Review Article

Probiotics: Health benefits in the mouth

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Abstract: Probiotics or health-beneficial bacteria have only recently been introduced in dentistry and oral medicine after years of successful use in mainly gastro-intestinal disorders. The concept of bacteriotherapy and use of health-beneficial micro-organisms to heal diseases or support immune function was first introduced in the beginning of the 20th century. Later the concept lead to the development of modern dairy industry and even today most probiotic strains are lactobacilli or bifidobacteria used in milk fermentation. The mechanisms of probiotic action are mainly unknown but the inter-microbial species interactions are supposed to play a key role in this together with their immuno-stimulatory effects. The introduction of probiotic bacteria in the mouth calls for ascertainment of their particular safety. Since acid production from sugar is detrimental to teeth, care must be taken not to select strains with high fermentation capacity. The first randomized controlled trials have nevertheless shown that probiotics may control dental caries in children due to their inhibitory action against cariogenic streptococci. Less evidence exists on their role in periodontal disease or oral yeast infections. Furthermore the best vehicles for oral probiotic applications need to be assessed. So far mainly dairy products have been investigated but other means such as probiotics in chewing gums or lozenges have also been studied. From the clinical practitioner's point of view direct recommendations for the use of probiotics cannot yet be given. However, scientific evidence so far indicates that probiotic therapy may be a reality also in dentistry and oral medicine in the future. (*Am J Dent* 2009;22:329-338)

Clinical significance: From the clinical practitioner's point of view direct recommendations for the use of probiotics cannot yet be given. However, scientific evidence so far indicates that probiotic therapy may be a reality also in dentistry and oral medicine in the future.

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Introduction

Probiotics have emerged as a fascinating scientific area, health-related and commercial target for the last two decades. Endorsed by the Food and Agriculture Organization and the World Health Organization, the definition of probiotics, in 2001, describes them as live microorganisms which when administered in adequate amounts confer health benefits on the host. Gradually, as the body of evidence of probiotic effectiveness accumulates, new features to the definition are appended broadening their implication. Commonly, most of the species ascribed as having probiotic properties belong to the genera *Lactobacillus* and *Bifidobacterium*. Those bacteria are generally regarded as safe (GRAS) because they can reside in the human body causing no harm and, on the other hand, they are key microorganisms in milk fermentation and food preservation and used as such from the dawn of mankind. Lactobacilli found in raw milk and fermented dairy products such as cheese, yogurt and fermented milk are ubiquitous in the diet and are found in the gastrointestinal tract soon after birth.

The role of beneficial bacteria on human health stems from the work of the bacteriologist and Nobel Prize laureate Ilye Metchnikoff in the turn of 20th century. Studying longevity and general health of a Bulgarian population dwelling in the Rhodopes Mountains and fed basically on dairy products, the scientist introduced the idea that lactic acid bacteria in yogurt may neutralize deleterious effects of gut pathogens thus extending life span. He further contributed to the adoption of the name of the species, *Lactobacillus bulgaricus*, one of the two essential yoghurt starter microorganisms. This also meant the birth of modern dairy industry.

Over the years, the scientific interest to discover, assess, and analyze species with probiotic properties has intensively grown and the research papers today outnumber 5820 (as of May 2009 in the PubMed search). Probiotic strains are now widely used to give consumers a health benefit substantiated in a range of randomized clinical trials. Table 1 summarizes the proven efficacy of probiotics in their most studied clinical applications. The list of disease conditions that may benefit from bacteria tend to increase with the advent of more sophisticated research methods utilized in studying the microbe-host interactions.

Mechanisms of action explaining beneficial probiotic effects include modulation of host immune response leading to strengthening of the resistance to pathogenic challenge, alteration of the composition and metabolic activity of host microbiota at the specific location. Among paramount selection criteria for probiotics are:

- Adhesion and colonization (at least transitory) of the human body. Adhesion may increase the retention time of a probiotic and place bacteria and host surfaces (body fluids and epithelial cells) in close contact thus facilitating further probiotic activity;
- Enhancement of the non-specific and specific immune response of the host;
- Production of antimicrobial substances and competition with pathogens for binding sites;
- Survival and resistance to human defense mechanisms during the oro-gastro-intestinal transit;
- Safety to the macro-organism.

Considering the particular activities of probiotics and their inhibitory effect on the growth of pathogens, research interest has been extended to the oral cavity where probiotics may also exert their therapeutic or preventive effect on the development and progression of common oral diseases. Figure 1 outlines the

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Table 1. Main areas of probiotic activity investigated.

Disease	Study design	Number of participants	Duration	Probiotics	Effect observed	Reference
Inflammatory bowel disease	Prospective	10	13 months	*B. breve, B. longum, L. casei*	Reduction of symptoms of Crohn`s disease in 7 out of 10 patients	Fujimori *et al*.[89]
	Randomized control trial	187	12 months	*L. rhamnosus* GG	Maintenance of remission in ulcerative colitis patients	Zocco *et al*.[90]
	Randomized double-blind study	120	12 weeks	*E. coli* Nissle 1917	Maintenance of remission of ulcerative colitis	Kruis *et al*.[91]
	Randomized double-blind trial	372	12 months	*E. coli* Nissle 1917	Maintenance of remission of ulcerative colitis equivalent to the therapy with mesalazine	Kruis *et al*.[92]
	Randomized double-blind controlled study	20	1 month	*L. rhamnosus* GR-1, *L. reuteri* RC-14	Increased proportion of putative CD4+ CD25+ Treg cells in the peripheral blood of IBD patients	Lorea Baroja *et al*.[93]
	Multicenter randomized double-blind controlled study	267	12 weeks	Symbiotic combination containing *L. paracasei*	Pain reduction and bowel movements in IDB patients	Andruilli *et al*.[94]
Virus/microbe- or antibiotic-associated diarrhea	Multi center, randomized, double-blind, placebo-controlled trial	113 infants	10 days	*E.coli* Nissle 1917	Shortening of diarrhea duration with 2.3 days	Henker *et al*.[95]
	Double-blind, randomized, placebo-controlled trial	240	Along with duration of antibiotic administration	*L. rhamnosus*	Reduction of the risk of diarrhea	Ruszczyński *et al*.[96]
	Double-blind, placebo-controlled trial	85	14 days	*L. casei* DN-114 001	Positive effect on stool consistency	Guralt *et al*.[97]
Lactose intolerance	Parallel single-blind study	33	10 days	*L. bulgaricus, Str. thermophilus*	Higher acute leucine assimilation	Parra & Martínez[98]
	Prospective study	11	2 weeks	*B. longum, B. animalis*	Alleviation of the symptoms	He *et al*.[99]
	Prospective study	20	3 days	*L. acidophilus* NCFM	Alleviated symptoms	Montes *et al*.[104]
Vaginosis	Randomized, placebo-controlled study	64	60 days	*L. rhamnosus* GR-1, *L. fermentum* RC-14	Yeast reduction	Reid *et al*.[100,101]
Allergies	Randomized, double-blind, placebo-controlled study	474	2 years	*L. rhamnosus, B. animalis*	Reduction of cumulative prevalence of eczema	Wickens *et al*.[102]
	Randomized, double-blind, placebo-controlled study	30	8 weeks	*L. paracasei* Lpc-37, *L. acidophilus* 74-2, *B. animalis* subsp. *Lactis* DGCC 420	Modulation of peripheral immune parameters	Roessler *et al*.[103]

plausible mechanisms by which probiotics may exert their beneficial effect in the mouth.

In the present review article we discuss the beneficial role of some probiotic species with the scope of activity in the oral cavity. We comment on their mode of action and clinical effectiveness, as well as evaluate the means of administration. This review is mainly based on our earlier contributions in the area and for more in-,depth view the reader is kindly asked to refer to these publications.[2-4]

PROBIOTICS AND THE MOUTH

Oral microbiota as a source of probiotics

The oral cavity is a rather intricate habitat providing the establishment of a great diversity of microbial species. Each environment within the mouth supports distinct yet overlapping communities of hundreds of species.[5-8] It has been recently estimated that over 1000 bacterial species are present in the oral cavity.[9] Furthermore, the tongue dorsum possesses a unique microbiotia: one-third of oral species is exclusively harbored on the tongue and cannot be isolated from any other oral niche.[10,11]

Bacteria reside in the mouth either in planktonic state or are finely integrated in oral biofilm on various oral surfaces. Oral biofilms are dynamically changing and develop increasingly complex structures as they mature. Interaction between species is characteristic in biofilms. Some species may depend on others to provide favorite environment for colonization. Furthermore, bacteria in biofilms differ physiologically from their planktonic counterparts and tend to be much more resistant to environmental factors and antimicrobial agents. It has been established that distinct genes become active when planktonic bacteria bind to surfaces and grow in biofilms.[12,13] On the other hand, saliva is the essential medium in the mouth contributing to the microbial diversity. It plays an integral role in propagating oral biofilms. Salivary flow can easily lead to detachment of some microbes from biofilm surfaces, and thus modulate microbial colonization. Furthermore, as a complex medium, saliva contains different proteins with bactericidal, bacteriostatic, or inhibitory activity that collectively may damage a variety of species in planktonic state.[14-17] Biofilm species composition can also depend on phenomena like auto-

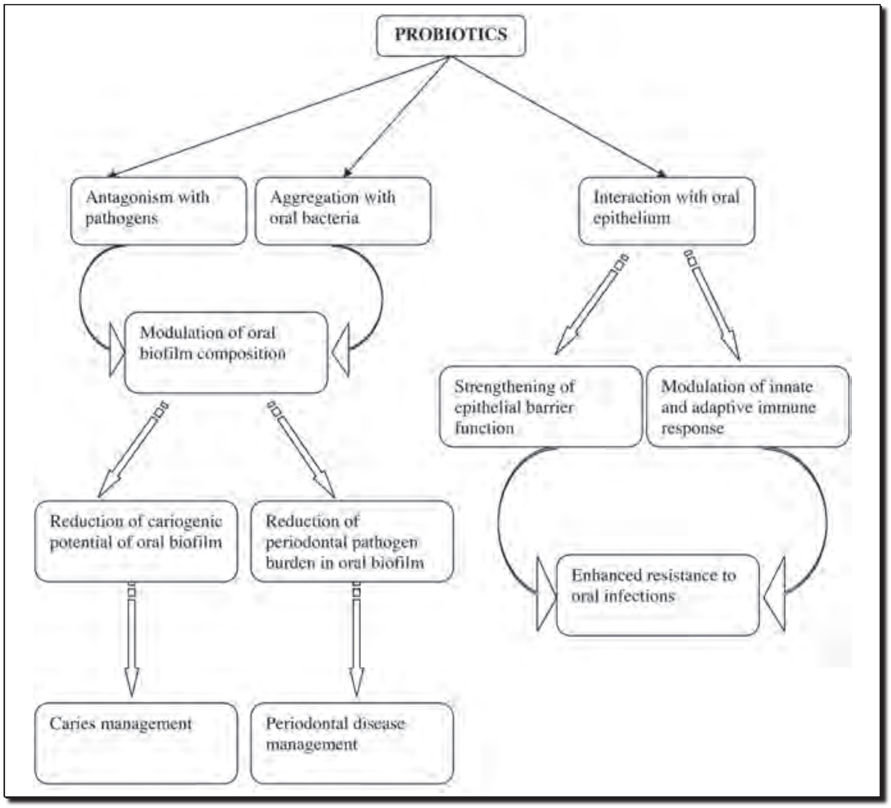

Figure. Possible probiotic activities in the oral cavity.

or co-aggregation that may prevent microorganisms from establishing themselves in the biofilms. Taking into consideration the multifaceted nature of biofilm development and multivariate species interactions, we can acquire better understanding and interpretation of studies with probiotics in the oral cavity.

Since probiotic species belong predominantly to the genera *Lactobacillus* and *Bifidobacterium*, it is of special interest to find out whether such microbes with beneficial properties naturally inhabit the oral cavity. Generally, there is scarce evidence that probiotics permanently reside in the human body and in the mouth, in particular.[18,19] However, it can be anticipated that among the 103-104 CFU/g lactobacilli found in the oral cavity.[20] There could be some species/strains capable of exerting probiotic properties. In a study[21] comparing species variability in the mouth and feces, it was discovered that species most frequently recovered from the rectal as well as from the oral mucosa were *L. plantarum* and *L. rhamnosus*, which were present in 52% and 26% of the individuals respectively. However, this study did not aim to define if those strains are permanent colonizers of the two sites tested and whether the mouth is their natural habitat. The most common species of lactobacilli recovered from saliva of a Thai population were *L. fermentum* and *L. rhamnsous*.[22] A promising finding was that lactobacilli population differed in healthy and individuals with periodontal disease. Koll-Klais *et al*[23] observed that healthy persons are populated by *L. gasseri* and *L. fermentum*, whereas the predominant species in periodontitis patients was *L. plantarum* while the first two were undetectable. The isolated lactobacilli suppressed growth of key periodontopathogens like *P. gingivalis*, *A. actinomycetem-comitans* and *P. intermedia* and the effect was strain- and individual-dependent. Observations by this study group showed that microorganisms with probiotic properties may indeed exist and reside in the oral cavity. However, the complexity of biofilm development and interspecies interactions require more thorough investigations in order to assert true probiotic candidates with activity in the oral cavity.

The intensive growth of functional food market worldwide has resulted in more probiotic species to be delivered to the macroorganism predominantly in different fermented foodstuffs and dairy products. Over the counter tablet forms containing various probiotic species also contribute to probiotic supply but it is yet questionable whether a tablet intake is a reliable source for probiotics to be established in the mouth due to the rapid transit.

Probiotic resistance to oral defense mechanisms

Considering the oral cavity as the main entry to the gastrointestinal tract, ingested probiotics are exposed first to saliva which mediates the contact with hard and soft oral tissues. During this first step of contact with the macro-organism, survival and resistance to environmental factors in the mouth are of paramount importance. Salivary proteins such as lysozyme, lactoferring, histatin, salivary peroxidase, cystatins, and secretory IgA can collectively affect viability or cell surface morphology of probiotic species, further affecting their adhesion and metabolic activity. The role of saliva on microbial establishment can be contradictory, however, inhibiting colonization on one hand (by growth inhibition, killing, or prevention of adherence to host tissues), and promoting microbial colonization, on the other hand.[24] *In vitro* studies[25] testing probiotic survival in saliva have shown that *Lactobacillus* and *Bifidobacterium* strains cannot grow in saliva but remain viable after 24 hours of incubation. Lysozyme pretreatment has been observed to significantly reduce the adhesion of *L. rhamnosus* GG, *L. rhamnosus* Lc705 and *L. casei* Shirota. However, the adhesive properties of *L. johnsonii* La1 and *B. lactis* Bb12 remained unaffected. These results emphasize the strain-specific response to proteolytic enzymes and this feature needs to be considered when selecting probiotics for the oral cavity. Studies in our laboratory[26] have shown that lysozyme pretreatment of lactobacilli can slightly increase their adhesiveness to saliva-coated surfaces. Viability of lactobacilli after lysozyme pretreatment was not significantly reduced but cell surface alterations might have contributed to the increased adhesion. Further studies on the mechanism whereby lysozyme affects adhesion are necessitated, however.

Saliva-mediated aggregation is another aspect to be considered when evaluating establishment of probiotics in the mouth. Aggregation ability is related to cell adherence properties. It has been well documented that bacteria can attach to immobilized salivary proteins, attach to epithelial cells, or aggregate with other bacteria already there.[27-29] The mechanisms of adhesion in lactobacilli involve hydrophobicity and surface charge, as well as specific carbohydrate and/or proteinaceous components.[30] Auto-aggregating strains express profound cell surface hydrophobicity that may improve colonization. Organisms able to co-aggregate with other bacteria, for example pathogens, may have greater advantages over non-co-aggregating organisms which are easily removed from the mouth. A common oral pathogen, *F. nucleatum*, which is regarded as a chain-microorganism in biofilm formation has shown a different pattern of co-aggregation with probiotic and putative probiotic lactobacilli which phenomenon correlated with various degrees of hydrophobicity (unpublished data in our laboratory). To emphasize the role of aggregation, recent results have shown that *L. salivarius* W24[31] was unable to form a biofilm when incubated as a monoculture in a microplate model, whereas when the species was added simultaneously with the inoculum of other commensal oral microorganisms, it established itself irrespective of pH. Similar findings were observed with *L. plantarum* SA-1 and *L. rhamnosus* ATCC 7469 that failed to form substantial biofilms in mono-culture but biofilm mass increased when co-cultured with *A. naeslundii*.[32]

Adhesion as a prerequisite for probiotic establishment

Among the various selection criteria, adhesion could be considered of primary importance that further favors the expression of probiotic activity. The capacity of probiotics to adhere to surfaces of the oral cavity can avoid or at least reduce rapid exclusion from the environment. In the mouth adhesion is a necessary phenomenon in the microbe-saliva interactions. Additionally, biofilm covering both mouth mucosa and dental hard tissues should be regarded as a mediator influencing adhesion. *In vitro* studies have assessed adhesion by measuring the attachment of bacteria to saliva-coated hydroxyapatite (HA) and oral epithelium.[26] HA beads or discs serve as a surface sharing chemical and structural similarity to tooth enamel, and results obtained could therefore reflect *in vivo* conditions. Probiotics and putative probiotic strains have been shown to vary extensively in their adhesiveness to saliva-coated HA. Among probiotics strains *L. rhamnosus* GG exhibited the highest values of adhesion, comparable to those of the early tooth colonizer *S. sanguinis*. Dairy starter *L. bulgaricus* strains adhered poorly to sHA.[26]

A significant weakness in the *in vitro* studies always is their limited ability to completely reproduce authentic environmental conditions. Studies using simulated biofilm formation to assess adhesion can provide more reliable results that explain the phenomena taking place *in vivo*.

Interspecies cross-talk is another feature that affects the composition and stability of microbiota in oral biofilms. It has been estimated that the beneficial role of probiotics is mainly based on their antagonistic effect on pathogens. This activity can be either due to competition for binding sites with pathogens, or production of antimicrobial substances. Probiotics produce lactic acid, hydrogen peroxide and bacteriocins or bactriocin-like substances that can inhibit growth of a wide range of pathogens. Inhibitory substances or bacteriocin-producing lactic acid bacteria, which aggregate with pathogens, may constitute an important host defense mechanism against infection in general.[33] Probiotic lactobacilli have shown various inhibitory activity *in vitro* against different oral pathogens.[23,34] However, in most of these studies, simple laboratory techniques have been used which in light of contemporary molecular methods necessitate more profound analyses elucidating the specific characteristics of microbial species interaction.

The adhesion of probiotic bacteria to oral soft tissues is another aspect that promotes their health effect to the host. Cell adhesion is a complex process involving contact between the bacterial cell and interacting surfaces. The epithelial lining of the oral cavity despite its function as a physical barrier, actively participates in immune response. It has been shown that probiotic bacteria stimulate local immunity and modulate the inflammatory response.[35,36] As Gram positive bacteria, lactobacilli express ligands for toll-like receptors (TLRs) which initiate immune responses enabling recognition of both pathogens and indigenous microbiota by epithelial cells. Recognition of commensal bacteria by TLRs is necessary for homeostasis, protection of epithelial cells from injury and stimulation of repair.[37] Although studies of immunomodulatory role of probiotics in the oral cavity are in their infancy, substantial information that allows prediction of key mechanisms of activity can be derived from studies within the gastrointestinal tract. Most probably in the oral cavity probiotic species bind TLR-2 on oral epithelial cells surface but here more in-depth studies are needed.

Epithelial cells play an essential role in providing innate defense against microbial challenge through the production of antimicrobial molecules, as well as cytokines and chemokines necessary for leukocyte recruitment.[38] Our recent studies (unpublished data in our laboratory) have shown that contact of probiotic bacteria with oral epithelium induces strain-, concentration-, and time-dependent IL-8 and TNF-α secretion. Both heat killed and live probiotic bacteria were able to stimulate cytokine secretion, whereas the effect was weaker for heat killed species. Another finding in our study was that only bacterial fractions but not the supernatants possessed immunostimulatory activity.

Probiotic effect on human β-defensin (hBD) secretion by oral epithelial cells may also serve as an evaluation criterion for immunostimulatory properties of these species. Human β-defensins have been identified in the oral cavity with broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, fungi and enveloped viruses.[39] However, results for probiotic activity on β-defensin production are yet only available from studies in the gastrointestinal tract. Active and heat inactivated *L. fermentum*, *L. acidophilus* PZ, *L. paracasei*, *L. plantarum*, *E. coli* Nissle 1917 and *P. pentosaceus* induced remarkable expressions of hBD-2 in Caco-2 cells.[40,41] The observed effect on hBD-2 secretion suggests that lactobacilli strengthen intestinal barrier function through the up-regulation of hBD-2 *via* induction of proinflammatory pathways including NF-kappaB and AP-1 as well as MAPKs. In the oral cavity hBD-2 is expressed in oral epithelium in normal uninflammed gingival tissue presumably because of the high level of exposure to commensal microorganisms.[42] However, a β-defensin mRNA has been less frequently found in periodontitis patients suggesting an important role of hBDs in innate host defense in the oral cavity.[43] Extending studies of the effect of probiotics on oral epithelium in terms of β-defensin secretion might broaden and affirm their health promoting activity in the mouth.

CLINICAL RELEVANCE OF PROBIOTICS IN ORAL AND DENTAL DISEASES

The first part of the review focused on probiotics as related to their possible modes of action. This section outlines the clinical effectiveness of probiotics as preventive or treatment strategies for common oral diseases.

Dental caries and periodontal diseases yet remain the most prevalent oral infectious diseases with significant social impact. The primary role of bacteria in their etiology is well established. Although bacteria are essential for disease initiation, the intricate interactions between microbiota, host and environmental factors, including diet, hygiene habits and physiological stress characterize susceptibility to oral and dental diseases and severity and progression.

Probiotics in caries management

Streptococcus mutans is the main causative microorganism in caries development because of its ability to produce highly branched, water-insoluble glucan, mutan, which facilitates its establishment in the oral biofilm.[44] Its acidogenic properties and rapid metabolism of sucrose, fructose and glucose generates low pH that challenges the homeostasis in the oral microbial community with a shift towards bacteria that induce caries.[45] Moreover, elevated salivary counts of *S. mutans* are associated with higher caries risk and disease progression.

Considering the essential role of *S. mutans* in caries development, various efforts have been made to affect its prevalence and cariogenic properties in the mouth. Several clinical studies have been executed with probiotic administration to reduce *S. mutans* bacteria in the oral cavity. The studies were conducted after the results from *in vitro* studies had shown that probiotic strains and putative probiotic candidates suppress the growth of *S. mutans* and other oral streptococci with cariogenic potential.[34,46,47]

The first randomized, double-blind, placebo-controlled intervention study examining the effect of milk containing *L. rhamnosus* GG on caries and the risk of caries in children when compared with normal milk was completed in 2001; the study included 594 children, 1-6 years old, who consumed milk for 7 months.[48] Probiotic milk was able to reduce *S. mutans* counts at the end of the trial and a significant reduction of caries risk was also observed. The putative caries prophylactic effect of probiotics has been also confirmed by daily intake of cheese containing *L. rhamnosus* GG and *L. rhamnosus* LC 705.[49] Despite the short duration and relatively small number of participants in this study, the probiotic cheese significantly reduced *S. mutans* counts in the intervention group during the post-treatment period when compared with the controls. Another probiotic species, *Bifidobacterium* DN-173 010, ingested once daily with yogurt demonstrated a significant reduction of salivary *S. mutans*, whereas no significant reduction was found in lactobacilli levels.[50]

Ice cream can be an attractive vehicle for probiotic intake combining both health-promoting and mood-boosting effects. A *B. lactis* Bb-12 containing ice cream, if eaten for 10 days, can lead to significant *S. mutans* level reduction.[51] In all studies discussed above probiotics were mostly delivered in fermented dairy products. It is conceivable that the means of administration might positively affect the effects observed as related to mutans streptococci reduction. To assess the role of other than dairy food vehicles for probiotic intake, Çaglar *et al*[52] administered *L. reuteri* ATCC 55730 in a tablet and in water taken *via* a telescopic straw for 3 weeks. The results obtained showed that irrespective of the means of delivery salivary counts of *S. mutans* were significantly reduced at the end of the intervention period. No statistically significant changes were found for lactobacilli isolated. When probiotic *L. reuteri* ATCC 55730 and ATCC PTA 5289 at concentration 108 CFU/g were given in a chewin gum for 3 weeks, salivary *S. mutans* reduction was significant and comparable to *S. mutans* reduction after xylitol chewing gum intake in the same test period.[53] However, no cumulative effect was observed when the chewin gum contained both xylitol and the probiotic mix. A lozenge with *L. reuteri* ATCC 55730/*L. reuteri* ATCC PTA 5289 taken by healthy individuals with high *S. mutans* counts resulted in significantly lower levels of *S. mutans* within 10 days.[54] The observed positive correlation between probiotic intake and caries pathogen reduction might be a useful strategy in caries prophylaxis in some special risk groups. Orthodontic patients wearing fixed appliances can experience higher caries risk during treatment and in them a probiotic intake of *B. animalis* subsp. *lactis* DN-173010 was shown to positively reduce salivary mutans streptococci.[55] The observed changes in salivary microbiota provide evidence to the clinicians for recommending to their patients the consumption of probiotics in addition to the "classical" oral hygiene practices and dietary counseling.

In none of the above studies, irrespective of probiotic species used, levels of lactobacilli have been reduced compared with baseline values. However, contradictory to the findings already discussed, Montalto *et al*[56] administered a probiotic preparation containing seven live probiotic lactobacilli in capsule or liquid form and found a statistically significant increase in the salivary counts of lactobacilli compared to baseline, while the counts of *S. mutans* remained unaffected. This is the only clinical trial presenting lack of probiotic effect on *S. mutans* levels. It might be attributed to the greater variety of probiotic species applied which may exert different effects than if the bacteria were given as a monoculture.

Plausible explanation for the clinical results of probiotic intake might be obtained from some *in vitro* studies. The observed reduction of mutans streptococci might be due to the competition for binding sites in oral biofilms. *L. casei* ATCC 11578 has shown to affect the adherence of the streptococci to saliva-coated HA, by slightly inhibiting the adherence of *S. mutans* and it could even release the already bound streptococci from the HA.[57] Various probiotics have demonstrated ability to modify the composition of salivary pellicle by binding and degrading proteins important for bacterial adhesion.

Aspects of safety from the caries prophylactic perspective

Although probiotics can affect main caries pathogens, lactobacilli themselves may associate with caries progression. Some strains of *Lactobacillus* spp., together with *S. mutans*, are known to play a key role in the etiopathogenic mechanisms leading to the development of dental caries.[56] The production of organic acids from dietary sugars is a leading factor also in dentin caries progression.[58] If probiotic lactobacilli taken orally are able to adhere or temporarily establish themselves in the mouth, their metabolism and acid production should not favor caries induction. Adhesion of two probiotics, *L. casei* Shirota and *L. acidophilus* in an artificial caries model have shown inconclusive results about the potential of those species to participate in caries progression; lactobacilli counts were higher in distilled water than in dentin samples under the terms of the study.[59] A probiotic *L. salivarius* LS 1952R administered to rats on 5 consecutive days possessed an inherent cariogenic activity after adherence to tooth surface and enhanced cariogenicity of *S. mutans.*[60] Reproducing oral biofilm model, Pham *et al*[31] observed that *L. salivarius* W24 could establish itself in the biofilm community if added simultaneously with the inoculum and could lower the pH of sucrose-exposed microbiota. These findings indicate that once established in the oral microbiota the presence of sucrose *L. salivarius* W24 might increase the cariogenic potential of the oral microbial community.

Six commercially available lactobacilli, *L. plantarum* 299v, *L. plantarum* 931, *L. rhamnosus* GG, *L. rhamnosus* LB12, *L. paracasei* F19, and *L. reuteri* were assessed for acid production from various sugars and sugar alcohols.[45] Among them, *L. plantarum* strains had the highest activity fermenting glucose, fructose, lactose, sucrose, maltose, trehalose, and arabinose. Fermentation of glucose, fructose, manitol, and trehalose by *L. rhamnosus* GG resulted in pH values between 5.2 and 6.8 following 24 hours of incubation. *L. paracasei* and *L. plantarum* displayed very slow fermentation and pH values reaching 5.2 – 6.8 after 72 hours of incubation. The inability of *L. rhamnosus* strains, *L. paracasei* F19 and *L. reuteri* to ferment sucrose adds valuable information about relative safety of probiotic strains in caries-prophylactic perspective. Another study addressing sugar fermentation has shown a strain-dependent pH drop and the decrease was the fastest with glucose for all 14 strains tested, thus highlighting the acidogenic potential of probiotics.[57]

The diversity of *in vitro* results does not allow clear conclusions and sound recommendations to be made about which probiotics may add benefit to the mouth. More large scale, multi-centered clinical investigations are needed to support the true effectiveness of probiotics in the prevention of oral and dental diseases. Moreover, inter-individual differences in oral microbiota compositions may be a key factor for the establishment of “good” bacteria that maintain the balance for oral health. It has been found that subjects without caries experience are colonized by lactobacilli that possess a significantly increased capacity to suppress the growth of mutans streptococci compared with subjects with arrested or active caries.[61] Generally, it can be concluded that the overall effect of lactobacilli on caries prevention seems favorable when probiotic candidates are carefully selected.

Probiotics and periodontal diseases

Despite substantial improvements in the oral health status of populations across the world, periodontal disease yet remains a significant social burden. Periodontal diseases are an end result of host response to the complex action of a group of periodontal bacteria, predominantly Gram negative anaerobes.[62] Accumulating evidence on the role of periodontal diseases on general health has related the chronic periodontal inflammation to various systemic diseases, diabetes mellitus being the most consistent.[63,64] On the other hand the aggravating effect of periodontal diseases on cardiovascular diseases,[65,66] pre-term and/or low birth weight,[67-69] Alzheimer’s disease[70] and renal disease[71,72] has been clinically observed.

Since the primary etiological factors for the development of periodontal disease are bacteria in supra- and subgingival biofilm, efforts for disease prevention and treatment are mainly focused on pathogen reduction and strengthening of the epithelial barrier, thus contributing to decreased susceptibility to infection. Probiotic bacteria, generally regarded as safe, may favor periodontal health if able to establish themselves in oral biofilm and inhibit pathogen growth and metabolism. Only few clinical studies outlining probiotic effectiveness in periodontal disease have been published. Therefore, data on probiotics with specific target periodontal structures are mainly from laboratory experiments.

From the periodontal perspective it should be noted that the composition of lactobacilli species differs in healthy and periodontitis patients and obligately homofermentatives are less prevalent in chronic periodontitis.[23] A 14-day intake of *L. reuteri* led to the establishment of the strain in the oral cavity and significant reduction of gingivitis and plaque in patients with moderate to severe gingivitis.[73] A *L. salivarius* WB21 containing tablet when administered to a test group compared with placebo demonstrated insignificant differences in pocket probing depth (PPD) and bleeding on probing (BOP) indices, but caused a significant change in those parameters when smokers and non-smokers were separately analyzed. Probiotic intake improved clinical condition in smokers and reduced salivary lactoferrin at the end of the 8-week trial.[74]

Periodontal inflammation has been reduced and also positively affected by the administration of two probiotic tablet forms Bifidumbacterin[a] and Acilact[b] available on the Russian market.[75] Studies from Russia have also shown that a periodontal dressing containing *L. casei* 37 can reduce the number of most common periodontal pathogens and extend remission up to 10–12 months.[76] Possible explanation to the results might be the inhibitory effect of probiotics on pathogen growth thus altering the composition of oral biofilm. Due to its ability to inhibit *P. gingivalis*, *L. salivarius* TI 2711 was given for 4 or 8 weeks in a tablet to healthy volunteers at a concentration 2x107 CFU/ml. A significant reduction of black-pigmented rods in saliva was observed, whereas the number of *S. mutans* and lactobacilli did not change.[77] Additional finding in this study was the convergence of pH to neutral after treatment, thus highlighting both caries and periodonto-prophylactic properties. The effectiveness of the latter *Lactobacillus* strain was confirmed by Matsuoka *et al*[78] who demonstrated by means of real-time PCR analysis that LS1 translocates to subgingival plaque decreasing the number of *P. gingivalis*. These data also suggest that LS1 is an effective probiotic.

A proposed mechanism of action of probiotics is strengthening the mucosal barrier *via* tropic effects on the epi-

Table 2. Clinical evidence of probiotic effectiveness in the mouth.

Probiotic strain	Vehicle of administration	Duration of probiotic intake	Dosage	Clinical effectiveness	Reference
L. rhamnosus GG	Milk	7 months		Less dental caries and lower *S. mutans* levels	Näse *et al.*[48]
L. rhamnosus GG and *L. rhamnosus* LC 705	Cheese	3 weeks	5x15 g cheese per day	Reduction of the highest levels of *S. mutans* and caries risk reduction	Ahola *et al.*[49]
Bifidobacterium DN-173 010	Yogurt	2 weeks	200 g yogurt daily	Reduction of *S. mutans*	Çaglar *et al.*[50]
B. lactis Bb-12	Ice-cream	10 days	53 g ice-cream	Reduction of salivary *S. mutans*	Çaglar *et al.*[51]
L. salivarius WB21	Tablet	8 weeks	3 x 1 tablet daily	Improvement of plaque index and PPD in smokers	Shimauchi *et al.*[74]
L. reuteri LR-1 or LR-2	*L. reuteri* LR-1 or LR-2 formulations	2 weeks	$2x10^8$CFU per day	Reduction of plaque and gingivitis in patients with moderate to severe gingivitis	Krasse *et al.*[73]
S. salivarius K12	Lozenge	3 days	Lozenge taken after chlorhexidine mouthrinse	Reduction of oral VSCs levels	Burton *et al.*[83]
L. rhamnosus GG (ATCC 53103), L. *rhamnosus* LC705 and *Propionibacterium freudenreichii* ssp *shermanii* JS	Cheese	16 weeks	50 g cheese	Reduction of high yeast counts	Hatakka *et al.*[86]

thelium, and stimulating both the innate and adaptive immune system. *In vitro* studies have shown that the ability to induce secretion of TNF-α and IL-8 by oral epithelial cells is strain-dependent and the response is generally low (data unpublished). However, a novel finding in our study was that the addition of P. gingivalis to the *in vitro* system was able to completely prevent the detection of cytokines tested. This result again underlines the intricate communication between species. A double-blind placebo-controlled clinical trial has provided evidence that probiotic bacteria, *L. reuteri* ATCC 55730 and ATCC 5289, taken in a chewing gum for 10 minutes twice daily can reduce the pro-inflammatory cytokines TNF-α and IL-8 in gingival crevicular fluid.[79]

Probiotics or putative probiotic species discussed above belonged mainly to the *Bifidobacterium* and *Lactobacillus* genera. However, due to the far-reaching diversity of species in the oral cavity, it can be anticipated that other genera might also possess probiotic activity under certain environmental conditions. A novel concept favoring periodontal health has been recently introduced by Teughels *et al*.[80] These authors have suggested that re-colonization of a gingival pocket after scaling and root planing might be tailored by introducing microbes capable of inhibiting adhesion of common periodontal pathogens, *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, and *T. forsythia*. The foundation of the re-colonization concept stands on the principle that subgingival application of oral streptococci, *S. sanguinis* KTH-4, *S. salivarius* TOVE and S. *mitis* BMS, would enhance the microbial shift away from periodontopathogens.[81] Better radiographic results of healing of periodontal pockets after scaling and root planing were registered when beneficial bacteria were applied compared to controls in a dog model.[82] However, more *in vivo* studies are needed to sustain the replacement therapy approach.

Use of probiotics in oral symptoms and mucosal diseases

The outlined clinical effectiveness of probiotics related mainly to the commonest oral diseases, dental caries and periodontal disease. However, both clinical and *in vitro* evidence suggests that probiotics can positively affect conditions such as halitosis and *Candida* infections in the mouth. Table 2 outlines probiotic species with the strongest evidence for clinical effectiveness in the mouth in general.

Halitosis

Halitosis, *foetor ex ore*, or bad breath, is a condition affecting comparatively large section of the population. Reports of its occurrence date back to ancient times. Documentation of early Egyptian and other cultures indicate that people were aware of this problem and sweetening their breath with various herbs and spices, a practice that continues to this day. Bad breath in the oral cavity is mainly ascribed to the production of volatile sulfur compounds (VSC) predominantly by Gram negative anaerobes residing in periodontal pockets and on the tongue dorsum. It can be assumed that bacteriotherapy can also improve this condition. The replacement of bacteria implicated in halitosis by colonization with probiotic bacterial strains from the indigenous oral microbiota of healthy humans may have potential application as adjuncts for the prevention and treatment of halitosis. Kazor *et al*[10] reported that *L. salivarius* was the most predominant species detected in healthy subjects, whereas it was detected in only one of the subjects with halitosis at very low levels.

The rationale of probiotic implementation in cases of halitosis has been documented in several studies. *S. salivarius* K12 taken in a lozenge after a mouthwash could reduce oral VSC levels in 85% of the subjects in the test group.[83] *Weissela cibaria* is another species being able to reduce VSC production both *in vitro* and *in vivo*.[84] A contributing factor to malodor reduction can be the ability of *W. cibaria* to co-aggregate with species renowned for their VSC production (*F. nucleatum*, for example), thus reducing the source for malodorous compounds in the oral cavity.[85]

Oral yeast infections

Candida albicans, a normal inhabitant of the oral cavity, is the most common cause of oral fungal infections. Age, genetic, hormonal, iatrogenic, systemic and local factors predispose clinical manifestations of the disease. Probiotic applications in the oral cavity may alleviate symptoms and reduce pathogenic potential of *Candida* species. A 16-week probiotic intervention study demonstrated a significant reduction by 75% of high yeast counts in the elderly.[86] Hyposalivation reduction was also observed by the intake of *L. rhamnosus* GG containing cheese associated with control of oral *Candida*. Although this is the only study published on the role of probiotics on yeast infection in humans, two other *in vivo* studies on mice have shown that lactobacilli might indeed be effective in controlling oral candidiasis. Elahi *et al*[87] demonstrated a higher clearance of *C. albicans* in mice fed with *L. acidophilus* compared to control group. However, no noticeable delay in colonization of the oral cavity by *C. albicans* of immunocompromized mice was achieved when heat killed *L. casei* and *L. acidophilus* cells were given.[88]

Conclusion

The oral cavity with a well maintained balance of the species and species interactions may be a potential source for health-promoting probiotic bacteria. On the other hand, daily intake of probiotic supplements may control common oral and dental infections. The data on probiotic effects in the mouth are accumulating but the exact molecular mechanisms of their action are still unclear. Furthermore, the dosage of probiotic administration in each indication needs to be defined. Finally, safety issues are of paramount importance with any kind of bacteriotherapy.

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b. Acilact, Alfarm Ltd., Moscow, Russia.

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