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The promise of probiotics in dentistry

A promessa dos probióticos em Odontologia

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Abstract

Objectives: The aim of this review is to examine the potential mechanisms of probiotic bacteria in the oral cavity and to summarize the observed effects of probiotics with regard to oral health. Additionally, safety concerns and future aspects are briefly considered. **Data sources**: Studies suggest that probiotics may function not only by direct inhibition of—or enhanced competition with—pathogenic micro-organisms, but also by more subtle mechanisms including modulation of the mucosal immune system. Clinical studies reporting the anticariogenic effects of probiotics and their use in the treatment of periodontal disease and in the treatment of halitosis and Candida albicans have been identified. **Study selection**: The studies focus on probiotic *Lactobacilli* and *Bifidobacteria* genera that are most used in various probiotic products. **Conclusions**: Although direct recommendations for the use of oral probiotics cannot yet be given, scientific evidence so far indicates that probiotic therapy may be a reality in dentistry in the future.

Keywords: Probiotics. Oral health. *Lactobacillus*. *Bifidobacterium*.

Resumo

Objetivo*: O objetivo desta revisão é analisar os mecanismos potenciais de bactérias probióticas na cavidade oral e resumir os efeitos observados de probióticos em relação à saúde oral. Além disso, preocupações com a segurança e os aspectos futuros são brevemente considerados.* **Fontes de dados***: Estudos sugerem que os probióticos podem funcionar não só pela inibição direta – ou aumento da concorrência com – os micro-organismos patogênicos, mas também por mecanismos mais sutis, incluindo a modulação do sistema imunológico da mucosa. Foram identificados estudos clínicos relatando os efeitos anticariogênicos dos probióticos e sua utilização no tratamento de doenças periodontais e no tratamento de halitose e Candida albicans.* **Seleção dos estudos***: Os estudos focam os probióticos lactobacilos e bifidobactérias, que são os mais utilizados em vários produtos* *probióticos.* **Conclusões***: Apesar de não haver ainda possibilidade de se recomendar diretamente o uso de probióticos orais, as evidências científicas até o momento indicam que a terapia probiótica pode ser uma realidade na Odontologia no futuro.*

Palavras-chave*: Probióticos. Saúde bucal. Lactobacillus. Bifidobacterium.*

Introduction

In recent years, there have been significant changes with regard to the effectiveness of—and attitudes towards—conventional antimicrobial therapy to combat disease. With the emergence of bacterial resistance to conventional antibiotics, there is an increased necessity not only to minimize antibiotic use and develop novel non-antibiotic-based treatments, but also to raise the profile of disease prevention. There arose an impetus to discover and implement new and preferably "natural" antibiotics to treat or prevent bacterial infections, a niche that bacterial interference therapy mediated by beneficial bacteria could easily fill. There is a public appetite for new therapies that recognize the importance of maintaining the natural balance of the resident microbiota and modulating the host immune responses to the microflora at a site. The concept of probiotics was thus born and a new field of microbiology was opened.

The term probiotics, the antonym of antibiotics, was introduced in 1965 by Lilly and Stillwell (1) as substances produced by microorganisms, which promote the growth of other microorganisms. Gradually, as the body of evidence of probiotic effectiveness accumulated, new features to the definition were appended, broadening their implication. Schrezemeir and de Vrese (2) defined probiotics as a preparation of—or a product containing—viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and as such exert beneficial health effects in this host. Endorsed by the Food and Agriculture Organization and the World Health Organization, the definition of probiotics, in 2001, described them as live microorganisms which when administered in adequate amounts confer health benefits on the host. (3)

The most commonly known probiotic bacterial strains belong to the genera *Lactobacillus* and *Bifidobacterium*. (4) These 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 humankind. Other microorganisms classified into this group include yeast and moulds, e.g., *Saccharomyces cerevisiae*, *Aspergillus niger*, *Aspergillus oryzae*, *Candida pintolopesii*, *Saccharomyces boulardii*.

Traditionally, probiotics have been associated with gut health, and most clinical interest has focused on the prevention or treatment of gastrointestinal infections and diseases. (4) However, these changing attitudes are also relevant to the prevention of dental diseases and there is an increased interest in the use of strategies that do not involve conventional antimicrobial agents for oral care. (5) There has been a paradigm shift away from treating dental diseases by targeting specific oral pathogens towards an ecological and microbial community-based approach to understand conditions such as caries and periodontal diseases. Considering the particular activities of probiotics and their inhibitory effect on the growth of pathogens, research interest has also been extended to the oral cavity where probiotics may exert their therapeutic or preventive effect on the development and progression of common oral diseases.

Probiotics and the mouth

The oral cavity is a rather intricate habitat providing the establishment of a great diversity of microbial species. It has been recently estimated that over a thousand bacterial species are present in the oral cavity. (6) Bacteria reside in the mouth either in planktonic state or are finely integrated as 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. Furthermore, bacteria in biofilms differ physiologically from their planktonic counterparts and tend to be much more resistant to environmental factors and antimicrobial agents. 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. (7) Biofilm species composition can also depend on phenomena like auto or coagreggation that may prevent microorganisms from establishing themselves in the biofilms. Thus the multifaceted nature of biofilm development and multivariate species interactions are important to assert true probiotic candidates with activity in the oral cavity.

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, lactoferrin, histatin, salivary peroxidase, cystatins and secretory immunoglobulin A (sIgA) can collectively affect viability or cell surface morphology of probiotic species, further affecting their adhesion and metabolic activity. *In vitro* studies testing probiotic survival in saliva have shown that *Lactobacillus* and *Bifidobacterium* strains cannot grow in saliva but remain viable after 24 hours of incubation. (8) 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. The mechanisms of adhesion in lactobacilli involve hydrophobicity and surface charge, as well as specific carbohydrate and/or proteinaceous components. (9) Species in the genus *Lactobacillus* have a superior adherence capacity to teeth and oral mucosal surfaces than *Bifidobacterium* species. (10) In vitro studies have assessed adhesion by measuring the attachment of bacteria to saliva-coated hydroxyapatite (sHA) and oral epithelium. (11) Probiotics and putative probiotic strains have been shown to vary extensively in their adhesiveness to sHA. Among probiotics strains, *Lactobacillus rhamnosus* GG presented the highest values of adhesion, comparable to those of the early tooth colonizer *Streptococcus sanguinis*. Dairy starter L. *bulgaricus* strains adhered poorly to sHA. Furthermore it was shown that lysozyme pretreatment of lactobacilli can slightly increase their adhesiveness to saliva coated surfaces. (11) Viability of lactobacilli after lysozyme pretreatment was not significantly reduced, but cell surface alterations might have contributed to the increased adhesion. Saliva-mediated aggregation is another aspect to be considered when assessing the establishment of probiotics in the mouth. Aggregation ability is related to cell adherence properties. Auto-aggregating strains express profound cell surface hydrophobicity that may improve colonization. Organisms able to co-aggregate with other bacteria may have greater advantages over non-coaggregating organisms which are easily removed from the mouth. To emphasize the role of aggregation, recent results have shown that *L. salivarius* W2431 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. (12) Similar findings were observed with *L. plantarum* SA-1 and *L. rhamnosus* ATCC7469 that failed to form substantial biofilms in mono-culture, but biofilm mass increased when co--cultured with *Actinomyces naeslundii*. (13)

Oral microbiota as a source of probiotics

In the oral cavity, lactobacilli usually comprise less than 1% of the total cultivable microbiota, and no species specific to the oral cavity has been found. Species commonly isolated from saliva samples include *L. paracasei*, *L. plantarum*, *L. rhamnosus* and

L. salivarius. (14) Culture-based studies suggest that *Bifidobacteria* are among the first anaerobes in the oral cavity. Bifidobacterial species isolated from oral samples include *Bifidobacterium bifidum*, *B. dentium* and *B. longum*. (15) Regarding normal microbiota and oral health, there seem to be differences in the ability of lactobacilli isolated from caries-active or healthy subjects to inhibit *S. mutans in vitro*. (16) In addition, the species composition of both *Lactobacillus* and *Bifidobacterium* microbiota is different between patients with periodontitis and the periodontally healthy ones. (17) These observations suggest that microorganisms with probiotic properties may indeed exist and reside in the oral cavity.

Potential mechanisms of probiotic effects in the oral cavity

The general mechanisms of probiotics can be divided in three main categories: normalization of the intestinal microbiota, modulation of the immune response and metabolic effects. The mechanisms of probiotic action in the oral cavity could be analogous to those described for the intestine. Possible ways that probiotic bacteria might affect oral health are summarized in Figure 1. (18)

Modulation of the immune system

Probiotics may provide beneficial effects by stimulating nonspecific immunity and modulating the humoral and cellular immune response. (19) Although the specific results varied, generally an enhanced salivary IgA production was observed during probiotic treatment. (20) Probiotic bacteria have been shown to influence immune responses non-specifically by enhancing the phagocytosis of pathogens and modifying cytokine production. Recent studies have highlighted mucosal dendritic cells as important arbiters of oral mucosal immune responses. (21) Probiotics stimulate dendritic cells

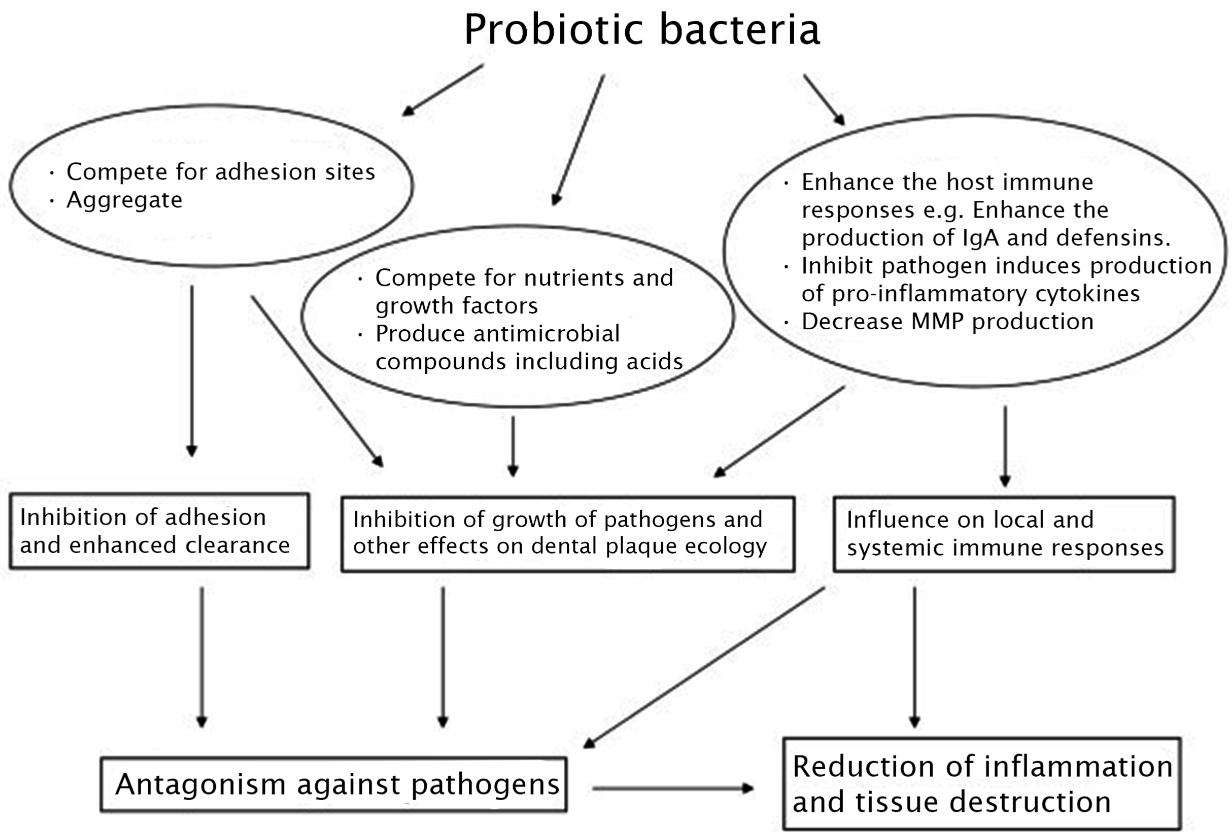

Figure 1 - Potential mechanisms of probiotic effects in the oral cavity

(antigen presenting cells) resulting in expression of Th1 (T-helper cell 1) or Th2 (T-helper cell 2) response, which modulates immunity. Intracellular pathogens are handled by Th1 response, while extracellular pathogens are taken care by Th2 response. Perhaps the most intriguing aspect of probiotic modulation of immune response is through its effects on cytokine production. Probiotics stimulate and modulate the mucosal immune system by reducing the production of pro-inflammatory cytokines through the action on Nuclear Factor-kappa B (NFkB) pathways. (22) Probiotics have also been shown to increase the production of anti-inflammatory cytokines as well as decrease matrix metalloproteinase (MMP) activity. (23) The epithelial lining of the oral cavity, despite its function as a physical barrier, actively participates in immune response. Probiotic bacteria 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. (24) Epithelial cells also 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. Human beta-defensins (hBDs) have been identified in the oral cavity with broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, fungi and enveloped viruses. Probiotic effects on hBD secretion by oral epithelial cells are under investigation. However, a beta-defensin mRNA has been less frequently found in periodontitis patients suggesting an important role of hBDs in innate host defense in the oral cavity. (25)

Competitive exclusion

It has been estimated that probiotics can exert their antagonistic effect on pathogens by competition for adhesion sites, or for nutrients and growth factors. Probiotics hinder pathogens by the production of biosurfactants that prevent adhesion. Van Hoogmoed et al. (26) observed that a biosurfactant generated by *S. mitis* BA and BMS cells was able to decrease the adhesion of not only *S. mutans* but also several periodontopathogens. Interestingly, probiotics have also been shown to inhibit adhesion by modifying the protein composition of the binding site. In this aspect, Haukioja et al. (8) have shown that certain probiotic strains modify the salivary pellicle protein composition by removing an important adhesion protein, salivary agglutinin gp340, which is necessary for adhesion of *S. mutans*. This resulted in a lower colonization efficiency of *S. mutans*. Bacteria can compete for certain essential nutrients or chemicals required for growth and in doing so can inhibit the growth of a pathogen. Hojo et al. (27) observed that salivary *Bifidobacterium* species can compete with *Porphyromonas gingivalis* for their mutual growth factor—vitamin K—which in turn inhibited the growth of *P. gingivalis* in a co-culture.

Direct interaction

Probiotic bacteria can produce a diverse range of compounds that act as antimicrobial agents such as lactic acid, hydrogen peroxide and bacteriocins. Lactic acid can pass across bacterial cell membranes and acidify the cytoplasm, which in turn can inhibit bacterial proliferation. In this respect, Sookkhee et al. (28) were able to isolate lactic acid bacteria from healthy oral cavities of Thai volunteers and showed that they had an antimicrobial activity against *P. gingivalis* and *S. mutans*. This activity was higher at an acidic pH, indicating that the antimicrobial effect was partly mediated by organic acids like lactic acid. Various *in vitro* and *in vivo* studies have shown that production of hydrogen peroxide by probiotic bacterial strains can inhibit the growth of pathogenic bacterial species. *Weissella cibaria* isolates generated a substantial quantity of hydrogen peroxide, which was sufficient to inhibit the proliferation of *Fusobacterium nucleatum*. (29) Bacteriocins are ribosomally synthesized proteinaceous antibiotics that kill or inhibit species closely related to the producer bacterium. Several bacteriocins derived from indigenous oral bacteria have been described. Studies have proved that *S. salivarius* is a safe species that not only produces broad-spectrum bacteriocins but harbors bacteriocin-encoding (and bacteriocin-inducing) transmissible DNA entities (megaplasmids). (30) A bacteriocin purified from *L. casei* killed *P. gingivalis* and its use has been proposed as a novel chemotherapeutic agent. (31)

Aggregation

Coagreggation and growth inhibition abilities of probiotic bacteria may play a key role in their interference with the oral biofilm. *Weissella ciberia* has the capacity to co-aggregate with F. nucleatum and to adhere to epithelial cells. These properties could enable *W. cibaria* to effectively colonize the oral cavity and limit the proliferation of pathogenic bacteria. (32) It has been observed that selected commercial probiotic lactobacilli displayed coagreggation activity and inhibited growth of clinical *mutans* streptococci. (33)

Observed effects on oral health

Caries and caries-associated microbes

The vast majority of the attention on the effect of probiotics in preventing or reducing the incidence of dental caries was directed towards the cariogenic bacterium *S. mutans*. 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. (34) 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* LC705. (35) 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 a day with yogurt demonstrated a significant reduction of salivary *S. mutans*, whereas no significant reduction was found in lactobacilli levels. (36) Ice cream can be an attractive vehicle for probiotic intake combining both health-promoting and mood-boosting effects. A portion of ice cream containing *B. lactis* Bb-12, if eaten once a day for 10 days, can lead to significant *S. mutans* level reduction. (37) In all studies discussed previously 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. (38) 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. In another study by Çaglar et al. (39), *S. mutans* count was reduced comparably in young adults who used xylitol or probiotics-enriched (*L. reuteri* ATCC 55730 and ATCC PTA 5289) chewing gum. However, no synergic effect was seen when combining both agents. 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. (40) 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. (41) 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. (42) administered a probiotic preparation containing seven living 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.

Periodontal diseases

The earliest studies on probiotics for enhancing oral health were for the treatment of periodontal inflammation. (43) Patients with gingivitis, periodontitis and pregnancy gingivitis were locally treated with a culture supernatant of *L. acidophilus* strain. Significant recovery was reported for almost every patient. Acilact, a Russian probiotic preparation of a complex of five live lyophilized lactic acid bacteria, has claimed to improve both clinical and microbiological parameters in gingivitis and mild periodontitis patients. (44) There also has been significant interest in using probiotics in treatment of periodontal disease recently. Krasse et al. (45) demonstrated that *L. reuteri* reduced the gingival index and bacterial plaque in the treated subjects. Twetman et al. (22) incorporated *L. reuteri* ATCC 55730 and ATCC PTA 5289 in chewing gum, which resulted in improvement of the gingival conditions manifested by reduction of the crevicular fluid volume and gingival bleeding, as well as the inflammatory mediators, tumor necrosis factor-α (TNF-α) and interleukin-8 level. Riccia et al. (23) studied the anti-inflammatory effects of *L. brevis* in a group of patients with chronic periodontitis. The treatment, which involved sucking on lozenges containing *L. brevis* over a period of 4 days, led to significant improvements in the targeted clinical parameters (plaque index, gingival index, bleeding on probing) and decreased MMP activity and other inflammatory markers in saliva for all patients. With *L. casei* Shirota and *Bacillus subtilis*, no difference in test and control groups in gingival bleeding or measured plaque index was observed, but the use of *L. casei* Shirota decreased polymorphonuclear elastase and MMP-3 activities in GCF, and gingival inflammation was lower in the group consuming the probiotic product, as measured by MPO activity after a four-day period of experimental gingivitis. (46) *B. subtilis* seemed to reduce the number of periodontal pathogens. (47) The recent research has shown that taking tablets containing *L. salivarius* WB21 significantly reduced the periodontal pathogens in subgingival plaque (48) and improved the periodontal health in volunteers. (49) A parallel open label study by Ishikawa et al. (50) observed that daily ingestion of tablets containing *L. salivarius* resulted in inhibition of *P. gingivalis*, *Prevotella* intermedia and *P. nigrescens*. A similar study by Matsuka et al. (51) reported decrease bleeding on probing and decrease in *P. gingivalis* count. Teughels et al. (52) conducted a split mouth design study on 32 beagle dogs with artificially created pockets; bacterial pellets *S. sanguinis KTH-4, S. salivarius TOVE and S. mitis BMS* were applied locally in designated periodontal pockets at baseline, 1, 2, and 4 weeks. The results showed a decrease in anaerobic bacteria and *Campylobacter* rectus with decreased pocket recolonization and bleeding on probing when compared with controls.

Oral candida

Candida species constitute part of the commensal oral flora in about 50% of healthy subjects, but able to cause a clinically apparent lesion if immune defenses were breached either on the local or systemic level. A study has shown that the subjects who consumed cheese containing the probiotic *L. rhammnosus* GG presented reduction in the prevalence of oral *Candida*, which subsequently may confer protective effect against oral candidosis. (53) However, others investigated the effect of various *lactobacilli* and could not find an effect on oral *Candida*. (54) This may be partly explained by the finding of the ex vivo experiment which demonstrated profound but variable abilities of commercially available strain of *lactobacilli* probiotics to inhibit the growth of *Candida albicans* possibly due to the low pH milieu produced by the lactobacilli. (55) Relevant to this is the laboratory study which demonstrated that the *Candida*-infected mice which were fed with *L. acidophilus* presented accelerated clearance of *C. albicans* from the mouth. (56)

Halitosis (Malodor)

“Physiologic” is a term used to describe halitosis as the result of imbalance of the microbiota in the oral cavity without any organic lesion, in contrary to “pathologic” halitosis, where patients usually presented with organic lesion most commonly periodontitis. (57) A recent study has shown that patients with genuine physiologic or pathologic halitosis benefited significantly from two-week therapy with tablets containing *L. salivarius* WB21 in addition to a significant reduction in the level of the volatile compounds and gingival bleeding on probing from periodontal pockets. (58) *Lactobacillus salivarius* TI2711 bacterium was also able to reduce the count of the oral black pigmented bacteroides, an organism that is strongly associated with production of the volatile sulphur compounds responsible for halitosis. (59) *Weissella cibaria* (29) and *L. salivarius* (60) were able to reduce the levels of volatile sulfide components produced by competing for colonization areas with volatile sulfide producing species.

Safety of probiotics in the oral cavity

It is worth sounding a note of caution concerning the use of probiotics for the purpose of preventing oral diseases. Different strains of same species may possess different characteristics, necessitating rigorous strain selection before being labelled as probiotic. (61) Some probiotic strains have been in use for many years and have excellent safety records. (62) However, there have been some cases of bacteraemia and fungaemia associated with probiotic use, although these have been in subjects who are immunocompromised or who suffer from chronic disease. (63) An individual who had been consuming *L. rhamnosus* in a probiotic preparation developed *Lactobacillus* endocarditis following dental treatment. (64)

The species that most commonly present probiotic benefits are lactobacilli and other lactic acid bacteria, and the production of acid is often thought to be an important component of their protection against pathogenic colonization. However, *Lactobacillus* species and acid production by acidogenic plaque populations may play a significant part in the development of caries, and a probiotic strain of **L. salivarius** has been shown to induce caries in an animal model, (65) and another is able to make a biofilm model more cariogenic. (66)

Future aspects

The new probiotic products targeted for oral health purposes do not necessarily comprise the same species as products now in market. Furthermore, the species might not necessarily belong only to genera *Lactobacillus* or *Bifidobacterium*. Preliminary results have been published on the safety and efficacy of a probiotic mouthwash containing three different oral streptococci for reducing the number of bacteria associated with dental caries and periodontitis. (67)

Genetically modified microbes bring a new dimension to the concept of probiotics. Their main approach is to reduce the harmful properties of pathogenic strains naturally colonizing the oral cavity. The modified strain could then be used to replace the original pathogen. One ambitious and promising example is the generation of an *S. mutans* strain with a complete deletion of the open reading frame of lactate dehydrogenase and thus significantly reduced cariogenicity. (68) Another option could be to enhance the properties of a potentially beneficial strain. One example is the construction of an *L. paracasei* strain with a functional scVF (single-chain variable fragment) antibody binding to the surface of *P. gingivalis*. (69) The concept of designer probiotics in oral applications is gaining momentum. The process involves equipping probiotic bacteria with genetic element necessary to overcome stress outside host, inside host and antagonize invading pathogens. Improving the stress tolerance profile of probiotic cultures significantly improves tolerance to processing stress and prolongs survival during subsequent storage. This contributes to a significantly larger proportion of the administered probiotics reaching the desired location (e.g., the gastrointestinal tract/periodontium) in a bioactive form. (70)

Probiotics are by definition viable, and until recently the viability of probiotic bacteria was usually ascertained by culture; however, both in the intestine and in the oral cavity, a significant proportion of bacteria are not yet cultivable. In addition, bacteria in biofilms can enter a dormant state; therefore, it can be speculated that bacteria with the ability to influence the microbiota in these sites need not necessarily be culturable. There has also been debate on the definition of “viable,” and even whether the definition of probiotic should be changed. Indeed, heat-killed beneficial oral *Streptococcus* strains have been shown to exert effects similar to those of a living bacterium. (71) Furthermore, viable but non-culturable probiotic bacteria maintain properties of viable bacteria. (72)

In field of oral immunology, probiotics are being used as passive local immunization vehicles against dental caries. (73) Bacteriophages, viruses that kill bacteria, have been detected in oral pathogens, such as *AGGregatibacter actinomycetemcomitans*, and they may play a role in the pathogenicity. Subsequently, future studies should be conducted to investigate if phage therapy might be applied for oral and dental diseases in the same way as has been attempted for systemic infections.

Summary and conclusion

Probiotics represent an upcoming field of research in oral medicine, the examination of the close relationships between oral health and daily diet. Most of the studies have been conducted with probiotic strains originally suggested for gut health; however, it is important to realize that each of the suggested health benefits should be studied for each bacterial strain individually. Thus, a probiotic bacterium in the mouth is not necessarily an oral probiotic. Furthermore, it is quite possible that the same species are not optimal for all oral health purposes; e.g., different properties might be desired in respect to dental and gingival health. At least some of the probiotic bacteria used in various probiotic products may colonize the oral cavity during the time they are in use; thus, the effects of probiotic bacteria in the oral cavity are important to understand. Probiotic bacteria seem to affect both oral microbiota and immune responses. On the other hand, the extent to which bacteria in food or in food ingredients can influence relatively stable oral microbiota is difficult to predict. Thus, research to unravel the mechanisms of possible probiotic action and long-term clinical trials are needed if probiotics are to provide a new scientifically proven means of preventing or treating oral diseases. It will also be a challenge to ensure that modes of delivery are developed that provide sufficient retention and exposure times in the mouth that will allow probiotics to colonize plaque or to enter into plaque or mucosal biofilms and influence microbial metabolism within them. Equally important concern is the dose of probiotics required for adequate action. Although the results of past studies are encouraging, much needs to be done for identification of the probiotics that are best suited to oral use, as well as the most appropriate vehicles for its delivery. Efforts should be made to increase the awareness of dental practitioners with this aspect of oral disease therapy and encourage the implementation of the concept of "food rather than medicine".

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