REVIEW ARTICLE

Prospects for the development of probiotics and prebiotics for oral applications

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There has been a paradigm shift towards an ecological and microbial community-based approach to understanding oral diseases. This has significant implications for approaches to therapy and has raised the possibility of developing novel strategies through manipulation of the resident oral microbiota and modulation of host immune responses. The increased popularity of using probiotic bacteria and/or prebiotic supplements to improve gastrointestinal health has prompted interest in the utility of this approach for oral applications. Evidence now suggests 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. Similarly, prebiotics could promote the growth of beneficial micro-organisms that comprise part of the resident microbiota. The evidence for the use of pro or prebiotics for the prevention of caries or periodontal diseases is reviewed, and issues that could arise from their use, as well as questions that still need to be answered, are raised. A complete understanding of the broad ecological changes induced in the mouth by probiotics or prebiotics will be essential to assess their long-term consequences for oral health and disease.

Keywords: *periodontal diseases*; *caries*; *probiotics*; *prebiotics*

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In recent years, there have been significant changes with respect to the effectiveness of, and attitudes towards, conventional antimicrobial therapy to combat disease. With the threat of widespread antibiotic resistance rendering many antibiotics useless against important diseases, there is an increased necessity not only to minimise antibiotic use and develop novel non-antibiotic-based treatments, but also to raise the profile of disease prevention. There is a public appetite for new therapies that are perceived to be natural through, for example, manipulation of the resident microbiota by the ingestion of probiotic bacteria or prebiotics. 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 (1–3).

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 (4,5). These approaches recognise the importance of maintaining the natural balance of the resident oral microbiota and the need to carefully modulate host immune responses to the microflora at a site.

One approach that has gained interest over recent years is the use of probiotic bacteria for oral applications. The rationale for their use in oral health care stems from the increase in evidence that supports their claims for benefit for a range of diseases, especially in the gastrointestinal tract (6–12). In this article, we will review the data on the use of probiotics for oral care or disease prevention, and discuss some of the issues that arise from their use, as well as identify questions that still need to be answered.

Probiotics and prebiotics

There is a long tradition, particularly in parts of Europe and Asia, of ingesting microbes or food products that affect the intestinal microbiota in ways that are believed to provide beneficial health effects, i.e. intake of probiotics and prebiotics. Probiotics are defined as viable micro-organisms that confer health benefit when administered in sufficient doses (6). The organisms that have been used as probiotics are primarily certain species of lactobacilli

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and bifidobacteria, and *Saccharomyces* spp., but some streptococci, enterococci and commensal *Escherichia coli* have also been claimed to have beneficial effects in certain situations (1, 6, 13, 14). Prebiotics (e.g. inulin-type fructans, maltodextrin, fructooligosaccharides and galactooligosaccharides) have been defined as non-digestible oligosaccharides that affect the proliferation of resident commensal bacteria that may then exert probiotic effects (15). More recently, the definition has been refined to include selectively fermented ingredients that allow specific changes in the composition and/or activity of the resident microflora that confer benefits upon host well-being and health (16). Studies of prebiotics have mainly been focused on gastrointestinal microbiota and health benefits; there has been little work in the oral cavity.

Much of the evidence for the health benefits of probiotics and prebiotics has been anecdotal, but the last decade has seen some developments in establishing the scientific base for administration of such agents and in understanding the mechanisms underlying their effects. This is reflected in the proliferation of reviews in this area in recent years (1, 6–14, 17–21).

Current applications of probiotics and prebiotics

Most of the applications and research into the mechanisms of action of probiotics and prebiotics concentrate on their roles in influencing intestinal health and function. Although some of the experimental evidence and data from clinical trials is conflicting, there is growing evidence for their efficacy in protecting against acute diarrhoeal disease in children, gastroenteritis and antibiotic-associated diarrhoea, inflammatory bowel diseases and pouchitis (6, 7, 10, 12). There is also evidence to support further investigation of the use of probiotics and prebiotics in the treatment of illnesses affecting sites other than the intestinal tract, e.g. urinary tract infections, vaginal infections, arthritis, atopic eczema, pharyngitis and otitis media (6, 7, 11, 22). Recently, *Lactobacillus rhamnosus* GG (LGG) administered in yoghurt was reported to enhance faecal clearance of vancomycin-resistant enterococci (23). The possibilities of applying probiotic therapy for other medical conditions are being investigated, including recovery from haemorrhagic shock, recovery from burn injury, cholesterol reduction and protection from coronary heart disease, effects on breast cancer cells, enhancement of tolerance of food allergens, protection from respiratory tract infections, liver conditions, skin infections, enhancement of bone health and reduction of obesity (18, 20, 21). However, the evidence-base for many of these is relatively under-developed.

The potential applications of probiotic bacteria have been further expanded by the development of strains that have been genetically engineered to produce the anti-inflammatory cytokine IL-10 (24), trefoil factor family proteins to enhance wound healing (25) or the 2D-CD4 receptor to try to reduce HIV infectivity (26).

Mechanisms of action

Probiotics

The diversity of conditions that may benefit from ingestion of probiotics illustrates the variety of mechanisms that may be involved in their actions and that some effects are systemic rather than only local. It is likely that these mechanisms vary according to the specific strain or combinations of strains used, the presence of prebiotics and the condition that is being treated, as well as the stage of the disease process in which the probiotic is administered (7). There are common themes emerging in studies of the modes of action of probiotics and numerous mechanisms have been proposed (7, 9–11, 13) including:

- Prevention of adhesion of pathogens to host tissues.
- Stimulation and modulation of the mucosal immune system, e.g. by reducing production of pro-inflammatory cytokines through actions on NFκB pathways, increasing production of anti-inflammatory cytokines such as IL-10 and host defence peptides such as β-defensin 2, enhancing IgA defences and influencing dendritic cell maturation.
- Modulation of cell proliferation and apoptosis through cell responses to, for example, microbially produced short chain fatty acids.
- Improvement of intestinal barrier integrity and up-regulation of mucin production.
- Killing or inhibition of growth of pathogens through production of bacteriocins or other products, such as acid or peroxide, which are antagonistic towards pathogenic bacteria.

Prebiotics

The ability of certain oligosaccharides to enhance the growth of resident commensal gut bacteria, particularly bifidobacteria and lactobacilli, is well documented (17). Thus, the major mechanism of action of prebiotics is assumed to be indirect, i.e. facilitating the proliferation of beneficial components of the resident microflora, with probiotic effects resulting from the actions of these bacteria as described above. Cellobiose has the additional property of down-regulating virulence factors of *Listeria monocytogenes* (27). There is evidence that some prebiotics also exert direct effects on the host, independent of their effects on resident bacterial populations (8, 15). These include stimulation of expression of IL-10 and interferon γ, enhancement of IgA secretion, modulation of inflammatory responses to pathogens and stabilisation of the gut mucosal barrier. Additionally, prebiotics with enhanced function have been designed. These oligosaccharide derivatives contain sugars that are specific epithelial cell receptors to which pathogens adhere and they, therefore, provide 'decoy' adhesion sites and cause pathogens to adhere to luminal contents rather than to epithelial cells (17).

The oral microbiota in health and disease

To be able to develop probiotic or prebiotic interventions for applications in oral health care and to understand their mechanisms of action and potential risks, it is essential to have a clear understanding of the oral microbiota and their functions in oral health and disease. This is not always easy, given the complexity of the oral microbiota; more than 700 species have been detected in the human mouth and the resident microbiota of one individual may comprise 30 to >100 species (28–30).

A wide variety of sites in the mouth are heavily colonised. Supragingival and subgingival plaque form through sequential and specific adhesive interactions that result in a complex climax community (5, 31). The tongue is heavily colonised and micro-organisms on the dorsum of the tongue are reservoirs for supragingival and subgingival plaque and salivary microbial populations (32–34). Many oral bacteria, especially streptococci, also survive within buccal epithelial cells (35, 36).

Functions of the resident microbiota

The main focus of research has been defining the micro-organisms and their traits that are responsible for disease, but there is an increased awareness that the resident microbiota does not play merely a passive role, but actively contributes to the maintenance of health. The large, diverse resident microbial communities that colonise mucosal sites co-exist with a host, with harmful effects only if the host becomes immunocompromised, if the resident microbial populations are suppressed or if micro-organisms reach sites to which they do not normally have access (e.g. through trauma). Studies, mostly of gastrointestinal bacteria, have shown that resident microbial populations contribute to host protection through blocking of colonisation by pathogens (37, 38), development of cell structure and function (39, 40), development of the immune system (41) and modulation of inflammatory responses (42–49). Evidence is accumulating to support a similar role for oral commensal bacteria in the development of the immune system (50), the maintenance of healthy oral tissue by influencing expression of mediators such intracellular adhesion molecule 1 (ICAM-1), E-selectin and IL-8 (51), modulating immune responses and enhancing cellular homeostatic mechanisms (52, 53).

Defining the resident microbiota

Technological improvements in the detection of culturable and non-culturable micro-organisms has led to the identification of increasing numbers of taxa in the mouth (54, 55) and have confirmed that resident oral microbial populations are site-specific as well as highly diverse, and the profile of the microbial community may be specific to an individual (28, 29, 33, 56). Species that predominate in disease can often be present in low numbers at healthy sites (5, 31).

In recent years there has been a greater emphasis, not only on defining resident microbial populations more fully, but also on identifying those that are significantly positively associated with health in an effort to better understand the processes that eventually lead to disease and the ways in which microbial populations may be manipulated to maintain host–microbe homeostasis and to develop novel prevention strategies. Kilian et al. (57) list the following species as 'true' oral commensal micro-organisms: *Streptococcus mitis*, *Streptococcus oralis*, *Actinomyces naeslundii*, *Fusobacterium nucleatum*, *Haemophilus parainfluenzae*, *Eikenella corrodens* and some species of *Prevotella*. Other studies have generated an increasingly long list of culturable and unculturable bacteria with a significant association with healthy sites (28, 30, 58–60).

Microbial populations associated with oral disease

The most common oral diseases are caries and periodontitis, which result from a shift in the balance of the resident microbiota at a site. The types and proportions of bacteria found in plaque taken from sites diagnosed with either caries or periodontal disease differ from one another and both are distinct from those that predominate at healthy sites. In caries, there are increases in acidogenic and acid-tolerating species such as mutans streptococci and lactobacilli, although other bacteria with similar properties can also be found and bifidobacteria, non-mutans streptococci, *Actinomyces* spp., *Propionibacterium* spp., *Veillonella* spp. and *Atopobium* spp. have also been implicated as significant in the aetiology of this disease (30, 61–65).

In periodontal diseases, there is an increase in plaque mass and a shift towards increases in obligately anaerobic and proteolytic bacteria, many of which are Gram negative and currently unculturable. The host damage that occurs during periodontal disease arises through the combined activities of subgingival biofilms and the host responses to these diverse bacterial populations. A number of reviews give excellent overviews of periodontal microbiology (5, 54, 57, 66–69) and these illustrate the significant paradigm shift that has occurred, away from concentrating on the roles of individual specific pathogens to recognising that periodontal disease results from the activities of successive consortia of organisms.

Other common oral infections also result from the activities of micro-organisms that are found in the resident microbiota. *Candida albicans* and other *Candida* species are present in low levels in oral microbial communities and can cause oral candidiasis and denture-associated stomatitis (70, 71). Halitosis is most often the result of production of malodorous metabolic end-products (especially volatile sulphur compounds) by oral bacteria, in particular Gram negative anaerobes (72, 73).

The potential for probiotics in prevention and control of oral diseases

Probiotics in prevention of caries

The oral health applications of either probiotics or 'replacement therapy' with *Streptococcus mutans* strains of attenuated virulence and increased competitiveness were first suggested for prevention of dental caries more than 20 years ago (74). Despite this, and the fact that some products have reached the market, there remains a paucity of clinical evidence to support the effectiveness of probiotics to prevent or treat caries (2, 3).

Many early studies concentrated on utilising bacteria that expressed bacteriocins or bacteriocin-like inhibitory substances (BLIS) that specifically prevented the growth of cariogenic bacteria (11, 74). Another approach has been to identify food grade and probiotic bacteria that may have potential in caries prevention. These have been selected because of their likely ability to colonize teeth and influence the supragingival plaque; *in vitro* models for this selection have included adhesion to hydroxyapatite, as a surrogate for colonisation of teeth, and mixed species biofilm models (75, 76). Also, strains have been screened for suitable antagonistic activity against relevant oral bacteria (77). *In vitro s*tudies of the antibacterial activity of live yoghurts showed inhibition of *S. mutans* but not some other oral streptococci, including *Streptococcus sobrinus*; this activity was heat-sensitive implying that the effect was not simply due to acid (77). Recently, oral lactobacilli have also been screened for their utility as potential probiotic strains (78–80) and strains of oral lactobacilli have been isolated that are inhibitory against *S. mutans*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*, as well as being tolerant of relevant environmental stresses (81). Another approach utilised a recombinant strain of *S. mutans* expressing urease, which was shown to reduce the cariogenicity of plaque in an animal model (82). Similarly, genetically modified probiotics with enhanced properties can be developed ('designer probiotics'). For example, a recombinant strain of *Lactobacillus* that expressed antibodies targeting one of the major adhesins of *S. mutans* (antigen I/II) was able to reduce both the viable counts of *S. mutans* and the caries score in a rat model (83).

Clinical studies have indicated that bacteria with established probiotic effects (lactobacilli and bifidobacteria) have some promise for prevention of caries. LGG ingested in dairy products (milk, cheese) reduced salivary mutans streptococcal counts in adults and protected against caries in children (84, 85). Other lactobacilli have also been shown to reduce mutans streptococcal counts in saliva. *Lactobacillus reuteri*, when delivered by yoghurt (86), straw or tablet (87), by chewing gum (88) or as a lozenge (89), significantly reduced the counts of mutans streptococci in saliva ($p < 0.05$). The short-term consumption of yoghurt (90) or ice cream (91) containing *Bifidobacterium* spp. resulted in a significant reduction in salivary mutans streptococci ($p < 0.05$) but not in lactobacilli. Other studies have reported reductions in mutans streptococci levels in saliva following use of probiotic-containing yoghurts (92).

Probiotics in prevention of periodontal diseases

There are fewer experimental studies exploring probiotic use in periodontal diseases, partly reflecting a poorer understanding of the precise aetiology of the disease and of the conditions that promote health. However, patients with moderate to severe gingivitis who were given either one of two *L. reuteri* formulations had reduced plaque and gingivitis scores compared to a placebo group (93). Similarly, the regular (three times daily for eight weeks) intake of tablets containing *Lactobacillus salivarius* resulted in benefits in terms of pocket probing depth and plaque index in individuals at high risk of periodontal disease (smokers) compared to a placebo control group (94). Other studies have aimed to identify organisms that have the potential for probiotic action that may protect against periodontal diseases. Some oral strains of lactobacilli and streptococci (81, 95–97) and bifidobacteria (98) have been reported to have *in vitro* inhibitory activity against periodontal pathogens, while others are more active against mutans streptococci (79–81). The subgingival application of beneficial oral bacteria (e.g. *Streptococcus sanguinis, Streptococcus salivarius* and *S. mitis*) (replacement therapy) has been shown to delay recolonisation by periodontal pathogens, reduce inflammation, and improve bone density and bone levels in a beagle dog model (99, 100). Koll-Klais et al. (97) observed that *Lactobacillus gasseri* strains isolated from periodontally healthy subjects were more efficient at inhibiting the growth of *A. actinomycetemcomitans* than strains from periodontally diseased subjects, and also inhibited the growth of *P. gingivalis* and *P. intermedia*; this correlated with an inverse relationship between carriage of homofermentative lactobacilli and subgingival colonisation by *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*. Ishikawa et al. (96) observed *in vitro* inhibition of *P. gingivalis, P. intermedia* and *Prevotella nigrescens* by *L. salivarius.* Daily ingestion of

L. salivarius-containing tablets resulted in reduced salivary counts of these black pigmented anaerobes.

The mechanisms of inhibition of periodontal pathogens have not been fully clarified. The inhibitory activity displayed by homofermentative lactobacilli against periodontal pathogens was principally related to their production of acid, not to H_2O_2 or bacteriocin production (97). Hojo et al. (98) suggested that bifidobacteria inhibit some black pigmented anaerobes by competing for an essential growth factor, vitamin K, although there was no significant relationship between higher bifidobacterial counts and lower black-pigmented anaerobe counts. Recently, a bacteriocin purified from *Lactobacillus casei* killed *P. gingivalis* but its use was proposed as a novel chemotherapeutic agent rather than as strain development for probiotic applications (101).

Probiotics in prevention of other oral diseases

Probiotics have also been reported to reduce yeast counts in the elderly, and may be a route to control *Candida* spp. and hyposalivation in this age group (102). There have also been clinical and laboratory studies of their potential for preventing halitosis. Peroxide production by strains of *Weissella cibaria* (commonly present in fermented foods) isolated from the mouths of healthy children, inhibited production of volatile sulphur compounds that contribute to oral malodour by *F. nucleatum in vitro* and in exhalations following mouth-rinsing by adult volunteers with a suspension of *W. cibaria* (103). The success of *W. cibaria* in reducing malodour may have also been because it coaggregated efficiently with *F. nucleatum* (103) and therefore competed with other late/secondary colonisers for adhesion sites. Thus, *W. cibaria* may have probiotic activities with potential for prevention of periodontal disease. Volatile sulphur compounds, such as H_2S and mercaptoethanol, are produced by a range of periodontal anaerobes (104). The inhibition of these micro-organisms by peroxide from *W. cibaria* may help reduce subgingival plaque pathogenicity while its competition for coaggregation sites may reduce the reservoir of micro-organisms available for transmission into plaque.

S. salivarius is one of the earliest colonisers of epithelial surfaces in the human mouth and nasopharynx, and its primary habitat is the dorsum of the healthy tongue (28, 73). *S. salivarius* K12 produces salivaricin, a lantibiotic with inhibitory activity towards most *Streptococcus pyogenes* (11). This strain has been commercially promoted as a probiotic that is reported to be protective against throat infections and oral malodour (11, 105). *S. salivarius* K12 displays other activities, not related to salivaricin production, that most likely contribute to its probiotic properties. It down-regulated IL-8 secretion by epithelial cells in response to pathogenic bacteria and to the immunomodulatory host defence peptide LL-37, and also influenced numerous cellular homeostatic pathways (53). *Streptococcus gordonii* was recently shown to also inhibit epithelial cell IL-6 and IL-8 secretion (52).

Many other strains of *S. salivarius* are reported to produce bacteriocins or BLIS, leading to suggestions for their usefulness as oral probiotics (106). Two salivaricin-producing strains, when administered to children in milk, promoted salivaricin A-like inhibitory activity in the indigenous, resident *S. salivarius* populations (107). The importance of strain selection for probiotic use is illustrated by the fact that some *S. salivarius* strains differ from K12 in some important activities; one strain increased production of malodorous products by facilitating *P. gingivalis* metabolism of salivary mucins (108) and another up-regulated IL-8 secretion by oral epithelial cells (109) in contrast to the down-regulation observed in response to K12.

Outstanding questions

Can probiotics colonise the oral cavity?

Most evidence indicates that probiotics in the gut are not able to permanently become part of the resident gastrointestinal microbiota and they disappear from faeces very soon after probiotic ingestion ends. Previous studies of the oral microbiota would indicate that it is very difficult to alter the composition of established plaque microbial communities (57). A number of studies of oral colonisation following probiotic ingestion have found similar patterns of lack of colonisation to those in the gut, in that ingested lactobacilli colonised only transiently and disappeared soon after administration of the probiotic ended (92, 110, 111). Colonisation with *L. reuteri* was achieved in the majority of periodontal patients given a probiotic, but the study only ran for 14 days (93). Stable and long-term colonisation by probiotic lactobacilli appears to have only been observed in an individual who received LGG probiotic therapy at the age of 10 years (111). The resident microbiota of children seems to be less stable and more subject to flux than resident microbial communities in adults (57), and perhaps it is in childhood that long-term influences on resident populations will be achieved.

Is colonisation of plaque essential for protection against caries or periodontitis?

There is some evidence that colonisation of the gut by probiotics may have beneficial systemic effects, enabling these organisms to provide protection against diseases at distant sites (22). Studies of the influence of *L. reuteri* ATCC55730 on salivary mutans streptococci and lactobacilli indicated that the benefits seen may have been due to systemic effects rather than to the colonisation of the mouth by the probiotic bacterium (87). Probiotics have been effective in some chronic inflammatory diseases that involve deregulation of the immune responses, e.g. arthritis and Crohn's disease. Some of the systemic effects claimed for probiotics are immunomodulation, alteration of mucin production, stabilisation of mucosal barriers, enhancement of IgA defences, effects on neutrophils and dendritic cells, (7, 9–11, 13) and enhancement of bone health through influencing bone mineral content and structure (20). Chronic inflammation and bone resorption contribute significantly to the pathogenesis of periodontal diseases, and it is possible to speculate that some of these systemic effects might provide concomitant protection against periodontal diseases. However, no studies have been carried out providing evidence for this.

It is also possible that colonisation of one site may provide indirect protection at other sites by mechanisms other than systemic effects. For example, reduction of colonisation of the tongue may reduce reservoirs for colonisation of plaque. Supragingival plaque and subgingival plaque are intimately connected, as supragingival plaque extends down the tooth to form subgingival plaque, so changes in supragingival plaque will influence the future composition of subgingival plaque. Lactobacilli associated with periodontal health were only rarely isolated from subgingival samples (97). However, these bacteria were found to inhibit the growth of certain periodontal pathogens; it was proposed that they may reduce the levels of these pathogens on the tongue, which constitutes a major reservoir for their transmission, and thereby indirectly reduce the colonisation of subgingival plaque by periodontal pathogens.

Are current approaches targeting the right micro-organisms?

Most oral diseases are polymicrobial in nature and result from complex ecological shifts in the resident microbiota. In caries, the ability of bacteria to colonize plaque, produce acid and survive under low pH conditions are of over-arching importance and these properties are not restricted to a few species (112). Thus, highly targeted approaches may have limited success as there are so many other micro-organisms that can occupy a similar niche. Also, there has been an emphasis on identifying probiotics that will have an effect on bacteria that have strong associations with established or severe disease; for example, strains are proposed to be potentially useful against periodontal disease if they have inhibitory activity against *P. gingivalis* or *A. actinomycetemcomitans.* By the time these species are prevalent, the disease is well established and the site is already in crisis; a more effective therapeutic approach than to target these late pathogens might be to inhibit the growth or activity of those microbial consortia that are associated with the transition from health to disease. The advances that have occurred in the technologies used to detect and characterise microbial populations are leading to a more detailed characterisation of the microbiota associated with specific phases of health and disease so this approach is becoming a realistic possibility. Finally, there is a widespread acceptance of the importance of oral ecology and maintenance of host–microbe homeostasis in oral health and disease (5, 113, 114). Recognition of the activities of bacteria that contribute to disease (e.g. acid production in caries, induction of inflammation and bone resorption in periodontal disease) may lead to therapies that target such activities, rather than certain species.

Are prebiotics a viable alternative or adjunct?

For a rational approach to the development of oral prebiotics and the manipulation of the resident microbiota, it is essential to know which species can be considered to promote health and to gain some understanding of their metabolic needs and interactions. It is recognised that the resident oral microbiota persists by catabolising endogenous nutrients such as salivary proteins and glycoproteins (115) and gingival crevicular fluid.

Clearly poor diet has an impact on oral health as well as general health, and controlling refined sugar intake has been used for many years to control the oral ecology and protect against caries. Similarly, xylitol has been used to reduce acid production by mutans streptococci, although this made no difference to the effectiveness of a probiotic-containing chewing gum (88). Algal lectins and cranberry juice have been suggested to reduce adhesion of oral streptococci (116, 117). Cocoa polyphenols can reduce the viability and acid production by cariogenic bacteria (118). However, while these all use (or suggest the use of) dietary components to influence the oral microbiota, they are not prebiotics as they inhibit potential pathogens rather than stimulate beneficial resident micro-organisms.

We know little about the impact of dietary components on subgingival plaque composition. In the gut there is some evidence collected over many years for the beneficial effects of promoting populations of bifidobacteria and lactobacilli. Koll-Klais et al. (97) found that homofermentative lactobacilli, particularly *L. gasseri*, were more prevalent in healthy rather than periodontally diseased sites; their presence was inversely associated with clinical parameters related to chronic periodontitis and also to subgingival colonisation by periodontal pathogens. Hojo et al. (98) also found *L. gasseri*, as well as *L. salivarius* and *Lactobacillus fermentum*, to be more prevalent in healthy sites but not exclusive to health. The same study found that *Bifidobacterium* spp., although not predominant in the mouth, were isolated from 80% of periodontally healthy subjects and *Bifidobacterium adolescentis* was isolated from 40% of healthy subjects and no periodontitis subjects. Counts of bifidobacteria were particularly high in a group of well-maintained ex-periodontitis subjects, indicating perhaps that these bacteria are better able to colonise sites that have undergone plaque removal. Thus, it is possible that prebiotic therapies that promote the growth of certain bifidobacteria and lactobacilli may enhance periodontal health. However, lactobacilli and bifidobacteria are themselves linked to caries aetiology, and it is also difficult to envisage how a prebiotic approach to enhance their growth would not encourage a general increase in prevalence of aciduric and acidogenic populations that are associated with an increased risk of dental caries.

Are there potential risks?

It is worth sounding a note of caution concerning the use of probiotics for the purpose of preventing oral disease. Different strains of a species may not all possess characteristics that enable them to be probiotics and rigorous strain selection for the disease concerned is complex but essential (7, 18). Some probiotic strains have been in use for many years and have excellent safety records (119–121). Most probiotic bacteria are weakly proteolytic and, for example, *Lactobacillus bulgaricus*, was shown to be incapable of degrading some host tissue components (122). However, there have been some cases of bacteraemia and fungaemia associated with probiotic use, although these have been in subjects who are immunocompromised (123, 124), or who suffer from chronic disease (119) or short gut syndrome (125). Other predisposing factors include prior prolonged hospitalisation and prior surgical intervention (124). An individual who had been taking *L. rhamnosus* in a probiotic preparation developed *Lactobacillus* endocarditis following dental treatment (126). In Finland, however, there has not been an increase in bacteraemia associated with probiotic lactobacilli following the increase in the use of these products since 1990 (127).

The species that most commonly exhibit 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 colonisation. However, *Lactobacillus* spp. and acid production by acidogenic plaque populations play a significant part in the development of caries, and a probiotic strain of *L. salivarius* has been shown to be cariogenic in a rat model (128). A number of probiotic lactobacilli and bifidobacteria produce acid from fermentation of dietary sugars *in vitro* (129). There are conflicting data on the salivary lactobacilli levels following probiotic usage. Some studies have reported no effects (91), others have found trends for an increase (1, 87), while others have detected statistically significant rises in counts of salivary lactobacilli (130). There is a converse risk in that the control or prevention of caries may indirectly affect periodontal pathogens. It has been known for many years that streptococci, through production of hydrogen peroxide, inhibit the growth of putative periodontal pathogens, leading to early proposals that interactions between groups of micro-organisms within plaque can influence the development of disease or actively contribute to the maintenance of health (131), and lactobacilli and bifidobacteria also inhibit the growth of a range of periodontal pathogens (81, 95–98, 131).

It is clear that careful selection of the strain to be ingested for a particular disease is essential and the mode and timing of administration can be crucial, as well as the age and health of the individual taking the probiotic. There is a sufficient knowledge base for major and minor risk factors to have been proposed for administration of probiotics to prevent intestinal conditions (119), but this knowledge base for oral applications is clearly more distant. One of the biggest problems to overcome may be that the probiotic activities and micro-organisms that protect against oral disease could increase the risk of development of dental caries. Therefore, a prebiotic-type approach to enhance endogenous beneficial commensals may be more attractive. 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 colonise plaque or prebiotics to enter into plaque or mucosal biofilms and influence microbial metabolism within them.

In conclusion, the use of probiotics for use in oral care applications is gaining momentum. There is increasing evidence that the use of existing probiotic strains can deliver oral health benefits. Further work will be needed to fully optimise and quantify the extent of this benefit. In parallel, the potential of prebiotics to maintain and enhance the benefits provided by the resident oral microbiota will be investigated. However, whether considering probiotics or prebiotics, it will be essential to develop an understanding of the broad ecological changes induced in the mouth by their ingestion and the long-term consequences of their use on oral health and disease.

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