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***Review article***

# OPTIMIZATION OF CULTURAL CONDITIONS FOR EXOPOLYSACCHARIDES PRODUCTION BY *Frateuria aurentia*

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**ABSTRACT:** The exopolysaccharides (EPS) producing ability was evaluated by nutritional defined media by the *Frateuria aurentia* was the highest yield EPS-producing strain, which was isolated from elephant dung in Srivilliputhur, Tamilnadu, India. It was identified as *Frateuria aurentia* with carbohydrate assimilation process and 16S rDNA gene sequencing. The favourable conditions for EPS yield by the microorganism growth in a chemically defined medium were tested with carbon, nitrogen, cultivation time, temperature, pH, hydrocarbon, surfactants, sodium chloride, metal ions and inoculums concentration in static and shaking conditions had the most significant influences. Among the various nutritional sources of interactive effects the maximum EPS production was recorded at pH 7.00 (1.431 ± 0.0096 OD); 30ºC (1.661 ± 0.028 OD); Jaggery (1.185 ± 0.003 OD); Tryptone (1.248 ± 0.011 OD); Ferric chloride (1.682 ± 0.022 OD); Glutamine (2.136 ± 0.067 OD); polyethyleneglycol (0.873 ± 0.041 OD); 2.5% NaCl (1.138 ± 0.023 OD); Neem oil (1.699 ± 0.176 OD); inoculum concentration 2.00% (1.625 ± 0.007 OD); The maximum EPS production was viewed at the 72hrs of incubation at static condition (1.58 ± 0.122 OD).
**Key words:** *Frateuria aurentia,* exopolysaccharides, optimization

## INTRODUCTION

Many microorganisms can synthesize exopolysaccharides (EPSs) and excrete them out of cell either as soluble or insoluble polymers. These EPS not only can protect the microorganisms
but also can be applied in many biotechnological applications, such as textile, pharmaceutical, cosmetics, food, metal mining, oil recovery and metal recovery. Common microbial EPSs, such as xanthan and dextran, have been used as commercial products for many years. Because of their novel properties, the bioactive microbial polysaccharides ß-D-glucans and bacterial cellulose have been used for immune modulation and tumour stasis and audio membrane. Thus, microbial EPSs have attracted more attentions from scientific and industrial communities (Satpute *et al*., 2010 and Staudt *et al*., 2011).

Most bacteria produce EPS under all conditions, but the quantities and the composition of EPS are strain dependent and affected by the nutritional and environmental conditions. So it is possible to increase the polymer production by manipulating the culture conditions (Looijesteijn 1999). In fact, mesophilic strains seem to produce maximum levels of EPS in suboptimal conditions for the bacterial growth, whereas EPS production appears to be growth associated in thermophilic strains (Degeest, 2001). The structure, composition and viscosity of the microbial
polysaccharides depend on several factors, such as the composition of the culture medium, carbon and nitrogen source, mineral salts, trace elements, type of strain, and fermentation conditions (pH, temperature, oxygen concentration, agitation (Conti *et al*., 1994 and Duta *et al*., 2004).

Optimization of the growth environment is important to achieving maximal EPS production by organisms such as *Xanthomonas*, *Pseudomonas* and *Rhizobium* sp. (Breedveld *et al*., 1993 and Morin *et al*., 1998). Published efforts to optimize EPS production in lactic acid bacteria have included studies evaluating the effects of such environmental conditions as temperature and pH. Greater specific polymer production (milligrams per gram of cells (dry weight) at a higher temperature (45°C) was also found and who examined EPS production by the same strain in defined medium. Correlation between the optimum growth temperature (37 to 42°C) and maximum polymer production was observed by strain CRL 870. It was found that maximum polymer synthesis (488 mg/l) observed by *L. casei* CRL 87 at 30°C occurred at 20°C and pH 6.0. However, optimum specific production (EPS produced per gram (dry weight of cells) and EPS yield (grams of EPS 3 100/grams of sugar consumed) were found at pH 4.0 (Mozzi *et al*., 1995; Van den Berg *et al*., 1995).

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For some EPS-producing bacteria, such as *Xanthomonas*, *Pseudomonas* and *Rhizobium* spp., nitrogen limiting conditions result in increased EPS production. The effect of nitrogen concentration on EPS production by Lactobacilli has not been examined. The yield and quality of microbial EPSs are greatly affected by the nutritional and environmental conditions. For instance, the EPS yield of strain *Rhizobium tropici* was significantly influenced by carbon, nitrogen and pH. The molecular weight of the alginate from *Pseudomonas fluorescens* produced on fructose was much higher than that produced on glucose (Cerning, *et al*., 1994; Kumar et *al*., 2007).

The aim of this study was to screen and identify the high yielding EPS producing strain and to better understand the concepts of the influence of different nutrients in the medium for cultivation conditions of EPS production.

## MATERIALS AND METHODS

### Collection and processing of the sample

Elephant dung sample was collected from Shenbagathoopu, Srivilliputhur, Tamilnadu North Latitude, 11° 00' and 12° 00 N, East Longitude, 77° 28' and 78° 50'. Isolates were obtained by serial dilution plating on nutrient agar medium. A total of 10 different colonies were isolated among them one colony was selected based on the exopolysaccharide production. Bacteria were screened based on their morphological characters, mucous and ropy appearances.

### Biochemical Characterization

The following biochemical test like Indole production test, Methyl red test, Voges Proskauer test, Citrate utilisation test, Catalase test, Oxidase test, Hydrogen sulfide production, Nitrate reduction test and Urease test were performed. Further the identification of bacteria was performed by 16S rDNA sequencing analysis. The strain was identified as *Frateuria aurentia.*

### Optimization of physiological factors for EPS

Some properties of the EPS obtained by cultivating the selected strains under the optimized conditions were investigated. The factors like pH, temperature, carbon, nitrogen, incubation time, amino acids, surfactants, hydrocarbon, inoculum concentration, NaCl concentration and metal ions concentration which were expected to affect the production of EPS by the selected strain were optimized by selecting one parameter at a time.

### Effect of different pH on bacterial growth

Different pH (3, 4, 5. 6, 7, 8 and 9) were adjusted into the production medium to determine the effect of pH on bacterial growth for EPS production. Growth of the organism was determined by optical density measured at 600 nm.

### Effect of temperature on bacterial growth

Different temperatures (10, 20, 30, 40, 50 and 60ºC) were used to prepare production medium to determine the effect of temperature on bacterial growth and EPS production. Growth of the organism was determined in terms of optical density measured at 600nm.

### Effect of incubation time on bacterial growth

In the production medium different incubation time (24, 48, 72, 96 and 120) hours were incubated to determine the effect of incubation time on bacterial growth and EPS production. Growth of the organism was determined in terms of optical density measured at 600nm.

### Effects of carbon sources on bacterial growth

Different carbon sources at 1% concentration (Dextrose, Jaggery, Sucrose, Maltose, Lactose and Molasses) were introduced to the production medium to determine the effect of carbon dose on EPS production. Growth of the organism was determined in terms of optical density measured at 600 nm.

### Effects of nitrogen sources on bacterial growth

Different nitrogen sources at 0.5% concentration (Urea, Tryptone, Glycine, Ammonium sulphate, Ammonium chloride and Ammonium carbonate) were introduced into the production medium individually to determine the effect of nitrogen source on microbial growth and EPS production. Growth of the organism was determined in terms of optical density measured at 600 nm.

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### Effects of metal ions on bacterial growth

Different metal ions at 0.02% concentration (Mercuric choride, Manganese sulphate, Ferric chloride, Zinc sulphate, Copper sulphate, Calcium chloride, Disodium hydrogen phosphate, Mercuric oxide and Ferrous sulphate) were introduced into the production medium individually to determine the effect of metal ions on microbial growth and EPS production. Growth of the organism was determined in terms of optical density measured at 600nm.

### Effects of amino acids on bacterial growth

Different amino acids at 0.2% concentration (Glycine, Glutamine, Cysteine, Alanine and Methionine) were introduced into the production medium individually to determine the effect of amino acids on microbial growth and EPS production. Growth of organism was determined in terms of optical density measured at 600nm.

### Effects of surfactants on bacterial growth

Different surfactants at 0.2% concentration (SDS, PEG, Tween 80 and Triton X 100) were introduced into the production medium individually to determine the effect of surfactants on microbial growth and EPS production. Growth of organism was determined in terms of optical density measured at 600nm.

### Effects of hydrocarbons on bacterial growth

Different hydrocarbons at 0.5% concentration (Toluene, Xylene, Liquid paraffin, Hexane, Olive oil, Neem oil, Coconut oil and Groundnut oil) were introduced into the production medium individually to determine the effect of hydrocarbons on microbial growth and EPS were examined. Growth of organism was determined in terms of optical density measured at 600nm.

### Effects of NaCl concentration on bacterial growth

Different concentrations of NaCl (1.5, 2.0, 2.5 and 3.0%) were introduced into the production medium individually to determine the effect of NaCl on microbial growth and EPS production. Growth of organism was determined in terms of optical density measured at 600nm.

### Effects of inoculums concentrations on bacterial growth

Different inoculums concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0%) were introduced into the production medium individually to determine the effect of inoculum concentrations on microbial growth and EPS production. Growth of organism was determined in terms of optical density measured at 600nm.

## RESULTS

### Optimization of cultural conditions for EPS production by Fraturia aurentia

EPS production by the *Frateuria aurentia* isolated from the elephant dung was adjusted using various cultural conditions. The EPS production was assayed after 72 hours of incubation at 30°C under various pH. Maximum EPS production was recorded at pH 7.0 (1.431 ± 0.0096 OD) next to that maximum EPS production was observed at pH 6.0 (1.245 ± 0.021). Minimum EPS production was recorded at pH 3.0 (0.879 ± 0.0141 OD) (Fig. 1.).

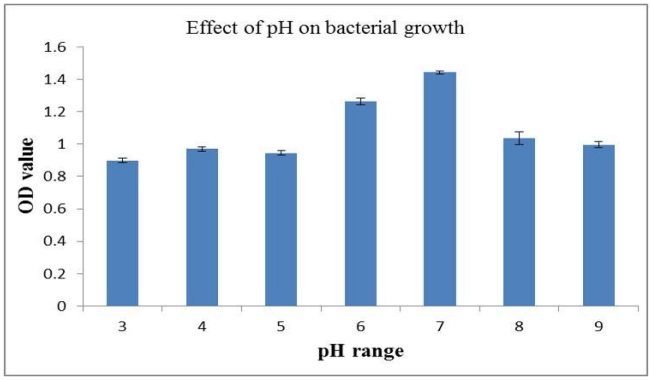


**Fig.1. Effect of pH on EPS production**

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## Effect of temperature on EPS production

Among the various temperature tested the maximum EPS production was obtained at 30ºC (1.661 ± 0.028 OD), followed by this at 40ºC (1.249 ± 0.007) was the second best temperature on EPS production. On the other hand, the minimum amount of EPS production was observed at 10ºC (0.952 ± 0.125 OD) (Fig. 2.).

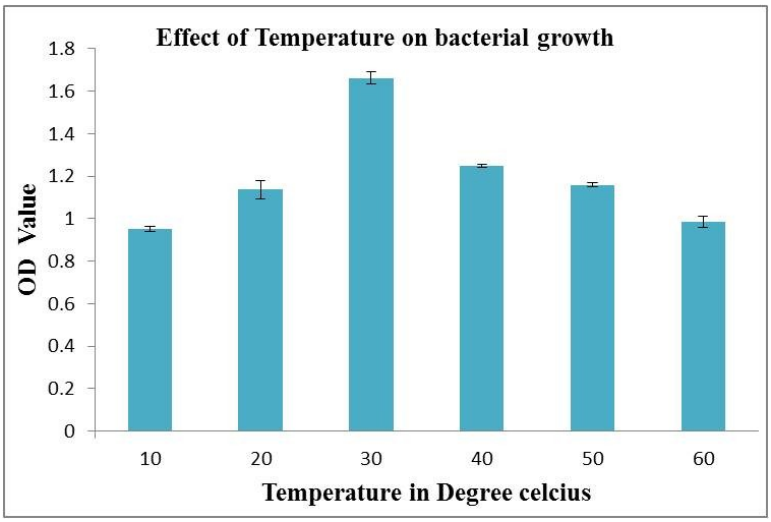


**Fig. 2. Effect of temperature on EPS production**

## Effect of carbon source on EPS production

The effect of carbon source on EPS production by *Frateuria aurentia* after 72 hours incubation at 30ºC. Here the maximum EPS production was observed in Jaggery (1.185 ± 0.003 OD) supplemented medium. The minimum EPS production was observed in Lactose (1.008 ± 0.005 OD) provided medium (Fig. 3.).

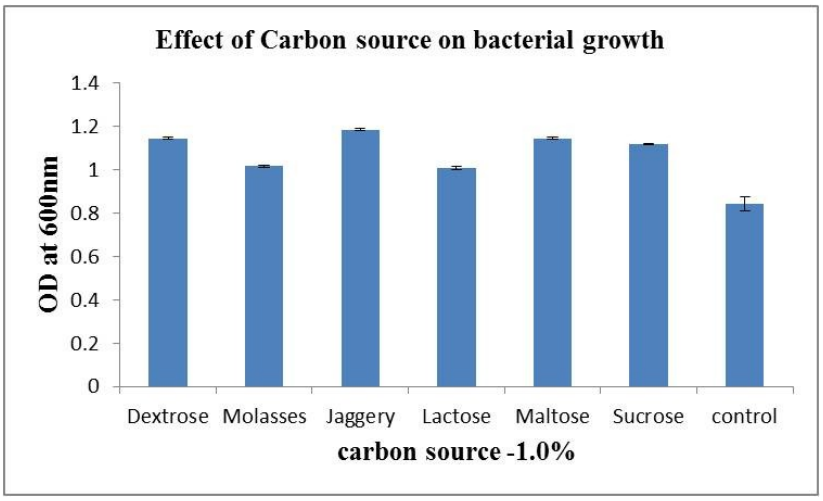


**Fig. 3. Effect of carbon source on EPS production**

## Effect of Nitrogen source on EPS production

The effect of different nitrogen sources on EPS production after 72 hours of incubation period at 30$^0$C showed maximum amount of EPS production on Tryptone (1.248 ± 0.011 OD) supplemented medium and minimum amount of EPS production in urea (0.125 ± 0.017 OD) (Fig. 4.).

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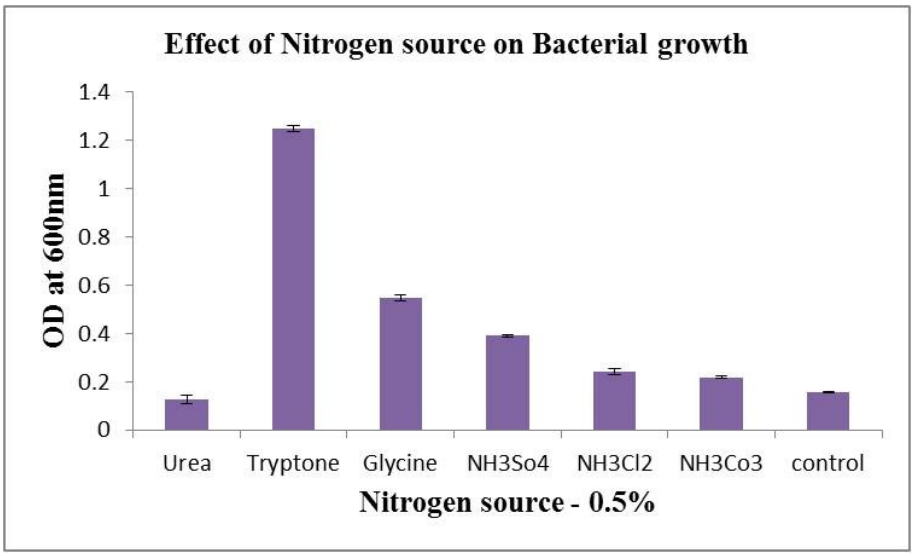


**Fig. 4. Effect of nitrogen source on EPS production**

## Effect of metal ions on EPS production

Among the tested metal ions, the maximum amount of EPS production was observed in Ferric chloride (1.682 ± 0.022 OD) supplemented medium. Followed by this, magnesium oxide was the second best metal ions on EPS production (1.137 ± 0.013 OD), whereas the minimum amount of EPS production was observed in zinc sulphate (0.793 ± 0.004 OD) (Fig. 5.).

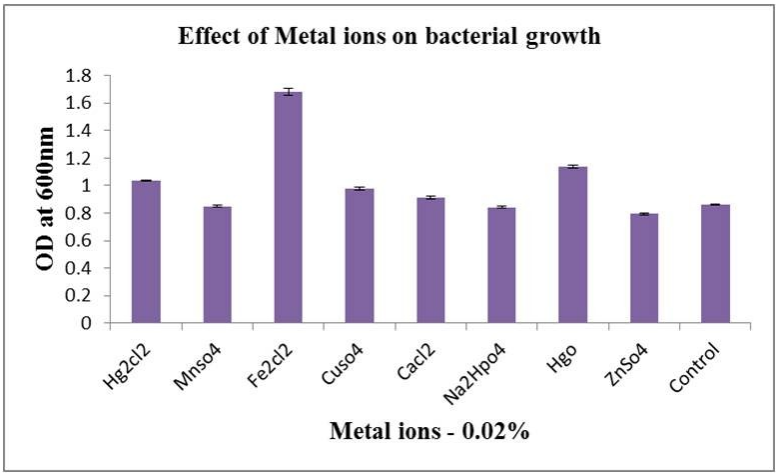


**Fig. 5. Effect of metal ions on EPS production**

## Effect of aminoacids on EPS production

The effect of various aminoacids on EPS production after 72 hours of incubation period at 30ºC showed maximum amount of EPS production observed in Glutamine (2.136 ± 0.067 OD) supplemented medium. Followed by this Alanine (2.036 ± 0.023 OD) was second best aminoacids in EPS production, whereas the minimum amount of EPS productin was observed in Cysteine (1.065 ± 0.041 OD) (Fig. 6.).

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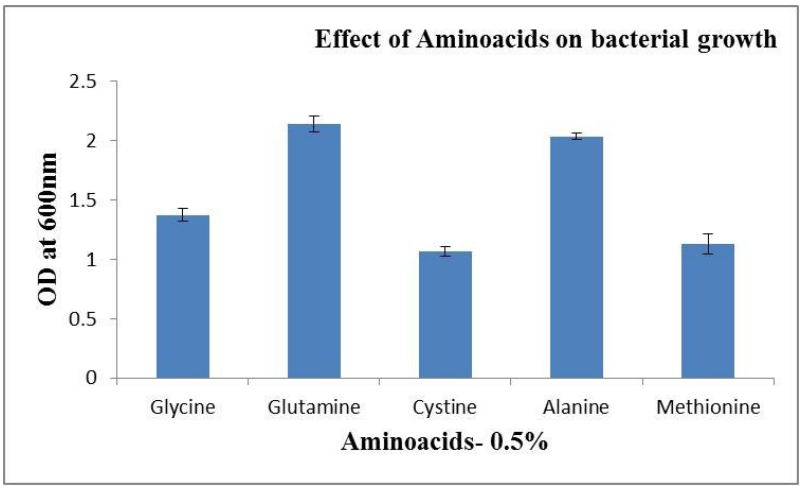


**Fig. 6. Effect of aminoacids on EPS production**

## Effect of surfactants on EPS production

The effects of different kinds of surfactants was tested on EPS production after 72 hours of incubation period at 30ºC. Among the tested surfactants, the maximum amount of EPS production was observed in polyethyleneglycol (0.873 ± 0.041 OD) supplemented medium. Followed by this Tween-80 (0.667 ± 0.033 OD) was second best surfactant in EPS production, whereas the minimum amount of EPS production was observed in SDS (0.0633 ± 0.0313 OD) (Fig. 7.).

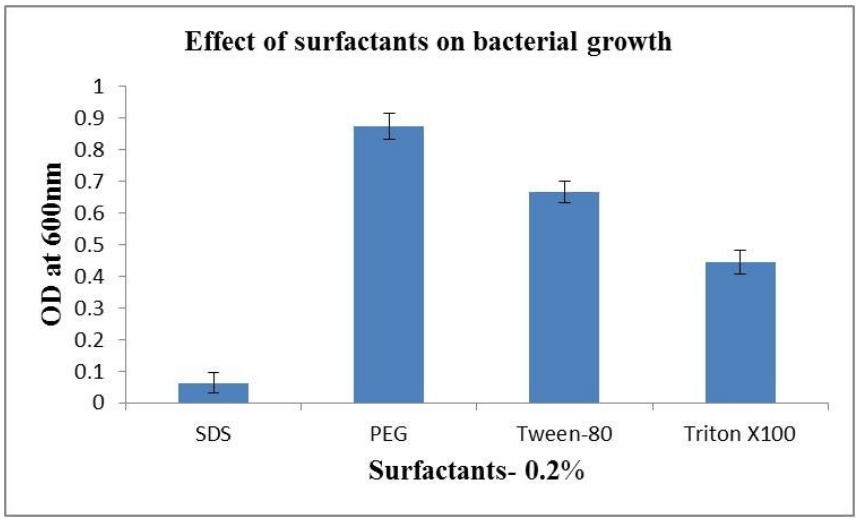


**Fig. 7. Effect of surfactants on EPS production**

## Effect of sodium chloride concentration on EPS production

The effect of various concentrations of sodium chloride was tested on EPS production after 72 hours of incubation period at 30ºC. Among the tested concentration, the maximum amount of EPS production was observed on 2.5% NaCl (1.138 ± 0.023 OD). The minimum amount of EPS production was observed on 3% NaCl (0.758 ± 0.035 OD) (Fig.8.).

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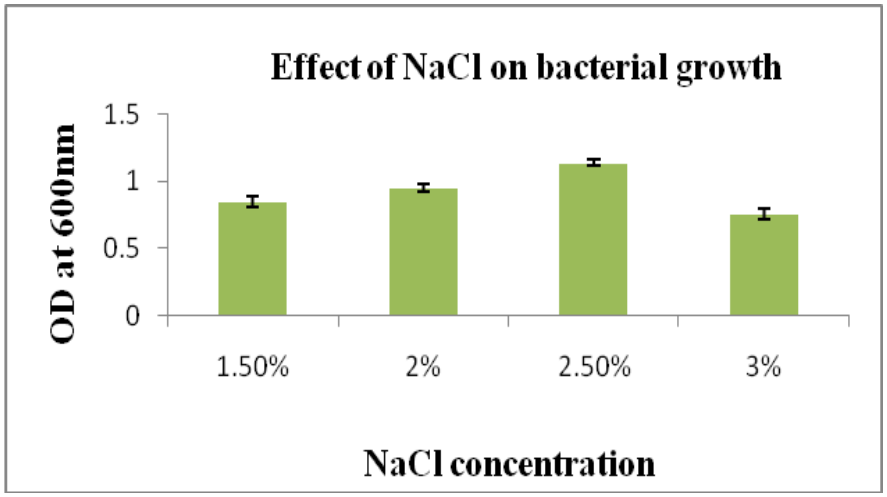


**Fig. 8. Effect of NaCl concentration on EPS production**

## Effect of hydrocarbons on EPS production

The effect of different kinds of hydrocarbons was tested on EPs production after 72 hours of incubation period at 30°C. Among the tested surfactants, the maximum amount of EPS production was observed in Neem oil (1.699 ± 0.176 OD) added medium. Xylene (1.629 ± 0.017 OD) comes second best hydrocarbon on EPS production. Groundnut oil (1.612 ± 0.009 OD) comes third best hydrocarbon on EPS production. The minimum amount of EPS production was observed in Hexane (1.022 ± 0.015 OD) (Fig. 9.).

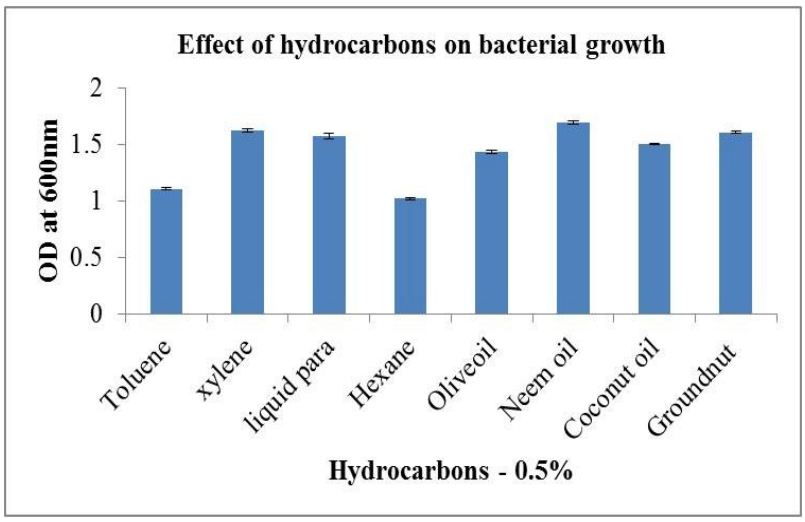


**Fig. 9. Effect of hydrocarbons on EPS production**

## Effect of inoculum concentration on EPS production

The initial inoculums level in the optimized basal media is a critical factor in EPS production process. The maximum EPS production was viewed at the 2.00% (1.625 ± 0.007 OD). 1.5% (1.623 ± 0.008 OD) was the second best inoculums concentration in EPS production. 1.00% (1.600 ± 0.004 OD) was the third best inoculums concentration in EPS production. The minimum amount of EPS production was observed in 4.00% (1.537 ± 0.0067 OD) (Fig. 10.).

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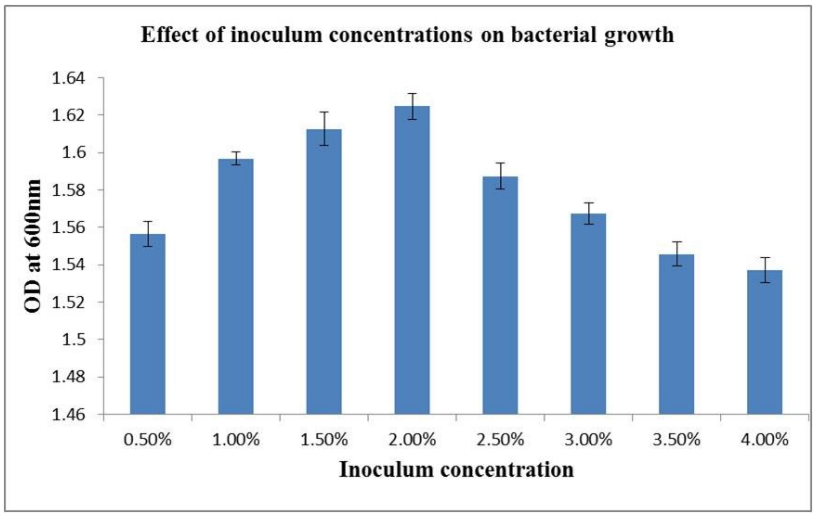


**Fig. 10. Effect of inoculum concentration on EPS production**

## Effect of static and shaking condition on EPS production

The maximum EPS production was viewed at the 72hrs of incubation at static condition (1.58 ± 0.122 OD). 48 hrs (1.28 ± 0.03 OD) was the second best in EPS production at static condition. The minimum amount of EPS production was observed in 120 hrs at shaking conditions (0.613 ± 0.006 OD) (Fig. 11.).

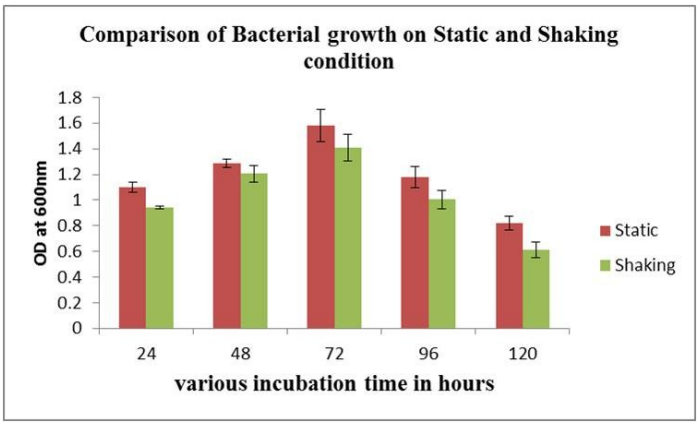


**Fig. 11. Effect of static and shaking condition on EPS production**

## DISCUSSION

In the present study the recovered maximum production of EPS observed from *Frateuria aurentia* in basal medium 90.66 ± 16.8mg/100ml of dry weight. The optimal culture media were determined as follows: After 72 hours of incubation at pH 7.0, 30ºC on optimized medium Jaggery- 1.0%, (carbon source), Tryptone – 0.5% (nitrogen source), Ferric chloride – 0.01% (metal ions), Glutamine – 0.2% (aminoacid), Inoculums concentration – 2.0%, Vitamin B complex – 1%.The bacterial EPS recovered from optimized medium was 231 ± 0.8 mg/100ml of dry weight respectively. *Frateuria aurentia* exopolysaccharide content under the optimised conditions was 2.5 times than that under the basic culture medium and initial conditions.

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Similarly, Gao *et al.* (2012) observed that, the optimal culture medium constituents were determined as follows: 30 g/L sucrose, 3.0 g/L soybean meal, 0.25 g/L $MgSO_4$, 1.5 g/L $K_2HPO_4$, 0.5 g/L $KH_2PO_4$, 0.03 g/L $ZnSO_4$ and 0.01 g/L $FeSO_4$. The optimum parameters for the liquid fermentation were as follows: temperature, 25°C; cultivation time, 6 d; initial pH, 8.0; volume of medium, 150 mL; and rotary speed, 180 rpm. GREP content and dry cell weight in optimized conditions were 540.1 ± 15.9 mg/L and 8.2 ± 0.3 g/L, respectively. GREP content under the optimized conditions was 2.5 times than that under the basic culture medium and initial conditions.

Similarly Kanmani *et al* .(2011) find out the optimal temperature, pH and NaCl for EPS production by *S. phocae* PI80, different temperature (25–50°C), pH (5.0–7.5) and NaCl concentrations (0–4%) were analyzed in MRS broth. The optimal temperature, pH and NaCl for cell growth and EPS production were 35°C, 6.5 and 2–3%, respectively with the corresponding cell growth (OD-1.333 ± 0.02, 1.335 ± 0.05 and 1.358 ± 0.02) and EPS (g/L) production (7.8 ± 0.29, 7.9 ± 0.34 and 8.1 ± 0.27) and also Ismail and Nampoothiri (2010) reported the maximum EPS production by *Lactobacillus plantarum* MTCC 9510 at 35°C. Wang *et al*. (2010) reported the effect of carbon sources on cell growth and EPS production by *S. phocae* was investigated in MRS broth. Among the carbons sources lactose (15 g $L^{-1}$) was found to be best for EPS production. EPS production was also studied at various concentration of lactose and it is found that maximum EPS production (11.75 ± 0.20 g $L^{-1}$) occurred at 20 g $L^{-1}$ of lactose. The amount of EPS production and properties are greatly dependent on the microorganisms and their culture condition such as temperature, pH and media composition by the amount of EPS production and properties are greatly dependent on the microorganisms and their culture condition such as temperature, pH and media composition. Ismail and Nampoothiri (2010) reported that maximum EPS production by *L. plantarum* MTCC 9510 was observed in presence of lactose (40 g $L^{-1}$). Growth and EPS production by lactic acid bacteria was also enhanced by nitrogen sources.

Lin *et al*. (2009), Ismail and Nampoothiri, (2010) and Wang *et al.,* (2010) stated that the effect of nitrogen sources on EPS production by *S. phocae* showed that yeast extract was most effective than other tested nitrogen sources. This may be due to the presence of larger quantities of free amino acids, short peptides and more growth factors in yeast extract. Among the various concentration, yeast extract at 20 g $L^{-1}$ showed maximum EPS (12.14 ± 0.31 g $L^{-1}$) production. Yeast extract was reported to be the most efficient nitrogen source, which greatly enhanced the EPS production by *L. plantarum* MTCC 9510 observed maximum EPS production was high in the presence of yeast extract by *Paenibacillus polymyca* EJS-3.

Similarly, Cerning *et al*. (1994) stated that the three growth conditions (temperature, pH, and Bactocasitone concentration) likely to affect EPS production. The influence of the carbon source on EPS production was not examined because other studies have shown in general, that glucose (10 to 20 g/l) provides the highest yield of EPS .Carbon source is one of the most important factors affecting EPS production. A wide variety of carbon sources, including sucrose, glucose, lactose, maltose, mannitol, sorbitol, whey, starch, and even non sugar sources like methanol and C9 to C16 n-alkanes, can be used to produce microbial EPS. Many studies demonstrate the influence of the type of carbon source on EPSs production (Wang *et al*., 2006; Miqueleto *et al*., 2010) and the carbon source that leads to high cell weight does not always result in EPS production increase. Lactose was the best carbon source for EPS production of strain SM-A87. Previous study showed that strain SM-A87 genome harbors 5 predicted galactosidase coding genes which suggests that it may efficiently use lactose as a carbon source for EPS synthesis. It is reported that EPS production is favored by a high carbon: nitrogen ratio and 10:1 is considered to be the most favourable for EPS production. Likewise, Kojic *et al*. (1992) was found that when the concentration of lactose, peptone and yeast extract were 32.22 g/L, 8.87 g/L and 5 g/L, respectively, and the carbon and nitrogen ratio was about 12:1, and the EPS production broth viscosity were reached maximum. Incubation temperature lower than the optimum may cause enhancement of EPS production and reduction of growth rate and cell mass, resulting in long logarithmic phase of growth and higher viscosity.

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In the present study carbohydrate content of EPS extract of *Frateuria aurentia* was 0.395± 0.198mg/100ml. Similarly, Vijayabasker *et al.* (2011) reported the carbohydrate estimation was done in *B. subtilis* in EPS basal medium and malt medium in which the optical density for carbohydrate was 0.91 ± 0.11mg/100ml and 0.43 ± 0.08 mg/100ml and also the amount of sulphate present in the EPS crude extract of *Frateuria aurentia* was estimated value was 0.312 ± 0.100mg/100ml and the amount of protein was present in EPS crude extract of *Frateuria aurentia* was 0.108 ± 0.162mg/ 100ml. Similarly Vijayabasker *et al.,* (2011) reported that Protein was higher for *B. subtilis* in sample malt medium, whereas the optical density was 0.11 ± 0.07 mg/100ml.

## CONCLUSION

A pure bacterial strain capable of producing EPS bacterial strain was isolated and identified as *Frateuria aurentia* by carbohydrate fermentation profile and sequence analysis of 16S rRNA. The important parameters had significant positive effects on the EPS production in different nutrients and cultivation conditions.

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## REFERENCES

Breedveld, M.W.; Zevenhuizen, L.P.T.M.H.; Canter Cremers, C.J.; Zehnder**,** A.J.B.; (1993). Influence of growth conditions on production of capsular and extracellular polysaccharides by *Rhizobium leguminosarum*. Antonie Leeuwenhoek., 64**,** 1–8.

Cerning, J.; Renard, C.M.G.C.; Thibault, J.F.; Bouillanne, C.; Landon, M.; Desmazeaud, M.; Topisirovic, L.; **(**1994). Carbon source requirements for exopolysaccharide production by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. Appl. Env. Microbiol., 60, 3914–3919.

Conti, E.; Flaibani, A.; O'Regan, M.; Sutherland, I.W.; (1994). Alginate from *Pseudomonas fluorescens* and *P. putida*: production and properties. Microbiology., 140, 1125–1132.

Degeest, B.; Vaningelgem, F.; De Vuyst, L.; (2001). Microbial physiology, fermentation kinetics, and process engineering of heteropolysaccharide production by lactic acid bacteria. Int. Dairy J., 11, 747-757.

Duta, F.P.; França, F.P.; Lopes, L.M.A.; (2006). Optimization of culture conditions for exopolysaccharides production in *Rhizobium* sp. using the response surface method. Electronic J Biotechnol., 9(4), 393-399.

Gao, C.; Wang, Z.; Su, T.; Zhang, J.; Yang, X.; (2012). Optimisation of exopolysaccharide production by *Gomphidius rutilus* and its antioxidant activities in vitro. Carbohydrate Polymers., 87, 2299– 2305.

Ismail, B.; Nampoothiri, K.M.; (2010). Exopolysaccharide production and prevention of syneresis in starch using encapsulated probiotic *Lactobacillus plantarum***.** Food Technol. Biotechnol., 48(4), 484–489.

Kanmani, P.; Satishkumar, R.; Yuvaraj, N.; Paari, K.A.; Pattukumar, V.; Arul, V.; (2011). Production and purification of a novel exopolysaccharide from lactic acid bacterium *Streptococcus phocae* PI80 and its functional characteristics activity *invitro*. Bioresource Technology., 102, 4827–4833.

Kojic, M.; Vujcic, M.; Banina, A.; Cocconcelli, P.; Cerning, J.; (1992). Analysis of exopolysaccharide production by *Lactobacillus casei* CG11, isolated from cheese. Appl Environ Microbiol., 58, 4086–4088.

Kumar, A.S.; (2007). Bacterial exopolysaccharides – a perception. J. Basic Microbiol., 47, 103–117.

Kumar, A.S.; Mody, K.; Jha, B.; (2007). Bacterial exopolysaccharides–a perception. J. Basic Microbiol., 47, 103–*117.*

*Lin, C.L.; Wang, C.C.*; Chang, S.C.; Inbaraj, B.S.; Chen, B.H.; (2009). Antioxidative activity of polysaccharide fractions isolated from *Lycium barbarum* Linnaeus. Int. J. Biological Macromolecules., 45**,** 146–151.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Looijesteijn, P.J.; Hugenholtz, J.; (1999). Uncoupling of growth and exopolysaccharide production by *Lactococcus lactis* subsp. *Cremoris* NIZO B40 and optimization of its synthesis. J. Biosci. Bioeng., 88, 178-182.
Miqueleto, A.P.; Dolosic, C.C.; Pozzi, E.; Foresti, E.; Zaiat, M.; (2010). Influence of carbon sources and C/N ratio on EPS production in anaerobic sequencing batch biofilm reactors for wastewater treatment. Bioresour Technol., 101, 1324–1330.
Morin, A.; (1998). Screening of polysaccharide-producing microorganisms, factors influencing the production and recovery of microbial polysaccharides. In: Dumitriu S, ed. Polysaccharides–Structural Diversity and Functional Versatility. New York: Marcel Dekker Inc. pp 275–296.
Mozzi, F.; Oliver, G.; de Giori, G.S.; de Valdez, G.F.; (1995). Influence of temperature on the production of exopolysaccharides by thermophilic lactic acid bacteria. Milchwissenschaft., 50, 80–82.
Satpute, S.K.; Banat, I.M.; Dhakephalkar, P.K.; Banpurkar, A.G.; Chopade, B.A.; (2010). Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. Biotechnol Adv., 28, 436–450.
Staudt, A.K.; Wolfe, L.G.; Shrout, J.D.; (2011). Variations in exopolysaccharide production by *Rhizobium tropici*. Arch Microbiol., In press.
Van den Berg, D.J.C.; Robijn, G.W.; Janssen, A.C.; Giuseppin, M.L.F.; Vreeker, R.; Kamerling, J.; Vliegenthart, J.F.G.; Ledeboer, A.M.; Verrips, C.T.; (1995). Production of a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of the polysaccharide. Appl. Env. Microbiol., 61, 2840–2844.
Vijayabaskar, P.; Babinastarlin, S.; Shankar, T.; Sivakumar, T.; Anandapandian, K.T.K.; (2011). Quantification and Characterization of Exopolysaccharides from *Bacillus subtilis* (MTCC 121**).** Adv. Biological Research., 5(2), 71-76.
Wang, J.M.; Hu, Y.L.; Wang, D.Y.; Liu, J.; Zhang, J.; Abula, S.; Zhao, B.; Ruan, S.L.; (2010). Exopolysaccharides in biotechnological Application. Cell. Immunol., 263, 219–223.
Wang, X.; Xu, P.; Yuan, Y.; Liu, C.; Zhang, D.; (2006). Modeling for gellan gum production by *Sphingomonas paucimobilis* ATCC 31461 in a simplified medium. Appl Environ Microbiol., 72, 3367–3374.