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REVIEW ARTICLE

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Probiotics for periodontal health—Current molecular findings

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1 | INTRODUCTION

Periodontal diseases, which include a range of conditions from gingivitis to periodonitits, are some of the most prevalent oral health diseases.[1-3] Specifically, periodontitis—a disease characterized not only by gingival inflammation but also by loss of connective tissue attachment, leading to resorption of alveolar bone and subsequent tooth loss[4-6]—affects approximately half of adults over 30 years of age in its mildest form and over 60% of individuals over 65 years of age.[7,8] Microbial biofilms are the primary etiology in chronic periodontitis; however, the condition can be modulated by many other local and systemic factors.[9,10]

In treating chronic periodontitis, the main goal is addressing the primary etiology.[11] Successful treatment of chronic periodontitis results in a reduction in periodontal pocket depth with gains in clinical attachment levels.[12] Traditionally, the main treatment modality for chronic periodontitis is nonsurgical periodontal therapy, including oral hygiene instructions and scaling and root planing.[13] Scaling and root planing therapy does not always result in improvements, especially for sites with deep probing depths and when patients suffer from comorbidities (diabetes mellitus, obesity, cardiovascular disease).[14-16] Adjunctive therapies, such as antibiotics or probiotics, may prove useful in these multifactorial conditions where scaling and root planing therapy alone is not successful.

In the past, systemic antibiotic therapy has been used to reinforce mechanical therapy and support the host defense by killing the subgingival pathogens remaining after nonsurgical therapy.[17] Systemic antibiotics may offer additional benefits in conjunction with scaling and root planing, but they are not innocuous drugs.[18] Antibiotics have side effects, and there is the potential for emergence of antibiotic-resistant bacterial strains.[18] As a result of these side effects, efforts have commenced to explore the use of probiotics as a method to modulate the composition of pathogenic biofilms in conjunction with scaling and root planing.[19]

The World Health Organization (2014)[20] defines probiotics as live cultures of microorganisms that confer a health benefit on the host when administered in adequate amounts. Research has highlighted success in many areas of medicine with the eight known classes of probiotics.[21] These successes include the treatment of diseases related to the gastrointestinal tract and oropharyngeal infections.[19,22] However, the role of probiotics in modulating periodontal diseases is not fully understood. The main focus of this literature review is to examine the in vitro and in vivo/preclinical, microbiological, and immunological effects of probiotics in the context of periodontal disease.

2 | IN VITRO MOLECULAR FINDINGS

In order for a probiotic candidate to be used orally in periodontal-disease–related conditions, it must first be tested using in vitro experimentation (Table 1). Similar to probiotics used in the gastrointestinal tract, probiotics for periodontal disease applications should demonstrate the absence of pathogenic gene sequences and the presence of antibacterial activity against periodontopathic bacteria, colonizing ability on oral epithelial cells, and the ability to modulate the immune response in the presence of periodontal pathogens.[23]

2.1 | Absence of pathogenic gene sequences

A healthy oral cavity consists of more than 700 bacterial species, predominantly colonizing the tooth and the surrounding structures, buccal epithelium, hard and soft palate, and tongue dorsum.[24] Given this bacterial richness, it is important to screen potential bacterial probiotic strains to ensure that they do not contain pathogenic gene sequences that may be harmful to the oral bacterial community, and thereby to oral health. Dental plaque, tongue coatings, saliva, and subgingival plaque have all been used to evaluate the different probiotic strains and their by-products that could be used in the context of oral diseases.[25,26] The potential pathogenicity of probiotic strains that were isolated from the oral cavity was identified using standard gene sequencing analysis. This analysis was used to generate a list of probiotic microorganisms that are safe for human use and that could be added to food or feed.[27] This list included a number of lactobacilli and streptococcal strains.[27]

2.2 | Antibacterial activity against periodontopathic bacteria

Many lactobacilli and streptococcal strains isolated from healthy oral cavities show antibacterial activity against *Porphyromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*,[25,26,28] and *Fusobacterium nucleatum*.[29] Studies also show that different lactobacilli strains can inhibit the ability of multiple microbial species. For example, *Lactobacillus fermentum* is active against microaerophiles, and *Lactobacillus gaseri* is active against anaerobes.[26] Other *Lactobacillus* strains, including *Lactobacillus paracasei* and *Lactobacillus acidophilus* have inhibitory effects on the growth of *Staphylococcus aureus*,[30] a pathogen found in aggressive periodontitis.[31] Streptococci inhibit anaerobes, such as *P. gingivalis* and *P. intermedia* through the production of lactic acid and other organic acids.[32] The growth of *P. gingivalis* is also inhibited by the production of hydrogen peroxide from some probiotic strains isolated from commercial yogurt products. One study[33] showed that the STYM1 strain of *Lactobacillus delbrueckii* releases intracellular proteins that can produce inhibitory amounts of hydrogen peroxide.[33] Hydrogen peroxide can be produced and generated by several different enzymes in many *Lactobacillus* strains, including pyruvate oxidase,[34] lactate oxidase, NADH oxidase [35] and NADH flavin–dependent reductase.[36] In this study,[33] the growth of *P. gingivalis* in agar plates treated with hydrogen oxidase from *L. delbrueckii* was inhibited significantly compared with the untreated plates.

2.3 | Antibacterial effects mediated by bacteriocins

Studies have found that bacteriocins contribute greatly to the antibacterial activities of many probiotics, including lactobacilli. Bacteriocins are antimicrobial proteins or peptides that are secreted by bacteria as a defense mechanism. Bacteriocins are classified into three main classes depending on their molecular size and function.[37,38] The bacteriocins relevant to the oral cavity include salivaricin from *Lactobacillus salivarius* and *Streptococcus salivarius*,[39] reuterin from *Lactobacillus reuteri*,[40] plantaricin from *Lactobacillus plantarum*,[41] and nisin from *Lactobacillus lactis*.[41] The bacteriocin plantaricin contributes to the improvement of systemic health.[42] In the context of oral health, plantaricin shows its antimicrobial activities by preventing the growth of *P. gingivalis*.[28] Salivaricin is known to suppress oral bacteria related to malodor,[39] whereas reuterin has potential applications for periodontal disease as it has antibacterial effects against *Tannerella forsythia* ATCC 43037.[40]

Similarly, nisin has antibacterial effects against multiple periodontal pathogens[43] in addition to its effects on gastrointestinal-associated gram-positive bacteria.[44] Nisin exhibits significant antibiofilm and antimicrobial effects against many gram-negative periodontal pathogens, including *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, *F. nucleatum*, and *Treponema denticola*.[45] Importantly, nisin also does not exhibit cytotoxic effects against various human cells that line the oral cavity or which are relevant to the periodontium[45]. Other drug-resistant oral bacteria, such as *S. aureus* can also be actively inhibited by the application of bioengineered and other forms of nisin produced by *L. lactis*.[46]

2.4 | Growth modulators and prebiotics to enhance antibacterial activities

The antibacterial activities of lactobacilli and streptococci are modulated by several factors, including growth media conditions. *L. salivarius*, *L. plantarum*, *Lactobacillus rhamnosus*, *L. fermentum*, and *S. salivarius* express better antimicrobial activity when cultured on media with additional glucose (1%-2%).[26] In contrast, anaerobic bacteria, including *P. intermedia*, *P. gingivalis*, and *F. nucleatum*, are inhibited in the presence of high glucose, compared with standard low-glucose–containing media (0.2%).[32] In addition, a nutritionally rich environment and use of prebiotics (compounds found in food that promote the growth and activity of beneficial microorganisms) promote antimicrobial activities of probiotics. This has been elucidated by studying probiotic strains co-cultured with pathogenic bacteria using different prebiotics, growth media, and conditions.[47,48] The probiotic strains tested included *Streptococcus gordonii* ATCC 49818, *Streptococcus mitis* ATCC 49456, *Streptococcus oralis*, *S. salivarius* TOVE-R, and *Streptococcus sanguinis* LMG 14657. The pathogenic bacteria tested included *A. actinomycetemcomitans* ATCC 43718, *F. nucleatum* ATCC 10953, *P. gingivalis* ATCC 33277, *P. intermedia* ATCC 25611, and *Streptococcus mutans* ATCC 25175. The results from these studies showed that treating biofilms with different prebiotics altered the proportion of beneficial bacterial species as well as that of pathogenic bacteria. Thus, prebiotics, specifically *N*-acetyl-D-mannosamine, showed a significant beneficial effect in shifting the bacterial communities toward a more beneficial composition while

TABLE 1 Summary of probiotic effects from in vitro studies

Author	Type of study	Oral biofilm model	Type of prebiotic/ probiotic	Groups (n)
2005				
Köll-Klais et al[26]	Screening of oral lactobacilli in chronic periodontitis and healthy patients and antimicrobial activity against *Porphyromonas gingivalis*, *Prevotella intermedia*, *Streptococcus mutans*, and *Aggregatibacter actinomycetemcomitans*	*P. gingivalis*, *P. intermedia*, *S. mutans*, and *A. actinomycetemcomitans* grow in suspension	Several lactobacilli strains	Two groups: healthy (n = 15) and chronic periodontitis (n = 20)
2010				
Zhu et al[29]	Screening of bacteria isolated from yogurt for antimicrobial activity against pathogens	*P. gingivalis*, *Fusobacterium nucleatum*, *A. actinomycetemcomitans*, *P. intermedia*, *Prevotella nigrescens*, *Peptostreptococcus anaerobius*, *Porphyromonas circumdentaria*, *Bacteroides fragilis*, and *Streptococcus sanguinis* grown on agar plates	Aliquots of yogurt containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* strains	—
2012				
Zhao *et al*[53]	Evaluation of the effect of *L. acidophilus* on secretion of interleukins by gingival epithelial cells exposed to *P. gingivalis*	Gingival epithelial cells + *P. gingivalis*	*L. acidophilus*	Four groups: control (gingival cells); gingival cells + *P. gingivalis*; gingival cells + *L. acidophilus*; gingival cells + *P. gingivalis* + *L. acidophilus*
Bosch et al[85]	Screening of acid lactic bacterial strains from healthy children (up to 10 y old) for probiotic candidates	*P. gingivalis*, *F. nucleatum*, *Treponema denticola*, *Prevotella denticola* and *S. mutans* grown on agar plates and tested for response to probiotics	Lactic acid bacteria	—
Chen et al[86]	Inhibition of periodontal pathogens by both *L. fermentum* and *Lactobacillus salivarius* and by their conditioned broth	*S. mutans*, *S. sanguinis*, and *P. gingivalis* grown on agar plates	*L. fermentum* and *L. salivarius*	—
2013				
Saha et al[87]	Development and characterization of carboxymethyl cellulose oral thin films for probiotic delivery	—	*L. fermentum*	—

Test method	Probiotics effects		
	Species composition	Antimicrobial activity	Immunology
Collection of saliva and subgingival samples, plated onto Man–Rogosa–Sharpe agar plates and incubated for 72 h at 37°C and 10% carbon dioxide. The isolated lactobacilli colonies were further analyzed by 16S ribosomal ribonucleic acid (RNA) gene sequencing	*Lactobacillus gasseri* and *Lactobacillus fermentum* were most prevalent lactobacilli identified in healthy patients, whereas *Lactobacillus plantarum* was the most prevalent strain in periodontal disease patients. Healthy patients exhibited higher counts of homofermentative strains than periodontitis patients did	The majority of probiotic strains significantly suppressed *P. gingivalis*, *P. intermedia*, *S. mutans*, and *A. actinomycetemcomitans* growth	—
Antimicrobial effects based on inhibition zone diameter (in millimeters) on agar plates	—	Aliquots of fresh yogurt significantly inhibited all the periodontal pathogens, whereas heat-treated yogurt inhibited all the periodontal pathogens except *F. nucleatum* and *P. gingivalis* Fresh yogurt but not heat-treated yogurt also inhibited the commensal bacteria *S. sanguinis* Probiotic antimicrobial activity varied depending on the sequence of inoculation (probiotics inoculated first, co-inoculated, or last)	—
RNA and protein levels for interleukin (IL)-1β, IL-6, and IL-8 at 2, 6, and 24 h after bacterial inoculation measured by reverse transcription polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay	—	—	Probiotic significantly decreased IL-1β, IL-6, and IL-8 levels in a dose-dependent manner at all time points
Antimicrobial effects based on inhibition zone diameter (in millimeters) on agar plates	—	From 46 lactic acid bacteria initially screened, 32 inhibited *P. denticola*, 29 inhibited *S. mutans*, 24 inhibited *F. nucleatum*, 24 inhibited *T. denticola*, and only seven inhibited *P. gingivalis* From 46 lactic acid bacteria, none were able to inhibit all five pathogenic strains, and 11 strains were able to inhibit four out of the five pathogenic strains	—
Antimicrobial effects based on inhibition zone diameter (in millimeters) on agar plates and the broth was evaluated by counting the number of bacterial colonies on agar plates after broth treatment	—	Both bacterial strains and their broth exhibited significant inhibition of periodontal pathogens; however, the broth presented a lower inhibition effect than the bacterial strains did	—
—	—	*L. fermentum* remained viable in the oral thin film for 142 d and its antioxidant activity was maintained for 150 d, peaking at 48 h	—

(Continues)

TABLE 1 (Continued)

Author	Type of study	Oral biofilm model	Type of prebiotic/probiotic	Groups (n)
2015				
Terai et al[26]	Screening of acid-producing bacterial strains isolated from healthy patients for probiotic candidates	*P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, *S. mutans*, and *Streptococcus sobrinus* grown on agar plates	14 lactobacilli and 36 streptococci strains isolated from 32 healthy patients	—
2016				
Amižic et al[88]	Screening of probiotic toothpaste and mouthrinse for antimicrobial effects	*Candida albicans*, *C tropicalis*, *E faecalis*, *S. salivarius*, and *S aureus* grown on agar plates	*L paracasei* or *L acidophillus*	12 groups: saline; control toothpaste (no probiotic); *L paracasei* toothpaste; *L acidophilus* toothpaste; mouthrinse 1; mouthrinse 2; and the combinations of the three toothpastes with the two mouthrinses
2018				
Slomka et al[47]	Screening of prebiotic substrates to modulate pathogens/beneficial bacteria in oral biofilm	Multispecies biofilm containing *A. actinomycetemcomitans*, *F. nucleatum*, *P. gingivalis*, *P. intermedia*, *S. mutans*, *S. sobrinus* as representative pathogens and *Actinomyces naeslundii*, *Streptococcus gordonii*, *Actinomyces viscosus*, *S. mitis*, *Streptococcus oralis*, *S. salivarius*, *S. sanguinis*, and *Veillonella parvula* as beneficial bacterial species	β-Methyl-D-galactoside; *N*-acetyl-D-mannosamine; L-aspartic acid; succinic acid; lactitol, turanose, methionine-proline, phenylalanine-glutamic acid	—
Yamada et al[89]	Effect of probiotic metabolite on *P. gingivalis* infection	*P. gingivalis* infection of gingival epithelial cells (Epi-4); *P. gingivalis* inoculation in mouse model	Metabolite generated by probiotic bacteria (10-hydroxy-*cis*-12-octadecenoic acid [HYA])	Four groups: control, *P. gingivalis*, HYA, *P. gingivalis* + HYA
Zupancic *et al*[23]	Probiotic incorporation into chitosan-poly(ethylene-oxide) nanofibers	*A. actinomycetemcomitans* grown on agar plates	*Bacillus* sp isolated from a healthy patient	Two groups: *A. actinomycetemcomitans*; *A. actinomycetemcomitans* + nanofibers
2019				
Cornacchione et al[30]	Identifying the different strains of *L. delbrueckii*, that inhibit and do not inhibit the growth of *P. gingivalis*	*P. gingivalis* grown on agar plates	*L. delbrueckii* isolated from commercial yogurt products	—

Test method	Probiotics effects		
	Species composition	Antimicrobial activity	Immunology
16S ribosomal DNA sequencing was used for bacterial identification; antimicrobial effects based on inhibition zone diameter (in millimeters) on agar plates; cariogenic potential was measured by demineralization of bovine tooth enamel; experimental endocarditis infection was assessed in rats by in vivo heart infection with the pathogenic bacteria	—	Isolated *Lactobacillus crispatus* YIT 12319, *L. fermentum* YIT 12320, *L. gasseri* YIT 12321, and *Streptococcus mitis* YIT 12322 showed no cariogenic potential	Isolated *L. crispatus* YIT 12319, *L. fermentum* YIT 12320, *L. gasseri* YIT 12321, and *S. mitis* YIT 12322 showed low endocarditis potential
Antimicrobial effects based on inhibition zone diameter (in millimeters) on agar plates	—	Probiotic toothpaste significantly inhibited *C. albicans* and *S. salivarius* compared to the toothpaste without probiotics and all the mouthrinses With some exceptions, the combinations of probiotic toothpaste and mouth rinses were not significantly different from probiotic toothpaste alone	-
Quantitative PCR used to measure bacterial levels at 3, 5, and 10 d	—	*N*-Acetyl-D-mannosamine, succinic acid, and methionine-proline prebiotics reduced pathogenic bacteria to <5% in the biofilm; *N*-acetyl-D-mannosamine resulted in a dose-dependent increase in the beneficial bacteria, reducing the pathogenic bacteria to 3% at the highest concentrations	—
Barrier disruption was measured by quantification of desmosomes visualized by transmission electron microscopy	—	HYA significantly inhibited gingival epithelial barrier disruption induced by *P. gingivalis*	HYA significantly suppressed *P. gingivalis* E-cadherin degradation and proinflammatory cytokines IL-1β, tmor necrosis factor-α, and IL-6 in mouse gingival tissue
Antimicrobial effects based on inhibition zone diameter (in millimeters) on agar plates	—	Probiotic significantly inhibited pathogen growth	—
Inhibitory effects based on the inhibitory zone diameter (in millimeters) on agar plates	—	The STYM1 strain of *L. delbrueckii* inhibited the growth of *P. gingivalis* by producing inhibitory amounts of hydrogen peroxide	—

TABLE 2 Summary of probiotic effects in animal studies

Author	Type of study	Defect site/size	Type of probiotic	Animal model (n)	Groups
2008					
Nackaerts et al[63]	Split-mouth double-blind randomized trial	Moderate periodontitis; bilaterally in the lower jaw	*Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus mitis*	Dog (n = 8)	Two groups with different treatments
2013					
Messora et al[61]	Randomized preclinical trial	Ligature in same position for 14 d on the mandibular right first molar (experimental periodontitis, EP)	*Bacillus subtilis*	Rat (n = 32)	Four groups: control, EP, probiotics, EP + probiotics
2014					
Foureaux et al[60]	Randomized 2 × 2 × 2 factorial scheme	Jaws removed, right hemimandibles used for histology/inflammatory cell count, left side used for protein assay by western blot	*B. subtilis*	Rat (n = 64)	Eight groups: control, stress, probiotics, EP, EP + probiotics, stress + probiotics, stress + EP, stress + probiotics+EP
Khasenbekova et al[90]	—	Low-protein diet for 3 mo, then moderate to severe chronical periodontitis was evaluated	*Lactobacillus casei* subsp *pseudoplantarum*; *L. casei* subsp *casei*; *Lactobacillus fermentum*; *Lactobacillus helveticus*; oral mucosal inoculation	Rat (n = N/A)	Four groups: control, EP, EP + probiotics, EP + Solcoseryl
Maekawa and Hajishengallis[59]	Randomized preclinical trial	Second molar; distal of first molar and mesial of third molar also included	*Lactobacillus brevis*	Mouse (n = 18)	Two groups: placebo vs *L. brevis* patch
2016					
Garcia et al[62]	Randomized trial, 2 × 2 design	Ligature around first molar	*Saccharomyces cerevisiae*; local irrigation	Rat (n = 72)	Four groups: control (n = 18), SRP (n = 18), probiotic (n = 18), SRP + probiotic (n = 18)
Messora et al[64]	Experiment 1 (E1): effects of probiotics on EP Experiment 2 (E2): effects of probiotics + SRP on treatment of EP	Ligature around mandibular first molar	*B. subtilis*; *Bacillus licheniformis* added to drinking water	Rat (n = 24)	E1, 4 groups: control, probiotics, EP and EP + probiotics E2, 4 groups: control, probiotics, EP-SRP, and EP-SRP + probiotics

Test method	Probiotics effects		
	Alveolar bone	Microbiology	Immunology
Radiography	Scaling and root planing (SRP) followed by local irrigation with probiotics significantly decreased bone loss and increased bone density	—	—
Histomorphometric analysis	Probiotics-only significantly reduced bone loss	—	—
Histomorphometric analysis; western blotting; enzyme-linked immunosorbent assay	Significantly reduced bone loss on unstressed rats Probiotics did not prevent bone loss on stressed rats	—	Significantly reduced levels of serum C-terminal telopeptide and increased levels of osteoprotegerin (OPG) on unstressed rats; significantly reduced the number of inflammatory cells in periodontal tissues in stressed rats
Hematological, biochemical, and morphological analyses	—	Complete clearance of periodontal pockets	Significantly reduced neutrophils and eosinophils; white blood cells returned to normal levels (no periodontitis); no significant changes on biochemical indicators
Bone measured by histological sections; dilutions of ligature biofilms plated on agar plates	Significantly reduced bone loss	Decreased the number of anaerobic bacteria and increased the number of aerobic bacteria	Significantly reduced tumor necrosis factor (TNF), interleukin (IL)-1β, IL-6, and IL-17A levels; anti-inflammatory effects heavily dependent on presence of arginine-deiminase
Histological analysis and immunolabeling	Significantly reduced bone loss at day 15 after SRP followed by local irrigation with probiotics	—	Probiotic-only treatment significantly reduced IL-1β at days 7, 15, and 30 and significantly reduced TNF-α by day 30 SRP followed by local irrigation with probiotics significantly reduced IL-1β at day 15 and significantly reduced TNF-α at days 15 and 30 Both treatments significantly reduced tartrate-resistant acid phosphatase (TRAP)–positive osteoclast cells at day 30 but increased IL-10 levels after 7 d
E1: histomorphometric, microcomputed tomography, immunological and hematological assays to evaluate periodontitis E2: immunohistochemical analysis	Probiotics-only and SRP followed by local irrigation with probiotics both significantly reduced bone loss	—	Probiotics-only significantly reduced eosinophils; reduced receptor activator of nuclear factor-kappa B ligand/OPG ratio to normal levels (no periodontitis) SRP followed by local irrigation with probiotics significantly reduced the number TRAP-positive osteoclast cells and IL-1β levels.

(Continues)

TABLE 2 (Continued)

Author	Type of study	Defect site/size	Type of probiotic	Animal model (n)	Groups
2017					
Oliveira et al[58]	Randomized preclinical trial	Ligature around mandibular first molar	*Bifidobacterium animalis* subsp *lactis*	Rat (n = 32)	Four groups: control, EP, probiotics, and EP + probiotics
Ricoldi et al[58]	Randomized preclinical trial	Ligature around mandibular first molar at baseline	*Bifidobacterium lactis*	Rat (n = 32)	Four groups: control, probiotics, EP-SRP, and EP-SRP + probiotics
2018					
Gatej et al[59]	Randomized preclinical trial	*Porphyromonas gingivalis* and *Fusobacterium nucleatum* swabbed onto molars with micro brush or via gavage	*Lactobacillus rhamnosus* GG	Mouse (n = 36)	Six groups: control, EP, gavage probiotics, gavage probiotics before EP, oral probiotics, oral probiotics before EP

reducing the number of pathogens.[48] The rationale for choosing this compound was based on its close relationship to metabolic enzymes derived from salivary mucin: *N*-acetylneuramine and *N*-acetylmannosamine. These enzymes are digested by and promote the growth of several *Streptococcus* strains, including *S. mitis*, *S. oralis*, *S. sanguinis*, and *S. gordonii*. Further, most probiotic strains are lactic acid bacteria, meaning that they produce lactic acid as the major metabolic end product of carbohydrate fermentation, and this acidic environment inhibits the growth of pathogenic bacteria. This acidification and the production of proteinaceous bacteriocins by several lactic acid bacteria support their growth and inhibit that of pathogenic microorganisms.

2.5 | Beneficial colonizing properties

The processing time of probiotics in the oral cavity is shorter than in other areas of the digestive tract.[49] Therefore, it is important that oral probiotics show good colonizing potential, including the ability to adhere to oral epithelial cells. Unlike the intestine, oral epithelial tissue is comprised of a thin mucin-covered mucosa, which allows microbes to attach directly to the oral epithelium.[49] Pili from some streptococcal strains, including *S. sanguinis* and *S. salivarius*, help the bacteria adhere to mucin, a major component of saliva, thus increasing the colonizing properties of these probiotic strains.[25] Within minutes after being consumed, several streptococcal strains, such as *S. sanguinis*, *S. gordonii*, *S. oralis*, and *S. mitis*, attach quickly to the pellicle on the tooth surface by connecting with the colonizing receptors on the pellicle.[25,50] However, compared with streptococci, lactobacilli generally have lower adherence ability when it comes to salivary-coated hydroxyapatite (human tooth simulation assay).[25] The surface of many lactobacilli exhibit traits that promote colonization, including high hydrophobicity, auto-aggregation, and high adhesive capacity to intestinal epithelial cell lines.[51] In the oral environment, lactobacilli and bifidobacteria compete with the pathogen *P. gingivalis* for adhesion to epithelial cells. These probiotics alter the interaction of epithelial cells with *P. gingivalis* by modulating the pathogen's ability to adhere to and invade these cells.[51]

2.6 | Immune modulation

The antimicrobial activity of probiotics also involves altering host-microbial signaling and the subsequent host immune response. Several studies found that lactobacilli modulate the inflammatory response to periodontal pathogens.[52,53] Specifically, when gingival epithelial cells were infected with *P. gingivalis* W83 and ATCC 33277 then treated with *Lactobacillus* and *Bifidobacterium* strains, different host immunological responses were noted.[52] While the levels of inflammatory cytokines, such as interleukin (IL)-1β,[52,53] IL-6,[53] and tumor necrosis factor-α,[52] increased in the presence of *P. gingivalis*, their levels were reduced following treatment with lactobacilli.

Test method	Probiotics effects		
	Alveolar bone	Microbiology	Immunology
Microbiological histomorphometric, immunohistochemical and microcomputed tomography analyses	Significant increase in bone density and decreased bone loss	Increased levels of *Actinomyces* and streptococci-like species while decreasing levels of *Veillonella parvula*, *Capnocytophaga sputigena*, *Eikenella corrodens*, and *Prevotella intermedia*-like species	Significant increase in expressions of OPG and β-defensins and significant decrease in IL-1β and nuclear factor-kappa B
Histomorphometric, microcomputed tomography and immunohistochemical analyses; microbiological effects of the probiotics on biofilms	SRP followed by local irrigation with probiotics significantly reduced bone resorption and attachment loss	Significant higher ratio between aerobic and anaerobic bacteria	Significant fewer TRAP-positive osteoclasts cells; significant increase in anti-inflammatory cytokines, and significant decrease in proinflammatory cytokines
The antimicrobial activity of probiotics; alveolar bone levels and gingival tissue changes were assessed using in vivo microcomputed tomography and histological analysis; serum levels of mouse homologues for IL-8 were measured using multiplex assays	Probiotic pretreatment (either via oral gavage or via oral inoculation) significantly reduced bone loss	*L. rhamnosus* GG showed no antimicrobial activity against *P. gingivalis* and *F. nucleatum*	Probiotics pretreatment via oral gavage significantly decreased the number of TRAP-positive osteoclasts cells

Although evidence regarding the change in levels of IL-1β, IL-6, and tumor necrosis factor-α following treatment with probiotics is generally consistent, data regarding the levels of IL-8 expression in a similar context are still controversial. Some studies reported an increased expression of IL-8 in gingival epithelial cells, which in turn attracts leukocytes and activates neutrophils, thereby mediating an inflammatory response to *P. gingivalis*.[54] However, in the presence of *Lactobacillus*, this increased expression was inhibited with increasing concentrations of the probiotic; showing Lactobacillus' ability to reduce the inflammatory response to the pathogen, *P. gingivalis*.[53] In contrast, other studies found that the IL-8 protein might be degraded by *P gingivalis* protease genes, especially in a mixed bacterial environment like the oral cavity, where there are multiple pathogens.[55] At the same time, lactobacilli can activate CXCL8, an IL-8–encoding gene, which increases the expression of IL-8 and counteracts its degradation by *P. gingivalis*, thus promoting an anti-inflammatory response against this pathogen.[52]

2.7 | Preclinical Studies

Whereas in vitro studies analyzed the molecular contributions of probiotics to periodontal-related conditions, preclinical studies focused on the effects of specific types of bacteria on experimental periodontal diseases, including gingivitis and periodontitis (Table 2). Most preclinical studies examined the antibacterial effects of lactobacilli, bifidobacteria, and streptococci.[56-61] Other probiotic strains that have been examined included the yeast *Saccharomyces cerevisiae*.[62] Most studies evaluated alveolar bone changes following probiotic treatment.[56,59,61,62] However, there has also been increasing interest in the immunomodulatory effects mediated by probiotics.[57-60,62] Most studies were performed using murine models,[56-62] which lend themselves to the study of both alveolar bone changes and immunomodulatory effects, although one study utilized the canine model to evaluate radiographic changes in alveolar bone levels.[63] In these animal models, periodontitis was often induced by placing ligatures around the first molar teeth[59,61] to mediate alveolar bone loss.

2.8 | Alveolar bone changes and other clinical outcomes

Several studies have shown that probiotic treatment generally prevents alveolar bone loss. Reduced alveolar bone loss was observed in ligature-induced periodontal sites that received the probiotic *Bacillus subtilis* treatment compared with those without.[61,62] In addition, changes to attachment loss and gut morphology have also been demonstrated through studies investigating probiotic administration. Specifically, probiotic supplementation with *B. subtilis* (CH201) not only reduced bone loss but also attachment loss, and it protected the small intestine from reactive changes induced by ligature-induced periodontitis.[61] Lactobacilli also significantly inhibited

inflammatory periodontal bone loss.[56,59] Treatment with *S. cerevisiae* as a monotherapy or in combination with scaling and root planing in rats was also effective in controlling periodontitis (alveolar bone loss and fewer tartrate-resistant acid phosphatase–positive cells) in rats.[62] Bone loss measurements were generally obtained using clinical microscopes, microcomputed tomography, and/or histomorphometric analyses. In a study using *B. subtilis* in a rat model of periodontitis, C terminal peptide levels were evaluated with and without probiotic therapy.[60] C terminal peptide, a marker of bone resorption, decreased in the presence of the probiotic, thus leading to decreased osteoclastic activity and bone loss.[60]

Studies examining the effects of probiotics in reducing alveolar bone loss and attachment loss or improving other clinical parameters suggest that the mechanisms of action are due to a combination of both local and systemic effects, including modulation of the local and systemic host immune response, antibacterial effects via different mechanisms, and stimulation of osteoblastic function (reviewed by Chatterjee et al.[65]).

2.9 | Microbiological changes

A main goal of using lactic acid bacteria to address periodontitis is to shift the oral microbiota so that there is a reduction in the number of anaerobic bacteria, which are strongly associated with the disease. When *L. brevis* or *B. lactis* were applied in a murine model of periodontitis, there was a significant decrease in the counts of anaerobic bacteria, leading to decreased inflammation.[59] Another major goal of using probiotics in the treatment of periodontitis is to promote the growth of commensal or beneficial bacteria, including species such as lactobacilli and streptococci, which trigger anti-inflammatory effects and promote a beneficial host response.[66] In humans, these beneficial oral commensal bacteria are largely known; however, the similarities and differences between the human and murine oral cavity remain unclear. Also, the long-term changes in the oral microbial composition mediated by probiotics are not well studied. Therefore, identification of beneficial probiotic bacteria for murine models and detailed analysis of shifts in microbial composition with probiotics warrant further investigation.

2.10 | Immunological effects

As previously discussed, probiotic therapy reduces alveolar bone loss in murine models with ligature-induced periodontitis. Immunological mechanisms may contribute to this, primarily through reduction of proinflammatory cytokines, macrophage and natural killer cell activation, and stimulated production of white blood cells and interferon.[67-69]

The probiotic *B. subtilis* is thought to mediate immunomodulation of both local and systemic effects in rats with induced periodontitis. Locally, probiotics modulate the innate immune response by mimicking periodontal pathogens in their interactions with toll-like receptors on dendritic cells, which stimulate a T-helper cell response. Systemically, *B. subtilis* works on the intestinal ecosystem—a primary part of systemic disease regulation. Rats treated with *B. subtilis* showed higher values of villous height and crypt depth in jejunum samples, indicating changes and improvements in host mucosal barrier function that led to diminished immunological reactivity.[61,70,71] Probiotics also led to increased production of intestinal anti-inflammatory cytokines (IL-10, transforming growth factor beta 1) while reducing the production of proinflammatory cytokines (tumor necrosis factor-α, interferon-γ, IL-8).[61,70,71]

The effects of probiotics on the host response are manifold. Compared with placebo, *L. brevis* (another probiotic studied in the context of periodontal disease in rats) contributed significantly to decreased bone loss and lower expression of tumor necrosis factor-α, IL-1β, IL-6, and IL-7.[59] This is in part explained by the actions of arginine deiminase, which is released by *L. brevis* in vivo. Arginine deiminase can inhibit nitric oxide production.[72] This is important, because previous studies in animal models suggest that nitric oxide plays a role in periodontal inflammation and bone loss.[73-76] High levels of nitric oxide could potentially facilitate the infiltration of inflammatory cells into periodontal tissues by inducing pathologic vascular permeability.[59] The immunological changes mediated by probiotics in treating periodontal diseases seem to occur not only in the periodontal tissue but also in other areas, including the intestinal lining and via changes in the intestinal microbiome.[77] Mice with ligature-induced alveolar bone loss showed intestinal changes along with improvement in alveolar bone outcomes in the oral cavity when treated with *L. rhamnosus*. The increased level in IL-6 protein expression caused by *P. gingivalis* and *F. nucleatum* was reduced after treatment with *L. rhamnosus*, especially in the ileum lining. IL-6 is an important mediator of bone resorption in periodontitis.[78] IL-6 is also a potential new target for gastroinstestinal inflammation therapy.[79] Changes in IL-6 mediated by treatment with *L. rhamnosus* supports the multiple effects of probiotics in modulating multiple inflammatory conditions occurring in a living body.

A randomized trial in rats using the probiotic *S. cerevisiae* showed decreased immunostaining for tumor necrosis factor-α in both the probiotic alone group and probiotic in conjunction with scaling and root planning group compared to controls.[62] In addition, in corroboration with previous studies, there was increased anti-inflammatory cytokine IL-10 levels and reduced levels of proinflammatory cytokines (tumor necrosis factor-α and IL-1β) in the probiotic groups.[61,62] Further, beta-glucan from *S. cerevisiae* induces the expression of immunoregulatory cytokines (IL-10, transforming growth factor beta 1, and IL-2) in bone marrow-derived dendritic cells.[80] Beta glucan also enhances wound healing by stimulating collagen synthesis.[81] Beta glucan works through enhancing phagocytosis and proliferation of professional phagocytes, including macrophages and dendritic cells.[82]

B. lactis, another probiotic candidate for periodontal disease, has shown an effect on immune control of bone remodeling through receptor activator of nuclear factor kappa B ligand protein expression.[58] Rats with experimental periodontal disease that received

probiotic treatment presented with levels of receptor activator of nuclear factor kappa B ligand and a receptor activator of nuclear factor kappa B ligand/osteoprotegerin ratio that was significantly reduced compared with those that did not receive probiotics.[58] Increased receptor activator of nuclear factor kappa B ligand ratios favor bone resorption via osteoclastogenesis and osteoclast activation.[83] Another study investigating *B. lactis* effects in rats found that groups treated with probiotics showed significantly fewer osteoclasts, increased expression of anti-inflammatory cytokines and reduced expression of proinflammatory cytokines compared to controls.[57]

Lactic acid bacteria, such as lactobacilli, also exhibit other immunomodulatory effects. A recent study showed that lactobacilli were used as a vehicle to deliver bacterial antigens that induce a protective immune response.[28,84] Specifically, *L. acidophilus* expressing FomA, an outer membrane antigen from *F. nucleatum*, increased immunity against *P. gingivalis* and *F. nucleatum* in the form of serum immunoglobulin G and salivary immunoglobulin A antibodies and an increased IL-1β inflammatory cytokine response.[84] Further, when *P. gingivalis* and *F. nucleatum* were co-cultured with KB (HeLa) epithelial cells, the number of bacteria that adhered to the cells in the presence of mouse serum positive for FomA was lower than that in the presence of negative serum.

3 | CONCLUSIONS

In summary, probiotics examined using in vitro and in vivo/preclinical models show promise for applications in a periodontal disease setting. In vitro models reveal that probiotics exhibit beneficial antibacterial and antibiofilm effects that are often mediated by bacteriocin production, permissive growth profiles under certain conditions that promote their preferential growth over pathogenic microbes, enhanced colonization and adherence potential, and beneficial modulation of host immune responses. Probiotics can also improve preclinical (alveolar bone loss and attachment loss), microbiological, and immunological outcomes in the treatment of experimental periodontitis in animal models. However, the effects of probiotics on long-term changes in microbiome composition merit further investigation.

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CONFLICT OF INTEREST

The authors report no conflicts of interest related to this study.

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