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EXPERT OPINION

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Probiotics as oral health biotherapeutics

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Introduction: Oral health is affected by its resident microorganisms. Three prominent oral disorders are dental caries, gingivitis and periodontitis, with the oral microbiota playing a key role in the initiation/progression of all three. Understanding the microbiota and the diseases they may cause is critical to the development of new therapeutics. This review is focused on probiotics for the prevention and/or treatment of oral diseases.

Areas covered: This review describes the oral ecosystem and its correlation with oral health/disease. The pathogenesis and current prevention/treatment strategies of periodontal diseases (PD) and dental caries (DC) are depicted. An introduction of probiotics is followed by an analysis of their role in PD and DC, and their potential role(s) in oral health. Finally, a discussion ensues on the future research directions and limitations of probiotics for oral health.

Expert opinion: An effective oral probiotic formulation should contribute to the prevention/treatment of microbial diseases of the oral cavity. Understanding the oral microbiota's role in oral disease is important for the development of a therapeutic to prevent/treat dental diseases. However, investigations into clinical efficacy, delivery/dose optimization, mechanism(s) of action and other related parameters are yet to be fully explored. Keeping this in mind, investigations into oral probiotic therapies are proving promising.

Keywords: dental caries, gingivitis, oral microbiota, periodontitis, probiotic carrier systems, probiotics

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1. Introduction

The oral cavity houses a microbial ecosystem accountable for influencing oral health and disease. The mouth's microbial diversity is contentious, cited differently by research groups [1,2]. Dewhirst *et al.* (2010) identified 1179 taxa in the human oral flora [3]. Keijser *et al.* (2008) have estimated 19,000 – 26,000 species in the mouth [4]. Jorn *et al.* (2005) identified 141 predominant species and Paster *et al.* (2005) detected 415 species in the subgingival flora [5,6]. These discrepancies may be caused by the use of different quantification and qualification methods, diversity among individuals and diversity between sampling sites.

Historically, the analysis of a bacterial population was done by growth on defined and selective media, but a large proportion of bacteria remain uncultivable [7]. Hence, research has turned to molecular techniques such as fingerprinting, terminal restriction fragment length polymorphism, ribosomal intergenic spacer analysis, 16S ribosomal RNA sequencing, microarrays, high-throughput genome sequencing, fluorescence in situ hybridization, catalyzed reporter deposition-fluorescence in situ hybridization, quantitative polymerase chain reaction and scanning electron microscopy in situ hybridization [8]. In general, metagenomics is an approach used to analyze the genomic content of heterogeneous populations of microbes.

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Article highlights.

- The oral microflora is important in maintaining oral health. Changes in the microflora play an important role in oral disease (OD) initiation and progression.
- OD poses significant health and economic burdens, with dental caries (DC) and periodontal disease (PD) as the most common. Current therapies for OD have limitations.
- This review introduces the oral microbiota, its role in oral health and disease, the pathogenesis/current therapies available for PD/DC, the current research on probiotic therapeutics to prevent and treat PD/DC and the potentials and limitations of probiotics in PD/DC.
- Probiotics are live microorganisms that could beneficially influence the oral microflora to maintain a healthy ecosystem, specifically in OD. Available literature suggests that probiotics in OD are proving promising with regard to inflammation, caries progression and bone resorption, to name a few.
- Further research is needed for the development of probiotic formulations for OD. Perhaps this research should focus on dose optimization, mechanism(s) of action of the probiotic, strain selection and well-designed animal and clinical trials.

This box summarizes key points contained in the article.

These techniques all vary in their sensitivity and specificity, possibly supporting the differing results with respect to the diversity of the oral microflora.

Regardless of the exact species number, the prominent genera that inhabit the oral cavity are *Streptococcus*, *Veillonellaceae*, *Neisseria*, *Haemophilus*, *Actinomyces*, *Prevotella*, *Capnocytophaga*, Treponema, *Eikenella*, *Staphylococcus*, *Porphyromonas* and *Fusobacterium,* described in another review [2,9]. **Table 1** highlights the main oral microflora organisms associated with DC and PD, noting that commensal microorganisms are typically in a synergistic relationship with the host [2,9,10]. Although the resident oral microorganisms are typically in homeostasis with the host, a disturbance in the balance can initiate and potentiate oral diseases [10,11]. Oral disturbances, which can promote the proliferation of opportunistic pathogens, include a decrease in oral pH, a presence of high levels of dietary carbohydrates (example sucrose) and dry mouth, termed xerostomia [12]. **Figure 1** provides a schematic representation of the pathogenesis of oral disease.

It has been shown that 60 – 90% of all schoolchildren have dental cavities, 5 – 20% of American middle-aged adults are affected by severe periodontitis, a disease that affects the supporting structures of the teeth, and 32% of American adults have gingivitis [13-16]. This review presents a summary of current prevention and treatment methods of the three most prominent oral diseases, specifying that there is no appropriate and successful method formulated to date, and so there is great interest in developing an efficient therapeutic. Probiotics are well established as effective therapeutics for a number of gastrointestinal tract (GIT) disorders, as discussed in other reviews [17,18]. Considering that the oral cavity is a continuation of the GIT, probiotics should have the potential to be developed as oral therapeutics. This review will introduce the pathogenesis of the three important oral diseases, in the context of the microflora, to better comprehend how probiotics could influence the microbial community. The focus will then turn to current research investigating probiotics as oral therapeutics. The different probiotic carrier systems, for delivery in the oral cavity, will be subsequently described. To conclude, we address the limitations and the potential future directions of probiotics for the treatment and prevention of OD.

2. Periodontal diseases and pathogenesis

Periodontal disease (PD) is subcategorized into gingivitis and periodontitis. Gingivitis is characterized by an inflammation of the gingiva and bleeding of the gums, which, upon progression, may result in periodontitis [13,19,20]. The latter involves the additional inflammation and infection of the supporting structures of the teeth, such as the periodontal ligaments and the alveolar bone. Gingivitis is reversible when efficiently treated early; however, upon progression to periodontitis, the disease is irreversible [21] and can result in tooth loss, tooth shifting, abscesses causing facial cellulitis, osteomyelitis and trench mouth [13,22].

Dental plaque, a contributor of PD, involves the formation of a bacterial biofilm on the oral surfaces (tongue, gingiva, teeth) (**Figure 2**) [11]. The biofilm matrix consists of bacteria and bacterial exopolysaccharides, high-molecular-weight polymers made up of sugar residues [23]. Saliva is unable to penetrate this layer to reach the tooth surfaces, further promoting the adherence, the proliferation and the colonization of opportunistic periodontopathogens [11]. Environmental changes around the gingiva during PD include an increased amount of gingival crevicular fluid (GCF), an inflammatory exudate and a slight rise in pH, even further encouraging the growth of periodontopathogens [11,24]. Activated host immune responses, to combat PD, include an increased leukocyte infiltration and cytokine production, correlated with PD progression and severity [25]. Receptor activator of nuclear factor κB ligand (RANKL), an osteoclast differentiation factor expressed on osteoblasts, T cells and B cells, is an important player in the activation of the immune-mediated disease progression [26]. In short, factors such as an altered subgingival microflora, increased inflammation and a hyperactive host immune system, all contribute, directly and indirectly, to the destruction of the tooth's supporting structures [11].

3. Etiology of dental caries and disease progression

Dental caries (DC), also termed dental cavities, is a chronic disease of childhood, five times more prevalent than asthma, that causes significant destruction to the hard surfaces of the tooth and, like periodontitis, is associated with dental plaque [27,28]. Certain commensal bacteria, including *Streptococci, Lactobacilli*

Table 1. The oral microbiota and its association with dental caries and periodontal disease.

Oral diseases	Pathogens	Ref.
Dental caries	*Streptococcus mutans*	[11,31,95]
	Streptococcus sobrinus	[96]
	Streptococcus sanguinis	[97]
	Streptococcus mitis	[97]
	Streptococcus pneumoniae	[97]
	Streptococcus infantis	[97]
	Streptococcus gordonii	[97]
	Enterococci	[31]
	Actinomycetes	[31,98]
	Veillonella atypica dispar parvula	[97]
	Lactobacillus gasseri johnsonii	[97]
	Lactobacillus casei paracasei	[97]
	Selemonas	[97]
	Neisseria	[97]
	Propionibacterium	[97]
Periodontal disease	*Fusobacterium nucleatum*	[99]
	Porphyromonas gingivalis	[71,100]
	Bacteroides forsythus	[71,100,101]
	Treponema denticola	[71,100,101]
	Actinobacillus actinomycetemcomitans	[100-102]
	Prevotella intermedia	[100]
	Prevotella nigrescens	[100]
	Peptostreptococcus micros	[103]
	Proteus mirabilis	[104]
	Pseudomonas aeruginosa	[104]
	Pseudomonas maltophilia	[104]
	Pseudomonas	[104]
	Enterobacter cloacae	[104]
	Enterobacter agglomerans	[104]
	Escherichia coli	[104]
	Klebsiella pneumonia	[104]
	Klebsiella oxytoca	[104]
	Serratia marcescens	[104]
	Campylobacter rectus	[100]
	Eikenella corrodens	[100]
	Porphyromonas endodontalis	[102]
	Candida albicans	[104,105]

and *Actinomycetes*, proliferate in dental plaque when dietary carbohydrates are not eliminated from the oral cavity following food intake, due to poor oral hygiene practices or xerostomia (dry mouth), leading to acid production [29-31]. Acidic conditions result in the demineralization of the tooth's outer layers (enamel and dentin), due to a leaching out of ions, promoting tooth decay and caries progression. Saliva normally acts as a buffer, neutralizing the acidic pH and providing ions such as calcium, fluoride and phosphorous, which promote tooth remineralization [30].

4. Current prevention and treatment methods for periodontal diseases

The therapeutic strategies of PD lie in its prevention, entailing good oral hygiene practices. Following disease onset, treatment practices include scaling (removal of plaque and tartar) and root planing (smoothing of rough surfaces on the root) to impede any additional bacterial adhesion and growth [13,19,20]. Ultimately, surgical procedures may be required to eliminate pockets, to support loosened teeth and to extract teeth to stop the disease from spreading to adjacent areas [13]. On the other hand, antiseptics such as chlorhexidine, povidone-iodine and stannous fluoride and antibiotics, such as minocycline, doxycycline, metronidazole and tetracycline, have been used [32,33]. However, there are several adverse reactions associated with antibiotic therapy including hypersensitivity, gastrointestinal disorders and antibiotic resistance [34,35]. The susceptibility to PD can be influenced by smoking and other underlying conditions such as diabetes, viral infections and stress [36-39]. In addition, PD has been associated with atherosclerosis and preterm babies, substantiating the importance for an efficient therapeutic [40,41].

5. Current prevention and treatment options for dental caries

Prevention and treatment strategies for DC involve proper oral hygiene, the addition of fluoride ions in countries drinking water and the use of other fluoride substitutes/supplements such as toothpastes, varnishes, gels, tablets, lozenges and chewing gums to promote remineralization [42-44]. The provision of dental sealants is a method currently used to inhibit the attachment and propagation of pathogenic bacteria responsible for caries progression [45]. However, a fluoride prevention regime may lead to fluorosis (staining, pitting of the teeth and enamel damage) while receiving dental sealants highly depends on the socioeconomic status of the patient [46]. Following disease onset, treatment methods focus on arresting caries progression and restoring the lost tooth structure [47]. Dental restorations are performed to replace the lost tooth structure and, with extensive loss, a prosthetic may be necessary [28]. Endodontic therapy and surgical intervention may be required when DC has extended to pulp tissues and periapical areas [28,48]. Complications arising from DC progression include severe tooth pain, tooth fractures, tooth loss and oral abscesses, which promote facial cellulitis and osteomyelitis [28,49].

With the etiology of oral diseases indicating that the oral microflora plays an important role, one may consider low levels of antibiotics as a treatment option. As previously mentioned, this is not an adequate therapy for chronic life-long disorders. On the other hand, probiotics have already shown potential for reducing the proliferation and adherence of cariogenic bacteria, suggesting their prospective use as a natural and safe oral therapeutic.

6. Introduction to probiotics and their potential in modulating the oral microbiota in prevention and/or treatment of oral diseases

According to the World Health Organization and the Food and Agriculture Organization of the United Nations, probiotics

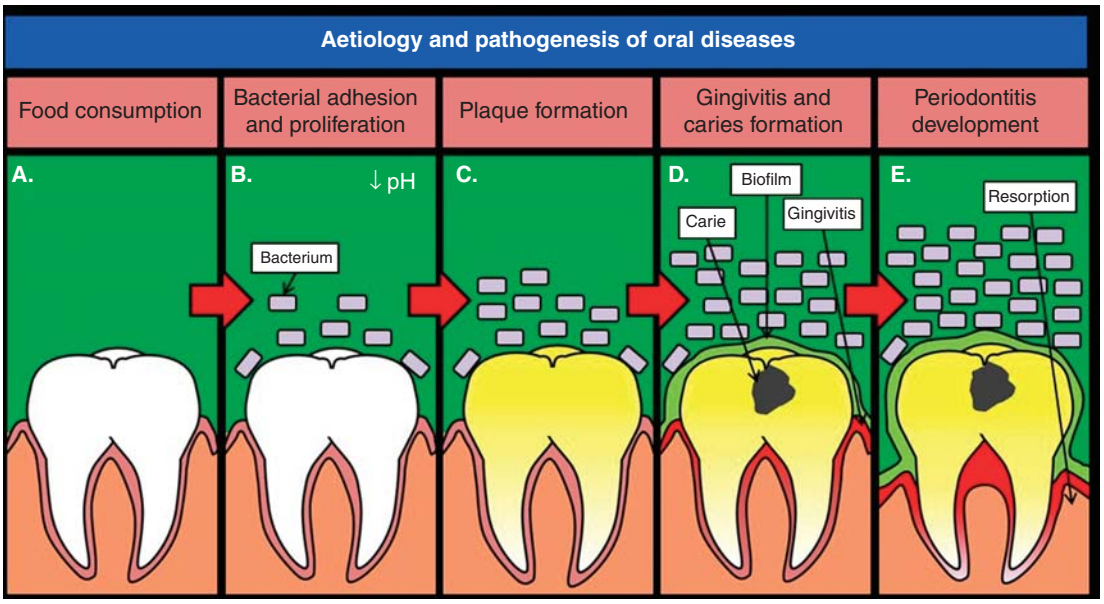


Figure 1. Oral disease development pathways. A. Consumption of food containing carbohydrates. **B**. Fermentation of dietary carbohydrates and development of an acidic pH enabling bacterial adhesion and proliferation. **C**. Formation of an oral biofilm known as dental plaque, which facilitates the survival and proliferation of pathogens residing in it. **D**. Development of gingivitis and dental caries due to established plaque. Gingivitis is recognized by red swollen gingiva and caries is recognized by tooth decay. **E**. Development of periodontitis due to a disseminated infection into the supporting structures of the tooth.

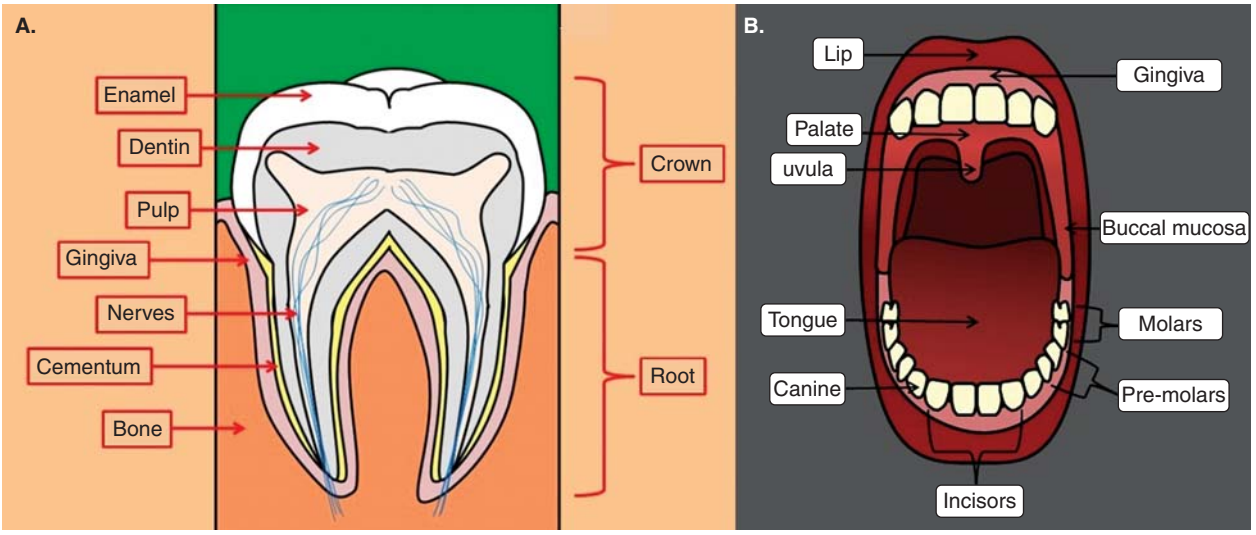


Figure 2. A. The anatomy of the tooth and susceptible mineralized layers namely enamel, dentin and cementum for oral disease. The innermost structure, the pulp consists of nerves, blood vessels and connective tissue where microorganisms can potentially reside and cause infection. **B**. The available surfaces (teeth, gingiva, palate, oral mucosa, uvula and tongue) in the oral cavity where microorganisms primarily colonize.

are 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host,' of which *Lactobacillus* and *Bifidobacterium* are the most commonly used [18]. There are numerous studies demonstrating the positive role of probiotics in disorders such as diarrhea, peptic ulcers, GIT cancers, inflammatory bowel diseases (IBD), constipation, hypersensitivity responses, cardiovascular diseases and urogenital tract disorders [18,50,51]. Probiotics have previously been shown to alleviate inflammation associated with both OD and GIT diseases [52,53]. Saliva appears to be an important

link between these two types of diseases. For example, Rezaie *et al.* have demonstrated that changes in salivary cytokine levels can be correlated to Crohn's and ulcerative colitis [54-56]. Specifically, with GIT-related inflammation, the salivary levels of nitric oxide and transforming growth factor-β_1 were also shown to be elevated. Keeping this research in mind, a probiotic therapeutic may prove beneficial for the simultaneous treatment of both types of disorders.

Additionally, probiotics are naturally found in food products such as yoghurt and milk and have yet to present serious side effects associated with currently available antimicrobials. A number of probiotic strains have been investigated for their overall safety, including their potential for transmissible antibiotic resistance [50,57]. Although probiotic research in the field of dental sciences is relatively new, preliminary studies suggest their beneficial influence on maintaining oral health. As aforementioned, the cause of oral disease is determined by a number of factors that include the proliferation of certain bacterial populations such as *Streptococcus* and *Actinomycetes*, their adherence to oral surfaces to facilitate amplified growth in dental plaque and disease-associated inflammation [31,58]. Probiotics have been shown to compete with pathogenic species in the GIT and have also shown remarkable anti-inflammatory properties [59]. Preliminary studies into probiotics have demonstrated positive results, particularly in the prevention and treatment of gingivitis, periodontitis and DC. **Table 2** provides a brief list of probiotics that have been investigated as therapeutics for combating oral disease.

A successful therapeutic for treating and preventing gingivitis, periodontitis and DC should target the etiologic factors discussed previously. For gingivitis and periodontitis, the mechanism of action of the therapeutic should focus on the diminution of dental plaque, the reduction of the number of periodontal pathogens and the modulation of the host immune response by controlling the release of inflammatory cytokines, which may slow down the destruction of supporting tissues. As for a DC therapeutic, the focus should lie in the reduction of the causative organism *S. mutans* and the reduction of dental plaque. This review presents research findings that have significantly contributed to the knowledge and understanding of probiotics for the prevention and treatment of oral diseases.

7. Potential for a probiotic therapeutic in gingivitis and periodontitis

As aforementioned, the etiology and progression of gingivitis and periodontitis are linked to a hyperactive host immune response, demonstrated by an increased level of inflammatory markers in diseased individuals [60]. There are methods used to qualify/quantify the capability of probiotics to inhibit the formation of dental plaque, to regulate the production of host inflammatory markers and to reduce the prevalence of periodontal pathogens [52,61-63]. Indexes such as Plaque Index (PI), Papilla Bleeding Index (PBI) and Gingival Index (GI) are approaches used to evaluate the capability of a therapeutic to arrest disease progression. PI, introduced by Silness and Loe in 1964, evaluates the state of oral hygiene by recording the amount of soft and mineralized deposits on tooth surfaces (levels of plaque). PBI is a scoring system for the inflammation of the interdental papillae by evaluating bleeding on probing. GI is an index that evaluates the gingival status by specifically investigating its swelling and redness. Using these established methods, researchers can qualify and quantify the effects of the administration of probiotics on overall oral health.

One can conclude that there are three main goals that probiotics should achieve to prove successful as therapeutics for gingivitis and periodontitis. The first goal involves the modulation of the host's inflammatory processes. The second goal involves the reduction of plaque formation. Finally, the third goal is to reduce the presence and counts of disease-promoting microorganisms. Grudianov *et al.* were one of the first who reported the beneficial effects of probiotics on PD [64]. They demonstrated that probiotic bacteria, as a biofilm, reduced the counts of pathogenic bacteria in humans [64]. A synopsis of other relevant research on probiotics, focusing on the aforementioned mechanisms of action, is described below.

7.1 Probiotics can be used to modulate oral inflammation

Slawik *et al.* conducted a clinical study to evaluate the effects of probiotics on gingivitis [65]. The test group consumed probiotic milk containing *Lactobacillus casei* strain Shirota at a concentration of 10^8 cfu/ml while the control patients did not consume any. At day 14 of the treatment, there was a significant reduction of BOP and GCF volume in the test group compared with the control group and also when compared with baseline [65]. In related research, Adam *et al.* performed an *in vitro* study demonstrating that *Streptococcus salivarius* K12 and M18 reduce inflammatory cytokines associated with PD [66].

With the onset of PD, there are elevated levels of inflammatory cytokines present in the GCF, including polymorphonuclear (PMN) elastase and matrix metalloproteinase-3 (MMP-3), to attempt to restrict disease progression [24]. Their levels are an important diagnostic parameter for PD, with their levels directly correlated with disease progression and severity. Staab *et al.* conducted a study investigating the effects of *Lactobacillus casei* strain Shirota on gingivitis by evaluating its ability to modulate the host immune responses [52]. The test group received 65 ml of probiotic drink containing *Lactobacillus casei* strain Shirota at a concentration of 10^9 cfu/ml for 8 weeks [52]. Following treatment, no significant differences were noted in the PI and the PBI of the test and the control groups [52]. However, significant decreases in GCF PMN elastase and MMP-3 levels in the test group patients were noted [52]. These results suggest that *Lactobacillus casei* strain Shirota may have the capacity to modulate the

Table 2. Probiotics and oral disease prevention/therapeutic modes of action: i) modulation of inflammatory responses, ii) inhibition of biofilm formation and iii) inhibition of disease-causing organisms. Research on specific strains and mechanisms of action is highlighted.

Probiotic	Mechanism(s) of action	Experimental design	Ref.
L. casei strain Shirota	Modulation of inflammatory responses	*In vivo* (human)	[52]
L. reuteri ATCC 55730	Inhibition of disease-causing organisms	*In vitro*	[78]
		In vivo (human)	[85]
		In vitro	[77]
	Modulation of inflammatory responses	*In vivo* (human)	[63]
	Inhibition of biofilm formation	*In vitro*	[77]
L. reuteri ATCC 5289	Modulation of inflammatory responses	*In vivo* (human)	[63]
		In vivo (human)	[70]
	Inhibition of disease-causing organisms	*In vitro*	[78]
		In vitro	[77]
	Inhibition of biofilm formation	*In vitro*	[77]
		In vivo (human)	[70]
L. lactis byproduct Nisin	Inhibition of biofilm formation	*In vivo* (human)	[61]
		In vivo (dog)	[84]
	Inhibition of disease-causing organisms	*In vitro*	[83]
L. rhamnosus GG	Inhibition of biofilm formation	*In vitro*	[77]
	Inhibition of disease-causing organisms	*In vitro*	[77]
		In vivo (human)	[106]
L. plantarum DSM 9843	Inhibition of biofilm formation	*In vitro*	[77]
	Inhibition of disease-causing organisms	*In vitro*	[77]
L. paracasei DSMZ16671	Inhibition of biofilm formation	*In vivo* (rats)	[79]
Bifidobacterium lactis Bb-12	Inhibition of disease-causing organisms	*In vivo* (human)	[80]
L. reuteri DSM17938	Inhibition of biofilm formation	*In vivo* (human)	[70]
L. salivarius WB21	Inhibition of disease-causing organisms	*In vivo* (human)	[72]
L. acidophilus La5	Inhibition of disease-causing organisms	*In vitro*	[78]
L. plantarum 299v	Inhibition of disease-causing organisms	*In vitro*	[78]
L. rhamnosus LB21	Inhibition of disease-causing organisms	*In vitro*	[78]
L. paracasei F19	Inhibition of disease-causing organisms	*In vitro*	[78]
L. rhamnosus LC 705	Inhibition of disease-causing organisms	*In vivo* (human)	[106]
Bifidobacterium DN-173 010	Inhibition of disease-causing organisms	*In vivo* (human)	[107]
S. salivarius K12	Inhibition of disease-causing organisms	*In vivo* (human)	[70]

plaque-induced inflammatory immune responses by reducing cytokine production.

Studies involving inflammatory markers in IBS and other diseases have attempted to investigate the effects of probiotics on GIT-related disorders, described in meta-analyses [67,68]. However, these studies remain inconclusive as to the exact role of probiotics on the modulation of systemic inflammatory markers. In terms of local inflammation involved in PD, Twetman *et al.* investigated the effects of probiotics on gingival inflammation [63]. This group investigated the use of a cocktail formulation consisting of *Lactobacillus reuteri* ATCC 55730 and *L. reuteri* ATCC PTA 5289 incorporated in chewing gums at a concentration of 10^8cfu/gum, with a treatment period of 4 weeks. Following treatment, there were significant decreases in BOP and GCF volume in the patients consuming active chewing gums when compared with those consuming placebo. Following 1 – 2 weeks of treatment, significant decreases in TNF-α and IL-9 were noticed for the patients consuming active chewing gums [63]. This study suggests that a cocktail formulation of probiotics is able to modulate immune responses linked to PD, by reducing BOP, GCF volume and inflammatory cytokines.

Meta-analysis has been used to assess the effectiveness of probiotics in the modulation of markers in GIT-related disorders. The same could potentially be used to elucidate the therapeutic effect of probiotics in OD, specifically on markers of inflammation involved in PD. Unfortunately, current meta-analyses on probiotic effects often remain inadequate. The studies often fail to correlate the exact role of probiotics on markers of pathogenesis [68]. Hence, more well-designed research needs to be done for proper investigations into the mechanism(s) of action of probiotics in OD (and other GIT-related disorders).

7.2 Probiotics can be used to reduce biofilm formation

In 2007, Noordin *et al.* conducted a clinical study investigating the effects of nisin on plaque accumulation [61]. Nisin is a bacteriocin, an antibacterial substance produced by lactic acid bacteria, which can be extracted from the probiotic *Lactococcus lactis*. More specifically, nisin is a lantibiotic, a class of bacteriocin obtained from gram-positive bacteria and considered safe by the American Food and Drug Administration (FDA) [69]. The patients rinsed twice daily with 15 ml of

solution for 60 s, 30 min following tooth brushing, for a period of 32 days. The test group received mouth rinse containing nisin while the other group received placebo for the first 14 days, followed by a wash-out period of 4 days after which the groups were interchanged. Significant decreases in the PI and the GI were observed in patients administered mouth rinse containing nisin. Hence, this study indicates the role of nisin in the reduction of plaque accumulation and gingivitis [61].

In another study, Vivekananda *et al.* demonstrated the effect of *L. reuteri* (Prodentis) lozenges on plaque accumulation and inflammation in a clinical trial [70]. The lozenges contained 10^8 cfu of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289. The study was performed for 42 days with the test patients receiving the probiotic lozenge twice daily from days 21 to 42. At the end of the trial, there were substantial decreases in PI, GI and gingival bleeding in patients treated with the probiotics compared with those administered placebo. This study demonstrates the ability of Prodentis to reduce dental plaque and gingival inflammation, suggesting its role as a potential oral therapeutic [70].

7.3 Probiotics can be used to inhibit disease-causing oral microbes

A probiotic therapeutic formulation for oral diseases should also be capable of reducing the prevalence of pathogenic microorganisms. An elaborate review has already been published on the oral microflora and its role in health and disease [71]. Mayanagi *et al.* demonstrated a significant probiotic inhibition of the pathogens: *Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia* [72]. The patients were administered *Lactobacillus salivarius* WB21 at a concentration of 6.7×10^8 cfu/tablet, three times a day for 8 weeks, with the control group receiving placebo tablets. The microbial population was analyzed using a quantitative real-time polymerase chain reaction. At the end of the study, there was a significant decrease in the counts of all of the above-mentioned periodontal pathogens in the test group compared with the control group [72].

As aforementioned, Vivekananda *et al.* demonstrated the ability of Prodentis to inhibit plaque formation and to reduce inflammation. In the same clinical trial, the group also demonstrated that the probiotic formulation inhibited the proliferation of the periodontal pathogens: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* [70]. At the end of the trial, day 42, there was a 10-fold reduction from 10^6 to less than 10^5 cfu/ml in the counts of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* compared with baseline [70].

In another study, Tsubura *et al.* demonstrated the effect of *Bacillus subtilis* E-300 on periodontal pathogens [73]. The red complex consists of *P. gingivalis*, *T. denticola* and *T. forsythia,* all of which have been associated with PD [74]. These periodontal pathogens have the ability to hydrolyze trypsin substrates such as *N*-benzoyl-DL-arginine-2-naphthylamide to form a color reaction, used for quantification in the BANA test [74]. In this study, the test group received the probiotic mouth rinse for 30 days while the control group received a commercially available mouth rinse, Neosteline Green (benzethonium chloride). Following treatment, a significant decrease in the number of organisms of the red complex was noted in the treated patients, using the BANA test [73]. This research further suggests the role of probiotics, like *Bacillus subtilis* E-300, to inhibit PD-associated pathogens.

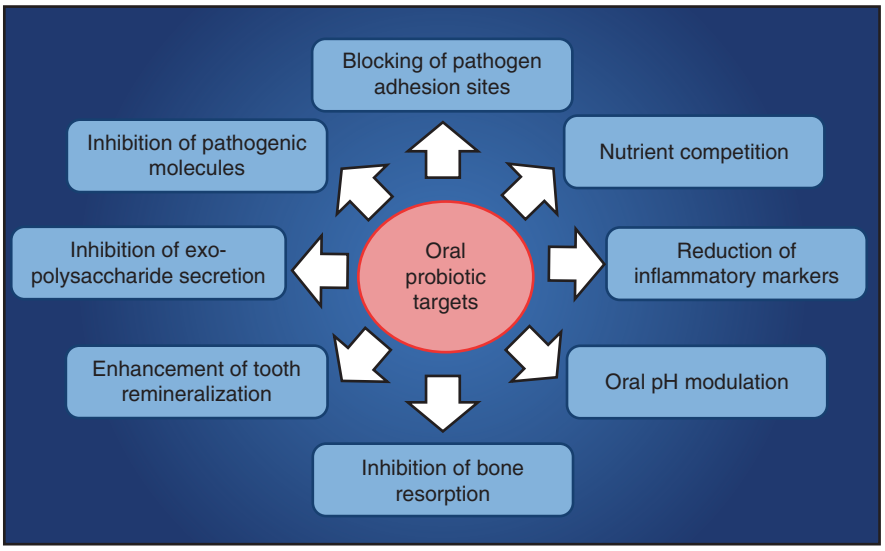
8. Potential for a probiotic therapeutic in dental caries

DC is a chronic disease initiated by the accumulation of dental plaque on the oral surfaces [75]. Recent studies investigate the use of probiotics for the prevention of DC. A number of dental plaque bacteria contribute to DC. As aforementioned, the goals for the successful prevention of DC by a therapeutic are to inhibit the proliferation of *S. mutans* and to inhibit its adherence to the oral surfaces.

The first study investigating probiotics for inhibition of oral streptococci was performed by Meurman *et al.*, in 1995 [76]. They performed an *in vitro* study and demonstrated the ability of LGG to inhibit oral *Streptococcus sobrinus* [76]. In a more recent *in vitro* study, Soderling *et al.* investigated the inhibition of *S. mutans* proliferation and plaque formation by the probiotic strains: LGG, *L. plantarum* DSM 9843, *L. reuteri* ATCC PTA 5289 and *L. reuteri* ATCC 55730 [77]. In terms of biofilm formation, all of the above probiotic strains demonstrated some inhibition; however, *L. reuteri* ATCC PTA 5289 and LGG demonstrated the highest inhibitory activities, measured by spectrophotometry and plate counts [77]. The probiotic strains also proved effective at inhibiting the growth of *S. mutans* in a pre-formed biofilm, with LGG and *L. plantarum* DSM 9843 as two of the strains demonstrating complete inhibition of *S. mutans* proliferation [77]. With these results, the probiotic strains capable of inhibiting *S. mutans* should be investigated further as potential DC therapeutics.

A number of probiotic strains were investigated, by Hasslof *et al.* for their *in vitro* inhibition of *S. mutans* [78]. The following *Lactobacilli* were investigated: *L. plantarum* 299v, *L. plantarum* 931, LGG ATCC 53103, *L. rhamnosus* LB21, *L. paracasei* F19, *L. reuteri* PTA 5289, *L. reuteri* ATCC 55730 *and L. acidophilus* La5 [78]. These were tested at four concentrations: 10^3, 10^5, 10^7 and 10^9 cfu/ml for inhibition of five streptococci strains: *S. mutans* NCTC 10449, *S. mutans* Ingbritt, *S. sobrinus* OMZ 176 and clinical isolates *S. mutans* P1:27 and *S. mutans* P2:29 [78]. Using an agar overlay interference test, all of the probiotic strains were shown to inhibit *S. mutans,* although the capacity for inhibition varied between each probiotic strain [78]. *L. plantarum* 299v and *L. plantarum* 931 were the most potent inhibitors, demonstrating complete inhibition of *S. mutans* at the lowest concentration

A.



B.

Mode of action	Oral probiotic targets	Reference(s)
Direct	Inhibition of pathogenic molecules	[70,72,78]
	Reduction of inflammatory markers	[24,52,66]
	Inhibition of exo-polysaccharide secretion	To be investigated
Indirect	Blocking of pathogen adhesion sites	[64]
	Enhancement of tooth remineralisation	To be investigated
	Inhibition of bone resorption	To be investigated
	Oral pH modulation	To be investigated
	Nutrient competition	To be investigated

Figure 3. A. Potential oral probiotic targets and mechanisms to enhance oral health. **B**. Potential direct and indirect modes of action of oral probiotic biotherapeutics.

of probiotic [78]. However, the inhibition for each of the *S. mutans* strains was variable [78]. Taking these results into consideration, this study suggests that selected probiotic strains have the ability to prevent DC by inhibiting the growth of *S. mutans,* but the extent of inhibition remains strain specific for the probiotic and for the pathogenic strain.

In an *in vivo* study, Tanzer *et al.* investigated *L. paracasei* DSMZ16671 for its potential to inhibit the development of DC [79]. Wistar rats were administered heat-killed *L. paracasei*, at a concentration of 5×10^8 cfu/g incorporated in a high-sucrose caries-inducing diet inoculated with *S. mutans* at a concentration of 10^{10} cfu [79]. The administration of the probiotic resulted in the inhibition of colonization by *S. mutans* 10449S, evaluated using plate counts [79]. In another human trial, there was a significant decrease in *S. mutans* following the consumption of probiotic ice-cream containing *Bifidobacterium lactis* Bb-12 at a concentration of 10^7 cfu/gram of ice cream, as reported by Caglar *et al.* [80]. Caglar *et al.* also demonstrated that the consumption of a probiotic chewing gum containing *L. reuteri* ATCC 55730 and *L. reuteri* ATCC PTA 5289, at a concentration of 10^8 cells per gum, reduced the levels of *S. mutans* in saliva when compared with placebo [81].

Turner *et al.* investigated the effect of nisin on *E. faecalis* and *S. gordonii* in the root canal system, relevant to the prevention of root caries [82,83]. A root canal is the inner tube of the tooth root, which contains the pulp tissue, the nerves, the blood vessels and the lymphatics. Nisin, at a concentration of 100 mg/ml, eliminated root caries-associated *Actinomyces* species, *Enterococcus faecalis* and *Streptococcus gordonii*, in pure culture [83]. Nisin was also investigated in beagle dogs,

Table 3. Formulation for probiotic deliveries for use in oral diseases.

Carrier	Probiotic	Dose	Duration	Ref.
Milk	*L. casei* strain Shirota	10^9 cfu/ml	8 weeks	[52]
Chewing Gum	*L. reuteri* ATCC 55730	10^8 cfu/gum	4 weeks	[63]
Straws/tablets		10^8 cfu once daily	3 weeks	[85]
Chewing Gum	*L. reuteri* ATCC 5289	10^8 cfu/gum	4 weeks	[63]
Lozenge		10^8 cfu/day	3 weeks	[70]
Milk	*L. rhamnosus* GG	$5 - 10 \times 10^5$ cfu/ml 5 days/week	7 months	[86]
Cheese		1.9×10^7 cfu/g; 5 x 15 g/day	3 weeks	[106]
Diet	*L. paracasei* DSMZ16671	5×10^8 cfu/g	-	[79]
Ice-cream	*Bifidobacterium lactis* Bb-12	10^7 cfu/g	1 – 2 weeks, Cross-over study	[80]
Lozenge	*L. reuteri* DSM17938	10^8 cfu/day	3 weeks	[70]
Tablet	*W. salivarius* WB21	6.7×10^8 cfu 3 times/day	8 weeks	[72]
Cheese	*L. rhamnosus* LC 705	1.2×10^7 cfu/g; 5 x 15 g/day	3 weeks	[106]
Yoghurt	*Bifidobacterium* DN-173	7×10^7 cfu/g; 200 g/day	4 weeks	[107]
Lozenge	*S. salivarius* K12	10^9 cfu once daily	3 weeks	[70]

where it reduced plaque and gingivitis when applied in the premolar area twice daily for 88 days [84]. With these data, nisin may prove beneficial as a therapeutic formulation against gingivitis and periodontitis.

In a clinical trial, Caglar *et al.* investigated the inhibitory potential of the probiotic strain *L. reuteri* ATCC 55730 on *S. mutans* proliferation [85]. Probiotic administration was achieved using straws and tablets containing 10^8 probiotic cells, administered once daily for 3 weeks. *S. mutans* and *L. reuteri* were quantified using Dentocult® SM and Dentocult® LB chair-side kits and cfu counts. This group demonstrated a reduction of *S. mutans* following probiotic consumption, highlighting the potential of *L. reuteri* ATCC 55730 for DC prevention [85]. In another clinical study, LGG, incorporated in milk, showed the potential to reduce DC in a population of 3- to 4-year-old children [86]. The treatment group received the probiotic formulation at $5 - 10 \times 10^5$cfu/ml for 7 months (5 days a week). The results demonstrated that the LGG treatment group had a lower incidence of DC associated with reduced *S. mutans* counts when compared with the control group [86]. This further confirmed LGG's ability to prevent childhood caries, proposing additional potential as a DC therapeutic [86].

9. Expert opinion

Recently, probiotics have been gaining interest for alleviating oral and other health disorders. The complex oral microflora is closely associated with the host's oral health. Adverse changes in the oral cavity are manifested by an imbalance in the microflora, potentially shifting the microbial population toward a pathogen-associated population. The increased presence of opportunistic microorganisms results in complications of tooth pain, tooth loss and abscesses. In addition, *S. mutans* has been shown as capable of disseminating in the systemic circulation leading to life-threatening conditions such as infective endocarditis, highlighting the impact of the oral microflora on overall health [87]. Contemporary prevention and treatment methods are not entirely successful and vary greatly among individuals. In addition, the oral cavity is exposed to disease-causing factors throughout the course of one's life, leading to the requirement for a long-term therapeutic, rendering antibiotics ineffective as a therapy.

Probiotics, unlike antibiotics, can be used to treat and prevent oral diseases as a natural and long-term approach. Results appear optimistic for this future therapeutic; however, there is a need for a better understanding of the mechanisms of action of probiotics to allow for an optimal therapeutic activity. **Figure 3** highlights the goals that should be met by a probiotic therapy for the successful prevention/treatment of oral disease. Future research should focus on probiotic inhibition of the growth and metabolic activity of pathogenic microorganisms for the development of an appropriate formulation to prevent/treat oral disease. Although there are a few clinical studies investigating probiotics, current studies have only been performed for short durations, whereas probiotic consumption is meant to be used as a long-term therapy. Moreover, present studies focus on inhibiting specific pathogens such as *S. mutans*, lacking investigations of the oral ecosystem as a whole. As discussed previously, a number of microorganisms are responsible for oral diseases (**Table 1**), hence future research should take a number of these organisms into account.

Recent research has also turned to replacement therapy for the treatment of DC. Replacement therapy is a term that can be used for probiotic therapy, but is more commonly used to describe the use of non-pathogenic genetically modified (GM) microorganisms to displace pathogenic microorganisms of a bacterial ecosystem. Hillman *et al.* prepared a GM avirulent bacterial strain of *S. mutans* that has a greater ability to colonize the oral cavity compared with a pathogenic strain

of *S. mutans* [88,89]. However, the use of GM organisms brings forth issues of safety and toxicity, as already discussed by Cummins *et al.* [90]. Hence, the future of replacement therapy will most likely lie with natural probiotic strains rather than genetically modified microorganisms.

Equally important is the need for the development of a delivery system that allows for a prolonged residence time of the delivered probiotic in the oral cavity. Previous and current research has focused on the use of chewing gums, mouth rinses, probiotic drops, chocolates and food products such as milk, cheese and yoghurt for probiotic delivery. **Table 3** provides a brief overview of the carrier systems previously investigated for probiotic delivery. Although these delivery systems have shown promising results, there remain a number of limitations to be addressed. For example, probiotics incorporated in food products, upon swallowing, result in rapid loss from the oral cavity. A loss of probiotics from the oral cavity means a loss of their efficiency in preventing and treating oral diseases. Developed delivery systems should, hence, focus at increasing the retention time of the probiotics in the oral cavity, increasing the efficiency of the probiotic therapeutic. Carrier systems, such as chewing gums, may increase the retention time of probiotics in the oral cavity; however, the retention time may need to be increased further for an optimal effect. Factors that need to be considered while developing a delivery system include the probiotic's targeted delivery while maintaining the viability and metabolic activity of the probiotic cells. A carrier system that would enable the release of probiotics in areas difficult to reach, such as the interdental spaces, pits and fissures of the teeth, should be developed. Current research into carrier systems such as collagen scaffolds and hydrogels may prove promising for probiotic delivery [91,92].

In other research, probiotics have also shown to be beneficial when administered in combination with prebiotics [93]. Prebiotics are 'nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species' [93]. The inclusion of prebiotics along with probiotics as a synbiotic may prove beneficial for oral health [94]. A carrier system that is made up of a prebiotic may just prove to be an appropriate technology. Lastly, the shelf-life of a carrier system and the probiotic incorporated in the system should be investigated, as bacterial viability is directly correlated with the efficacy of any developed therapeutic.

In terms of toxicity concerns, the safety of all the probiotic strains should be investigated prior to any clinical studies, as the delivery of any microorganism can potentially prove harmful. The safety studies should involve dose optimization studies, where only the required number of bacterial cells would be delivered, to avoid any bacterial overload. Reliable and reproducible methods for sampling and quantifying oral bacteria *in vivo* should be investigated. In terms of PD, quantifying inflammatory cytokines and bone resorption are among the most important investigations. Procedures that would enable accurate sampling and analysis of inflammatory cytokines are of prime importance. Techniques such as radiography and micro-computed tomography could prove advantageous to study bone resorption *in vivo*. Lastly, well-designed and lengthier human trials should be performed in order to produce an appropriate probiotic therapeutic for combating oral diseases.

Declaration of interest

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