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HOT TOPIC

Probiotics: contributions to oral health

JH Meurman[1], I Stamatova[2]

[1]*Institute of Dentistry, University of Helsinki and Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Finland;* [2]*Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Medical University, Plovdiv, Bulgaria*

Probiotics have been extensively studied for their health-promoting effects. The main field of research has been in the gastrointestinal tract. However, in the past few years probiotics have also been investigated in the oral health perspective, which is the topic of the present review. We discuss the mechanisms of bacterial adhesion, potential of probiotics in oral cavity colonization, interspecies interactions, and possible effects on immunomodulation, and means of probiotic administration. We suggest that probiotic treatment of diseases other than dental caries and periodontal disease should also be systematically investigated. In general, hardly any randomized controlled trials have been conducted in this area and the studies on probiotics *vs* oral health are still in their cradle. Hence, much more investigations are called for before any evidence-based conclusions can be drawn: if or not probiotic therapy can be recommended for oral health purposes.
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Introduction

Over the past 5 or 6 years there has been a near-exponential increase in publications on probiotics. A Medline search indicates that for the period since January 2006 more than 360 articles addressing probiotics have been published. The term 'probiotics' has undergone several definitions arriving at the final one, officially adopted by the International Scientific Association for Probiotics and Prebiotics term, outlining the breadth and scope of probiotics as they are known today: 'Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host' (Guarner *et al*, 2005). The idea of probiotics dates back to the first decade of 1900 when the Ukrainian bacteriologist and Nobel Laureate Ilya Metchnikof (1908) studying the flora of the human intestine developed a theory that senility is caused by poisoning of the body by the products of some of these bacteria. To prevent the multiplication of these organisms he proposed a diet containing milk fermented by lactobacilli which produce large amounts of lactic acid and for a time this diet became widely popular. Probiotic organisms are thought to act through a variety of mechanisms including the competition with potential pathogens for nutrients or enterocyte adhesion sites, including degradation of toxins, production of antimicrobial substances, and local and systemic immunomodulation (Silva *et al*, 1987; Lewis and Freedman, 1998; Isolauri *et al*, 2001). The latter definition is better because it does not restrict the application of the term only to probiotics with intestinal outcomes. Table 1 presents some of the main fields of activity of probiotics in general medicine. Discussing these investigations in detail, however, is beyond the scope of the present review.

There are a number of different organisms that can be classified as probiotics. The most common probiotic strains belong to the genera *Lactobacillus* and *Bifidobacterium*. *Lactobacillus* species from which probiotic strains have been isolated include *L. acidophilus*, *L. johnsonii*, *L. casei*, *L. rhamnosus*, *L. gasseri*, and *L. reuteri*. *Bifidobacterium* strains include *B. bifidum*, *B. longum*, and *B. infantis*.

Within dentistry, studies with *L. rhamnosous* GG (Meurman *et al*, 1994; Näse *et al*, 2001; Ahola *et al*, 2002), *L. reuteri* (Nikawa *et al*, 2004) have defined their potential in interacting with *S. mutans* by reducing the number of this caries pathogen, thus suggesting a role of probiotics in caries prophylaxis. Similarly, our group has recently observed that probiotic administration reduced oral *Candida* counts in the elderly – a finding that might offer a new strategy for controlling oral yeast infections (Hatakka *et al*, 2007).Yet, there is a paucity of information regarding the contributions of probiotics to oral health. The present article aims at summarizing the literature published in the past few years with respect

Correspondence: Prof. JH Meurman, PB 41, FI00014 University of Helsinki, Helsinki, Finland. Tel: +358 9 19127 272, Fax: +358 9 19127 517, E-mail: jukka.meurman@helsinki.fi
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Table 1 Examples of recent studies where probiotics have been investigated

Scopes of activity	*Reference*	*Result*
Cancer risk reduction	El-Nezami *et al* (2006)	Possible reduction of liver cancer
	Mego *et al* (2005)	Interaction with pathogenic gastrointestinal bacteria leading to decreased cancer risk
	Commane *et al* (2005)	Multifactorial anticarcinogenic activity
Gastrointestinal health	Brzozowski *et al* (2006)	Attenuation of the adverse effects of *Helicobacter pylori*
	Bergonzelli *et al* (2005)	General review over the issue defining the positive effect of probiotics
Urinary tract health	Reid *et al* (2001)	Coaggregation with uropathogens
	Falagas *et al* (2006)	Prevent urogenital infections in women
Immune response induction	Christensen *et al* (2006)	Insufficient data to prove the positive probiotic effect
	Rinne *et al* (2005)	Elevated numbers of IgM, IgA, IgG-secreting cells
Antimicrobial potential	Olivares *et al* (2006)	Antibacterial activity against different pathogenic bacteria
	Hutt *et al* (2006)	Strain-dependent antibacterial effect
Cardiovascular system	Aihara *et al* (2005)	High blood pressure reduction
	Jauhiainen *et al* (2005)	Blood pressure lowering effect

Table 2 Test strains considered probiotics in the oral cavity

Test strain	*Reference*	*Type of experiment*	*Feature tested*	*Result*
S. salivarius	Burton *et al* (2006a)	*In vivo*	Reduction of VSC	Reduced VSC levels
L. rhamnosus GG *L. acidophilus* *L. casei*	Busscher *et al* (1999)	*In vitro* *In vivo*	Inhibition of *S. mutans*	Positive correlation to *S. mutans* inhibition
L. reuteri	Çaglar *et al* (2006)	*In vivo*	Inhibition of *S. mutans*	Reduced *S. mutans* levels
Bifidobacterium DN-173 010	Çaglar *et al* (2005a)	*In vivo*	Inhibition of *S. mutans*	Reduces levels of caries pathogens
L. rhamnosus GG *Propionibacterium freudenreichii* ssp. *shermanii* JS	Hatakka *et al* (2007)	*In vivo*	Inhibition of *C. albicans*	Reduce high yeast counts
L. rhamnosus *L. paracasei* *L. johnsonii*	Haukioja *et al* (2006a)	*In vitro*	Adherence Survival in saliva	Better adherence than bifidobacteria
L. rhamnosus GG *L. casei* *L. reuteri*	Haukioja *et al* (2006b)	*In vitro*	Inhibition of *S. mutans*	Inhibit *S. mutans* adhesion to salivary pellicle
W. cibaria	Kang *et al* (2005)	*In vitro*	Adherence	S-protein positively affects adhesion
W. cibaria	Kang *et al* (2006)	*In vivo*	Reduction of VSC	Inhibited production of VSC
L. casei Shirota *L. acidophilus*	Lima *et al* (2005)	*In vitro*	Adhesion	Different pattern of adhesion according to the test strain
L. rhamnosus GG	Yli-Knuuttila *et al* (2006)	*In vivo*	Adherence	Only temporary colonization in oral cavity

VSC, volatile sulfur compounds.

to the possible role of probiotics on oral and dental health. Table 2 presents the possible probiotic strains in the oral cavity. In 2005 two reviews on probiotics in the oral health perspective were published (Çaglar *et al*, 2005a,b; Meurman, 2005). The reader of the current text is advised to refer to these recent reviews for earlier data.

Probiotic strains in the oral cavity

Figure 1 presents the ultrastructure of one of the most studied probiotic bacterium, *Lactobacillus rhamnosus* GG. An essential requirement for a microorganism to be 'an oral probiotic' is its ability to adhere to and colonize surfaces in the oral cavity. Microorganisms generally considered as probiotics may not have oral cavity as their inherent habitat and, subsequently, their possibility to confer benefit on oral health is then questionable. Paster *et al* (2001) in an attempt to determine bacterial diversity in the human subgingival plaque by using culture-independent molecular methods have estimated that the total species diversity in the oral cavity ranges between 500 and 600 species. This number was further extended by Kazor *et al* (2003), who detected 200 additional unknown species on the dorsum of the tongue, making the number of species in the mouth to reach 700. Lactobacilli make approximately 1% of the cultivable oral microflora (Marsh and Martin, 1999).

The most common lactobacilli species recovered from saliva in a study by Teanpaisan and Dahlen (2006) were *L. fermentum*, *L. rhamnosus*, *L. salivarius*, *L. casei, L. acidophilus* and *L. plantarum*. Three of them are probiotic strains used in dairy products. A similar diversity in the oral lactobacilli flora was observed by Colloca *et al* (2000) who found *L. fermentum*, *L. plantarum*, *L. salivarius* and *L. rhamnosus* to be the predominant species in healthy human mouth. Kõll-Klais *et al* (2005) found no differences in salivary lactobacilli counts between chronic periodontitis and

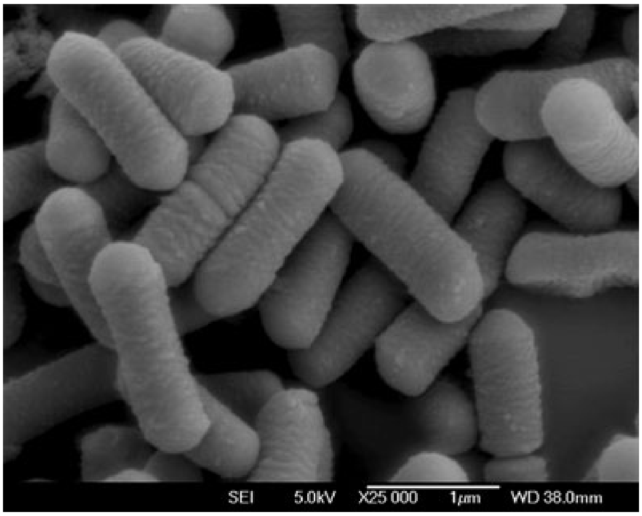

Figure 1 Scanning electron micrograph showing the typical form of probiotic strain *Lactobacillus rhamnosus* GG successfully tested in oral health trials (photograph: Kari Lounatmaa)

healthy patients, *L. gasseri* and *L. fermentum* being the predominant species among other isolates: *L. oris*, *L. plantarum*, *L. paracasei*, *L. rhamnosus*, *L. gasseri*, *L. acidophilus* and *L. cispatus*. However, in a later study the same authors observed a higher prevalence of homofermentative lactobacilli in healthy mouths compared to samples from patients with chronic periodontitis (Kõll-Klais *et al*, 2006). These findings indicate that lactobacilli as members of resident oral microflora could play an important role in the microecological balance in the oral cavity. These studies further demonstrated that lactobacilli strains with probiotic properties may indeed be found in the oral cavity. Yet there is no evidence whether these lactobacilli strains were detected due to the frequent consumption of dairy products leading to temporary colonization only, or if the oral environment is their permanent habitat. There are no long-term follow-up studies published to answer this question.

One mechanism of action of probiotics is suggested to be their modulation of host immune response. Immune inductive sites in the oral cavity are within the diffuse lymphoid aggregates of the Waldeyer's ring. Lingual and pharyngeal tonsils and adenoids contain most of the lymphatic tissue. The role of these anatomic structures as inductive sites of mucosal immunity has been shown by intranasally delivered vaccines (Wu *et al*, 1997). Dendritic cells scattered in mucosal surfaces are pivotal in the front-line bacterial recognition (antigen presentation) and in activating T-cell responses. Depending on the signals from dendritic cells either immune tolerance or active immune response toward a specific antigen may occur (Banchereau and Steinman, 1998). A marked production of interleukin-10 by dendritic cells in gut mucosa has been registered after administration of a probiotic mixture (Hart *et al*, 2004). However, more studies on activation of the oral immune inductive sites after probiotic administration are needed before further conclusions can be drawn. Such investigations might even cast light on probiotic effects in general and substantiate their specific applications in the future.

Probiotic activity in the oral cavity

Attachment, adhesion, and oral colonization of probiotics
The mechanism of adhesion to oral surfaces is an issue of importance for the long-term probiotic effect of the microorganisms. Among the different assays available to study the adhesion phenomenon, two model systems predominate: systems using saliva-coated hydroxylapatite, and hydroxylapatite coated with buffers, proteins, and other substances (Ostengo and Nader-Macias, 2004). The pattern of adhesion of different probiotic strains to oral epithelial cells has been tested as well. Most of the experiments on adhesion have been carried out with strains broadly used as probiotics in dairy products such as yogurt and cheese (Table 3).

Yli-Knuuttila *et al* (2006) assessed colonization of *L. rhamnosus* GG (LGG) in the oral cavity of healthy students. After the 14-day trial period, the occurrence of LGG in the oral cavity decreased gradually, indicating that no permanent colonization had occurred and that the oral persistence of LGG was only temporary. However, further colonization studies with larger materials and in different patient groups are still needed.

A relatively new strain and a potential candidate for a probiotic, *Weissella cibaria*, isolated from humans and animals worldwide, as well as from fermented foods, was tested for co-aggregation ability with *Fusobacterium nucleatum* and their attachment to epithelial cells (Kang *et al*, 2005). *Fusobacterium nucleatum* plays an important role as a bridge-organism that facilitates the colonization of other bacteria by co-aggregation

Table 3 Different means of probiotic administration for oral health purposes

Vehicle	*Strain*	*Outcome*	*Reference*
Lozenge	*S. salivarius*	Reduces oral VSC levels	Burton *et al* (2005)
Straw, tablet	*L. reuteri* ATCC 55 730	*S. mutans* level reduction	Çaglar *et al* (2006)
Yoghurt	*Bifidobacterium* DN-173 010	Reduction of salivary *S. mutans*	Çaglar *et al* (2005b)
Cheese	*L. rhamnosus* GG; *Prorionibacterium* JS	Reduced risk of high yeast counts and hyposalivation	Hatakka *et al* (2007)
Rinse solution	*W. cibaria*	Reduction of VSC	Kang *et al* (2006)
Capsule, liquid	*L. sporogenes*, *L. bifidum*, *L. bulgaricus*, *L. thermophilus*, *L. acidophilus*, *L. casei*, *L. rhamnosus*	Increased salivary counts of lactobacilli without significant decrease in *S. mutans* counts	Montalto *et al* (2004)
Yogurt drink	*L. rhamnosus* GG	Temporary oral cavity colonization	Yli-Knuuttila *et al* (2006)

(Kolenbrander, 2000). Many authors have reported that the co-aggregation abilities of lactobacilli species might enable them to form a barrier that prevents colonization of pathogenic bacteria (Reid *et al*, 1988; Boris *et al*, 1997), due to the production of a microenvironment around these pathogens in which inhibiting substances were generated by *Lactobacillus* species. Kang *et al* (2005) reported that *W. cibaria* efficiently co-aggregated with *F. nucleatum*. Pronase treatment led to additional reduction in co-aggregation between both species, thus indicating the proteinaceus character of the interspecies interaction. Heat-resistant components firmly attached to the cell surface of *W. cibaria* were responsible for the co-aggregation with *F. nucleatum*. The results of this study clearly showed that the S-layer proteins of the bacterial cell wall may play an important role in the adherence of *W. cibaria* to the epithelial cells.

In a study addressing the survival of bacteria in saliva and their adherence to oral surfaces, Haukioja *et al* (2006a,(b)) tested the colonization potential of different commercially available probiotics and *Lactobacillus* and *Bifidobacterium* strains obtained from the dairy industry. The results cast light on several controversial points reflecting mechanisms of colonization in the oral cavity. All test strains demonstrated 24-h survival rates in saliva but with great variations among the strains in their binding capacity to the saliva-coated surfaces. Lactobacilli showed better adherence than bifidobacteria. Thus, lactobacilli may compete for the same binding sites on saliva coated hydroxylapatite with *F. nucleatum* which explains their lower colonization capacity. This phenomenon indicates that probiotics might affect the formation of oral biofilms and modify resident microflora. Haukioja *et al* (2006a,b) defined a novel mechanism whereby lactobacilli and *B. lactis* Bb12 affected the composition of salivary pellicle on hydroxyapatite and thereby inhibited *S. mutans* adherence *in vitro*.

Studies on probiotics and dental caries

The impact of oral administration of probiotics on dental caries has been studied in several experiments utilizing different test strains. *Lactobacillus rhamnosus* GG (Meurman *et al*, 1994; Näse *et al*, 2001; Ahola *et al*, 2002) and *L. casei* (Busscher *et al*, 1999) have proved their potential to hamper growth of these oral streptococci. Çaglar *et al* (2006) registered definite *S. mutans*-count reduction after a 2-week consumption of yoghurt containing *L. reuteri*. A temporary reduction in *S. mutans* was observed during the period of yogurt intake and few days after cessation of consumption, indicating the necessity of continual administration of the probiotic in order to achieve an effect.

Little information is available about the relationship between probiotic bifidobacteria and counts of *S. mutans*. The only study addressing this study question tested *Bifidobacterium* DN-173 010 (Çaglar *et al*, 2005a,b). A statistically significant reduction in salivary mutans streptococci was observed. Due to the limitations of the study protocol with bifidobacteria, however, further investigations are needed for drawing final conclusions.

Considering the growing body of evidence about the role of probiotics on caries pathogens, however, it has been suggested that the operative approach in caries treatment might be challenged by probiotic implementation with subsequent less invasive intervention in clinical dentistry (Anderson and Shi, 2006). However, we strongly feel that more studies are definitely needed before this goal could be achieved. Most of the studies cited above do not meet the criteria of investigations for evidence-based medicine.

Probiotics and periodontal disease

Another issue in oral pathology, chronic periodontitis, could also benefit from orally administered probiotics. The presence of periodontal pathogens could be regulated by means of antagonistic interactions. A decrease in gum bleeding and reduced gingivitis has been observed by Krasse *et al* (2006) with the application of *L. reuteri*. Kõll-Klais *et al* (2006) reported that resident lactobacilli flora inhibits the growth of *Porphyromonas gingivalis* and *Prevotella intermedia* in 82% and 65%, respectively.

Probiotic strains included in periodontal dressings at optimal concentration of 10^8 CFU ml^{-1} were shown to diminish the number of most frequently isolated periodontal pathogens: *Bacteroides* sp., *Actinomyces* sp. and *S. intermedius*, and also *C. albicans* (Volozhin *et al*, 2004). These authors registered a 10- to 12-month remission period after periodontal treatment by application of the periodontal dressing that comprised collagen and *L. casei*. Nevertheless, similar to the case with dental caries, however, there is not yet any true evidence on the effect of probiotic therapy on periodontal disease.

Probiotics and imbalanced oral ecosystem

Halitosis, the oral malodor, is a condition normally ascribed to disturbed commensal microflora equilibrium. It has recently been positively affected by regular administration of probiotics. Kang *et al* (2006) have shown a definite inhibitory effect on the production of volatile sulfur compounds (VSC) by *F. nucleatum* after ingestion of *Weissella cibaria* both *in vitro* and *in vivo*. In children, a marked reduction in the levels of H_2S and CH_3SH by approximately 48.2% ($P < 0.01$) and 59.4% ($P < 0.05$), respectively, was registered after gargling with *W. cibaria* containing rinse. The possible mechanism in the VSC reduction is the hydrogen peroxide generated by *W. cibaria* that inhibits the proliferation of *F. nucleatum*. *Streptococcus salivarius*, also a possible candidate for an oral probiotic, has demonstrated inhibitory effect on VSC by competing for colonization sites with species causing an increase in levels of VSC (Burton *et al*, 2005, 2006a,b). Burton *et al* (2006a,b) further reported that *S. salivarius* strain K12 produced two lantibiotic bacteriocins, compounds that are inhibitory to strains of several species of gram-positive bacteria implicated in halitosis. However, the few studies published on the role of probiotics in the treatment of halitosis do not entitle any evidence-based conclusions. Nevertheless, we think that this might be an area where probiotic therapy indeed could bring something new if the preliminary observations on the 'balancing' effect of probiotics on VSC-generating microflora are confirmed. Randomized, blinded, and placebo-controlled studies with large enough patient materials are also needed in this area.

Probiotics and yeasts
Oral cavity with its variety of functions and complex structures is a specific site with its inherent pathology and diseases although the mouth is of course closely related to other parts and systems of the body. *Candida albicans* is among the most common infectious agents in the oral cavity. The incidence of yeast infections is higher at older age and under conditions of impaired immunity. Testing the pattern of colonization of *L. acidophilus* and *L. fermentum*, Elahi *et al* (2005) showed a rapid decline in *C. albicans* in mice after the intake of probiotic strains. Continuous consumption of probiotics led to almost undetectable numbers of fungi in the oral cavity, maintaining the protective effect for a prolonged period after cessation of application. The capacity of different lactobacilli to stimulate cellular and humoral factors of mucosal protection varies particularly in terms of salivary nitrous oxide and γ-interferon levels. Elahi *et al* (2005) have observed a correlation between the highest peak of interleukin-4 secretion and complete eradication of *C. albicans*. The results obtained in animal studies, however, require further testing of the effect of the strains on cases with clinically manifested *C. albicans* infection in humans.

A reduction in the prevalence of *C. albicans* in the elderly after consumption of probiotic cheese containing *L. rhamnosus* GG and *Propionibacterium freudenreichii* ssp. *shermanii* JS has been registered by Hatakka *et al* (2007) which was as an interesting observation in this randomized placebo-controlled trial. A concomitant feature of the probiotic activity observed in this study was the diminished risk of hyposalivation and the feeling of dry mouth of the subjects. The authors had no explanation to this and the finding certainly needs to be confirmed in further investigations.

It could be hypothesized that extending research on oral pathology, such as yeast infections, with respect to probiotics, and analyzing the molecular mechanisms of probiotic activity, might further broaden the field of their potential applications.

Administration of probiotics
Appropriate forms of administration of probiotic strains have been discussed in several articles. Dairy products supplemented with probiotics are a natural means of oral administration and easily adopted in dietary regime. However, for the purposes of prevention or treatment of oral diseases, specifically targeted applications, formulas, devices, or carriers with slow release of probiotics might be needed.

Montalto *et al* (2004) administered probiotic mix both in capsules and in liquid form without observing statistically significant difference, however, in the *S. mutans* counts between the two test groups. A specially designed straw with a reservoir containing probiotics has also been presented by Çaglar *et al* (2006) who compared the effect of two non-dairy delivery methods, a Life top straw (BioGaia AB, Stockholm, Sweden) and a lozenge on the effectiveness of *L. reuteri* to reduce the number of *S. mutans* . Both means of administration showed significant reduction in salivary *S. mutans* levels in half of the patients when compared with subjects who received placebo.

A recent invention for caries prophylaxis is a chewing gum containing *L. reuteri Prodentis*. Consumed twice daily this was marketed to regulate *S. mutans* counts in the oral cavity (http://www.biogaia.se). The average content of *L. reuteri* was 10^8 CFU ml^{-1}. However, we conclude that the most suitable means of delivery and dosages of probiotics for various oral health purposes have not been defined. Table 2 summarizes the variety of vehicles used so far for the administration of probiotics for oral health purposes.

Safety aspects

The issue of safety is of special concern during the past few years due to the increased probiotic supplementation of different food products. From the safety point of view, the putative probiotic microorganisms should not be pathogenic, should not have any growth-stimulating effects on bacteria causing diarrhea, and should not have an ability to transfer antibiotic resistance genes. The probiotics should rather be able to maintain genetic stability in oral microflora (Grajek *et al*, 2005).

The increased probiotic consumption inevitably leads to increased concentrations of these species in the host organism. *Lactobacillus* bacteremia is a rare entity, and data on its clinical significance are mainly found through case reports. For the last 30 years there have been approximately 180 reported cases (Boriello *et al*, 2003). Clinical characteristics of *Lactobacillus* bacteremia are highly variable, ranging from asymptomatic to septic shock-like symptoms. Any viable microorganism is capable of causing bacteremia, however, especially in patients with severe underlying diseases or in immunocompromised state. Nevertheless, the present literature supports the conclusion that the incidence of *Lactobacillus* bacteremia is unsubstantial and that all the cases where it has been registered are individuals with other systemic diseases such as diabetes, cardiovascular diseases, gastrointestinal disorders, malignancies, or organ transplant patients (Husni *et al*, 1997; Cannon *et al*, 2005). However, it is evident that careful monitoring is needed in this regard in the future.

Several studies have been carried out in immunocompromised patients. In a controlled study exposing 35 HIV-positive patients to *L. reuteri*, no clinically significant side effects were noted (Wolf *et al*, 1998). Salminen *et al* (2002) found no increase in *Lactobacillus* bacteria in blood culture samples when screening the Finnish population for the period 1990–2000. Specifically, their study showed no increase related to the increasing probiotic use of LGG-containing commercial dairy products during that period. Further, Salminen

(2006) has recently reported no adverse effects caused by LGG ingestion, or LGG treatment in general, on HIV-positive patients. $CD4^+$ cell counts or viral load levels were analyzed and all these patients received highly active antiretroviral therapy. Consequently, LGG-containing products are not likely to exert any major health risks among HIV-positive patients (Salminen, 2006).

An indirect proof of safety might be the results of studies investigating lactobacilli species as live vectors in delivery of antigens at mucosal sites. Animal experiments have shown that *L. lactis*, *L. casei*, *L. plantarum*, *L. helveticus* and recombinant *L. plantarum* are capable of inducing both systemic and mucosal immune response against *S. pneumoniae* antigens and tetanus toxin, respectively, delivered by an intranasal route (Grangette *et al*, 2001; Oliveira *et al*, 2006).

The absence of acquired antibiotic resistances is another safety criterion to be tested in potential probiotic candidates. Some probiotics are closely related to opportunistic bacteria and this may also cause transferral of antimicrobial resistance genes in between microorganisms (Lester *et al*, 2006). Several results from antibiotic susceptibility tests claim that the *tet*(W) and *tet*(S) genes in some probiotic lactobacilli and bifidobacteria strains are responsible for gentamycin, sulfamethoxazole, polymyxin B, and tetracycline resistance (Huys *et al*, 2006; Masco *et al*, 2006). These investigations emphasize the need for a minimal safety evaluation during the selection of strains for probiotic use. However, further studies are also needed in this area because the increasing number of species that develop resistance to commonly used antimicrobial drugs is of great global concern. Hence, before any recommendations can be given for probiotic therapy in preventing and/or treating microbial infections instead of using antibiotic or antifungal drugs, transferral of resistance genes needs to be carefully investigated.

Conclusions and recommendations for future research

The present review briefly outlines the potential for probiotic strains in improving oral and dental health. Similar to their better known actions in the gastrointestinal tract, probiotics exert their effects in many ways also in the oral cavity. The mechanisms of probiotic action in the mouth are anticipated to be similar to that observed with gastrointestinal indications. However, data on 'oral probiotics' are yet insufficient, and it is not known whether the putative probiotic strains could modulate, for example, immune response in the oral cavity as has been suggested to take place in the gut mucosa. The epithelial structure and chemical composition of excretions in the gastrointestinal tract differ from those in the mouth mucosa and saliva. The resident microbiota is also different in these anatomic

Table 4 Problems and recommendations in oral probiotic research

Problem	*Recommendation/comment*
Complex microbiology of the oral cavity	Systematic screening for potential resident probiotic strains. Interactions between microorganisms of the mouth are poorly understood
Different microbial attachment sites	Investigating microbial (probiotic) attachment separately on the teeth, and on keratinized and non-keratinized epithelium. Probably different probiotics are needed for therapy in dental and oral mucosal diseases
Saliva	Salivary defense mechanisms, both specific and nonspecific, should be investigated in relation to potential probiotics. Data from gastrointestinal studies are not directly applicable in saliva parameters
Safety	Strains that readily ferment dietary carbohydrates and decrease pH in the mouth are not suitable probiotics for oral health purposes. In addition, general safety aspects such as those related to potential invasiveness and antibiotic resistance genes must be screened
Means of administration and dosage	Slow-release approach should be investigated. It appears that probiotic therapy in order to be effective needs to be continuously administered. Optimal dosages of probiotics in oral health indications need to be assessed
Trials	Randomized controlled trails are needed with patients materials based on proper power calculations. Probiotic intervention should be tested in the clinical setting using potential strains for specific oral health purposes
Genetically modified microorganisms	Whether or not potentially probiotic microbial strains can or should be genetically modified in order to strengthen their beneficial potential or characteristics needs to be investigated. In the first hand, this calls for extensive studies on the mechanisms of probiotic action

sites. Consequently, results from studies conducted in patients with gastrointestinal diseases cannot be directly adopted in oral medicine and dentistry.

The oral cavity with its diversity of microbial species has been shown to harbor strains also distinguished as probiotics as such. In this regard further studies identifying resident probiotics of the mouth, clarifying the mechanism of their colonization, and the eventual effect on the oral environment are needed. Studies of the probiotic effect on the balance of the oral ecosystem would also be needed. Of particular interest might be studies on the combined effect of different probiotics applied simultaneously, thus testing the possible additive, cumulative, or competitive modes of action in the oral environment.

The studies quoted here for the safety of probiotics may be regarded as a starting point for further and more thorough research. We recommend focusing both on novel strains and conducting studies where currently known probiotic microorganisms would be investigated in the oral health perspective. For example, many starter cultures have been studied for probiotic effect with non-contributory results but this area is still far from proper coverage. Consequently, more studies are needed here, too. Further, the basic criteria a strain should meet in order to be considered as an 'oral probiotic' might vary depending on the specific indications for which it is anticipated. When compared with the criteria appropriate for respective strains in other parts of the gastrointestinal tract, oral applications may need modification. In other words, for an 'oral probiotic' different criteria may be needed than for those of other health indications. Systematic screening and discovery of 'latent' or 'resident' probiotic microorganisms is needed to identify the best candidate probiotics for oral and dental diseases.

Understanding the mechanisms whereby probiotic species modulate oral immunity is important, and the role of probiotic therapy in the treatment of oral manifestations of other diseases such as cutaneous diseases should also be investigated. There are no data as to whether probiotics exert any effect on oral manifestations of autoimmune diseases. In this regard it might be interesting to conduct studies on patients with lichen planus, pemphigus vulgaris, cicatricial pemphigoid, or aphthous stomatitis.

So far the vehicles for administration of probiotics have mainly been dairy products, most of which are produced by lactic acid fermentation. Species that ferment sugar and lower oral pH are detrimental to the teeth. Hence, systematic studies and randomized controlled trials are called for to find out the best probiotic strains and means of their administration in different oral health indications. Finally, possibilities to genetically modify or engineer potential probiotic strains may offer totally new visions and need to be studied. Table 4 briefly summarizes some of the aspects we have discussed here.

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