.

The growth and differentiation of bone cells is controlled by various factors which can be modulated by heparan sulphates. Here, we investigated the effects of the derivative named OS-EPS on bone. We compared the effect of this compound with that of a low molecular weight native EPS. The observed data show different levels of bone resorption regulation by GAGs or OS-EPS, most of them leading to proresorptive effects.

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Keywords Exopolysaccharides; glycosaminoglycan, heparin-like, derivatives, sulphation, bone metabolism, bone remodeling

Bioadhesion and biofilms formation: Impact of supports mechanical properties

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Adhesion of bacteria to surfaces represents a significant interest in many biotechnological applications but also causes enormous health and economic problems. In marine environment, attachment of bacteria can lead to biofouling. Thus, understanding mechanisms governing bacterial adhesion is a crucial issue. Until present, nearly all researches have focused on physicochemical properties of material surfaces and bacteria but not directly on mechanical characteristics such as stiffness of supports. However, this parameter is well-known to influence the eukaryotic cells adhesion. According to this observation, the objective of this study is to observe the impact of stiffness of materials on bacterial adhesion and biofilms formation.

Adhesion supports – To test this parameter, one support with adjustable stiffness was chosen: agarose gel. Stiffness variability of agarose gels was determinated by rheometry.

Bacteria models - Three marine bacteria: a positive gram, *Bacillus* 4J6 and two negative gram *Pseudoalteromonas* 3J6 and *Pseudoalteromonas* D41 were chosen like models. A characterization of these bacteria was achieved (chart of growth, morphology by scanning electron microscopy and mobility tests). Their physicochemical surface properties were determinated: hydrophobicity and acid / base character by microbial adhesion to solvents (MATS) and net charge by electrophoretic mobility assays.

Bacterial attachment and biofilms formation assays - Tests were made on two agarose gels (0,75 and 3%). Adhesion and biofilms formation were analyzed by confocal laser scanning microscopy and by counting of viable and farmable cells.

Keywords : mechanical properties, bioadhesion, bacteria

Biodegradable Edible Film Based on Kefiran: Development and Characterization

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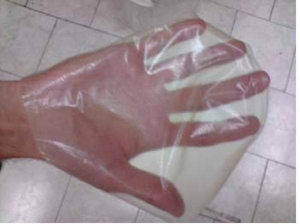
In recent years, the food and packaging industries have been joining their efforts to determine new ways to protect food from environmental conditions and mechanical stresses. This attitude is closely connected with the growing importance of consumer demand for high quality and long shelf-life products and the increased awareness of environmental issues. As an answer to these issues, biopolymers, including proteins, polysaccharides and lipids, are being implemented, based on the research efforts of many groups worldwide. Edible, biodegradable films and coatings can act as barriers to control the transfer of moisture, oxygen, carbon dioxide, lipids, and flavour components, and thus maintain the quality and increase the shelf life of food products. Kefiran is finding increasing use in the food industry as a texturing and gelling agent. It is a water-soluble polysaccharide containing approximately equal amounts of glucose and galactose residues in a chain sequence. This polysaccharide has attracted considerable interest, because several functional properties such as antibacterial, antifungal and antitumor activities have been reported for it. This study examined the feasibility of using kefiran, an exopolysaccharide obtained from kefir grains, as a new film-forming material. Kefiran-based films, with and without glycerol as plasticizer, were prepared by a casting and solvent-evaporation method. To study the impact of the incorporation of glycerol into the film matrix, physical, mechanical, and thermal properties of the films were investigated. As expected, the increase of glycerol concentration from 15 to 35% (w/w) increased extensibility but decreased tensile strength, implying higher mobility of polymer chains by the plasticizing effect of glycerol. Water vapor permeability of films was found to increase as the plasticizer content increased. Glass transition temperatures decreased as a result of plasticization as glycerol content increased. The properties of the films were related to their microstructure, which was observed by scanning electron microscopy. Thus, it was observed that plasticizer is a significant factor in the properties of these films and their food technology applications.

Keywords: Kefiran; Edible film; Plasticizer; glycerol

Figure: Biodegradable edible film based on kefiran and glycerol



Biofilm (biocellulose membrane) production by *Glucoacetobacter xylinum* from waste residues of fruits and tea leaves

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The biofilm (biocellulose membrane (C6H1005) n), produced over the static culture of *Glucoacetobacter xylinum* associated to *Saccharomyces cerevisiae,* was obtained from fruit residues (orange, papaya, pineapple juices) and tea leaves (green, black and mate teas). For green, black and mate teas, the average biofilm yields for 10 day cultures were 1.6 g fiber/day; 1.0 g fiber/day and 0.5 g fiber/day, respectively. The addition of (20%) orange juice or (30%) red wine to the green tea culture increased the biofilm yield up to 2.6 g fiber / day for 07 day culture. The addition of (1.2%) collagen to the mate tea culture increased productivity by 5 times. Comparing all media tested, the best productivity of 2.9±0.5 g cellulose/day was attained for green tea added with orange juice. DSC analysis for the biofilm confirmed thermal degradation up to 130°C and a melting point of (-15°C). The infra-red spectroscopy (FTIR) confirmed the cellulose properties with a peak at 1644. The Elasticity to indentation (similar to Young's model for indentation) varied from 250-300 MPa, and confirmed the bio-membrane (80% RU and 1.5 mm thickness) strength and flexibility. The biofilm showed a water absorption capacity of 9.5 times over its dry weight; and an alcoholic calendula extract diluted (1:1) in water (added with nisin) absorption capacity around 7.5 times over its dry weight. The incorporation into the bio-membrane of *Calendula officinalis* hydro alcoholic preparations aims at either the antioxidant, anti-inflammatory or wound healing activities on topical applications. Alternatively, nisin may be added into the biofilm to increase the membrane preservation.

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Biological control of some native biofilm forming and surfactin producing *Bacillus subtilis* strains against six pathotypes of *Rhizoctonia solani*

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A screening strategy was developed to assess the potential of some Iranian native Bacillus sp. isolates collected from rice rhizosphere and phylosphere to control different pathotypes of six pathotypes of *Rhizoctonia solani* using a hierarchical combination of different assays. Among the 704 bacterial isolates, 240 isolates (22% of phylosphere and 38% of rhizosphere isolates) showed more than 40% antifungal activity against the six pathotypes of *R. solani.* The antagonistic ability among the isolates had a normal distribution and antagonistic potential of the majority of the isolates tends to the mean (μ=49.58). This demonstrates that the distribution of probability of antagonistic potential in rice fields may be equated as $p(x) = e^{-(x-\mu)^2/2\sigma^2} / \sqrt{2\pi\sigma^2}$. The biofilm forming isolates showed the highest antagonistic potential ranged between 70-90%, however the probability distribution of them was low value (5.92%). The selected isolates showed significantly different mycelial growth inhibition on different pathotypes of the fungus, which showed the significant effects of the genotype of pathotype on the antagonistic ability of the selected bacteria. About 82% (197 isolates) of the selected bacterial strains showed biosurfactant production, while only 18.2% (44) of them exhibited both biofilm formation and surfactant production and exhibited the highest antagonistic activities. Five the most powerful antagonistic strains, in addition to biofilm and surfactants, produced different volatile and diffusible antifungal metabolites with different antifungal activities against the pathotypes of *R. solani*, and were able to colonize the rice root surface at a level higher than the required threshold level. Biochemical and molecular (16SrDNA) characterization of three selected bacteria revealed that they belong to *Bacillus subtilis* species*.* Regarding the difference of optimal growth conditions for the selected antagonistic bacteria, it seems that simultaneous application of the three strains lead to enhance an effective biological product.

Keywords Antagonistic Potential; *Bacillus subtilis*; Biofilm; surfactin; Probability Distribution; *Rhizoctonia solani;* Pathotype*,* 16SrDNA

Biological Response of oral microorganisms/Human Gingival Fibroblasts co-culture in the presence of 2-hydroxyethyl methacrylate

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2-hydroxyethyl-methacrylate (HEMA) is one of the major components of dental polymerized adhesive and composite resins. The polymerization process *in situ* is incomplete and HEMA is released in a free monomeric form and interferes in the oral cavity environment.

This study aimed to evaluate HEMA monomeric effects on the co-culture of *Streptococcus mitis* and Human Gingival Fibroblasts (HGFs).

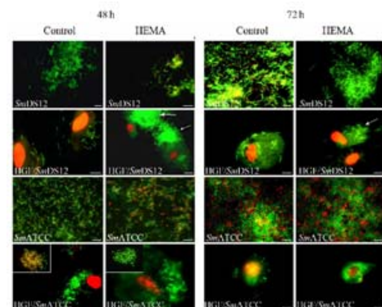
In the first part of the study, we tested the effect of HEMA on biofilm formation and preformed biofilm of Streptococcal strains alone or combined to each other, and the HEMA prevalent effects were both the reduction of bacterial adhesion to a polystyrene surface and the increase of bacterial death also characterized by an aggregative behavior.

Then, the clinical strains *S. mitis* DS12 and the reference strain *S. mitis* ATCC 6249 were co-cultivated with HGFs in the presence of HEMA 3 mM, for 48 and 72 h; the amount of sessile and planktonic cells, as well as the prokaryotic and eukaryotic cell viability were analyzed in treated and untreated samples.

The treatment of *S. mitis*/HGFs co-culture with HEMA did not produce significant effects on the bacterial adhesion and induced an increase in the planktonic *S. mitis* ATCC 6249 population after 48 and 72 h in respect to the untreated samples. The HEMA effect on the bacterial viability was shown through a significant increase in planktonic *S. mitis* ATCC 6249 cells when co-cultured with HGFs, while a cytotoxic effect on HGFs, without bacteria, was recorded. A general increase of bacterial aggregation on HGFs was also detected. Moreover, the presence of prokaryotic cells protected HGFs against cytotoxic effect of HEMA (Fig. 1).

Data obtained in this study suggest that HEMA exhibits a toxic effect mainly on eukaryotic cells and this effect can be modulated by co-cultivation with the *S. mitis* cells that, in presence of HEMA, enhance their aggregation rate on HGFs.

The association between HGFs and *S. mitis* strains favours their cross protection suggesting beneficial effects on healthy oral status.



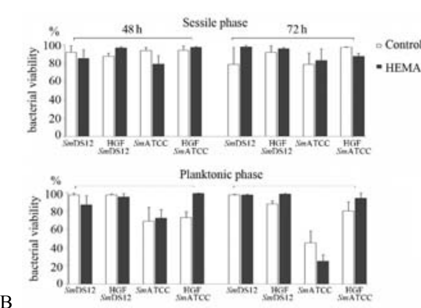


Fig. 1.Effect of HEMA on the viability of *Streptococcus mitis* DS12 (*Sm*DS12) and *S. mitis* ATCC 6249 (*Sm*ATCC) alone and co-cultured on Human Gingival Fibroblasts (HGFs). A) Representative images of sessile streptococcal strains alone (rows 1 and 3) and co-cultured with HGFs (rows 2 and 4). (B) Percentage of streptococcal viability on sessile and planktonic growth phases.

Keywords: Co-culture; HEMA; Human Gingival Fibroblasts; *Streptococcus spp.* m.digiulio@unich.it

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Burkholderia kururiensis biosurfactant: anti-adhesive properties to inhibit biofilm development from *Listeria monocytogenes* pathogen on 304 stainless steel

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Listeria monocytogenes is a pathogenic microorganism, mainly found in food industries as biofilm former. This microorganism is present in products from animal and vegetal origin, such as dairy and meat products. When this microorganism is swallowed with food, can cause problems to humans as septicemia and meningitis. It can adhere and multiply on a wide variety of surfaces, including stainless steel 304 and under drastic growth conditions. Due to problems caused by bacterial adhesion to surfaces and subsequent food contamination, it is extremely important to develop new products to enhance the surfaces cleaning. The use of biosurfactants, compounds of tensoactive properties, is emerging as an alternative towards to synthetic origin products. Their main advantages over the synthetic detergents are the low toxicity and highly biodegradable nature and anti-adhesive activity, among others. *Burkholderia kururiensis* KP23 is a non-pathogenic microorganism, a producer of biosurfactant from the class of glycolipids, still in the characterization process. Due to the non-pathogenicity of this microorganism, it has an excellent potential for use in industrial processes. This work aims to apply the biosurfactant to inhibit the adhesion of the *L. monocytogenes* ATCC 7644 pathogen in 304 stainless steel surfaces. Microplates of 304 stainless steel were preconditioned with purified biossurfactant in four different concentrations (5.0 g / L, 2.5 g / L, 1.0 g / L, 0.5 g / L) for 24 hours. After this time, the biosurfactant was removed from the microplates and the wells were washed with distilled water. Standard suspensions of *L. monocytogenes* were inoculated in wells containing TSYE broth for different times to determine the anti-biofilm activity of the biosurfactant. The same test was carried out with sodium dodecyl sulfate (SDS), a chemical surfactant from synthetic origin, in order to obtain a comparison between a surfactant from synthetic source and one of renewable source. SDS was used in the same concentrations of the biosurfactant. The most anti-adhesive activity of biosurfactant was showed in the concentration of 5.0 g / L at 30h, where was observed 83.9% of inhibition in the biofilm formation. In opposite, SDS induced adhesion in all used concentrations, reaching a maximum induction (470%) when a concentration of 0.5 g / L at 6h was used. To verify the anti-adhesive and antimicrobial activities of the biosurfactant, standard suspensions of *L. monocytogenes* were inoculated with serial dilutions of purified biosurfactant to determine the minimum concentration able to inhibiting the microbial adhesion (anti-adhesive) and / or the bacterial growth (antimicrobial). When added to the medium, the biosurfactant had an anti-adhesive activity of 95.0% at a concentration of 3200 µg/mL. Under these conditions, there was no adhesion to the surface, but planktonic growth was the same of the one reached in the control. In antibiofilm activity assays, the biosurfactant inhibited adhesion of the microorganism to the surface. In this way, this work showed the potential use of this biosurfactant as anti-adhesive and can be applied in different industrial fields, such as in the food industry. Furthermore, the studied biosurfactant was more efficient than the surfactant of chemical origin.

Keywords biofilm; biosurfactant; *Listeria monocytogenes*; microbial adhesion

Cell-to-cell aggregation in *S. epidermidis* and its effect on quantification of total and viable bacteria within biofilms

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Biofilms forming on the surface of indwelling medical devices by microorganisms such as *Staphylococcus epidermidis*, act as a source of acute infections. Since colonization of medical devices represents a serious problem in public healthcare-associated infections, bacteria forming biofilms have been an important issue often studied. Proper quantification of viable bacteria within *S. epidermidis* biofilms can however be challenging. Often, biofilm quantification of *S. epidermidis* is performed with colorimetric methods but these do not provide information regarding viable bacteria. CFU counting is often used but in the case of *S. epidermidis*, a bacteria that normally grows in clusters of cells, sonication is always required in order to obtain individual cells. In older biofilms, the number of dormant bacteria is expected to be higher than in young biofilms. Therefore, disrupting a biofilm structure without damage the cells in older biofilms can be a challenge.

Here, biofilm samples of *Staphylococcus epidermidis* 9142 strain grown for 24, 48 or 72H in TSB supplemented with 0,5% glucose were ressuspended 1 mL of physiological saline solution and sonicated at different cycles. Following sonication biofilms were quantified using three different approaches: colorimetric methods, CFU counting, and microscopic evaluation using a Neübauer chamber coupled with staining with fluorescence dye *LIVE/DEAD*® BacLight ™ Bacterial Viability Kit (Molecular Probes Inc). Cell counting was optimized using Sigma Scan Pro and validated against manual counting of the images.

In the conditions used, higher numbers of sonication cycles prevented any clustering of cells but were affecting cell viability. On the other hand, lower numbers of sonication cycles were not effective in completely eliminating cell clusters, especially in 72H-old biofilms. The presence of the cell clusters at the lower sonication cycles resulted in high variability of CFU counting. On the other hand, cell counting with a Neübauer chamber was the best way to proper quantify the total and viable bacteria within the biofilms. By using the automatic counting software and validating the methodology, quantification of biofilms was relatively fast and reliable to perform.

Keywords Biofilm; *Staphylococcus epidermidis*; Sonication, automatic cell counting

Colistin surface conditioning impairs *Pseudomonas aeruginosa* biofilm formation and enhances ciprofloxacin antimicrobial activity

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Bacterial biofilms on medical devices (MD) are commonly associated with persistent infections. Biofilm formation is central to pathogenesis due to the ability of the biofilm-entrapped bacteria to evade the host immune responses and the increased antimicrobial resistance phenotype often shown by sessile microorganisms. In order to control the infections related with MD, antimicrobial peptide conditioning of MD surfaces can be an attractive answer. Colistin (COL), an important cationic antimicrobial peptide (AMP) produced by *Bacillus polymyxa* strains, was used to coat polystyrene (PS) surfaces. This work aims at characterizing the antimicrobial effect of COL surface coating to impair *Pseudomonas aeruginosa* adhesion and subsequent biofilm formation. The response of *P. aeruginosa* 24 h biofilms treatment with Ciprofloxacin (CIP) in non-conditoned and COL-conditioned surfaces was also assessed

P. aeruginosa from collection (ATCC 10145) (PAC) and *P. aeruginosa* isolated (PAI) from a medical device (endoscope) were used as biofilm producers. PS surfaces were pre-conditioned with 64 mg/L of COL during 30 min. Biofilms were then developed in unconditioned and COL-conditioned surfaces, being after treated with Ciprofloxacin (CIP) at 0.75 mg/L. Biofilms were phenotypically characterized in terms of biomass, respiratory activity and cell number.

Results showed that, in general, the MD isolate produces biofilms with more mass and activity but less number of cells than the reference strain, being the action of COL conditioning or CIP treatment similar for both strains. The surface conditioning with COL was very efficient, as it impaired significantly biofilm formation in terms of mass and activity, allowing the adhesion of just 3-log of cells. The CIP treatment of biofilms developed in unconditioned and COL-conditioned surfaces, promoted reduction of biofilm mass, activity and 2-log of number of biofilm cells. Concerning the combined application of COL surface conditioning and biofilm treatment with CIP it was observed an increase in CIP efficacy in biofilm sanitation, especially regarding biofilm-entrapped cell reduction. In fact, the combination of conditioning/treatment promoted an accentuated reduction of the biofilm mass and activity and caused a reduction of 4-log of biofilm-entrapped cells.

This study demonstrates the potential use of COL surface conditioning since this surface treatment impairs biofilm formation, probably interfering in the transition from irreversible attached cells to mature biofilms. Moreover, and as consequence of the reduced amount of biofilms attached to COL-conditioned surfaces, adhered cells or thin biofilms become more exposed to the subsequent action of CIP. This study highlights a promising use of COL as MD coating and a synergistic effect between COL surface conditioning and CIP antimicrobial activity.

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Keywords biofilm control, surface conditioning, Colistin, *Pseudomonas aeruginosa*

Crystal deposits on the surface of antimicrobial central venous catheters derived from administration of total parenteral nutrient solution impair antimicrobial efficacy

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Background. Central venous catheters (CVCs) are commonly used for the monitoring and management of critically ill patients care. However, bacterial colonisation of these devices precedes catheter-related infection (CRI) and the process of bacterial colonisation have not been well-studied. Antimicrobial-coated CVCs appear to be effective in reducing CRIs. We previously reported that crystal deposits on the surface, possibly derived from exposure to solutions such as total parenteral nutrition (TPN) in clinically used non-antimicrobial CVCs, could act as hotbeds of bacterial propagation.[1] However, on the intraluminal surface of antimicrobial CVCs, it is unclear whether such crystal deposits would appear and play an identical role on the intraluminal surface of antimicrobial catheters.

Subject. The purpose of this study was to investigate wheter 1) crystal deposits on the surface of antimicrobial catheters would occurr thorough TPN fluids administration and, if such deposits occurred, 2) such deposits would decrease the antimicrobial durability *in vitro*.

Materials and Methods. Two types of antimicrobial polyurethane CVCs (the antiseptics chlorhexidine and silver sulfadiazine; CHX-CVCs, Arrow Guard Plus; Arrow, Reading, PA, and with silver, platinum, and carbon lack; SP-CVCs, Edwards Life Science, Irvine, CA), in comparison with that of a control, uncoated polyurethane CVC were investigated. All types of CVCs (n=4; each type) were administrated with or without 3L of TPN fluids (flow rate; 63 mL/h). Bacterial adhesion testing, antibiotic sensitivity testing, and observation on the intraluminal surface of CVCs by scanning electron microscopy (SEM) were performed. Bacterial adhesion testing; we used a modification of a previously published method by Hanna et al.[2] In brief, four 1 cm-long segments of each catheter were tested for clinical isolates of MRSA or *Acinetobacter baumannii,* which has been confirmed the cause of outbreaks of the nosocomial infection last year in Japan. The steril CVC segments were placed into 12 well tissue culture plates containing of 2 mL of incubation medium that had been inoculated with organisms to a 0.5 McFarland standard.The plates incubated at 37 ℃ and the segments were sampled for day1, 3 and 5. Then the number of bacteria was determined. Antibiotic sensitivity testing was used a modified Kirby-Bauer method.

Results. After TPN fluids administration, crystal deposits and enoumous bacterial propagation on the intraluminal surfaces were confirmed in all typed CVCs by SEM. In bacterial adhesion testing, both antimicrobial CHX-CVCs and SP-CVCs without TPN fluids administration demonstrated the long antimicrobial durability more than 5 days, however, after administration of TPN fluids the durability was rapidly and markedly reduced within 5 days. In-vitro antibiotic sensitivity testing revealed a high antimicrobial activity in both antimicrobial CVCs after TPN fluid administration as well as without TPN administration, which supposed that bacterial adhesion testing would susceptibly reflect the impact of bacterial propagation on the surface of CVCs more than antimicrobial activity testing. The deposits derived from TPN fluids acted as hotbeds of bacterial propangation and decreased the antimicorbial activity even in antimicrobial CVCs.

Conclusion. Crystal deposits appeared on the intraluminal surface of all types of CVCs during administration of medication, and seemed to markedly impair the antimicrobial efficacy of antimicrobial CVCs. The results suggested that the deposits facilitated bacterial colonisation of even antimicrobial CVCs, and this would be one of the critical causes of CRI.

Keywords: antimicrobial central venous catheters; catheter-related infection, MRSA, *Acinetobacter baumannii*

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Differences in biochemical compounds in batch culture grown planktonic and biofilm cells of *Amphora rostrata Wm.Sm.*

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Diatoms are abundant in biofilms developed on surfaces immersed in sunlit waters. In both planktonic and biofilm mode of growth, diatoms produce biochemical compounds which perform several functions including motility, protection, maintenance, macro aggregates production, and detoxification. However, little is known about the differences, if any, in the production and molecular level characterization of carbohydrates in planktonic and biofilm cells of diatoms. In order to identify the differences in these two modes of growth, we measured concentrations of total carbohydrates, carbohydrate fractions, neutral carbohydrates, uronic acids, and amino sugars in planktonic and biofilm cells of *Amphora rostrata.* Distribution of carbohydrate fractions, uronic acids and amino sugars was different between biofilm and planktonic cells of the diatom. Cell normalized concentrations of these biochemical components were 2 to 5 times greater in the planktonic as compared to the biofilm cells of the diatom. Concentrations of glucose and glucosamine decreased, whereas fucose increased in planktonic cells over the period of cultivation. Conversely, concentrations of glucose and glucosamine increased while that of fucose decreased in attached cells. Our study suggests that there exist marked differences in carbohydrates of planktonic and biofilm cultures of *A. rostrata*.

Keywords: carbohydrate; glucose; fucose; glucosamine; protein; planktonic cells; biofilm cells; *Amphora rostrata*

Does co-association in dual-species biofilms between *Listeria monocytogenes* and *Pseudomonas putida* determine antimicrobial resistance?

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Comparison between the resistance to benzalkonium chloride (BAC) between mixed-species biofilms formed by different strains of *L. monocytogenes* and *Pseudomonas putida* CECT 845 under different scenarios and the obtained by the corresponding mono-species *L. monocytogenes* biofilm was carried out. The association of *P. putida* with *L. monocytogenes* quickens biofilm formation and increases significantly ($p<0.05$) the BAC-resistance of the biofilm after 4 days of incubation at 25 ºC respecting to that formed by monospecies biofilms.

According with the adherence profiles of *P. putida*, two different patterns of association between both species (A and B) were identified, being type A pattern found in the mixed biofilms much more resistant to BAC.

Obtained results clearly highlight that to improve disinfection protocols for assuring food safety, it is necessary to mimick those bacterial association that occur in nature.

Keywords biofilm; disinfection; *Listeria monocytogenes*; *Pseudomonas putida*; benzalkonium chloride; electronic microscopy.

Effect of Biofilm Formation of dental Plaque Isolates on the Surface of Artificial Teeth

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Four biocides (Benzidamine hydrochloride 0.15%, Chlorohexidine 0.2%, Sodium floride 0.05% and potassium chloride 0.05%, and Benzidamine hydrochloride 0.15% and Chlorohexidine 0.2%) singly or in combination were supplemented in N-agar medium to monitor the efficacy of bioocides and resistance pattern of dental plaque isolates. Resistant profile from all the dental plaque isolates . *Acenitobacter sp.* (accession no. JF837190), *Moraxella sp*. (accession no. JF837191), *Bacillus sp.* (accession no. JF837192) showed maximum resistance against all the biocides at the concentration of 1000 μgml^{-1}.Artificial teeth were constructed. *Acenitobacter sp.* (accession no. JF837190), *Moraxella sp*. (accession no. JF837191), *Bacillus sp.* (accession no. JF837192) which were isolated from dental plaques were used for inoculation and biofilm formation on acrylic teeth. Acrylic resin teeth are naturally more compatible with the denture base than porcelain teeth. Acrylic resin teeth are naturally more compatible with the denture base than porcelain teeth. After thirty days artificial teeth were drawn aseptically from 12 test (25ml media+ artificial tooth+25ml biocide) and 2 control (50ml media+ artificial tooth) flasks and subjected to different procedure to find out the effect of bacterial biofilm on artificial teeth. Artificial teeth were subjected for the study procedure of surface microscopy, Tooth hardness, and different color parameters.Surface microscopy of artificial teeth was done with the help of Metallurgical Microscope (model, Leica DM 4000M) that equipped with Camera (Model, sony DSPC3CCD video camera).Microscopic 5×10 magnification reveals significant microscopic change on the surface of artificial teeth from test flask compared with the surface of artificial teeth from control flask .Microscopic view depicted clear difference on the surface (before and after bacterial inoculation) of artificial teeth. Hardness of artificial teeth was measured with the help of hardness tester (Zwick/ Roell ZHV). With probe load of 200 gm and indentation time 5 sec hardness was measured. Due to the impact of bacterial biofilm over all hardness of all the artificial teeth was decreased. Color parameters (brightness ISO, gloss and color difference) of artificial teeth were measured with Spectrophotometer (model, CM 2600 Konica Minolta). Significant changes was observed in the color brightness, and gloss of artificial teeth surface due to the impact of bacterial biofilm .

Effect of iron concentration in growth medium on poly β-hydroxybutyrate production in *Azotobacter chroococcum* and physical and mechanical properties of the polymer

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Poly-β-hydroxybutyrate (PHB) is a biodegradable thermoplastic polyester produced by variety of bacteria under conditions of deprivation of oxygen, nitrogen, phosphate, sulphur, magnesium or potassium. *Azotobacter chroococcum* is obligatory aerobic nitrogen-fixing bacterium and a sufficient supply of air in fed-batch cultivation of *Azotobacter chroococcum* 23 in medium supplemented with ammonium significantly enhanced both the cell growth and PHB accumulation. Aerobic respiration of obligatory aerobic nitrogen-fixing bacteria results in the generation of highly reactive and toxic free radicals and obligate aerobes requires iron for protection from toxic oxygen product during active respiration, as well as for numerous other iron-dependent redox reactions and nitrogen fixation.

The present work focused on an effect of different concentrations of iron ($FeSO_4$ 36, 108 and 180 μM) in a modified Burk's medium on the *Azotobacter chroococcum* growth and PHB accumulation as well as on the characterization of the isolated native PHB granules and the purified polymer. 3-piperidinobenzanthrone (P8), a new hydrophobic fluorescent probe dye, was used to study an effect of iron ion concentration on the bacterial cell and the isolated native PHB granules. The luminescence signal of probe P8 can indicate hydrophobicity of PHB samples.

It was found, that the initial iron salt concentration in growth medium affected iron ion consumption rate and accumulation in the *Azotobacter chroococcum* cells. Under conditions of fed-batch cultivation high iron concentration positively affected PHA yield. PHA latex of PHB granules isolated from the biomass with the highest iron content was much more contaminated with iron and proteins than the other latexes in our study. This impurity negatively affected PHB latex films elasticity. Differences were observed in physical and mechanical properties of pure PHB samples recovered from biomass of *A. chroococcum* in dependence of iron concentration in growth medium too. *A. chroococcum*/P8 fluorescent probe system fluorescence intensity was lowering with increasing of iron concentration in the cells.

Keywords: *Azotobacter chroococcum*, poly-β-hydroxybutyrate, iron, physical and mechanical properties, 3-piperidinobenzanthrone

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Effect of pH on surface physicochemical properties of *Acinetobacter baumannii*

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The first step in biofilm formation is bacterial attachment to solids surfaces, which is dependent on the cell surface physico-chemical properties. The purpose of this work was to analyze the effect of pH on the physico-chemical cell surface characteristics of *A. baumannii* (hydrophobicity, electron donor- electron acceptor). These characteristics were evaluated by the microbial adhesion to solvents method (MATS) and contact angle measurements (CAM). Using MATS technique we found that *A. baumannii* was hydrophilic at the different values of pH studied. The pH seemed to have little or no influence on cell hydrophobicity. Excepting of pH 6.5, the electron donor character was stable, whereas the electron acceptor increased as the pH increased. At pH 6.5, *A. baumannii* strain presents a clear maximum value of electron donor.

However, the results of contact angle measurements show that *A.baumannii* was hydrophobe at pH 6.5, pH 4, and hydrophilic at the other pH. Moreover, increases of pH provoke augmentation of electron donor character and a diminution in the electron acceptor character.

Regardless of the method utilized, the obtained results of MATS and CAM confirmed the influence of pH on the surface physicochemical properties and show a similar effect of the pH 6,5 on cell surface electron donor-electron acceptor properties of *A. baumannii*, which could playing a role in adhesion of *A. baumannii* to urinary catheters.

Keywords*: A. baumannii*; pH; Physico-chemical properties; MATS; CAM; Adhesion.

Effect of Various Polyols and Polyol Contents on Properties of Kefiran-Based Edible Films

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Kefiran is finding increasing use in the food industry as a texturing and gelling agent. It is a water-soluble polysaccharide containing approximately equal amounts of glucose and galactose residues in a chain sequence. In recent decades, food and packaging industries have been joining their efforts to find new ways to protect food from environmental conditions like oxygen, light, moisture, microbes, and mechanical stresses. The use of synthetic polymers and plastics for packaging has grown tremendously in the last century; however, this increase has created serious environmental problems due to the materials' inability to biodegrade. Moreover, the insecurity of oil and petroleum resources – the raw materials from which such packaging is derived – is encouraging the food industry to explore the use of natural bio-based materials and polymers in packaging. The feasibility of using kefiran as a new film-forming material was studied. Edible kefiran films were produced using glycerol and sorbitol as plasticizers at the ratio of 10, 15, and 25% (w/w) by the casting method. Physical, mechanical and water vapor permeability (WVP) properties were determined as a function of plasticizer concentration. Dynamic mechanical thermal analysis was used to determine the glass transition temperature. Increased glycerol concentration caused an increase in flexibility and WVP, as expected. However, the plasticizing effect of glycerol became less significant, particularly for puncture deformation and tensile strength. Glycerol was found to be a more suitable plasticizer for kefiran films than sorbitol. The properties of the films were related to their microstructure, which was observed by scanning electron microscopy. It was determined that plasticizers can play an important role in adapting the properties of these films to make them more suitable for food technology applications.

Keywords: Kefiran; Edible film; Plasticizer; Exopolysaccharide

Effectiveness of topical novel nanohybrids of silver particles in a mice *Pseudomonas aeruginosa* keratitis model

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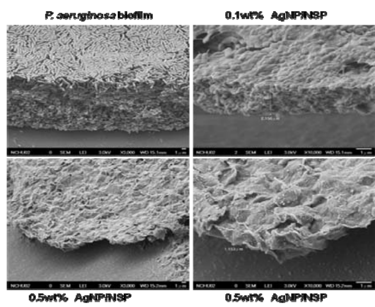
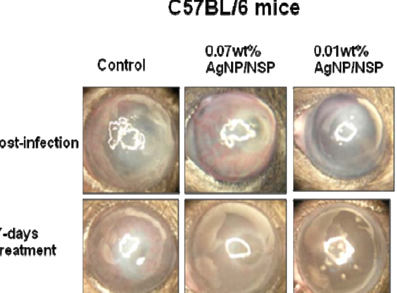
Pseudomonas aeruginosa keratitis is an acute sight-threatening infection disease. We previously reported that the novel nano-materials AgNPs/NSP successfully inhibited the growth of planktonic *P. aeruginosa*. In the present study, the effects of AgNPs/NSP on the *P. aeruginosa* biofilms were further investigated *in vitro* and *in vivo*. Biofilms were grown on Calgary Biofilm Device (CBD) for 96 hours as mature biofilms. Studied using the crystal violet and alamarBlue® cell viability assays showed 0.25wt% and 0.5wt% of AgNPs/NSP could eradicate at least 99.9% of biofilm bacterial population after 1-h exposure ($p<0.01$). Treatment with AgNPs/NSP at concentration 0.01wt% and 0.07wt% could reduced 50% and 80% bacterial formation biofilms ($p<0.01$). Scanning electron microscope (SEM) revealed that 0.5wt% AgNPs/NSP were adherent on the surface of biofilms and reduced thick biofilm approximately from 9µm to 1.153µm. Confocal Laser Scanning Microscopy (CLSM) was used to generate the 3D images of AgNPs/NSP treated *P. aeruginosa* biofilms. Therefore, using a mouse model of *Pseudomonas* keratitis, right corneas of all C57BL/6 mice were scratch injury and delivery with 10^6 colony-forming units of *P. aeruginosa.* Eyes were topically treated four times a day with 0.07wt% AgNPs/NSP (n=7), and significantly attenuated clinical symptoms were observed by slit lamp and stereomicroscope ($p < 0.05$). After 7 days treatment, corneas were examed by histopathology and corneal that heals in treatment with AgNPs/NSP. Our study suggest that AgNPs/NSP treatment may help to eradicate the biofilm associated keratitis caused by *P. aeruginosa.*

Keywords: nanoscale silicate platelets, silver nanoparticles, biofilm, bacterial keratitis

SEM



Scanning electron microscope (SEM) revealed that 0.5wt% AgNPs/NSP were adherent on the surface of biofilms and reduced thick biofilm approximately from 9µm to 1.153µm.



Evaluation of biofilms on functional coatings

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The settlement of living organisms on immersed surfaces takes place following four successive steps. These are i) formation of a conditioning film, ii) attachment of bacteria to the surface, iii) settlement of unicellular species, and iv) settlement of multicellular eukaryotes. This community is characterized by intricate interactions.

It is generally agreed that the prevention of marine fouling can be achieved by coatings from which a release of biocides prevents the growth of organisms (bacteria, algae, mollusks). This strategy was applied by conceiving self-polishing paints. In the early 70s, organostannous erodable paints revolutionized fouling prevention by their efficiency. They were composed of tributyltin (TBT) grafted to a polyacrylic backbone via an ester linkage. TBT was found to be a harmful molecule to marine ecosystems, and so its removal from paint formulae is planned.

In order to develop less toxic paints, it is necessary to further investigate the properties of antifouling coatings. The use of confocal laser scanning and scanning electron microscopies was investigated to facilitate the observation of adhered microfouling on functional coatings.

We have formulated several coatings by varying binder and surface properties:

- Antifouling paints based on polyacrylic and rosin binders
- Poly(ethylene oxide)-grafted silicones
- Bio-based films prepared from chitosans by ionic interactions between the cationic biopolymer and polyphosphate ions. Antifouling efficiency could be obtained when films entrapped copper.

To understand the effects of coatings on biofilm formation, we have used different conditions of immersion:

- In natural seawater to evaluate the kinetics of colonization of natural microufouling onto coatings. Results have confirmed that a link exists between the microfouling observed and the antifouling activity of the coatings: the microfouling adhesion kinetic was sensibly decreased on a coating with good antifouling properties.
- In vitro, by evaluation of bacterial initial adhesion and biofilm formation in static conditions. Two strains of bacteria were selected due to their specific characteristics: The first was *Pseudomonas aeruginosa* PAO1, a hydrophobic Gram-negative bacteria which is known to frequently adopt a biofilm lifestyle, both in the environment and in the course of pathogenesis. The second was *Bacillus 4J6,* a hydrophilic Gram-positive bacteria chosen for its relevance in the marine environment.
- In vitro, to evaluate the attachment of a diatoms (*closterium cylindrotheca*) onto coatings
- In a bioreactor, to verify the cellular arrangement between bacteria (*Bacillus 4J6*) and diatoms (*closterium cylindrotheca*) over time by studying the dynamics and architecture of multi-species biofilms.

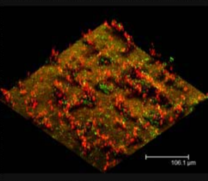

Figure: Observation of multi-species biofilms onto antifouling paint

The study revealed the utility of CLSM for the evaluation of antifouling paints and reports on the efficacy of CLSM to study the initial microfouling layer. This method yields important data relating to biofilm morphology, particularly film thickness and biomass measurements. This non-invasive technique enabled us to obtain qualitative and quantitative data about the biofilm formation.

Keywords : biofilms, antimicrobial surfaces, antifouling, confocal scanning microscopy

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Evaluation of chitosan to control biofilm formation by oral pathogens

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Biofilms play a key role in the colonization of the oral cavity by microorganism and as such are fundamental in the aetiology of oral diseases, such as dental caries and periodontitis. In light of this, new approaches have been sought in the treatment of oral diseases focusing more in the control of biofilms formation than in the combating of planktonic microorganism. As these biofilms constitute an advantage for microorganisms, protecting them from traditional antiseptics and antibiotics, while providing a stable platform for microbial growth, new approaches, capable of controlling or impeding the formation of these biofilms, have been sought. Nowadays, the focus is on biomaterials and biopolymers as these may present a natural alternative, without the downsides and secondary effects of traditional antibiotics and treatments. As such, chitosan with its renowned antimicrobial activity, biocompatibility and biodegradability may be a promising solution.

In our study we assessed the capability of sub minimal inhibitory concentrations (MIC) of non-altered acid soluble high molecular weight (HMW) and low molecular weight (LMW) chitosan in controlling and inhibiting the formation and growth of oral biofilms of several pathogenic bacteria, including *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Prevotella intermedia* and mixtures of two or more of these bacteria in mediums supplemented with 5 % (v/v) sucrose.

Results showed that sub-MIC concentrations of both molecular weights (MW) were very effective in inhibiting biofilm and controlling the development of already formed biofilms. Bacteria in the presence of sub-MIC concentrations of chitosan presented reduced capability for biofilm formation (reduction percentage reaching a maximum of approximately 90 % when compared to a positive control) with no significant differences ($p < 0,05$) in behaviour being present between MW. On the other hand chitosan concentrations presented significant differences ($p < 0,05$). In addition, monitorization of pH levels showed that chitosan lowered initial pH levels and then had a buffer effect stabilizing the pH of mediums. This functionality is of great importance as it hampers sucrose degradation and the formation of exopolysaccharides, crucial for biofilms establishment. Furthermore, it was possible to assess that sub-MIC concentrations did not affect bacterial viability as showed by enumeration of viable counts at the end of every assay.

As such is only possible to conclude that chitosan is a viable and promising alternative to traditional oral antimicrobials and presents great efficiency in controlling a key step in the colonization of the oral cavity.

Keywords Oral pathogens, biofilm formation, sub-MIC, chitosan

Exopolysaccharide Producing Enzymes Identified from Enriched Genomic Libraries of Sugarcane Associated Bacteria

Gavin George, Kyle Willard, Charl Marais, Jens Kossmann

There exists a vast diversity of naturally occurring carbohydrate biopolymers several of which are emerging as important industrial components.These polymers are finding application in a range of industries including, but not limited to, the food, pharmaceutical, textile, and cosmetics industries.

Polysaccharide polymers can be composed of a range of subunits, including glucose, fructose, mannose, galactose, etc., linked in alpha or beta conformations through several possible glycosidic linkages between units. The monomer composition, steriochemical conformation, linkage as well as secondary structures, such as branching and chain length, all affects the physicochemical properties of the polysaccharide.

While polysaccharide polymers are abundant in nature, *viz*. cellulose which is one of the most abundant organic compounds on earth, we chose to examine bacterial exopolysaccharides (EPS) which present significant variation in the carbohydrate polymers that are produced. Bacteria populations vary considerably depending on the niche conditions and so, in the search for specific substrate utilization, samples were collected from areas rich in sucrose with the aim of identifying polyglucan and -fructan producing enzymes. To this end, water run-off from a sugar mill was collected and cultured on high sucrose medium.A large number of EPS producing bacteria were identified and initial polymer analysis showed great diversity in physicochemical properties of the produced glycans. Genotyping too has revealed that a large range of EPS producing bacteria, including several previously uncharacterised strains, have been collected.

A significant hurdle exists in the downstream application of bacterial EPS in that the purification costs from certain organisms can make large scale production prohibitively expensive. This problem can be avoided through the production of the polysaccharides in in more industrially appropriate organisms. In order to do this the genes encoding the active enzymes must be identified; however, the lack of sequence data makes PCR based discovery difficult.An efficient method for gene discovery is the construction and screening of genomic and metagenomic libraries. With this poster we report on the production and screening of libraries which we have constructed as well as the identification and expression of gluco- and fructosyltransferases.

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Film Forming Solutions Based on Kefiran and Various Plasticizers: Rheological Characterizations

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Rheological properties are useful in providing a broad understanding of a film's microstructure. Some researchers have reported that a high-viscosity film-forming solution would make it difficult to remove air bubbles and hinder casting in thin layers. The low-viscosity film-forming solutions have also attracted attention of other researchers. To make a level coating on solid surface, the viscosity of film-forming solutions should prevent sagging by gravity effects and allow capillary leveling. It is noted that rheological properties, which are sensitive to variations in molecular structure, are useful in developing structure-function relationships for systems of polysaccharide solutions. In this sense, the characterisation of the film-forming solution contributes to the understanding of the differences in the final properties of the film. Kefiran is finding increasing use in the food industry as a texturing and gelling agent. It is a water-soluble polysaccharide containing approximately equal amounts of glucose and galactose residues in a chain sequence. The rheological properties of kefiran film-forming solutions, as well as the structural characterisation of the resulting films, were investigated as a function of various plasticizer types. The behaviours of the storage (G') and loss (G'') moduli as a function of frequency were typical of gel-like material, with the G' higher than the G''. Plasticization effectiveness could be compared by determining viscoelastic properties. On application of dynamic rheological tests, all film-forming solutions showed physical gel-like behaviour.

Keywords: Rheological properties; Loss modulus; Storage modulus; Plasticizer

Helicobacter pylori biofilm: a natural environment to favour the genomic variability

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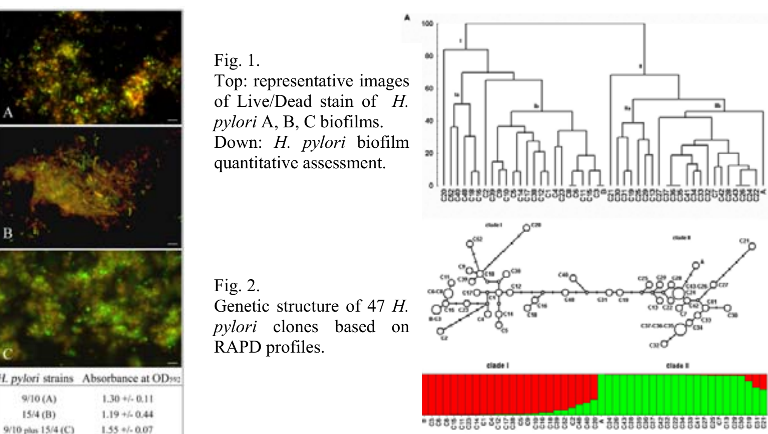
Helicobacter pylori persistence might be associated to genetic variability and biofilm development. In particular, the 'biofilm niche' might represent a suitable environment in which gene transfer could take place promoting recombination events widespread in *H. pylori* species. *H. pylori* forms a structured biofilm characterized by an extracellular polymeric substances (EPS) matrix, in which extracellular DNA (eDNA) fragments are involved in the dynamic transfer of genome in a protected environment. In this way, the released DNA might be involved both in the exchange of genetic material and in the development of biofilm structure.

In the light of the recent detection and characterization of the eDNA in the biofilm EPS matrix of *H. pylori* strains, the aim of the present work was to investigate the interactions between two clinical *H. pylori* strains, to better understand the dynamic balance among strains which can coexist in the same biological niche during colonization. For this purpose the biofilms produced by *H. pylori* 9/10 (A),*H. pylori* 15/4 (B) and their mixture(C) were studied for the biomass production and cell viability by Microtiter biofilm assay and Live/Dead staining, respectively. Moreover, the genetic heterogeneity of 45 clones, coming from a 48 hours mature biofilm of the co-cultured *H. pylori* clinical strains (C) was studied by both Random Amplification of Polymorphic DNA (RAPD) and *cagA* (EPIYA motifs) and *vacA* (s/i/m regions) virulence genes analysis.

Helicobacter pylori 9/10 (A) and 15/4 (B) as well as their mixture (C) were capable of developing a well structured biofilm although no statistical significant differences were recorded in the viability of cells belonging to A, B or C biofilms. With regard to the biomass measurement, no significant differences were recorded between A and B biofilms; on the contrary, C biofilms were able to express more adhesive capability displaying a remarkable (P<0.001) difference when compared with the biomasses of the corresponding parental strains (Fig. 1).

The DNA fingerprintings associated to the 45 clones isolated by C biofilms showed an high genetic heterogeneity with an overall mean Jaccard similarity value of 0.528. In particular, the cluster analysis displayed two main groups including clones with RAPD patterns similar to the ones of the parental strains (Fig. 2). With regard to virulence factors allelic combination, interestingly the 60% of clones displayed an allelic combination s1i1m1m2 of *vacA* gene associated to *cagA* EPIYA motif pattern P1P2P3P3P3.

In the present study, we provide evidence that the presence of recombinant clones produced a self-organized population, more stabilized and more able to develop a well-structured biofilm arisen by a balance between the two parental strains. Therefore, these data confirm that *H. pylori* genome variability represents a successful strategy for the evolution and persistence of the bacterial population.



H. pylori strains	Absorbance at OD_{592}
9/10 (A)	1.30 +/- 0.11
15/4 (B)	1.19 +/- 0.44
9/10 plus 15/4 (C)	1.55 +/- 0.07

Fig. 1.
Top: representative images of Live/Dead stain of *H. pylori* A, B, C biofilms.
Down: *H. pylori* biofilm quantitative assessment.

Fig. 2.
Genetic structure of 47 *H. pylori* clones based on RAPD profiles.

Keywords: biofilm; *Helicobacter pylori*; inter-strain recombination

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High variability of gene expression in *S. epidermidis* biofilm population

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In the past two decades *S. epidermidis* has emerged from a commensal microorganism into a predominant opportunistic pathogen associated with nosocomial infections due to the ability to adhere to abiotic surfaces and form biofilms. The increasing use of indwelling medical devices has influenced the rise of *S. epidermidis* to a major medical research topic. *S. epidermidis* biofilms are well known to be resistant to both the host immune response and antimicrobial therapy, making these infections hard to treat and often resulting in recurrent infections. To better understand why biofilms have evolved in this manner, many comparative studies have been performed using biofilms and planktonic cultures. However, since biofilm cultures are fundamentally different from planktonic cultures, some concerns have been raised in the past for such studies. While it seems reasonable to compare biofilm cultures with stationary planktonic cultures, recently it has been suggested that biofilms cultures could be compared directly to the bacteria in suspension, grown in the vicinity of the biofilms. Nevertheless and however interesting, this suggestion fails to accommodate the fact that mature biofilms will release bacteria from within the biofilm to the suspension. This phenomenon was suggested to be responsible for colonization of further niches. Therefore, such population can both contain biofilm outbound bacteria as well as plantkonic free floating bacteria.

In an attempt to better understand the possible differences between cell populations, we selected 5 distinct bacterial isolates previously characterized for biofilm formation and compared the expression of some genes of interest, namely *atlE* (involved in initial adhesion) and *icaA* (involved immune evasion and biofilm maturation). Three populations were characterized: (1) late exponential planktonic cultures grown on Erlenmeyer flasks, (2) biofilm populations attached to polystyrene 24-well culture plates and (3) the bacteria grown in suspension on the same well of culture plates as the biofilms (non-adherent cells), all grown in TSB supplemented with 0,4% glucose. Differences in the gene expression profile were observed between *S. epidermidis* strains. The *icaA* expression values were generally higher in biofilms as compared with planktonic cultures. However, when comparing with the non-adherent cells grown in the vicinity of the biofilms, some strain to strain variation was observed, as in some cases the non-adherent cells has lower *icaA* expression but in other instances the opposite occurred. A similar effect occurred with *atlE* expression. A possible explanation for the higher variation on the non-adherent cells has to do with the washing step required before resuspending the biofilm: while more tenacious biofilm forming strains will withstand better the washing step, some weaker biofilm forming strains will be washed away. In the latter cases the bacterial population described as non-adherent cells will be very heterogeneous. Thus, with the variation found in the non-adherent bacteria, it seems that in order to study the physiological differences that occur when bacteria are living in a biofilm, planktonic cultures grown independently of biofilms should be used to better understand the pathophysiology of the biofilm-related infections.

Key-words: *S. epidermidis*; Biofilms; Gene expression

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Histological description of membranes of chitosan in oral cavity

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Guided tissue regeneration can succesfully retrieve periodontal ligament and bone lost in the periodontal disease. Today, thanks to advances in biotechnology have been developed biopolymers such as chitosan. It has the ability to form films with good mechanical, antimicrobial and permeability properties. It has been used to treat wounds and burns; because of it is hemostatic capacity and accelerating effect on scaring. Chitosan is a natural, low-cost, renewable and biodegradable biopolymer. The most important indication for their use is represented by the biocompatibility with the live tissue.

Hypothesis
Chitosan membrane is able to promote new tissue formation in oral cavity.

Objective
The purpose of this research was to observe under the microscope the formation of new tissues using chitosan in oral cavity.

Materials and methods: This research aims to analyze chitosan promoting activity in the formation of new tissue placed in class II furcation lesions into 2 beagle dogs. We analyzed seven chitosan membrane, 4 are placed in the experimental model 1 (EM1) and control tooth membrane polylactic-polyglicolic acid while in the experimental model 2 (EM2) using 3 membranes of chitosan and control tooth membrane of polylactic-polyglycolic acid. The specimens were preserved in 10% formol and stored for five months in decalcifiying solution of trichloracetic acid (EDTA 20%). after the softening stage, each tooth was included into paraffin blocks. The different cuts were visualized by optical microscopy (Carl Zeiss) and analyzed employing the AxioVision software.

Results
The results showed good acceptance of chitosan by tissues as well as control membrane (polylactic-polyglicolic acid). The findings in histological preparations were absence of bacteria and inflammatory infiltration. It was observed the presence alveolar bone, periodontal ligament,cementum, connective tissue collagenized hyperplasic, metaplastic bone formation and extravasation.

Conclusions
Chitosan membrane constitutes an excellent biopolymer to he application of guided tissue regeneration improves wound healing of class II furcation defects. It is a low-cost biopolymer and therefore it is accessible for all people.

In situ assessment of antibacterial activity of dermaseptine S4 derivatives against *Pseudomonas fluorescens* nascent biofilms by using ATR-FTIR spectroscopy

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Formation of biofilms, complex assemblages of microorganisms embedded in a self-produced exopolymer matrix attached to material surfaces, is a major economic and health problem in numerous areas from food production to medical implants or water distribution systems. Compared with planktonic counterparts, biofilm bacteria can tolerate up to 1000 times more antibiotic and disinfectants. Consequently, their eradication requires higher antibiotic concentrations that unfortunately favour the emergence of multiresistant strains. An alternative could be to use antimicrobial peptides (AMPs). AMPs play a central role in the innate immunity of system of many organisms and are active towards a wide range of microorganisms. In addition, due to their membanolytic activity, development of microbial resistance to AMPs should be rarer. Nevertheless several issues need further development. Their minimum inhibitory concentrations (MICs) are relatively high compared to those of conventional antibiotics and a wide application of AMPs is hindered by their high manufacturing cost and the cytoxocity of some AMPs. It is necessary to optimize peptide structure and size in order to improve their antimicrobial activity and toxicity profile. This can only be possible by gaining more insight into their precise molecular mechanisms of action, still largely in dispute, not only against planktonic bacteria, subject of most studies, but also against sessile bacteria inside biofilms. How? By probing and tracking *in situ*, at the cellular and molecular scales, and in real time the changes induced by AMPs in bacterial cells. Among the possible tools, the Attenuated Total Reflectance - Fourier Transform InfraRed (ATR-FTIR) spectroscopy could be a valuable tool. Indeed, because its analysis depth is very thin, typically of the order of 1-2 µm, the ATR-FTIR technique enables us, *via* the ATR-FTIR fingerprints of biomolecules, to monitor *in situ* not only initial stages of biofilm formation but also, subsequently, response of the base sessile bacteria monolayer to environmental condition changes and to study the influence of environmental conditions on bacterial adhesion, biofilm growth or detachment processes [1, 2].

This communication illustrates this original approach with the study of the activity of three analogues of dermaseptine S4 (S4(1-16)M4K, S4(1-28)M4K and S4(5-28)) against 6h-old *Pseudomonas fluorescens* (*Pf*) biofilms. The ATR-FTIR fingerprints of bacteria were monitored *in situ* and in real time during 24-hour treatment with AMPs by using an ATR-FTIR flow-cell. AMP concentrations were near of MICs determined on planktonic *Pf* bacteria. Biofilms were also analyzed by epifluorescence microscopy using the live/dead bacterial viability kit in order to determine bacterial surface coverage and the viability of biofilm cells. Important and rapid changes over time were observed in ATR-FTIR fingerprints of biomolecules revealing major biochemical and physiological changes, bacterial detachment but also, at some time, bacterial regrowth. These changes and the difference in behavior between the three AMPs are analyzed and discussed.

Keywords: Nascent biofilm, antimicrobial peptides, FTIR-ATR spectroscopy, *in situ* monitoring, *Pseudomonas fluorescens,*

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In vitro antimicrobial susceptibility of single and mixed populations in Cystic Fibrosis: the role of novel microorganisms

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Pseudomonas aeruginosa is the dominant pathogen associated with bacterial infections occurring in Cystic Fibrosis (CF) patients, resulting in 80% of mortality in adults. However, pulmonary infection has recently been defined as polymicrobial, involving classical and other unusual bacteria, which may play a crucial role when associated with the conventional ones. This work aims to evaluate the susceptibility patterns of mono and dual-species biofilms encompassing traditional and emerging microorganisms from CF.

The traditional pathogen, *P. aeruginosa* PA14, and two novel microorganisms, *Inquilinus limosus* M53 and *Dolosigranulum pigrum* CIP104051 were used to form single and dual-species biofilms. These were developed on the Calgary Biofilm Device and their susceptibility profiles were estimated against eight antibiotics (Tobramycin, Gentamicin, Levofloxacin, Ciprofloxacin, Clindamycin, Cefotaxime, Chloramphenicol and Rifampicin), by measuring the minimum inhibitory concentrations (MIC) and minimum biofilm eradication concentration (MBEC).

Data showed that most antibiotics were effective in inhibiting planktonic bacterial growth at low concentrations, mainly in mono-populations. Single biofilms involving novel bacteria were more sensitive to virtually all antibiotics than *P. aeruginosa*. However, when in mixed biofilms, those organisms acted synergistically with *P. aeruginosa*, attaining additional antibiotic resistance and requiring higher doses of antibiotics to eradicate them. From these results, it can be concluded that the presence of unusual bacteria and their complex interactions with conventional organisms might not be ignored thereby enabling to develop suitable therapy strategies to combat CF.

Keywords Cystic fibrosis; *Pseudomonas aeruginosa*, antimicrobial susceptibility

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Influence of ageing time on antibacterial packaging from Sodium and Calcium caseinate incorporated with Carvacrol

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Research on biopolymers as an alternative to petroleum-based thermoplastics for packaging materials is constantly increasing. In addition, antimicrobial food packaging is one of the most updated tendencies in research for food industry because it can minimize the microbial contamination of food products during storage, transportation and handling [1], controlling spoilage and pathogenic microorganisms [2], resulting in an increase in shelf-life of fresh products. The formulation of edible films incorporated with essential oils offering antimicrobial properties could have an important effect on shelf-life extension and safety of foods [1,3]. Particularly, carvacrol is a major volatile aromatic compound obtained from oregano essential oil, with high antimicrobial activity and totally harmless to human health [4]. Proteins, such as caseinates, are considered as potential candidates to produce biofilms because they show advantageous properties for packaging biomaterials. Some of these properties are their ability to form networks, plasticity and elasticity; good barrier to oxygen, carbon dioxide and aromas [5]. They are inexpensive and abundant raw materials obtained from renewable sources and they also present the opportunity to be biodegradable. However, caseinates show an intrinsic rigid structure and consequently plasticizers should be incorporated into the protein matrix to permit films manufacturing. Glycerol has been selected since it has been successfully used in protein-packaging applications [5,6].

In this study, caseinates edible films plasticized with glycerol and incorporated with 10 wt% of carvacrol were successfully obtained by a casting method. The influence of the addition of two different plasticizer concentrations (25 wt% and 35 wt%) has been evaluated by tensile testing according to the ASTM-D882 standard. Two bacteria whose presence in food could lead to a hazard to consumer health, *Staphylococcus aureus* and *Escherichia coli*, were selected to evaluate the antibacterial effectiveness of these edible films by using the agar diffusion method. Optical properties such as transparency and color co-ordinates in the CIE L*a*b* space to evaluate their total color differences were also determined. The aim of this study was to evaluate the influence of glycerol at two different concentrations as well as the carvacrol activity on mechanical properties and antibacterial effectiveness against *Escherichia coli* and *Staphylococcus aureus*, of bioactive edible films based in sodium (SC) and calcium (CC) caseinates. All the tested formulations resulted in completely transparent and homogeneous films. Ductile properties were significantly improved with the addition of high concentrations of glycerol and films remained flexible after 35 days with SC films showing higher flexibility. Formulations incorporated with carvacrol presented inhibitory effects on both bacteria tested, even after 35 days. In conclusion, caseinate based films showed potential to be considered good supports for carvacrol to be used as antibacterial film in multilayer packaging systems.

Keywords: biofilm; sodium caseinate; calcium caseinate; carvacol; *E. coli, S. aureus*

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Influence of carbon source on bacterial cellulose production by *Gluconacetobacter xylinus* CECT 7291

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The main source of cellulose is the plant kingdom. However there are also different bacterial species with the ability to synthesize cellulose. Since 1886 it is known the capacity of the Gram-negative bacterium *Gluconacetobacter xylinus* to form an extracellular gelatinous layer. It has been described as the specia with the greatest ability to produce cellulose and has been taken as a model organism for basic and applied studies on the bacterial cellulose.

The bacterial cellulose is one of the specific products of primary metabolism of the bacteria. It forms a film on liquid and solid media that protects the bacteria from mechanical and chemical agents. It also facilitates cell adhesion to host tissues and increases its ability to colonize substrates. This film acts as a mechanism of "float", allowing the bacteria to more easily obtain the oxygen needed for its growth. Unlike plant cellulose, bacterial cellulose has got high purity. This peculiarity, along with an ultrafine network structure, gives it some differential properties, like high crystallization degree, elasticity and durability, greater tensile strength than plant cellulose, high capacity to absorb water and compatibility with plant cellulose. It is also metabolically inert and produces hydrophilic polysaccharides that bind to the cellulose forming a compact and less dense than water matrix. Because of these features, bacterial cellulose provides a stronger paper than plant cellulose, which is useful to reinforce conventional paper pulp and that allows paper manufacturing for some fibrous material.

Cellulose production depends, among other factors, on the medium carbon source. Although there are several studies of this influence, there is no study that correlates the carbon source with the properties that can present the cellulose produced to use it as an aid for the restauration of degraded papers.

This study determines the effect of carbon source in the cellulose formed by varying the culture medium of *Gluconacetobacter xylinus* CECT 7291. The base medium used is always HS (Hestrin-Schramm) which only varies the carbon source (glucose, sucrose, fructose, mannitol and glycerol). Leaving constant the initial pH of the medium (6,3), the temperature (30ºC), and absence of agitation. Cellulose layers are obtained at four different culture times (4, 7, 10 and 13 days). The medium is characterized in each of those four times in terms of pH and consumption of carbon source. The cellulose layers obtained in each case are washed, pressed and dried, and its pH, dry weight, thickness and optical and mechanical properties are characterized.

The results indicate significant variations in the parameters studied depending on the carbon source, allowing to select the optimal conditions for using bacterial cellulose in subsequent applications in degraded papers restauration.

Keywords: *Gluconacetobacter*, bacterial cellulose, paper restauration.

Influence of *Lactobacillus acidophilus and Actinomyces naeslundii* in the biofilm formation of *Streptococcus mutans*

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Streptococcus mutans is the most important etiological agent of dental caries in humans worldwide. The main pathogenic factor of *S. mutans* is their high capacity to adhere to solid surfaces. It is a key contributor for extracellular polysaccharide matrix development in dental biofilm. It has been reported that *Lactobacillus acidophilus and S. mutans* are the two species most frecuently detected into dental caries, while *Actinomyces naeslundii* was found in patients with no caries lesions. The aim of the study was to determine the influence of *Lactobacillus acidophilus and Actinomyces naeslundii* in the biofilm formation of *Streptococcus mutans.* The biofilm formation of *S. mutans* was measured by fluorescent microscopy and compared with the biofilm developed when *S. mutans* was co-cultured with *L. acidophilus* or *A. naeslundii*. The expression of adherent genes like spaP, gtf, ftf and gbpB of *S. mutans* was determined by qPCR. The obtained results showed that biofilm formation was higher in the presence of *Lactobacillus acidophilus,* while in the presence of *Actinomyces naeslundii* the quantity of biofilm formed was less. These results correlate with the level of expression of spaP, gtf, ftf and gbpB genes of *S. mutans.* We conclude that S. mutans compite with *A. naeslundii* for the surface to form biofilm, while *L. acidophilus* increase the biofilm formation of S. mutans.

Keywords *S. mutans,* biofilm, *L. acidophilus*, *A, naeslundii*, expression gene, adherent genes.

Influence of long-range van der Waals forces on biological adhesion - The importance of the subsurface composition

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Understanding and controlling the adhesion of biological objects to inorganic surfaces are important tasks that involve multiple different disciplines and find application in various topics such as the development of antimicrobial surfaces, biosensors or artificial adhesives.

To characterize biological adhesion, most studies describe a substrate solely by its surface energy, which is mainly determined by the surface properties. The composition of the material beneath the surfaces is frequently overlooked. That way, long-range van der Waals interactions are disregarded. These interactions, however, can have a significant influence on the shape of the interface potential in the vicinity of the surface. We could show that microscopic, mesoscopic and even macroscopic biological objects are influenced by differences in the microscopic interface potential.

By using tailored silicon wafers with different thick oxide layers, we were able to tune the van der Waals part of the interface potential independently of the surface properties. On these substrates, we performed adsorption experiments with different proteins (α-amylase, BSA, lysozyme) as well as adhesion measurements with bacteria of the *Staphylococcus* genus and with a species of tropical gecko (*Gecko gecko*). The protein adsorption was studied by X-ray reflectivity. Using X-ray energies of 27 keV we could access the structure and thickness of the adsorbed protein films *in situ*. The bacterial adhesion was explored by using atomic force microscopy. We conducted force spectroscopy measurements using cantilevers on which living bacteria were immobilized. To characterize the gecko adhesion we mimicked the typical gecko movement with a custom mechanical testing platform (“Robotoe”). While dragging setal arrays across the substrates, we determined the involved forces.

In all cases, we observed significant differences depending on the subsurface composition of the substrates. The final adsorbed amount of proteins as well as the adhesion forces of both, bacteria and geckos, were substantially influenced by the thickness of the oxide layers. That is to say that microscopic proteins, mesoscopic bacteria and macroscopic gecko ‘feel’ not only the surface properties but also differences in the microscopic van der Waals potentials.

Keywords van der Waals forces; bacterial adhesion

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Isolation Characterization and Localization of Exo-Polysaccharides from cyanobacterium *Arthrospira platensis* strain MMG-9

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Arthrospira platensis is a well known cyanobacteria for its nutritional value and diverse secondry metabolites. Exo-polysaccharides (EPS) is an important trait of majority of cyanobacteria. EPS from locally isolated *A. platensis* strain was isolated and analyzed. Three different EPS fractions i.e. released (REPS), loosely bound (LEPS) and closely bound (CEPS) were isolated separately. Total protein and carbohydrate content was estimated. Eight different monosaccharides were analysed in all EPS of fractions using HPAE-PAD technique. Localization of EPS layers outside the cyanobacterial filaments was determined by confocal laser scanning microscopy (CLSM) after staining with different fluorescent dyes. *A. platensis* showed thick smooth deposition of EPS layers around the spiral filaments when observed under CLSM. LEPS was the least isolated fraction of EPS. Maximum amount of total carbohydrates and total proteins was measured in CEPS as compared to other fractions. Fructose, mannose and ribose were the rare monosaccharide residues in EPS and except fructose all other monosaccharides were detected in CEPS. Quantitatively all monosaccharides were higher in amount in CEPS especially galactose, xylose and glucose. The EPS was found to be very diverse in nature and different fractions showed specific characteristics. This can be a source of interesting polysaccharides of desirable characteristics in biotechnology.

Keywords *Arthrospira platensis*; Confocal laser scanning microscopy, Exopolysaccharides, Monosaccharide analysis

Mathematical modelling and numerical simulations for multispecies biofilm formation

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Mathematical models for multispecies biofilm growth are presented in the framework of continuum theory. The mathematical problem consists of a system of nonlinear partial differential equations of hyperbolic and parabolic type, as described below

$$\frac{\partial f_i}{\partial t} + \frac{\partial}{\partial z}(u f_i) = r_{M,i}(z,t,f,S), 0 \le z \le L(t), t > 0, i = 1,...,n,$$

$$\frac{\partial u}{\partial z} = \sum_{i=1}^{n} r_{M,i}(z,t,f,S), 0 \le z \le L(t), t > 0,$$

$$\dot{L}(t) = u(L(t),t), t > 0,$$

$$\frac{\partial S_j}{\partial t} - D_j \frac{\partial^2 S_j}{\partial z^2} = r_{S,j}(z,t,f,S), 0 < z < L(t), t > 0, j = 1,...,m.$$

In the system above:

z is the space variable and t the time variable;

$f_i(z,t)$ is the volume fraction of the microbial species i, $i = 1,...,n$; $f = (f_1,...,f_n)$;

$S_j(z,t)$ is the concentration of the substrate j, $j = 1,...,m$; $S = (S_1,...,S_n)$;

$r_{M,i}(z,t,f,S)$ is the specific growth rate of the microbial species i;

$r_{S,j}(z,t,f,S)$ is the conversion rate of substrate j;

$u(z,t)$ is the velocity of the microbial mass;

$L(t)$ is the biofilm thickness (free boundary);

D_j is the diffusivity coefficient and σ the biomass flux between biofilm and bulk liquid.

The mentioned system was firstly derived in [1]. It is very general and can be used for applications of engineering and industrial interest, e.g. reactors where biofilms are used to clean waste waters safely and effectively. Existence and uniqueness theorems are proved and properties of solutions are shown [2]. Efficient numerical methods will be illustrated, based on the method of characteristics. Simulations are proposed related to the anaerobic digestion process in biofilm, particularly the competition between acidogenic and methanogenic bacteria.

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Membrane tubules 60-90 nm in diameter interconnect *Salmonella* Typhimurium in biofilms and attach bacteria to substrata and host cells

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Adhesive tubular appendages interconnect bacteria in biofilms and attach them to substrata and host cells. Using scanning electron microscopy techniques we measured the diameter of tubular appendages interconnecting *Salmonella enterica* serovar Typhimurium in biofilms grown on gallstones or cover slips. The tubular appendage diameter of bacteria of virulent flagellated C53 strain varied in the range between 60–70 nm, thus strongly exceeding in size the bacterial flagella (11-20 nm) or pili (6-7 nm). Non-flagellated bacteria of mutant nonflagellated SJW 880 strain in biofilms were also interconnected by 60-90 nm tubular appendages. Tubular appendages connecting bacteria of C53 strain to host cells (human neutrophil) had the same diameter. Transmission electron microscopy studies of thin sections of *S.* Typhimurium biofilms grown on agar or cover slips revealed numerous fragments of membrane tubular and vesicular structures between bacteria of both flagellated and non-flagellated strains. The membrane structures concurred in diameter with tubular appendages observed by scanning electron microscopy, thus indicating that tubular appendages represent membrane tubules (tethers). Membrane tubules in size correspond to so called “membrane sheaths” of bacteria but relationship between membrane tubules and flagella remains to be elucidated. Previously we have shown that neutrophils can contact cells and bacteria over distance via membrane tubulovesicular extensions 150-250 nm indiameter (membrane tethers, cytonemes). Present electron microscopy study revealed the prominent similarity in behaviour and functions between bacterial tubular appendages and neutrophil cytonemes. Long-rang communications via membrane tubules appear to represent a new adhesive mechanism common for eukaryotic cells and bacteria.

Keywords: samonella; biofilms; membrane tubules, long-range adhesion

Microbial production of novel polyhydroxyalkanoate bearing functional side groups by mixture carbon source

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Polyhydroxyalkanoates (PHA) are synthesized by many bacteria under unbalanced growth condition. They are intracellular energy storage. PHA exhibits thermoplastic and elastomeric properties similar as polyethylene; therefore it has been applied as bioplastics, biomaterials and biofuels. PHA is generally divided into two groups, short-chain-length (SCL) phase and medium-chain-length (MCL) phase. SCL-PHA consists of (*R*)-hydroxyalkanoate of C3-C5, while MCL-PHA is comprised of C6-C14. In our laboratory, one strain of *Pseudomonas* sp. TO7 production of approximate homo-polyoctanoate has been isolated. PHA containing functional side groups can improve their intrinsic novel properties greatly and leading to expansion of PHA applications... The purpose of this study is to design and produce unusual PHA with the expected structures, such as PHA containing double bonds as well as phenyl side groups. Mixed carbon source of sodium octanoate/11-undecylenic acid and sodium nonanoate/11-undecylenic acid as well as sodium octanoate/5-phenylvaleric acid with varies combination ratios were used for accumulation of unsaturated PHA or phenyl group bearing PHA in *Pseudomonas* sp. TO7*.* The mixtures of alkanoate and 11-undecylneic acid supported the growth and PHA production. The mixtures of octanoate (0.5%) and 5-phenylvaleric acid (>0.4%) supported the growth, but not PHA production. The ^{1}H NMR spectrum corroborates the composition and the expected structure of expected polyester produced by *Pseudomonas* sp. TO7. The number average molecular weights of all of the PHA obtained were approximately 200,000 Da and the polydispersity index was variety and approximate 3.0 in each case. The glass transition temperature and the melting temperature of P(HO-co-HU) and P(HN-co-HU) were near -45°C and 50°C respectively, as measured by calorimetric analysis. The glass transition temperature of P(HO-co-HPV) was -30°C. These novel PHAs containing double bonds in the side chains can be easily modified by chemical functionalization. Our results suggest that new PHA monomer based polymers can be designed and expected by feeding mixed carbon source with wild type strain *Pseudomonas* sp. TO7.

Keywords polyhydroxyalkanote; *Pseudomonas* sp. TO7; functional side groups

Molecular analysis of bacterial biofilm communities in response to environmental perturbations within drinking water distribution systems

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Drinking water distribution systems (DWDS) contain microorganisms which survive disinfection and under certain environmental conditions attach to the internal surfaces of pipes and form biofilms (Simões *et al*., 2007). Microbial biofilms can generate various problems in DWDS such as changes in water quality (e.g. discolouration, taste, odour), promotion of opportunistic pathogens survival and corrosion of pipes (Szewzyk *et al*., 2000; Beech and Sunner, 2004).

Most of the previous research in relation to microbial communities in DWDS has been based on culture-dependent methods, which underestimate the actual bacterial diversity within environmental samples (Amann *et al*.,1995). In order to circumvent the limitations of these methods we have applied two different DNA-based molecular techniques to study microbial biofilms in DWDS. Biofilm samples were obtained from coupons inserted into an internationally unique temperature controlled pipe-rig test facility at the University of Sheffield (Denies *et al*., 2010). To reproduce the dynamics occurring in real systems, the facility operated at three different hydraulic regimes (0.11 N/m^2, 0.22 N/m^2 and 0.44 N/m^2) and at a temperature of 8°C. DNA was isolated from biofilms formed on the pipe-rig walls after 28 days of growth and specific regions of the 16S rRNA gene were amplified by polymerase chain reaction (PCR). To characterize the bacterial communities inhabiting the biofilms, the amplified DNA was analysed using a molecular fingerprinting technique, terminal restriction fragment length polymorphisms (T-RFLPs), in combination with a high-throughput sequencing technique (pyrosequencing). At the end of the monitoring period, changes in the microbial community structure in response to a discolouration event were assessed.

The use of T-RFLPs allowed for the characterization of the dominant bacterial groups within biofilms under different hydraulic regimes. However, pyrosequencing analysis generated a more accurate description of the bacterial communities and is a more powerful technique to detect changes at species level in response to environmental change within DWDS.

This study yielded new knowledge of the microbial ecology within DWDS. Such knowledge is vital to improve current operation, control and management strategies to help safe guard drinking water quality and ultimately public health.

Keywords: drinking water distribution systems, biofilms, T-RFLPs, pyrosequencing.

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Molecular mass characterization of the capsular polysaccharide produced by *Haemophilus influenzae* type b during fermentation

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Haemophilus influenzae type b (Hib) is a Gram negative bacterium responsible for invasive diseases as pneumonia, bacteremia and meningitidis in infants, elders and immunodeficients. Prevention against Hib infections can be achieved with vaccination using the capsular polysaccharide as antigen, though it must be linked to a carrier protein to induce T dependent immune responses. The polysaccharide, a linear chain of ribosylribitol phosphate, is released into the culture broth. In this laboratory, purification process has been established based on tangential micro and ultrafiltrations, ethanol precipitation and enzymatic hydrolysis. Purification presents about 50% of polysaccharide concentration loss in the ultrafiltration steps using 100 kDa cut-off membranes, suggesting that the molecular mass of the polymer is low. In this work, we demonstrate that the molecular mass decreases over time during fermentation, which can be due to hydrolysis.

Fermentations were conducted on Bioflo2000 NBS bioreactors (10 and 5 liters) at 37°C and pH 7.5 using MP medium. Three base solutions were used to adjust pH during the culture, in order to evaluate the influence of alkaline hydrolysis on polysaccharide phosphodiester linkage: sodium hydroxide (pKa = 13.00), sodium carbonate (pKa = 10.33) and ammonium hydroxide (pKa= 9.25). Batches were fed either with concentrated medium or glucose 50% (w/v). Samples were collected for dry cell mass, polysaccharide concentration (by Bial`s method), organic acids and carbohydrates concentration (HPLC) and molecular mass determination (HPSEC).

Relative molecular mass values varied from nearly 1,000 kDa at 5 hours of fermentation to values as low as 350 kDa at later samples, decreasing constantly throughout the culture. Respective samples for the different base solutions had no significant variation, suggesting that local alkaline hydrolysis has little or no effect whatsoever. One specific batch was kept on a starvation state once glucose was totally depleted, presenting no variation on polysaccharide concentration over nearly 2 hours. Though, molecular weight profiles show a decreasing tendency during that period, as values diminish on size about 100 kDa. This is an evidence of a hydrolyzing agent in the broth, possibly an enzyme. Other batches were fed with pulsed glucose, and this addition seemed to momentarily cease the hydrolysis, suggesting that the agent might have its expression or activity regulated by the availability of substrate. Determination of this hydrolyzing agent and its activity will be considered for the enhancement of cultivation process to synthetize high molecular mass polysaccharide and improve the polysaccharide recovery on the purification process.

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Keywords: *Haemophilus influenzae* b, capsular polysaccharide, Molecular mass, size exclusion chromatography

Monitoring impact of carbamate pesticides on bacterial community structure within natural river biofilms using DGGE technique

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The technique of Denaturing Gradient Gel Electrophoresis (DGGE) has been successfully applied to analyze the genetic diversity of natural microbial communities corresponding to environmental variations. Bacterial community in river biofilms contributes significantly on river self-purification processes. Environmental stresses such as metals and organic pollutants have been found to influence the bacterial community diversity and function of river biofilms. Thus this study would like to employ DGGE technique to determine the effects of carbamate pesticides (i.e., methomyl, carbaryl and carbofuran) onto bacterial community structures within natural river biofilms under the condition of single, two-, and three pesticide systems.

The DGGE gels showed 5 to 19 distinguishable bands per gel strip for river biofilms. The Shannon-Weaver diversity index calculated on the basis of the number and relative intensities of bands on a gel track ranged from 1.34 to 2.81. With decreasing in concentrations of pesticides during the testing period, the number of bands and Shannon-Weaver diversity index decreased with time. It indicated that bacterial species in biofilms were sensitive to pesticides tested and resulted in collapse of microbial community diversity.

The cluster analysis of DGGE banding patterns revealed two major groups which corresponded to the influence of different pesticides to bacterial communities. The low similarity between biofilms without pesticides and with single pesticide or the mixture of carbaryl and carbofuran suggested that bacterial communities within biofilms were easily affected by the presence of these pesticides. DNA sequence analysis of the predominant 8 bands on the DGGE gel found three bacterial groups of α-proteobacteria, δ-proteobacteria and Firmicutes. *Bacillus cereus* AH820 (Firmicutes) and uncultured clone SP27 (δ-proteobacteria) increased significantly after adding the mixture of carbaryl and carbofuran for 3 days, indicating they were tolerant to this type of pesticide mixture and possibly had the ability to degrade these pesticides. These species may play an important role on biogeochemical elemental cycling in riverine systems.

Keywords: Denaturing Gradient Gel Electrophoresis; River biofilms; Bacteria; Carbamate pesticides; Shannon-Weaver diversity index; Tolerant

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Nitrate effect on sulfate reducing and nitrate reducing sulfide oxidizing bacteria in experimental bioreactors

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Sulfate reducing bacteria (SRB) are present in a wide range of environments like marine sediments, oil reservoirs and sewers. In anaerobic conditions they contribute to the water depuration by oxidizing organic matter with sulfate and with the release of sulfide, which is unhealthy, corrosive, damages the facilities of wastewater treatment plants (WWTP) and reduces the efficiency of the depuration process. Among the methods proposed to control sulfide production, nitrate addition is considered one of the most suitable, although the mechanism is scarcely understood. Our aims were to determinate the minimum effective nitrate concentration that reduces sulfide production, study the kinetics of nitrate and sulfide in experimental bioreactors and confirm the changes in the microbial community induced by nitrate addition.

Two pilot scale anaerobic bioreactors with separated atmospheric phases were set up at Guadalete-WWTP (Jerez, Spain). One of them was fed with nitrate at four different concentrations (0.12, 0.24, 0.50 and 1.0 mM) for periods of three days and the other used as a control. Physicochemical variables were measured in water and on air until initial sulfide concentration was recovered. Activity and presence of microorganisms implicated in the sulfur and nitrogen cycles were also analyzed by molecular biology techniques.

Biofilms were a net source of sulfide for the water and gas phases (7.22 ± 5.3 μmol·s^{-1}) in the absence of nitrate dosing. Addition of nitrate resulted in a quick (within 3h) decrease of sulfide both in the water and in the atmospheric phase. Sulfide elimination efficiency in the water phase increased with nitrate concentrations following Michaelis–Menten kinetics (Km = 0.63 mM NO_3^-). Suppression of nitrate addition resulted in a recovery or an increase of initial net sulfide production in about 3h. Species of nitrate-reducing sulfide-oxidizing bacteria like *Sulfurimonas denitrificans* and *Paracoccus denitrificans*, increased its activity during nitrate addition and the majority of sulfate-reducing bacteria, reduced its number with treatment. Results confirmed that nitrate efficiently stimulated sulfide oxidation by NR-SOB and reduced the effect of SRB, decreasing the concentration of dissolved H_2S in the water phase and consequently its release to the atmosphere.

Keywords: nitrate; sulfide; SRB; NR-SOB; wastewater; biofilm

Plasma induced modification of ultrafiltration membranes for viral removal in drinking water treatment

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Currently the plasma treatment is a tool widely used in surface modification of different materials to optimize their properties against a particular application. In the present work, plasma technique was used to deposit a layer of hydrophilic polymer on asymmetric ultrafiltration (UF) membranes. Theses membranes were synthesized for the phase inversion method. Polyethersulfone (PES) membranes were prepared using polyethylene glycol (PEG) as additive, with several values of molecular weight (400, 1000 and 10.000 Da), being dimethylacetamide (DMAc) the solvent and water as the non-solvent. The membranes synthesized were first submitted to plasma of argon gas, non – polymerizable, and subsequently other plasma atmosphere generated with argon and vapor of acrylic acid (AA). The aim was enhance hydrophilicity and uniform surface pores of the UF membranes leading to less fouling and subsequent flux enhancement. The characterization of the membranes before and after the surface modification was done using contact angle to determine its effect on the surface hydrophilicity. Also SEM and AFM images have been used to know the membrane morphology and Liquid-liquid Displacement Porosimetry (LLDP) was used to quantify the pore size distributions. The pure water permeability and tests of retention of viral model, bacteriophage PP7 using the host Pseudomonas aeruginosa, were measured showing a proper performance. Finally, the fouling was evaluated by measuring the relative water reduction (RFR). The membranes modified by plasma had high permeability and smaller RFR values after filtration with PP7.

Keywords*: Polyethersulfone ultrafiltration membrane, plasma treatment, membrane characterization, water disinfection.*

Polyhydroxyalkanoates production by a new isolated *Pseudomonas aeruginosa* from cassava wastewater utilizing soybean oil

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Nowadays, the total global capacity of plastic production increased from 1.5 million tons in 1950 to over 245 million tons. These materials are generally petrochemical-based polymers and so they are not biodegradable. Thus, the synthetic polymers are extremely persistent and accumulate in the ecosystems. It is very difficult to reduce the consumption of plastic products due to their versatile properties, from this is desirable the replacement of petroleum-based polymers with alternative material, that have plastic-like properties and degradability after being discarded. Among various types of biodegradable polymers, the polyhydroxyalkanoates (PHAs) are well known, they are microbial polyesters produced by a wide range of bacteria, being recognized as completely biosynthetic and biodegradable. *Pseudomonas aeruginosa* accumulates the medium-chain-length PHAs that are composed of 3-hydroxy fatty acid monomer units ranging in length from six up to fourteen carbon atoms. Plants oils are related as good carbon sources for PHA production by this bacterial specie, which has been isolated from waste materials and industrial byproducts. In this work, *P. aeruginosa* was isolated from cassava wastewater and identified by 16S rDNA sequence analysis. Colonies from nutrient agar incubated for 72h at 30°C was scraped to inoculate 100 ml of nutrient broth (3 $g.l^{-1}$ meat extract, 5 $g.l^{-1}$ peptone, pH 7.0 ± 0.1) at 30°C on a rotary shaker for 24h. The mcl-PHAs production experiments were carried out in 200 ml of mineral salts medium, containing $(NH_4)_2SO_4$ and soybean oil as a carbon source at concentrations between 1% and 5% (w/v). The shaker flasks were inoculated with 3.0ml of the nutrient broth culture and incubated at 30°C with shaking at 150rpm for 72h. After incubation, the cells were harvested by centrifugation (10600 x g, 10min, 4°C), washed twice with distilled water and lyophilized to a constant weight for PHAs determination. The cell dry weight was determined gravimetrically. The soybean oil concentration and residual oil was determined gravimetrically after hexan extraction of the acidified culture samples. For PHAs determination, samples of about 10mg of freeze-dried cells were subjected to propanolysis and the propyl esters were assayed by gas chromatography with Agilent 7890A GC System. In this work, *P. aeruginosa* showed maximum polymer yield ($1.42g.l^{-1}$) and PHA cellular productivity (35%) at 2% soybean oil. The composition analysis of the mcl-PHA showed a polymer composed primarily of monomers 3-hydroxydecanoate and 3-hydroxydodecanoate with low incidence of monomers 3-hydroxyhexanoate and 3-hydroxyoctanoate. In these experiments, increasing concentrations of soybean oil had an effect in the polymer composition with an increase of the monomers 3-hydroxyoctanoate and 3-hydroxydecanoate and decrease of the monomer 3-hydroxydodecanoate.

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Keywords: PHA; soybean oil

Polyhydroxybutyrate production from biodiesel glycerol by *Burkholderia cepacia*

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The polyhydroxyalkanoates (PHAs) are biodegradable polyesters that are synthesized intracellularly and deposited as granules in bacterial cultures in the presence of excess carbon source and a growth limiting nutrient. These biopolymers are attractive substitute for petrochemical-based plastics due to their similar material properties to various thermoplastics and elastomers. The PHB is the most common type of PHAs and this homopolymer of 3-hydroxybutyric acid has been studied extensively. Actually, the biofuels production is another great alternative to replace the petroleum, specially the biodiesel generated from the transesterification of vegetable or animal fats and oils. The crude glycerol is the main byproduct of biodiesel production, which has a relative low value due to the presence of impurities. The challenge in the PHAs production is the competitive prices of petroleum polymers. A solution for this problem has been proposed with the implementation of biorefineries that co-produce additional value-added products along with biodiesel, such as bacterial biopolymers production utilizing crude glycerol as a carbon source. Therefore, it is necessary to discover a new promising PHAs-producing bacterial strain. In this work, the *Burkholderia cepacia* was isolated from a tropical moist forest soil (Atlantic forest) in Ubatuba (SP, Brazil) and identified by 16S rDNA sequence analysis. Bacterial growth from nutrient agar incubated for 72h at 30°C was used to inoculate 100 ml of nutrient broth (3 $g.l^{-1}$ meat extract, 5 $g.l^{-1}$ peptone, pH 7.0 ± 0.1) at 30°C on a rotary shaker for 24h. The polymer production experiments were carried out by inoculating each test flask with 3.0ml of the nutrient broth culture in 200 ml of mineral salts medium, containing $(NH_4)_2SO_4$ and biodiesel glycerol as sole carbon source at concentrations between 1% and 5% (w/v). The erlenmeyers flasks were incubated at 30°C with shaking at 150rpm for 72h. After incubation, the cells were harvested by centrifugation (10600 x g, 10min, 4°C), washed twice with distilled water and lyophilized to a constant weight for PHAs determination. The cell dry weight was determined gravimetrically. The glycerol concentration was determined by liquid chromatography with Prominence UFLC apparatus (Shimadzu®). Samples of about 10mg of freeze-dried cells were subjected to propanolysis for PHAs determination.The propyl esters were assayed by gas chromatography with Agilent 7890A GC System. *B.cepacia* showed maximum polymer yield at 2% crude glycerol (2.23g. l^{-1}) with a PHB cellular productivity of 48.57% cell dry weight.

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Keywords: PHA; biodiesel glycerol

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Preparation and Characterization of Edible Film Based on Kefiran and Oleic acid

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Kefiran, a microbial polysaccharide obtained from the flora of kefir grains, is finding increasing use in the food industry as a texturing and gelling agent. It is a water-soluble polysaccharide containing approximately equal amounts of glucose and galactose. Recent studies have shown that high yields of these exopolysaccharides can be easily isolated from the grains in deproteinised whey. Thus exopolysaccharides from kefir grains might be an affordable alternative to synthetic packaging in food applications. In addition, kefiran has several important advantages over other polysaccharades, such as antibacterial, antifungal, and antitumour properties. Kefiran can produce films with good appearance and satisfactory mechanical properties. However, kefiran films are highly permeable to water vapour, which is an important drawback that limits its application, since the effective control of moisture transfer is a desirable property. In order to improve water barrier properties, hydrophobic compounds, such as lipids, are frequently incorporated into hydrocolloid-based films. New edible composite films based on kefiran and oleic acid (OA) at the ratio of 15, 25, and 35% (w/w) were prepared using emulsification with the aim of improving their water vapour barrier and mechanical properties. Film-forming solutions were characterised in terms of rheological properties and particle-size distribution. The impact of the incorporation of OA into the film matrix was studied by investigating the physical, mechanical, and thermal properties of the films. The water vapour permeability (WVP) of the emulsified films was reduced by approximately 33% by adding OA. The mechanical properties of kefiran films were also affected by adding OA: tensile strength was diminished, and elongation increased considerably. Differential scanning calorimetry showed that the glass transition temperature (Tg) of the kefiran film was -16°C and was not considerably affected by adding OA. Therefore, OA could be incorporated into these films for some food-technology applications that need a low affinity toward water.

Keywords: Kefiran; Edible film; Oleic acid; Water vapour permeability

Preparation Polypropylene and corn starch biocomposites: Investigation the mechanical properties, morphology and biodegradability

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The environmental impact of persistent plastic wastes is raising general global Concern and disposal methods are limited. In addition, petroleum resources are finite and are becoming limited. A biodegradable polymer that degrades more rapidly in the environment is a solution of this problem. Among the biomaterials present today in the market, those derived from renewable resources such as starch based products are the most widespread and economic biomaterials.

In this study, biodegradable composites of polypropylene/thermoplastic starch (PP/TPS) with different content of TPS (100/0, 90/10, 70/30, 50/50), in presence of 6 wt% Ethylene Vinyl Acetate (EVA) copolymer as a coupling agent, were prepared using co-rotating twin screw extruder. The mechanical properties, morphology and biodegradation of composites were investigated. Scanning Electron Microscope (SEM) was used for the investigation of the particle distribution and phase morphology before and after biodegradation. Biodegradation of composites were done in fungal culture medium and was assessed by SEM images, monitoring the structural changes in the FT-IR spectra and weight loss of specimens after 3 weeks culturing. The mechanical properties of tensile strength and Young's modulus increased with increasing in starch content but elongation at break decreased. SEM images revealed that with increasing starch content more holes found on the surface of specimens after biodegradation. In the FTIR spectra, decreasing in intensity of hydroxyl regions after biodegradation indicates that starch domains of blends have eroded by fungi. The biodegradation was also monitored by the increase in the carbonyl index. Increasing in starch content helped to speed up the weight loss of PP/TPS blends. The weight loss of PP/TPS (90/10), (70/30) and (50/50) blends were 1.0, 10.9 and 28.8%, respectively.

Keywords: Polypropylene, corn starch, biocomposites, biodegradability, morphology

Preparation, characterization and antibacterial activity of photocured thymol-doped acrylic resins

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Incorporation of antibacterial agents in coatings applied to food-packaging is an open approach to prepare active antibacterial packaging able to prevent the surface growth of bacteria and fungi and therefore the risk of food borne illness. In recent years there has been an increasing interest in the development of new types of effective and non toxic antimicrobial compounds which would not be considered as chemicals, but as natural ingredients, by the consumers. Among them, thymol, one of the most active antimicrobial constituents of essential oils, can be easily derived from plants (thyme and oregano) and it has been accepted by FDA and has received a positive opinion by Europe.

In this context, the preparation of thymol-doped antibacterial coatings based on a photo-crosslinkable diacrylic resin has been investigated. The antibacterial activity of thymol-doped coatings has been evaluated against Gram-positive (*Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. This activity is expected to depend on the thymol concentration in the coating and to its ability to move to the surface and to be released in the surrounding medium. For this purpose, release tests have been performed with the aim to give a quantitative description of the migration of thymol from the photocured diacrylic resin containing increasing amount of thymol into various media (air, water, ethanol, methanol, ethyl acetate and *n*-hexane) under different conditions.

Keywords antibacterial coatings; thymol; photocured resins; food-packaging

Production and chemical composition of extracellular poymers produced by *Bacillus subtilis*, *Bacillus licheniformis*, *Bacilllus pumilus* and *Bacillus* sp.

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Four marine fouling bacterial strains, *Bacillus subtilis* (CE-2), *Bacillus licheniformis* (Bac), *Bacilllus pumilus* (SS-14) and *Bacillus* sp. (Ti-28) were examined for their extracellular polymer (EPS) production ability at the laboratory level. These carbohydrate rich polymers help microbial communities to tolerate extreme conditions and biofilms formation. These polymers contain acidic polysaccharides such as uronic acids. The carbohydrates and uronic acids were produced during logarithmic and stationary phases of growth and were higher during the latter growth phase. The present study demonstrates that *Bacillus subtilis* (CE-2), *Bacillus licheniformis* (Bac), and *Bacilllus pumilus* (SS-14) are effective producer of EPS, which are rich in uronic acids. The capillary gas chromatographic analyses revealed the presence of glucose, galactose, mannose, xylose, arabinose, ribose, fucose in the EPS isolated from *Bacillus* spp. Glucose was most abundant (38 to 94 mol %) monosaccharide present in the EPS produced by *Bacillus* spp. Monosaccharide compositions of EPS produced by four bacterial strains were chemically different. EPS was heteropolysaccharides and acidic in nature.

Key words: Extra-cellular polymers, Carbohydrates, Uronic acids, Monosaccharides, *Bacillus* spp.

Production and Molecular Characterization of Bioplastic Producing Bacteria Using Mustered Oil and Potato Extract

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Biodegradable plastics from renewable resources, such as PHAs, are alternative to petroleum-based plastic materials, which are non-biodegradable. Biodegradable plastic was extracted from bacterial strains, which were isolated from different contaminated environments of Pakistan. The characterization of bioplastic producing bacteria isolated from minimal environmental conditions and to develop a biological process to produce PHAs from cheap carbon source. Bacterial strains were isolated and purified from different regions of the Pakistan. Strains were isolated from industrial and domestic waste water and sewage sludge. Fermentation and PHA production was done in batch culture in 5 liter fermenter to optimize the PHAs production under various experimental conditions. Samples subjected to PHA production were checked by using inoculating on PHA detection agar and PHA broth. Quantitative analysis for biodegradable plastic produced by different bacterial species was performed by Modified surfactant hypochlorite. High PHA production was detected in strains belonging to *Pseudomonas, Bacillus* and *Enterobacteriales* as predominant genera. PHA production under different carbon sources, nitrogen concentration, pH and temperature was estimated. The PHA production of *Pseudomonas aeruginosa* by mustered oil cultivation was studied under six experimental conditions, such as air flow rates, pH, Temperature, Optical density, substrates concentration and dry cell weight. The isolated strains of *Pseudomonas* showed the ability to synthesize polyhydroxyalkanotes in batch culture. Synthesis of PHA was observed in exponential growth and it depended on carbon/nitrogen ratio in the culture. It can be concluded that the PHAs storage capacity was higher two to three times in aerobic compared to anoxic conditions. PHA production of *Pseudomonas* species were subsequently authenticated at molecular level by PCR analysis and the *phaC* gene (540 bp) encoding PHA synthase was amplified. After *phaC* gene sequencing and *16SrRNA* ribotyping, most of the *phaC* gene containing strains showed homology to *Pseudomonas aeruginosa*. Positive samples yield a specific 540-bp PCR product representing partial coding sequences of the phaC1/C2 genes. PHA polymerase 1 (*PhaC1*) and PHA polymerase 2 (*PhaC2*) from *Pseudomonas aeruginosa* were screened and sequenced and submitted to gene bank. with accession number *bankit1367455, bankit1367459* and *bankit1367463*. *PhaC* gene operon was amplified. PHA Polymerase synthaseI (*PhaC*1) was amplified by 179-L primers with ORF primers for *Pseudomonas aeruginosa* gave the PCR product of 1300 bp while PHA polymerase synthase II (*PhaC*2) was amplified by combining 179-R with ORF primers for *Pseudomonas* gave the PCR product of 1700 bp.

Production of crude gellan gum powder from deproteinized whey and use as a novel thickener/viscosifier/stabilizer in solutions and food products

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OBJECTIVES: The utilization of deproteinized cheese whey as a substrate for an efficient production of gellan gum, and the application of the crude lyophilized product (which is also rich in protein, vitamins and minerals) as a new food thickener/viscosifier/stabiliser with low production cost and multiple technological advantages.

METHODOLOGY: Deproteinized cheese whey was used a substrate for the growth of Sphingomonas paucimobilis, producer of gellan gum. The microbial strain, which normally grows on glucose, was gradually adapted to lactose after repeated liquid and solid cultures in lactose media, before used as inoculum. Fermentations were carried out in shake flasks and in 15L fermentor in order to optimise gellan biosynthesis on whey medium. The "deproteinized" whey (produced after ultrafiltration and removal of proteins) was further deproteinized in some samples after a preheating process (100C x 30´) prior to sterilization to remove any remaining proteinaceous compounds. The effect of agitation, temperature, lactose concentration, glycerol addition, fed-batch processes, bi-staged processes (with a pH or temperature shift), etc upon gellan and biomass production, sugar utilisation, respiratory quotient (RQ) and broth intrinsic viscosity was investigated. The entire fermentation broth, which is very viscous after the end of the fermentation, was lyophylized and tested for its viscosifying/stabilising ability and compatibility in water solutions and in yoghurt production at several concentrations (0.1-2.0%).

RESULTS: The gradual adaptation of S. paucimobilis to lactose concentrations, as well as the complete removal of proteins in whey was crucial in attaining a high gellan biosynthesis (approximately double gellan concentration). Previous studies focused on gellan production using deproteinized whey had encountered a bottleneck limiting gellan synthesis, possibly due to high lactose concentration (~45 g/l in whey) and high nitrogen content. In our study, the complete removal of proteins (which are useful for growth, but limit gellan biosynthesis) in fully-deproteinized whey (FDP) and the dilution of whey (50% with water) led to higher gellan concentrations and complete sugar utilisation. Fed batch processes with one or two stages of addition of FDP did not improve gellan accumulation in the fermentation broth, in contrast with previous result with glucose-based media, showing that the organism is sensitive to high concentrations of whey-based media. However, when additional pure lactose was supplemented to 50% diluted FDP, gellan production rate and broth viscosity increased significantly, showing that it is not the lactose but another substance in the deproteinized whey (e.g. salts and minerals) that hinders gellan biosynthesis. Interestingly, the addition of 5g/l glycerol in the FDP medium doubled the viscosity of the process medium and led to a distinct increase in growth rate and gellan formation rate (maximum gellan concentrations was observed in only ~30h as compared to ~60h in the process without glycerol addition). The process with 50% FDP and 5g/l glycerol was optimal for crude whey-based gellan production (12g/l of gellan), and the viscosity of this broth was equivalent to that obtained with the industrial, synthetic glucose-based medium (over 200mPas at 200rpm). This is probably because glycerol may act as a precursor of lipid carriers for gelllan biosynthesis, and also improves mass transfer in the fermentation broth. After the end of the fermentation the yellow, viscous, sterile, crude gellan broth was lyophilized and the powder was added in water solutions and yoghurt desserts. The results showed that the viscosifying ability of 1% - 2% crude whey-based gellan is comparable to the effect of a 0.1 - 0.2 % of a pure gellan gum powder, showing a potential for an industrial applications. Additionally, the whey-based crude gellan powder offered a nice, distinct flavour which received a better sensorial score compared to the commercial pure gellan powder, probably due to the content of whey aromatic compounds.

CONCLUSIONS: These results show that a novel crude polysaccharide product produced from the waste of dairy industries can be obtained via the optimized fermentation of fully deproteinized whey, especially after glycerol addition and microbial strain adaptation. This novel crude gellan product, which needs no purification steps, and no synthetic media, offers great sensory attributes (as concerns texture and flavour) and can act as an efficient thickener and stabiliser in solutions and food products, where it may replace pure gellan (or other thickeners), which is costly and scarcely used in the food industry, partly due to high separation-purification costs.

Keywords: crude gellan gum, deproteinized whey, viscosity, food thickener

Production of fructooligosaccharides by *Bacillus subtilis* natto

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Fructooligosaccharides (FOS) are oligosaccharides of fructose containing a single glucose moiety. FOS are produced by the action of fructosyltransferase (FTase) found in many plants and microorganisms. FOS have a number of interesting functional properties that make them important food ingredients, such as modulator of lipid metabolism, increase intestinal calcium absorption, non cariogenic effect and are prebiotic as selectively stimulate the growth and/or activity of potentially health-enhancing intestinal bacteria. The present strain of *Bacillus subtilis* natto was isolated at Department of Biochemistry and Biotechnology of State University of Londrina from a traditional Japanese fermented food and it is able to selectively produce levan in rich sucrose medium. The levan production is due to the enzyme levansucrase – Ftase. The presence of this enzyme enables *B. subtilis* natto as a potential producer of fructooligosaccharides. The aim of this work was to evaluate by statistical methodology, using a central composite factorial design, the influence of the following variables: sucrose concentration (X_1), pH (X_2) and agitation (X_3) at three levels: 250, 300 and 350 g/L; 5, 6 and 7 and 100, 200 e 250 rpm, respectively. The fermentation was carried out in 250mL Erlenmeyers flasks containing 50 mL of culture medium. Flasks were inoculated with 0.2g/L of cells and they were incubated in a rotary shaker at 37°C for 24hours. The others parameters were adjusted according to the factorial design. After fermentation the reducing sugar were analyzed by Somogyi-Nelson and total sugar by Dubois method. The production of fructooligosaccharides was accompanied by high performance liquid chromatography (HPLC) with a refractive index detector RID 10-A (Shimadzu) under the following conditions: column Bio-Rad HPX 87C; temperature 80°C, mobile phase, water; flow rate, 0.6 mL/min. using 1-kestose and nystose as standard. All statistical analyses were conducted using STATISTICA7.0 (data analyses software system by StatSoft, Inc. 2004, USA). The statistical analyzes showed that pH was significant on FOS production and had a positive effect on it. The prediction of optimized condition for maximum production was sucrose concentration of 342g/L, pH 7.7 and agitation of 207 rpm, and which the production expected was of 93.41 g/L of fructooligosaccharides. The validation of optimal condition has been confirmed.

Keywords: *Bacillus subtilis* natto, fructooligosaccharides, levansucrase

Quorum sensing-, efflux pump- inhibition and matrix-dispersing enzyme treatments as potential control strategies to limit *Flavobacterium johnsoniae*-like biofilm formation

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The biofilm-forming ability of *Flavobacterium johnsoniae*-like isolates associated with outbreaks of fish disease in South Africa is linked to their pathogenicity and persistence, which facilitates their survival in aquaculture systems. The effect of matrix-dispersing enzyme, DNase I; quorum sensing inhibitors [(Z-)-4-bromo-5-(bromomethylene)-2(5H)-furanone, S-adenosylhomocysteine, and *trans*-cinnamaldehyde] and efflux pump inhibitors [1-(1-naphthylmethyl)-piperazine (NMP) and phenylalanine arginine β-naphthylamide (PAβN)] on initial attachment and detachment from mature biofilms of 15 *F. johnsoniae*-like isolates and five *Flavobacterium* sp. type strains, were investigated using modified microtiter plate assays. Treatments were added at the time of inoculation to determine their effect on initial attachment and to pre-formed biofilms to determine their detachment effect. The percentage biofilm reduction was also assessed for each treatment. Decreased adhesion was observed for *F. johnsoniae*-like isolates, following treatments at the time of inoculation, in the following order: DNase I > furanone > *trans*-cinnamaldehyde > S-adenosylhomocysteine > PAβN > NMP. However, increased detachment from mature biofilm was observed in the following order: *trans*-cinnamaldehyde > S-adenosylhomocysteine > furanone > PAβN > NMP> DNase I. The specific treatment results obtained were isolate-specific, especially in the initial attachment assays due to different phenotypes, genotypes, origin and biofilm-forming abilities of these isolates. Individual isolates displayed statistically significant differences following treatments. The isolate-specific response to treatments was evident when critically examining two isolates YO64 (a strong biofilm-former) and YO12 (a poor biofilm-former), which demonstrate differential gene expression in both planktonic and in biofilm states of growth using two-dimensional gel electrophoresis and selective subtractive hybridization. For *F. johnsoniae*-like isolates the most effective biofilm removal strategy was cinnamaldehyde exposure, while DNase I treatment was most effective to prevent attachment and thus biofilm formation. Thus alternative strategies to antimicrobial agent use could be proposed to control biofilm formation by *F. johnsoniae*-like isolates, to potentially limit outbreaks of disease in aquaculture systems.

Keywords: biofilm inhibition, *Flavobacterium* spp. DNase I, quorum sensing inhibitors, efflux pump inhibition

Relation between different pathogenicity factors of *Staphylococcus epidermidis*

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Background: The infections related with artificial devices implanted in the human body are very frequent and constitute a serious problem at present. *Staphylococcus epidermidis* is an important cause of these infections, which are attributed to its ability to adhere and form a multilayered *biofilm* on polymeric surfaces. For this reason, it is necessary to know the relation between the pathogenicity factors to develop subsequent measures that inhibit the adherence and microorganisms organization process in *biofilms*. Our objectives were 1) to characterize genetically the *S. epidermidis* strains included in this study, and detect to the genes related to adhesion and *biofilms* formation; 2) to analyze the bacterial superficial characteristics, such as cellular surface hydrophobicity (CSH); 3) to determine the capacity of *biofilm* formation and the composition of the same; and 4) to study the initial adhesion phase of *S. epidermidis*, differentiating between bare artificial surfaces and those previously covered with proteins derived from the host.

Methods: A total of 29 *S. epidermidis* strains were used. The amplification of genes was made by PCR with specific primers. Quantitative measurement of *biofilm* production was determined. PIA/PNAG production using antibodies specific by Dot-blot and the different *biofilm* components of *S. epidermidis* by microtiter detachment assay with dispersin B (Dsp B), sodium metarperiodate ($NaIO_4$) and proteinase K (PK) was carried out. CSH was assessed using the MATH (Microbial Adhesion To Hydrocarbons) method. Adhesion to polystyrene was studied, under different conditions.

Results: In *icaADBC*-positive strains a greater prevalence exists of the *aap*, *bhp*, *mecA* and *IS256* genes, which are predominant in the invasive *S. epidermidis* strains. The majority of *icaADBC*-positive strains are *biofilm* producers, although the presence of this operon in the *S. epidermidis* genome does not always indicate which of these strains produce "in vitro" *biofilm*. Dot-blot experiments reveal an accumulation of PIA/PNAG in *icaADBC*-positive *biofilm*-producing strains. Also, these *biofilms* are resistant to removal by PK but sensitive to detachment by DspB and $NaIO_4$, suggesting that a polyssacharidic compound is responsible for the *biofilm* produced by these strains. A greater proportion of hydrophobic strains between the non-*biofilm*-producing strains is observed. No correlation between unspecific or specific adhesion with *biofilm* production or CSH of *S. epidermidis* strains was detected.

Conclusions: In all *S. epidermidis* strains that contained *icaADBC* operon and were *biofilm* producers, this *biofilm* was constituted mainly by PIA/PNAG. Conversely, the strains that lacked *icaADBC* operon and was *biofilm*-producing, this *biofilm* being rich in proteins and other polysaccharides. The exopolysaccharide produced by *S. epidermidis* increases hydrophilicity of the cellular surface. The hydrophobic surface could facilitate adhesion and later colonization of biomaterials by non-*biofilm*-producing strains, and would explain the pathogenicity of the same, traditionally considered as less pathogenic. The unspecific initial adhesion of *S. epidermidis* to biomaterials was not exclusively dependent on CSH, but was also influenced by other factors. The mechanism of the adhesion process is very complex.

Keywords: *Staphylococcus epidermidis*; Biofilm; Medical-device infections.

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Resistance to the thermochemical disinfection of the spores of *Bacillus cereus* organized in biofilm on a stainless surface

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After adhering to the surfaces in dairy processing, the bacteria form biofilm. Generally this bacterian biofilm gives a greater resistance to disinfectants. The main aim of this study is to evaluate the efficiency of the thermochemical disinfection on an experimental biofilm formed on stainless steel surfaces.

One strain was of *B. cereus* used in this study: *B. cereus* ATCC11728.

Overnight precultures were used for inoculation. The cultures of spores were performed in a medium with following composition (10 g /L peptone, 10 g /L yeast extract and 10 g /L glucose in distilled water). At 30°C with slow shaking, the cultures lasted for 5-7 days. The vegetative cells were killed by heating for 10 min at 80°C. The cultures were then harvested by centrifugation (9630×*g* for 10 min at room temperature) and washed three times with distilled water. The suspension thus obtained was stored at 4°C until use **Ahimou and al (2001)**.

Stainless steel chips were prepared according to the procedure of **Ren and Frank (1993)** with little modifications. Stainless steel chips (5x1cm) were submerged in a 1/1(v/v) mixture of ethanol and acetone for 1 h to remove grease. The chips were then rinsed with distilled water, immersed in 2% NaOH solution for 5 min at 75°C, rinsed again with distilled water, immersed in 1% HNO_3 for 5 min at 75°C, and then given a final rinse with distilled water. For sterilization, the stainless steel chips were autoclaved at 121°C for 15 min.

Stainless steel were then placed in the suspension for 4h at 30°C.The suspension was then removed and replaced with Luria broth 1/10 .The stainless steel samples with spore attached for 24h at 30°C. After this incubation the stainless steel were washed with distilled water to eliminate the not adhered spores.

Samples were serially diluted with physiologic solution and the detached spores were enumerated on nutriment agar after 24h of incubation at 30°C.

We treated the biofilm of *Bacillus cereu*s (24h) by 2% of sodium hydroxide at 70 °C for 10 min, after this step the coupons of stainless steel rinsed, and we treated by Divosan QC® at different temperatures (70,90 and 110°C), contact time (5,10 and 15 minutes),and concentration (0,6, 0,8 and 1%). Inhibitor-mediated reduction of biofilm formation was correlated to the value obtained without addition of the compounds.

The results of each combination are like these :(0, 6%, 70°C, 5min) 1,107 log_{10}, (0, 6%, 70°C, 10min) **1,162** log_{10}, (0, 6%, 70°C, 15min) **1,243** log_{10}, (0, 6%, 90°C, 5min) **1,215** log_{10}, (0, 6%, 90°C, 10min) **1,243** log_{10}, (0, 6%, 90°C, 15min) **1,274** $log_{10,}$ (0, 6%, 110°C, 5min) **1,285** log_{10}, (0, 6%, 110°C, 10min) **1,391** log_{10}, (0, 6%, 110°C, 15min) **1,527** $log_{10,}$(0, 8%, 70°C, 5min) **1,120**log_{10}, (0, 8%, 70°C, 10min) **1,193** log_{10}, (0, 8%, 70°C, 15min) **1,408** log_{10}, (0, 8%, 90°C, 5min) **1,262** log_{10}, (0, 8%, 90°C, 10min) **1,374** log_{10}, (0, 8%, 90°C, 15min) **1,483** $log_{10,}$ (0, 8%, 110°C, 5min) **1,359** log_{10}, (0, 8%, 110°C, 10min) **1,425** log_{10}, (0, 8%, 110°C, 15min) **1,563** log_{10}, :(1%, 70°C, 5min) **1,193**log_{10}, (1%, 70°C, 10min) **1,343**log_{10}, (1%, 70°C, 15min) **1,453** log_{10}, (1%, 90°C, 5min) **1,366** log_{10}, (1%, 90°C, 10min) **1,453** log_{10}, (1%, 90°C, 15min) **1,463** $log_{10,}$ (1%, 110°C, 5min) **1,471** log_{10}, (1%, 110°C, 10min) **1,550** log_{10}, (1%, 110°C, 15min) **1,692** log_{10}.

The effect of thermochemical disinfection applied to the *Bacillus cereus* spores in biofilm is strongly linked to the temperature and contact time. It has a major effect in contrast to the increased concentration of the chemical agent,

Key words: Biofilm, cleaning and thermochemical disinfection spores, *Bacillus cereus.*

Single and sequential application of electrolyzed oxidizing water with benzalkonium chloride or peracetic acid for removal of *Staphylococcus aureus* biofilms

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Staphylococcus aureus is a common food-borne pathogen that can form biofilms on surfaces of food processing plants. Biofilms protect bacteria against environmental stresses, such as the application of disinfectants, and this increases the risk of food contamination. Improper disinfection can lead additionally to emergence of biocide resistance. Electrolyzed oxidizing water (EOW) has been regarded as a promising sanitizer in recent years. EOW has been reported to be less detrimental against environment and users than traditional disinfectants.

The present study has been therefore aimed to evaluate the potential application of EOW against biofilms formed by *S. aureus* on stainless steel surfaces.

Firstly, the effectiveness of acidic, neutral and alkaline EOW (pHs 3.0, 6.0 and 8.0, respectively, 500 $\mu g \cdot mL^{-1}$ active chlorine) against 48h-biofilms formed by four different *S. aureus* strains (St.1.01, St.1.04, St.1.07 and St.1.08) was evaluated. Logarithmic reduction in biofilm viable cells was the highest for acidic EOW in all cases, though differences were rather small (4.61, 4.50 and 4.38 log $UFC \cdot cm^{-2}$ for acidic, neutral and alkaline EOW, respectively). The resistance of biofilms formed by the different strains to all three EOW decreased in the order St.1.01 > St.1.07 > St.1.04 > St.1.08.

Acidic EOW was also most effective against planktonic cells, with minimal bactericidal concentration (MBC) ranging from 450-500 $\mu g \cdot mL^{-1}$. MBCs of neutral EOW were higher, ranging from 650-700 $\mu g \cdot mL^{-1}$. Planktonic cells of St.1.01 were the most resistant to both acidic and neutral EOW. In comparison, the maximum active chlorine concentration that could be produced in alkaline EOW (550 $\mu g \cdot mL^{-1}$) was too low for MBC values to be determined. For the same reason, minimum biofilm eradication concentration (MBEC) of acidic, neutral and alkaline EOW could not be determined. Thus, biofilms were clearly much more resistant to EOW than planktonic counterpart cells.

Next, given the highest efficiency of the production unit (Envyrolite EL-400) when neutral EOW was generated and that differences between acidic and neutral EOW were small, the effectiveness of neutral EOW (800 $\mu g \cdot mL^{-1}$ active chlorine) against 48 h-old St.1.01 biofilms was assessed at different exposure times (5-30 min) with the aim of reducing the period of disinfection. An exposure time of 5 min active reduced biofilm viable cells by approximately 4.36 log $UFC \cdot cm^{-2}$, whereas 20 min were needed for logarithmic reduction to be higher than 5 $UFC \cdot cm^{-2}$ after 20 min.

Lastly, the effectiveness of sequential application of neutral EOW and either benzalkonium chloride (BAC) or peracetic acid (PA) (5-min each) against 48 h-old *S. aureus* St.1.01 biofilms was examined in order to enhance the potential application of EOW as a disinfectant.

By using a first order rotatable factorial design, the effects of neutral EOW, BAC and PA were characterized by a positive first-order term and a negative second-order term in all cases. Sequential application was highly effective in all cases, with reductions over 5-log units in a large part of experimental range. Differences between applications were rather small. The effect of neutral EOW was very similar whether applied before or after BAC or PA. In contrast, PA seemed to depend upon the sequence of application, and was thus more effective if applied secondly. When first, no advantage over neutral EOW was noted. BAC showed a slightly higher effect than neutral EOW, particularly when applied first. Interestingly, a significant negative interaction effect was found for all cases.

When EOW was applied twice, no rinsing was needed. As a result, low concentrations of EOW had a higher effect than when applied with BAC or PA. The advantage is clear, but it must also be considered that using only EOW could facilitate the emergence of bacterial resistance.

Keywords *Staphylococcus aureus*; biofilm; electrolyzed water.

The action profile of intrinsic antimicrobial polymers at conditions typical for the perishable food chain

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Sustainable antimicrobial (SAM) polymers are a new class of functional surfaces (Kossmann and Ottersbach 2009). According to Buranasompob (2005) these polymers are intrinsic antimicrobial thus not migrating out of the surface. Furthermore, the mammal toxicity of this material is very low (Buranasompob, 2005). Thölmann et al. (2003) reveal that the antimicrobial activity of these polymers is based on the helical structure and the high concentration of functional amino groups and their three dimensional structure. First investigations provide good antimicrobial properties against a range of microorganisms (Buranasompob, 2005). However, till now there are no scientific data concerning the applicability of SAM polymers in contact with food.

Thus the objective of this study was the investigation of sustainable antimicrobial surfaces in terms of their ability to reduce surface bacteria in contact with perishable food.

The antimicrobial activities of SAM polymers were analyzed by adapting the test method JIS Z 2801 (2000) to the special application conditions in contact with perishable foods. Hence, the influence of temperature, time, bacterial strain, food residuals and the composition of different material modifications on the rate of antimicrobial activity has been investigated. As a comparison material for the rate of activity, prevalent used surfaces containing silver sulfadiazine were applied.

Investigations showed a high antimicrobial capability of SAM polymers. The high extent of antimicrobial activity of these materials becomes obviously by comparing it with the rate of activity of surfaces containing silver sulfadiazine. Within two hours the SAM-polymer prototypes are able to reduce the surface concentration of *Staph. aureus* about 4 log CFU/ml at 35°C. In the same time no reduction can be observed on silver containing surfaces. After eight hours, silver containing surfaces showed a reduction rate of only 2 log CFU/ml.

These differences are even more significant at chilled temperature. At these temperatures SAM polymer prototypes reduced bacterial counts of *Staph. aureus* about 4 log CFU/ml after only two hours of incubation, whereas silver containing surfaces showed a low reduction of 0.5 log CFU/ml after 48 hours incubation. Also the factors food residuals and variations in the bacterial strain affect the antimicrobial effectively of SAM polymers in a minor way than that of surfaces containing silver.

The results show the high reduction potential of SAM polymers. The material shows great promising for a wide range of applications, e.g. in the sanitary sector, in medical applications or food packaging. However, before introducing SAM-polymers to these areas, further developments of the physical and chemical properties are necessary.

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Keywords antimicrobial surfaces, food contact materials, polymers, hygiene

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The control of oral Streptococci biofilm formation by the help of probiotic strains

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Oral Streptococci especially *Streptococcus mutans* are the major cause of dental caries and periodontal diseases. Going along with the increasing antibiotic resistance of bacteria and also biofilm forming bacteria, new methods for decreasing of oral cavity pathogens must be investigated. The aim of this study was to determine the effect of *Lactobacillus fermentum ATCC9338*, *Lactobacillus acidophilus DSM 20079* and *Lactobacillus rhamnosus ATCC* 7469 as probiotic strains on the adhesion of oral streptococci as the pioneer strains of biofilm formation to the tooth surfaces.

S.mutans ATCC35668 and other oral streptococci isolated from dental plaque were studied. The ability of biofilm formation was investigated with colorimetric method and the strongest isolates were selected. Then the effect of probiotic strains on the adhesion of streptococci isolates were determined in polystyrene microtiter plate simultaneously (m1) and 30 minutes before streptococci entrance to the system (m2).

The results showed that in the presence of probiotic strains the streptococcal adhesion, then it`s biofilm formation, were reduced. The highest adherence reduction of mutans streptococci (31.44%) and non mutans streptococci (23.52%) were related to the presence of *L. acidophilus* and *L. fermentum*, respectively. The adherence reduction of streptococci was significantly stronger if the probiotic strain inoculated to the system before the oral bacteria. The effective pattern of probiotic strains on the adherence of mutans and non mutans Streptococci were as follows:

On mutans Streptococci: L. acidophilus> L.rhamnosus > L. fermentum

On non mutans Streptococci: L.fermentum> L.rhamnosus> L. acidophilus

It can be concluded that Adhesion reduction is likely due to bacterial interactions and colonization of adhesion sites with probiotic strain before the presence of streptococci. Adhesion reduction can be an effective way on decreasing dental plaque formation and also the cariogenic potential of oral Streptococci.

Key words: Biofilm, Dental plaque, Probiotic, Streptococci

The degradation potential of carbamate pesticides by natural river biofilms in single and multi pesticide systems

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River biofilms form on almost submerged surfaces and have diverse microbial communities which may provide highly active microorganisms and extensive surface areas for sorption and metabolism of contaminants. The wide spread use of carbamate pesticides in agriculture has led to their presence in different environmental compartments such soil, water and air, posing an ecotoxicological threat to organisms. Thus, this study would like to investigate the degradation capacity of river biofilms to carbamate pesticides in single, two- and three-pesticide systems. The target carbamate pesticides were methomyl, carbaryl and carbofuran.

In the river die-away test, removal of methomyl and carbofuran in the presence of natural river biofilms was higher than those without biofilms, indicating that biofilms play a significant role on the removal of these pesticides. Maximum removal by river biofilms was 99.2% for methomyl, 54.1% for carbofuran, and 0% for carbaryl. No pesticide was detected in biofilms at the end of experiment, reflecting the removed pesticide was degraded by microorganisms within biofilms. Removal of methomyl by biofilms was not affected by the presence of the other pesticides. However, an increased trend of removed carbaryl and carbofuran was observed in the three-pesticide system in contrast to the single pesticide system.

Experimental data fitted well ($p<0.05$) with the first order model except for the data from control set containing methomyl(i.e. pesticide addition without biofilms). Experimental groups (with biofilm addition) showed higher dissipation rates (k =0.12-1.15/day) and lower half lives ($t_{1/2}$ =1-6 days) of pesticide removal than control groups (without biofilms, k =0.02-0.45/day and $t_{1/2}$ =2-28 days). It revealed that biofilms would take part in and speed up the pesticide removal from sterilized river water. After adding the other pesticides, the dissipation rates of methomyl decreased. However, the dissipation rates of carbaryl with biofilm addition increased in two-pesticide system compared to that in single pesticide system. The results suggested that microorganisms within biofilms behaved differently to pesticide removal in the presence of the other pesticides.

Keywords: River biofilms; Carbamate pesticides; Removal; Degradation; First order model

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The role of van der Waals forces on the dynamic adhesion of bacteria

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Bacterial adhesion is –after protein adhesion- the second step in the formation of biofilms and can be a crucial problem in everyday life, healthcare and industrial application. The bacterial adhesion process is complicated and depends on many factors, e.g. on surface properties such as surface chemistry, hydrophobicity and surface roughness, which influence mainly the short-range interactions, and on subsurface properties that effect the long range van der Waals (vdW) forces. Most studies primarily discuss the influence of the surface properties and prevalently overlook the structure of composite materials. We focused on the effect of the subsurface composition on static bacterial adhesion and dynamic bond maturation.

In order to tune the long-range vdW interactions, we used Si wafers with oxide layers of 2 nm and 150 nm thickness. Both substrates have the same surface properties, but different vdW potentials due to their different subsurface composition. By hydrophobizing both wafer types with a self assembled silane monolayer we obtained a second pair of substrates with a different surface chemistry.

To probe the bacterial adhesion, we performed AFM force spectroscopy measurements using tipless cantilevers covered with bacteria. This allows for statistical measurements of the maximum adhesion force between the bacteria and the substrates. Immobilization of the bacteria was achieved by two ways: electrostatic fixation by Poly-L-Lysine and covalent binding with Polydopamin. Both fixation types do not alter the bacterial surface structure. That way, bacterial adhesion is tested for a pseudo-planctonic state.

As model bacteria, we used the nonpathogenic species *Staphylococcus carnosus* and the pathogenic *Staphylococcus aureus*.

The determined adhesion forces reveal significant differences due to the different subsurface composition. The force on the wafers with the thin oxide layer is about two times stronger than on the wafers with the thick oxide layer. This ratio was observable on both, hydrophilic and hydrophobic wafers. By varying the contact time between bacteria and substrates, we could moreover observe differences in bond maturation due to the different substrates. These findings suggest that i) surface properties (e.g. surface energy) are not sufficient to describe bacterial adhesion and ii) van der Waals forces have to be taken into account.

Keywords bacterial adhesion; van der Waals forces; bond maturation

The use of composite fibers for production of biomass carriers

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Growth in biofilms is a general ability of microbial populations which are historically used in wastewater treatment. The basic aim of biofilm formation is the fixation of microorganisms at a given place as a means of stabilizing living conditions. In a biofilm environment, organisms are protected against negative environmental influences. Compared to dispersion growth, biofilms offer many advantages that allow their use in specific biological treatment of industrial wastewater. The crucial advantage is the increase in the residence time of biomass in biofilm reactors allowing concentrations of slow-growing microorganisms and the diffusion barrier of the biofilm which reduces the effects of toxicants and suboptimal physico-chemical conditions. The development of the biofilm depends on many factors, from the surface properties to the supply of nutrients and hydrodynamic forces in the bioreactor. The objective of many research projects in the field of wastewater treatment is to technologically improve the biomass carrier. The final requirements are excellent colonization, high cleaning efficiency given the maximum specific surface area, optimal density and ease of production. Based on these crucial parameters the Technical University of Liberec has begun the development of a new type of carrier which is based on the use of polymeric nanofiber materials. This has resulted in a yarn which consists of a carrier fiber with a layer of nanofibers (the diameter of the nanofibers is in the order of hundreds of nanometers). The clear advantages of nanotechnology are a high protected specific surface area which promotes the microbial population during initial adhesion to the surface of the carrier and also during future development, protection of the population from surrounding adverse effects, promoting the supply of nutrients and supporting the compactness of the biofilm. The aim of the article is to highlight the possibilities and potential of biofilms with the presence of a composite nanofiber carrier.

Keywords nanofibrous technology; carrier of biomass; immobilization microorganisms; biodegradation; wastewater treatment

Universal system for cultivation and proteomic studies of bacterial biofilm communities

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Gene expression changes accompanying formation of bacterial biofilm communities are efficiently studied using the proteomic approach. There, the quantitative and reproducible way of protein samples preparation is an obvious prerequisite. Similarly, the studies of major attributes of biofilms, such as cell signalling and antibiotic resistance, require availability of flexible and easy to modify laboratory cultivation system. To meet these requirements we adapted cultivation system originally developed for studies of Streptomycetes differentiation (Nguyen et al., 2005) and tested in *Mycobacterium smegmatis* biofilm studies. The system uses cheap glass micro-beads (0.3 μm) immersed in a liquid medium and can be used for static cultivation in a Petri dish or assembled into the flow-through reactor. Presence of glass beads facilitates disintegration of biofilm mass during the protein samples preparation. We followed the formation of biofilm on glass beads by observation of whole cultivation dishes in an AQUASEM scanning electron microscope (TESCAN, Brno, Czech Republic). We observed that biofilm formation is very fast, a homogenous biofilm layer is formed within 24 hours on the surface of glass beads. For adhesion of the cells it is necessary to incubate the cells at 37ºC, the control sample incubated at 4ºC did not show any cells adhering to the beads. We compared proteomes isolated from biofilm populations of *M. smegmatis* on glass beads with those from control samples from beads with culture incubated at 4ºC and those from planktonic populations in cultures shaken at 37ºC. Proteins were separated by 2D electrophoresis and proteome data were analysed by PDQuest software (BioRad). Comparison of proteomes revealed number of differences in protein profiles between biofilm and planktonic cultures. When the factor of 8 fold difference in spot density was used in proteome data evaluation we found 20 different proteins between the culture shaken at 37ºC and 4ºC stationary control culture, 16 different proteins between biofilm and 37ºC shaken culture and only 6 different proteins in comparison of biofilm and 4ºC control. Unique proteins were found in all compared cultures ranging in numbers from 6 to 19. Venn graphs presenting intersections of protein groups obtained from all the comparisons resulted in a list of candidate proteins important for both planktonic (13) and in biofilm (2) growth. Identification of defined proteins by mass spectrometry is underway. Presented cultivation system was proved as very efficient for biofilm development and regulation “omic” studies, and it also holds the potential for testing of the effect of different materials and antibacterial compounds on biofilm formation when coated beads and modified media are used.

Nguyen L.D. et al. *Appl Environ Microb*. **71,** 2848-2852 (2005).

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Keywords: Biofilm, *Mycobacterium smegmatis*, glass beads, proteomics,

Yeast biofilm-like behaviour in rich or poor media in the presence of polyphenols

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The yeast cell wall is a dynamic structure in which changes in protein composition occur depending on nutrient availability and environmental conditions. Cell wall mannoproteins are responsible both for yeast interaction with various environmental substances, such as wine phenolic compounds, and cell-cell recognition events which lead yeast to grow as a biofilm, mat colonies, or display invasive and pseudohyphal forms, here defined as biofilm-like behaviour. Significant differences in colour, polyphenolic index and anthocyanin content of wines fermented with different yeast strains have been reported. The use of the chromogenic medium Grape skin agar (Graski) to evaluate the aptitude of yeasts to adsorb coloured phenolic compounds has allowed the inheritability of the trait Wine Colour Adsorption to be demonstrated. Yeast biofilm-like phenotypes depend on the expression of the FLO gene family but also on carbon and nitrogen availability.

The aim of this work was to study changes both in yeast biofilm-like behaviour and in Wine Colour Adsorption ability in order to understand the link between these phenotypes.

The study was carried out in rich or poor media, concerning carbon and nitrogen concentration, supplemented with polyphenols. Eleven wine autochthonous *Saccharomyces cerevisiae* strains, belonging to the microbial collection of the Department of Scienze e Tecnologie Agro-Forestali e Ambientali, and two control strains *S. cerevisiae* Σ1278b, (MATa), which has adhesive and filamentous phenotypes and *S. cerevisiae* BY4742, (MATα, Δflo8), which has a non-adhesive phenotype, belonging to the Department of Agriculture and Food Systems, were used. The strains were tested for: a) biofilm formation in synthetic complete medium with 2% or 0.1% of dextrose, in Synthetic Low Ammonium Dextrose (SLAD) with 2% or 0.1% of dextrose, and in the same media supplemented with 100 mg/L of (+)-catechin; b) invasive growth in solid YPD with 2% or 0.1% of dextrose, in solid SLAD with 2% or 0.1% of dextrose, in the same media supplemented with 100 mg/L of (+)-catechin, and in solid Graski with 2% or 0.1% of dextrose, in solid SLAD with 2% or 0.1% of dextrose supplemented with skins from black grapes; c) growth as mat colonies in the same media used for invasive growth but using 0.3% of agar; d) polyphenolic compound adsorption in solid Graski with 2% or 0.1% of dextrose and in solid SLAD with 2% or 0.1% of dextrose supplemented with skins from black grapes. In order to check the experimental condition influences on cell morphology, the strains were microscopically observed.

Results demonstrate that polyphenolic compounds modify in a different way the biofilm-like yeast phenotypes, and this behaviour varies amongst the yeast strains. The genetic basis of this difference is currently being explored and elucidated (Fig. 1). These phenotypes and the Wine Colour Adsorption phenotype could be considered not fully correlated strain-specific phenotypes, probably due to the simultaneous or not presence of the responsible cell wall proteins which determines a variety of phenotype combinations.

Keywords biofilm; polyphenols; wine colour adsorption; yeast cell wall

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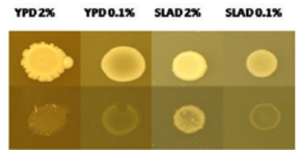

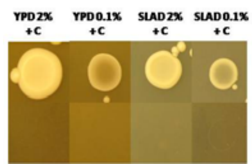

Fig. 1 - Invasive growth of the strains Sc1321 in YPD and SLAD 2% or 0.1% of dextrose (on the left) and in the same media supplemented with 100 mg/L of (+)-catechin - C - (on the right). Upper row: before washing plates; bottom row: footprints after washing plates.

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"Advances in feeding strategies of high cell density cultivation of *E.coli* and *Pichiapastoris* for production of recombinant proteins"

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High cell density cultures offer an efficient means for the economical production of recombinant proteins. However, there are still some challenges associated with high cell density cultivation (HCDC) techniques. A variety of strategies in several aspects, including host design consideration, tuning recombinant protein expression, medium composition, growth methodologies, and even control and analysis of the process have been successfully employed by biotechnologists to increase yield in high cell density cultures. Although most researchers have focused on *E.coli*, other microorganisms have the potential to be grown at high density and need further investigation. In recent years, information on physiological changes of hosts during different phases of cultivation derived from functional genomics, transcriptomics and proteomics are being used to overcome the obstacles encountered in high cell density cultivation and hence increase productivity.

Different novel feeding strategies for production of various recombinant therapeutic proteins, including human interferon gamma (hIFN-gamma), human granulocyte colony stimulations factor (h G-CSF), human growth hormone (hGH) using an isopropyl β-D-thiogalactoside- inducible expression system in recombinant *E.coli* BL21 were studied. Fed-batch modes were designed to compare the effect of μ (specific growth rate) on recombinant-protein production, substrate consumption, by-product formation and plasmid stability during pre- and post-chemical induction in high cell density cultures of *E.coli*. It was found that $Y_{P/S}$, the product/substrate yield of recombinant protein was significantly affected by μ throughout the process, but product/biomass yield ($Y_{P/X}$) was influenced by μ at the pre-induction stage. By applying an efficient feeding strategy, in which the μ was maintained at the maximum attainable level, recombinant protein was accumulated up to a level of 60% of the total cell protein and its productivity was increased significantly. In this case, the overall productivities of biomass and recombinant protein were 6.36 and 2.1 respectively, in comparison with 1.91 and 0.16 gh^{-1} $litre^{-1}$ during exponential feeding, in which μ was kept constant throughout the entire process.

By using a two-stage glycerol feeding method for HCDC of recombinant *Pichiapastoris*, the final dry cell weight and rhG-CSF yield achieved was close to 120 gl^{-1} and 30 mgl^{-1} respectively. Furthermore, three types of conventional methanol feeding strategies, including μ-stat, DO-stat and constant methanol concentration were investigated and compared with respect alcohol oxidase (AOX), formate dehydrogenase (FDH) and β- Gal activities, and all dry weight (CDW) , methanol and formaldehyde concentration during the production phase.

Keywords: High Cell Density Cultivation; Recombinant Proteins; Feeding Strategies; *E.coli*; *Pichiapastoris*

Construction of analysis method for tetrachloroethylene dissimilate-degrading microbes

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【Introduction and Purpose】 Tetrachloroethylene (PCE) is degraded by dehalorespiring bacteria via the dissimilatory pathway under an aerobic condition. Stable isotope probing (SIP) method is effective for deep understanding of the assimilatory degrading microbial population. However, the SIP method cannot be applied to the study of dissimilation of a pollutant. In this study, we improved the SIP method for constructing an analytical technique that could be used for identifying microbes involved in dissimilation. **【Method】** To modify the SIP method, we first focused on the carbon and energy sources of the dehalorespiring bacteria. We thought that incorporation of ^{13}C in dehalorespiring bacteria might be affected by the presence or absence of PCE and that the PCE-degrading dehalorespiring bacteria could be easily identified via microbial community structure analysis. To confirm the applicability of this modified SIP method in identifying dehalorespiring bacteria, we analyzed a microbial consortium including a well-known dehalorespiring bacterium. A consortium containing *Dehalococcoides ethenogenes* 195 was introduced as a model case in which the dehalorespiring bacteria were included. The treatments with and without PCE addition were developed, and ^{13}C-fumaric acid was used for labeling. After 7 days of incubation, we extracted total DNA and separated the ^{13}C-DNA by density gradient ultracentrifugation. The microbial community structure was analyzed by denaturing gradient gel electrophoresis (DGGE). The dehalorespiring bacteria were identified by comparing the DGGE profiles of the bacteria exposed to treatments with and without PCE. **【Result and Discussion】** After incubation with ^{13}C, labeled heavy DNA appeared in the bacteria exposed to treatments with and without PCE. Moreover, the DNA of the dehalorespiring bacteria in the consortium could be identified by comparing DGGE profiles of the two treatments. Sequence analysis confirmed that the identified DNA had a sequence similar to the *Dehalococcoides ethenogenes* 195 sequence. To conclude, the modified SIP method we developed was effective for analyzing PCE-dissimilating microbes, irrespective of the culture method used. We named this modified SIP method stable isotope probing for dissimilation (SIP-D).

Genomic analysis of exopolysaccharide biosynthesis by *Vibrio diabolicus*

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Natural glycopolymers are widely distributed in plants, animals as well as micro-organisms. Many of them have already found applications in several industrial fields such as food texture, human health,…. The demand in such molecules is constantly increasing, leading to the interest in new polysaccharides with innovative properties. *Vibrio diabolicus* is a marine bacterium producing an exopolysaccharide (EPS), HE800, which shows structural homology with hyaluronic acid. As a glycosaminoglycan mimetic, its properties in bone and cartilage regeneration, angiogenesis, and osteosarcoma treatment are currently studied.

In competition with the polymers from plants or animal, microbial polysaccharides offer many advantages in terms of biotechnological exploitation (non dependent upon climatic or seasonal variability, easier extraction, degree of regularity of structure, more safety regarding viral risks…). The use of polysaccharides for human health requires the control of characteristics of produced batches ; in particular, the molecular weight is an important parameter that needs to be stable between lots of production. Indeed, the biological activity is dependent upon the molecular weight ; subsequent eventual modifications (functionalisations such as sulfation…) are also better carried out if the molecular weight is controled.

It has been shown for several bacteria that the features of the produced polysaccharide are dependent on the growth conditions especially during fermentation. In this context, the aim of our study is to gain insight in the molecular mechanisms of the biosynthesis of polysaccharides. Bioinformatic analysis of the genome of *Vibrio diabolicus* has resulted in the identification of genes potentially coding for the proteins involved in the exopolysaccharide biosynthesis. The analysis also put forward the hypothesis that the biosynthesis mechanism in *V. diabolicus* is approaching that of groups 1 and 4 production of lipo- and capsular polysaccharides in *E. coli*.

Keywords polysaccharide, biosynthesis, modifications, applications

Overcoming limiting steps to optimize secretory protein production in streptomycetes

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Streptomycetes are Gram-positive soil bacteria which are efficient producers of secondary metabolites of a biocide nature as well as efficient secretors of a wide range of hydrolytic enzymes of industrial and environmental use.

Streptomyces lividans is thepreferred host for the overproductionof homologous and heterologous secretory proteins. The *S. lividans* main secretory pathway, the Sec route, does not have the first chaperon (SecB) of the *Escherichia coli* equivalent pathway, therefore, the Signal Recognition Particle (SRP) triggers pre-secretory proteins to the cellular membrane.

S .lividans harbours four genes encoding four different type I signal peptidases (SipW-Z), all working in a cooperative manner. Mutations in the major type I signal peptidase SipY or in a main component of the translocation machinery (SecG) showed to influence the bacterial metabolism in a similar manner as determined by genome-wide transcriptional studies and proteomic analyses.

The obtained results have permitted the identification of a subset of genes and extracellular proteins which could be used to monitor an efficient secretion process in streptomycetes.

Keywords *Streptomyces lividans*. Secretion.

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Antimicrobial And Anticancer Agents Among Novel Tetrazolo[1,5-*c*]Quinazolin-5-Thion S-Derivatives

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Problem statement: Investigations for new effective and less toxic antimicrobials and anticancer drugs are always up-to-date. The tetrazole derivatives are quite interesting objects as for synthesis and for pharmacological screening. Despite they weren't found in the nature and were synthetically obtained, among their fused analogs there are a lot of biologically active substances. We were interested in tetrazolo[1,5-*c*]quinazoline derivatives, because they are presented only in few examples and their physico-chemical and biological properties were evaluated not sufficiently. Various biological applications were reported for tetrazoloquinazolines, including fungicides, pesticides, antiallergics, antiulcer agents, bactericides, bronchodilators, anti-inflammatory agents, analgesics, antianphylactics and antihypertensive. Investigation of the antimicrobial activity of the 9-bromo–(chloro)-5-morpholin(piperidin)-4-yl-tetrazolo[1,5-*c*]quinazolines to *Esherichia coli* et *E. faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* revealed that sensitivity of Gram positive bacteria of the compounds was higher than that of Gram negative bacteria. 5-Phenyl-tetrazolo[1,5-*c*]quinazoline was effective against *St. aureus* and *E. faecalis* (MIC= 250 mg/L). None of the mentioned tetrazolo[1,5-*c*]quinazoline derivatives influenced *E. coli*. More than that, 9-bromo-5-morpholin-4-yl-tetrazolo[1,5-*c*]quinazoline demonstrated antitumor activity against leukemia L1210 and column cancer Caco-2 cell lines. **Approach**: Introduction of Sulfur atom in the 5 position of the tetrazolo[1,5-*c*]quinazoline nucleus could afford the broadening of the biological activity range. It's known that, the main way of formation above mentioned skeleton is cyclisation of 4-hydrozinoquinazolines with nitritic acid or diazotizing 4-halogenoquinazolines with sodium azide or cyclisization of the 5-(2'-aminophenyl)-*1H*-tetrazoles with acetic acid anhydride to result proper tetrazolo[1,5-*c*]quinazolines. **Results**: We obtained tetrazolo[1,5-*c*]quinazoline by treatment of the 4-hydrazinoquinazoline with sodium nitrite in the acetic acid at 0ºC with subsequent nucleofilic degradation of it's pyrimidine ring by refluxing in the 50% hydrochloric acid water solution to 5-(2'-aminophenyl)-1*H*-tetrazole. The latter was cyclisized with potassium ethylxantoganate in propanol-2 or with carbon disulfide in equimolar potassium ethylate solution resulting the tetrazolo[1,5-*c*]quinazoline potassium salt. The structures of all newly synthesized compounds were confirmed by FT-IR, ^{1}H, ^{13}C NMR, LC-MS data and elemental analysis. The screening for antibacterial and antifungal activities against *Escherichia coli, Staphylococcus aureus, Enterococcus aeruginosa and E. faecalis, Pseudococcus aeruginosa, Klebsiella pneumonia* and *Candida albican* in concentration 100 µg revealed, that the most active substances were N,N-dimethyl-, N,N-diethyl-, N,N-isopropyl-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)ethylamine and 1-(4-methoxyphenyl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)ethanon. "Structure-activity" relationship revealed that shortening of dialkylamino fragment of syntheised substances leads to moderate decreasing of antimicrobial activity against *Enterococcus faecalis*, and otherwise leads to increasing of activity against *Staphylococcus aureus*, *Escherichia coli* and antifungal against *Candida albicans.* Introduction of the 4-methoxyphenyl radical at 5 position of tetrazolo[1,5-*c*]quinazolin-5-thione resulted synthesis of the best antimicrobial among all investigated substances, that even moderately inhibited the growth of *Pseudococcus aeruginosa* and *Klebsiella pneumonia.* The research of the antitumor activity showed that in concentration 1.0 µM 3-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)propanoic and isobutanoic acids, 1-(4-methoxyphenyl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)ethanone and ethyl tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetate exhibited lethal activity against acute lymphoblastic leukemia CCRF-CEM cell line, and moderate anticancer properties against leukemia MOLT-4 and HL06-(TB) and renal cancer UO-31 cell lines. **Conclusion**: The results of the investigation could be considered as promising lead for the further investigations and modifications of the novel tetrazolo[1,5-*c*]quinazolin-5-thion S-derivatives to improve their biological properties.

Keywords: 5-R-Tetrazolo[1,5-*c*]quinazolin-5-thiones / Antifungal / Antibacterial / Anticancer

Plant growth regulators of *Cucumis sativus* L. roots among ([1,2,4]triazolo[1,5-*c*]–quinazolin-2-ylsulfanyl)carboxylic acids and amides

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Problem statement: It is reported, that plant growth retardation can be a side activity of some triazole-type fungicides such as triadimenol, triadimefon or ipconazole, which inhibit the oxidative demethylation in the fungal ergosterol biosynthesis path and also reduce the formation of 14-demethylatedrols in higher plants blocking the obtusifoliol 14-demethylase. For the last week Germany suffers 330 cases of serious infection, most of which have occurred in the north of the country. The cause has been identified as Shiga toxin-producing *Echerichia coli* O157:H7, that was firstly said to be found on Spanish *Cucumis sativus,* which is a member of the *Cucurbitaceae* plant kingdom family, and one of the widely eaten foods, especially in the hot summer months and finally - on the German bean sprouts. Shiga toxin-producing *E. coli* causes: diarrhea (frequently bloody), vomiting and fever. So, we decided to investigate retardant GAs-like activity of the already proved to be antifungals ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)carboxylic acids and amides at the *Cucumis sativus* L. roots to prevent people from other food diseases and farmers from sales loss. **Approach**: The influence of the synthesized ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)carboxylic acids and amides at the length of hypocotyl, main root, growth area and number of adventitious roots of the *Cucumis sativus* were measured to the nearest millimeter in the 5 concentrations (0.001, 0.005, 0.02, 0.1, 0.5 mg/ml). Explants were cultured on Petri dishes, containing 10 ml of water with 0.01 ml of Tween-80. The cultures were incubated in a growth room at 30±2 ºC and at relative air humidity 80% for 72 h in darkness. Control solution contained water with 0.01 ml of Tween-80. Gibberelline was used as reference. **Results**: The effect of hormesis, a dose-response relationship phenomenon characterized by low-dose stimulation and high-dose inhibition, was observed for the synthesized compounds (Fig.). Almost all investigated substances stimulated values of above-mentioned parameters of growth in the concentration 0.005 mg/ml. Exceptions were the amides of acetic (5) and α-propionic acids (7), that showed the strongest cytotoxic activity inhibiting the cell growth of main and adventitious roots, demonstrating growth retardant properties. The best growth stimulators appeared to be acid 3 and 4 in the concentration of 0.02 mg/ml, which promoted *Cucumus sativus* L. hypocotyl growth at 47.29% and 26.21%, main root at 33.17% and 23.90%, adventitious roots at 18.18% and 24.24% accordingly, exceeding the activity of gibberellin.

Cmpd.	n	R	R_1
1	0	H	OH
2	0	CH_3	OH
3	1	H	OH
4	3	H	OH
5	0	H	$N(C_2H_5)_2$
6	0	H	$-N(CH_2)_5-$
7	0	CH_3	NHC_4H_9
8	0	CH_3	NH(m-OCH_3-Ph)

Fig. Structure of the ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)carboxylic acids and amides.

Conclusion: The investigated compounds can be used as novel plant growth regulators or as inhibitors in high concentrations (0.5 mg/ml) or as promoters in low concentrations (0.001-0.02 mg/ml) like refined and selective agrochemicals possessing the antifungal activity against *Candida albicans*.

Keywords *Cucumis sativus* L., growth regulation activity, [1,2,4]triazolo[1,5-*c*]quinazoline-2-thiones.

3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-one S-derivatives – new perspective chemotherapeutical agents

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Problem statement: The quinazoline derivatives is a classical group of biological active substances in medical chemistry, which is characterized by wide and diverse spectrum of biological activity. It's necessary to mention that the prior way of quinazoline heterocycle modification is synthesis of the antimicrobial and anticancer agents. It's important that condensation of quinazoline fragment with other heterocycle is a known approach of formation the biological active substances with the aim to increase their pharmacological activity and decrease their toxicity. Synthetic and screening researches in the field of quinazoline condensed analogs were carried out by our group together with leading Ukrainian scientists and National Cancer Institute (NCI). It allowed us to identify significant antimicrobial and anticancer potential among S-derivatives of 3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones. **Approach**: Substance **II** was synthesized by [5+1]cyclocondensation of 6-R-3-(2-aminophenyl)-[1,2,4]triazin-5-ones (**I**) as 1,5-NCCCN-binynuclefiles with carbon disulfide in ethanol in with potassium hydroxide (Method A), by interaction of compounds **I** with potassium ethylxantogenate in the propanol-2 (Method B, Scheme). The presence of thiol fragment in structure of compound **II**, and established fact of influence of substituent at 6 position at antimicrobial and anticancer activity, appeared to be the reason for synthesis of new 6-{[ω-(dialkylamino(heterocyclyl)alkyl]thio}-3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones (**III**) and N-cycloalkyl-(cycloalkylaryl-, aryl-, aralkyl-, heteryl-)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetamides (**IV**). The latter compounds were obtained by alkylation of substance **II** with proper halogen derivatives in propanol-2 : water mixture ((2:1), Scheme).

Method A: CS_2, KOH, EtOH; Method B: $KSC(S)OC_2H_5$, i-PrOH; R=Me, Ph, 4-MePh, 4-EtPh, 4-PrPh, 4-BuPh, 4-FPh, 4-MeOPh, 4-EtOPh, 2-tihenyl; R_1=R_2=Alk, heterocyclyl; R_3=Ar, Het; n=2, 3, 4

Scheme. Synthesis of the 3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones S-derivatives (**II-IV**).

Results: Bioluminescence tests on luminescent bacteria *Photobacterium leiognathi* Sh1, antibacterial activity against *E. coli*, *St. aureus*, *M. luteum*, *C. tenuis* and *A. niger* showed that the most active substances were compounds **III**. Thus, substances **III** inhibited growth of *M. luteum* and *A. niger*, at the same time showing no activity against *E. coli, St. aureus* and *C. tenius*. Pharmacological screening of the anticancer activity in concentration 10^{-5} M demonstrated that the most sensitive cancer cell lines to the substances **III** and **IV** were leukemia (K-562, MOLT-4, RPMI-8226, SR), non-small cell lung cancer (A549/ATCC, HOP-62, HOP-92, NCI-H322M, NCI-H460), colon cancer (COLO 205, HCT-116, HCT-15, HT29, KM12, SW-620), CNS cancer (SF-539, U251), melanoma (LOX IMVI, MALME-3M), ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-8, NCI/ADR-RES, renal cancer (ACHN, RXF 393, SN12C, UO-31, prostate cancer (PC-3, DU-145) and breast cancer (MCF7, MDA-MB-231/ATCC). Then, according to the standard procedure of NCI, 10 substances were researched for 5 concentrations (100 μM-0,01 μM) at 57-59 cell lines of 9 cancer types to investigate dose-dependency. The investigated substances showed anticancer activity against cell lines of leukemia, melanoma, renal, colon and CNS cancers. **Conclusion**: So, moderate antimicrobial and anticancer potential of 3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones S-derivatives is confirmed, which was the reason for further modification of above mentioned skeleton with purpose to research pharmacological data of the potential drugs.

Keywords: 3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones, S-derivatives, antibacterial, anticancer, Bioluminescence inhibition