Concise Review

Probiotic Bifidobacteria in Managing Periodontal Disease: A Systematic Review

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ABSTRACT

Although various probiotic organisms have been evaluated for their utility in the management of periodontitis, their strain-specific mechanisms of action are still unclear. We aimed to systematically review the effect of bifidobacterial probiotics on periodontopathogens and host immune responses in periodontal diseases. An electronic search of articles published until June 2022 in Medline, PubMed, Web of Science, and Cochrane Library databases was performed. Randomised controlled trials (RCTs) and in vitro and animal studies were assessed, and the data regarding antimicrobial properties, immunomodulation, and clinical outcomes were analysed. A total of 304 studies were screened, but only 3 RCTs and 6 animal and in vitro studies met the inclusion criteria. The use of different strains of bifidobacteria led to (1) a reduction of key players of the red complex periodontopathogens; (2) reduced levels of pro-inflammatory cytokines (eg, interleukin [IL]1-β and IL-8) and higher levels of anti-inflammatory cytokines (IL-10); (3) enhanced levels of osteoprotegerin and reduced levels of receptor activator of nuclear factor kappa-B ligand; and (4) a reduction of the dental plaque, bleeding on probing, alveolar bone loss, and clinical attachment loss. Bifidobacterial probiotic adjuvant supplementation, especially with *Bifidobacterium animalis* subspecies *lactis*, appears to help improve clinical periodontal parameters and develop a healthy plaque microbiome through microbiological and immunomodulatory pathways. Further human and animal studies are warranted prior to the therapeutic use of bifidobacteria in the routine management of periodontal infections.

Introduction

Several oral and systemic diseases, including periodontal diseases, are associated with dysbiosis of the oral microbiome.[1,2] Therefore, the current mainstay therapeutic approach for periodontal disease is to shift a dysbiotic biofilm of the foregoing periodontopathogens to a more health-associated eubiotic entity through a combination of improved oral hygiene measures and subgingival debridement.[3,4] This shift is almost always rather transient due to the dynamics of the recolonisation process.[4-6] Similarly, therapeutic antibiotics or antiseptics used for a short period have little impact on the long-term effectiveness of periodontal therapy.[4,6]

Different probiotic bacteria have been investigated as adjunct treatment modalities in the management of periodontal diseases.[7] It has been hypothesised that sufficient numbers of healthy probiotic bacteria could counter the injurious toxic effects of periodontopathogens and restore the diseased site to health.[8] Probiotics are known to restore health not only by suppressing periodontopathogens but also by modulating immunologic responses, epithelial permeability, bacterial translocation, and the provision of regulatory metabolites.[9] In this context, several probiotics such as

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Lactobacillus and *Bifodobacterium* genera have been investigated for their utility as useful adjuncts in the management of periodontal diseases.[7,10]

Members of the genus *Bifidobacterium* (Gram-positive anaerobic bacteria) are initial colonisers of the human gastrointestinal tract and are known to confer positive health benefits on their host.[11] Previous workers have noted that whilst bifidobacterial probiotics demonstrate consistent beneficial effects,[12,13] *Lactobacillus* species had dubious and inconsistent clinical effects in ameliorating periodontitis.[14] However, none of the previous reviewers have discussed in detail the mechanisms of action of these probiotics and, in particular, how bifidobacterial species and their varying strains confer the observed salutary effect. Therefore, the main objective of this study was to systematically review the current literature on the strain-specific effects of bifidobacterial probiotic therapy in the management of periodontal diseases.

Materials and methods

Search strategy and data extraction

The electronic search used different databases to find randomised controlled trials (RCTs) and in vitro and animal studies published until June 2022. PICO (P = Population/Patient/Problem, I = Intervention, C = Comparison, O = Outcome) question: Do genus *Bifidobacterium* probiotics (I) compared to placebo/antibiotics/without probiotics (only scaling and root planing [SRP]) (C) result in modulation of periodontopathogens and host immune responses (O) in managing periodontal disease (P)?

A series of search terms was used to garner clinical evidence and in vitro and animal studies. Additional publications were identified in published review articles and reference lists of previously identified articles by handsearching. A detailed description of the search strategy, including inclusion and exclusion criteria, is provided in the Supplementary Material.

PRISMA criteria[15] were followed to establish a comprehensive and systematic procedure for data extraction (Figure 1).

Quality and the overall risk of bias assessment

The methodological quality of eligible clinical and in vitro studies was assessed using the Cochrane Collaboration risk of bias assessment instrument.[16] SYRCLE's risk of bias tool containing 10 entries[17] was used for animal studies (Table 1).

Results

A total of 9 studies[12,13,18-23] were deemed eligible for the current systematic review (3 each of RCTs,[12,13,18] animal studies,[21-23] and in vitro studies[12,19,20]; Figure 1).

The included RCTs encompassed a total of 122 patients with generalised chronic periodontitis. All 9 included studies described the probiotic effects of *Bifidobacterium* species, assessing their impact on gingival or periodontal health and disease. The characteristics and outcomes of the included studies using bifidobacterial probiotics, either alone or in conjunction with oral hygiene and/or SRP, are summarised in Tables 2, 3, and 4. A methodological quality assessment of studies is provided in the Supplementary Material.

Bifidobacterial probiotics and their effect on periodontopathogens

The probiotic bacteria used in the included in vitro studies were 3 reference laboratory strains of *Bifidobacterium longum* subspecies *longum*, *Bifidobacterium longum* subspecies *infantis*, *Bifidobacterium animalis* subspecies *lactis*, and 2 clinical isolates: *Bifidobacterium dentium* and a *B longum* (unknown subspecies). These were tested against 4 different anaerobes (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Actinomyces naeslundii*) and 2 facultative anaerobes (*Aggregatibacter actinomycetemcomitans*, *Streptococcus oralis*).

According to studies using the agar diffusion models, *B lactis* HN019 was able to inhibit the growth of main periodontopathogens ($P < .05$). However, no relative differences in the degree of growth inhibition of pathogens could be discerned.[12]

In another in vitro study,[20] *B animalis* subspecies *lactis*, *B longum*, and *B dentium* were shown to invade and integrate into polymicrobial biofilms of *P gingivalis*, *A naeslundii*, and *F nucleatum*, even though their antimicrobial effect was mainly limited to *P gingivalis* ($P < .05$). The results were strain-dependent as *B animalis* Bb12 was the only strain that significantly reduced the viability of *P gingivalis* in the biofilm at both 18- and 42-hour time points ($P < .05$), whereas the other tested strains had no such impact.

Another experiment using oral bifidobacteria from periodontally healthy individuals demonstrated their significant activity against *P gingivalis*.[24] However, the growth suppression was only observed when the bifidobacteria were precultured in the suspending medium prior to inoculating *P gingivalis*.[24] These results imply either a possible competitive inhibition of *P gingivalis* due to nutrient depletion or, alternatively, impedance of its growth due to the toxic metabolites of bifidobacteria.

The antimicrobial effect of *Bifidobacterium* species has also been evaluated using different combinations of probiotic bacteria. The combinatorial effect of *B infantis*, *B logum*, and *B lactis* on *P gingivalis*, *F nucleatum*, and *S oralis* biofilms, either singly or in combination, has been evaluated in vitro.[19] *B lactis* and *B infantis*, when singly inoculated, showed the largest and the swiftest antimicrobial effect against *F nucleatum*, whereas it took a longer period, 168 hours, to significantly inhibit the growth of *P gingivalis*. Similarly, a triple probiotic combination of *B longum*, *B lactis*, and *B infantis* inhibited the growth of *F nucleatum* after 24 hours, whereas significant inhibition of *P gingivalis* was noted only after 72 hours. Interestingly, none of the 3 bifidobacteria significantly impacted the growth of *S oralis*, a beneficial organism related to periodontal health.

In an RCT in humans, bifidobacteria were shown to modulate the composition of subgingival plaque derived from deep pockets.[13] Thus, Invernici et al[13] noted that periodontopathogens including *P gingivalis*, *T denticola*, *F nucleatum*, *Campylobacter showae*, and *Eubacterium nodatum* were significantly

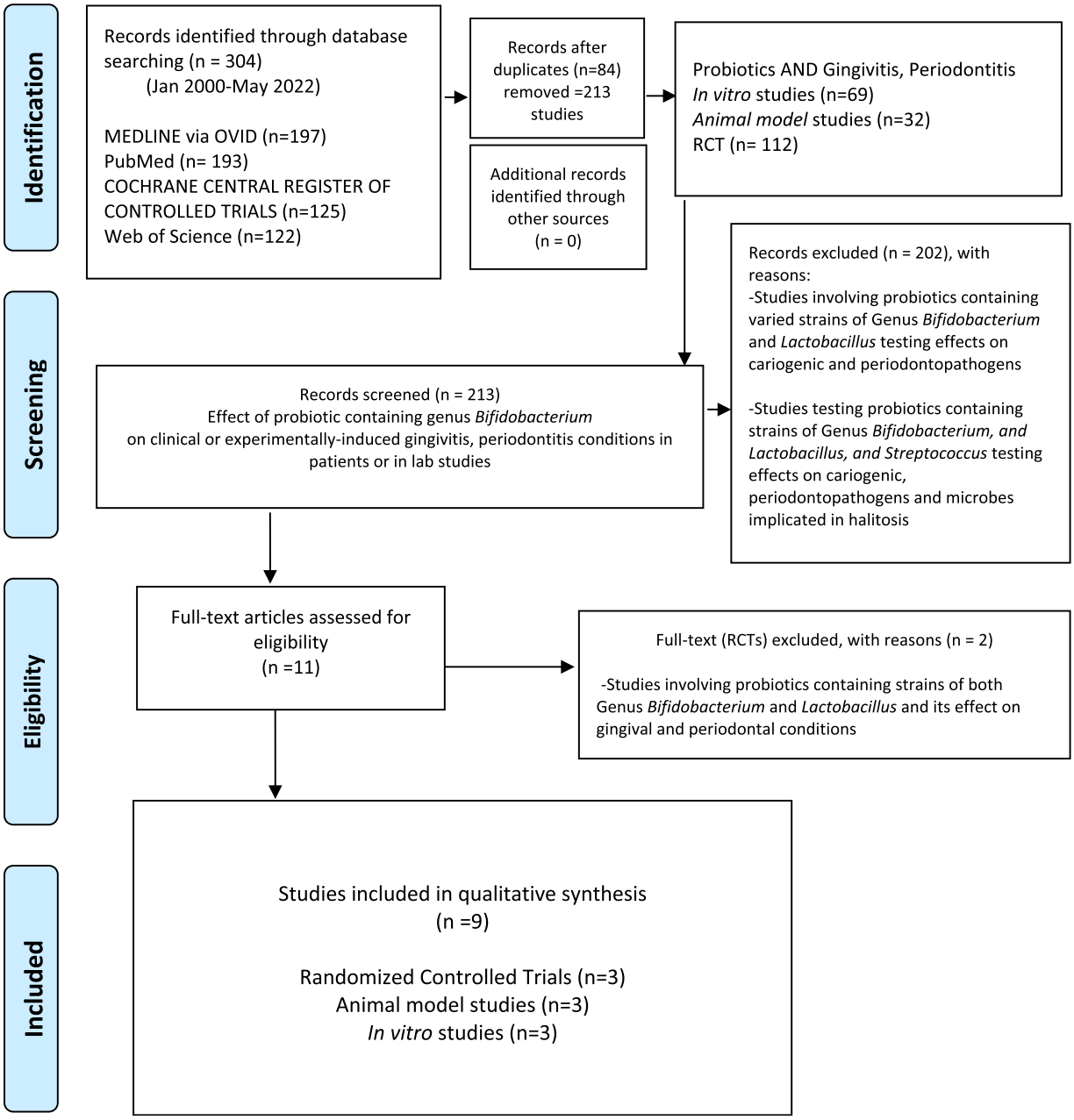

Fig – Flow diagram of the screening and selection process.

suppressed after 30 days of probiotic application, as opposed to subpopulations of *Actinomyces naeslundii* and *Streptococcus mitis associated with gingival health* ($P < .05$). This indicates that bifidobacteria favour the growth of microbiota associated with gingival/periodontal health, with simultaneous suppression of the proportion of periodontopathogens.

A similar beneficial effect of bifidobacterial probiotics has been reported in animal studies, which used experimental periodontitis models induced by ligatures in the mandibular first molars of rats.[22,23] The topical application of *B animalis* subspecies *lactis* HN019 in rats led to increased levels of health-associated *Actinomyces*- and *Streptococcus*-like species, whilst reducing the disease-associated *Capnocytophaga sputigena*, *Eikenella corrodens*, and *P intermedia* species.[22] In another animal study using the identical *B animalis* strain, a higher ratio of aerobic to anaerobic bacteria was found in the ligature-associated periodontitis in rats treated with the bifidobacterial probiotic ($P < .05$).[23]

Bifidobacterial probiotics and immune modulation

RCTs in humans have shown the immunomodulatory effects of bifidobacterial applications. When volunteers with experimental gingivitis were treated with *B animalis* subspecies *lactis* for 28 days,[18] lower levels of pro-inflammatory cytokine IL−1β were noted in their gingival crevicular fluid compared with control group. In a similar RCT,[13] the use of *B. lactis*-containing lozenges by patients with chronic periodontitis for

Table 1 – Risk of bias assessment using Cochrane Collaboration risk assessment tool for randomised controlled trials and in vitro studies and risk of bias assessment using SYRCLE's risk of bias tool for animal studies.

Clinical and in vitro study	Selection bias		Performance bias	Detection bias	Reporting bias	Confounding bias
	Baseline characteristics similarity/appropriate control selection	Allocation concealment / Randomisation	Blinding of researchers	Blinding of outcome assessors	Selective outcome reporting	Account for confounding variable
Randomised controlled trials						
Invernici et al (2020)[12]	+	? / +	?	?	?	+
Invernici et al (2018)[13]	+	? / +	?	?	?	–
Kuru et al (2017)[18]	+	? / +	–	?	?	–
In vitro studies						
Argandoña Valdez et al (2021)[19]	+	? / +	?	+	+	?
Invernici et al (2020)[12]	+	? / +	?	?	+	?
Jasberg et al (2016)[20]	+	? / +	?	?	+	?

Animal studies

Animal model study	**Selection bias**			**Performance bias**		**Detection bias**		**Attrition bias**	**Reporting bias**	**Other**
	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessment	Blinding	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Silva et al (2022)[21]	Y	Y	Y	Y	UC	Y	UC	Y	Y	Y
Oliveira et al (2017)[22]	Y	Y	Y	Y	UC	Y	UC	Y	Y	Y
Ricoldi et al (2017)[23]	Y	Y	Y	Y	UC	Y	UC	Y	Y	Y

Risk of bias legends: +, low risk; –, high risk; ?, unclear risk; Y, yes (low risk of bias); N, no (high risk of bias); UC, unclear (moderate risk of bias).

Table 2 – Characteristics of the included studies on genus *Bifidobacterium* probiotics used in chronic periodontitis cases in RCTs.

Study and country	No. of participants; *groups* sex (M/F); age (y)	Professional prophylaxis oral hygiene instructions	Tested probiotic *Bifidobacterium* strain(s)	Mode of delivery	Dosage (CFU); frequency and duration	Assessment time points	Measured outcome(s)
Invernici et al (2020)[12] Brazil RCT	N = 30 (GCP); *SRP + probiotic group* = 15 (5 M/10 F); *SRP + placebo (control)* = 15 (8 M/7 F); age (>30)	Supragingival plaque control and OHI 7 days prior to probiotic therapy	*Bifidobacterium animalis* subspecies *lactis (B lactis)* HN019	Lozenges	1×10^9; twice daily for 30 days	D0 (pre-intervention), D30, and D90 (postintervention)	Clinical: PI, BoP; immunologic: βD-3, TLR4, CD-57, and CD-4 expressions
Invernici et al (2018)[13] Brazil RCT	N = 41 (GCP); *SRP + probiotic group* = 20; *SRP + placebo (control)* = 21; age (>30)	Supragingival plaque control and OHI 7 days prior to probiotic therapy	*Bifidobacterium animalis* subspecies *lactis (B lactis)* HN019	Lozenges	1×10^9; twice daily for 30 days	D0 (pre-intervention), D30, and D90 (postintervention)	Clinical: PI, CAL, BoP, PPD, GR; immunologic: IL-8, IL-1β, and IL-10 levels
Kuru et al (2017)[18] Turkey RCT	N = 51 (HP); *probiotic group* = 26 (19 M/17F); *control group* = 25 (10 M/15F); age (16–26)	Supragingival plaque control and OHI 7 days prior to probiotic therapy (no brushing was advised between D28 and D33)	*Bifidobacterium animalis* subspecies *Lactis* DN-173,010	Yoghurt	1×10^8; once daily for 28 days	D0 (pre-intervention), D28, and D33 (postintervention)	Clinical: GI, PI, PPD, BoP, GCF (vol.); immunologic: IL-1β levels

BoP, bleeding on probing; CAL, clinical attachment loss; CD, cluster of differentiation; D, day; GCF, gingival crevicular fluid; GCP, generalised chronic periodontitis; GI, gingival index; GR, gingival recession; HP, periodontically healthy patient; IL, interleukin; OHI, oral health instructions; PI, plaque index; PPD, probing pocket depth; RCT, randomised controlled trial; SRP, scaling and root planing; TLR4, toll-like receptor 4.

30 days, before and after SRP (adjunct therapy), led to a favourable immunological outcome. Thus, the mean ratios of pro-inflammatory cytokines interleukin (IL)-1β or IL-8 in the gingival crevicular fluid (GCF) were lower in the probiotic group than in the control group. Furthermore, the test group had higher levels of the anti-inflammatory factor IL−10 after probiotic therapy.

Adjuvant therapy post-SRP has also been investigated in humans. Probiotic treatment with *B lactis* HN019 for 30 days significantly increased the expression of β-defensin-3 (antimicrobial peptide); toll-like receptor 4, which is involved with pathogen recognition and activation of innate immunity; and cluster of differentiation−4 (an important ligand for the function of immune cells) in gingival tissues of patients with generalised chronic periodontitis.[12]

Furthermore, in an animal study evaluating the effect of *B lactis* on nonsurgical treatment of periodontitis, the litters treated with probiotics showed increased expression of anti-inflammatory cytokines and reduced expression of pro-inflammatory cytokines compared to the control group.[23]

Recent molecular studies have shown that one possible mechanism underlying bone resorption associated with chronic periodontitis is osteoclastogenesis through increased receptor activator of nuclear factor kappa-B ligand (RANKL) production.[25] Interestingly, *B lactis* HN019 administration may regulate bone remodelling through RANKL protein expression pathway.[22] Rats with experimental periodontitis that received *B lactis* HN019 presented with higher levels of osteoprotegerin (OPG) and β-defensins (βDs) that assist bone formation, as opposed to lower levels of RANKL and IL-1β, involved in limiting bone growth.[22] In addition, *B lactis* HN019 was found to markedly reduce IL-1β levels and the ratio of RANKL/OPG in rats with metabolic syndrome and experimental periodontitis.[21] *B lactis* treatment also downregulated the expression of tumour necrosis factor-alpha (TNF-α) and IL-6 in rats with periodontitis.

Bifidobacterial probiotics and tooth-supporting tissues

RCTs reviewed here assessed a raft of periodontal health/disease associated indices: plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD), gingival recession (GR), and the quality of the GCF. However, only PI and BOP were evaluated in all 3 RCTs.

In 2 studies of patients with generalised chronic periodontitis, SRP was performed in both the test and control groups, and thereafter probiotics were administered twice daily via bifidobacteria-laced lozenges.[12,13] In another study, standardised bifidobacteria-laced yoghurt was provided to periodontally healthy patients.[18] The duration of probiotic therapies varied from 28 to 30 days, and pre- and postintervention assessments were performed in all clinical studies.

Kuru et al[18] induced experimental gingivitis in a cohort of healthy volunteers, and then volunteers consumed yoghurt containing *B animalis* subspecies *lactis*. At days 0, 28, and 33, GCF samples and PI, GI, PD, and BOP measurements were obtained from identical teeth and periodontal sites. No intragroup or intergroup differences in PI, GI, or BOP between the probiotic and control groups were noted during the allocated toothbrushing period. However, on day 33, when brushing

Table 3 – Characteristics of the included in vitro and animal model studies examining genus *Bifidobacterium* probiotics.

Study and country	Test strain(s)	Methodology	Assessment tool	Measured outcome
		In vitro studies		
Argandoña Valdez et al (2021)[19] Brazil	**PROB strains:** *Bifidobacterium longum* subspecies *longum* (ATCC 15,707), *Bifidobacterium longum* subspecies *infantis* (ATCC 15,697), and *Bifidobacterium animalis* subspecies *lactis* (ATCC 27,673) vs **periodontal pathogens:** *Fusobacterium nucleatum* subspecies *nucleatum* (ATCC 25,585), *Porphyromonas gingivalis* (33,277), and *Streptococcus oralis* were grown in brain heart infusion agar	Bacterial strains were incubated for 24 h, 72 h, and 168 h at 37 °C	Checkerboards DNA–DNA hybridisation	The absolute counts of each bacterium in combination were presented in the percentage of bacterial growth
Invernici et al (2020)[12] Brazil	**PROB strains:** *B lactis* HN019 vs **periodontal pathogens:** *Prevotella intermedia* (ATCC 25,611), *Porphyromonas gingivalis* (W83), *Fusobacterium nucleatum* (ATCC 25,586), and *Aggregatibacter actinomycetemcomitans* (ATCC 33,393)	Bacterial strains were incubated in 15-mm wells in Tryptic soy agar at 37 °C for 72 h under anaerobic conditions	**Sensitivity analysis:** the mean (± SD) of the inhibition zones observed for various periodontopathogens' sensitivity to *B lactis* HN019	Diameter (mm) of inhibition halos
Jasberg et al (2016)[20] Finland	**PROB strains:** *Bifidobacterium animalis* subspecies *lactis Bb12*, oral *B dentium*, and *B longum* isolates vs **periodontal pathogens:** *Porphyromonas gingivalis*, *Actinomyces naeslundii*, and *Fusobacterium nucleatum*	In vitro biofilms assays and agar-overlay interference assays	The samples were serially diluted and were cultured on the agar plates in an anaerobic atmosphere	The mean values with SD of either log10 (CFU+1) or the pH of the biofilm medium at the end were evaluated
		Animal model studies		
Silva et al (2022)[21] Brazil	**PROB strains:** *Bifidobacterium animalis* subspecies *lactis* HN019	PROB administration started on the 8th week of the study PE was induced on the 14th week by placing ligature on the animals' lower first molars	Biomolecular analyses; immunoenzymatic assays; microtomographic analyses	IL-1β, TNF-α, and IL-6; RANKL/OPG ratio
Oliveira et al (2017)[22] Brazil	**PROB strains:** *Bifidobacterium animalis* subspecies *lactis* HN019	32 rats were divided into groups: **C** (control; without EP); **EP** (EP only); **C-HN019** (control+PROB); **EP-HN019** (EP+PROB); **in the test group:** 1 mL of suspensions containing PROB topically administered in the subgingival region of the mandibular first molar on days 0, 3, and 7; **control group:** placebo gingival tissue, hemimandibles, and oral biofilm were collected and analysed	**Microbiological analysis** using checkerboard DNA–DNA hybridisation technique; **immunologic analysis, histomorphometric analysis, immunohistochemical analyses, micro-CT analyses**	IL-1β, IL-10, RANKL, and OPG; attachment loss
Ricoldi et al (2017)[23] Brazil	**PROB strains:** *Bifidobacterium animalis* subspecies *lactis (B lactis)* HN019	At baseline, 32 rats were assigned to 4 groups: **C** (control); **PROB; EP-SRP; and EP-SRP-PROB**; in groups EP-SRP and EP-SRP-PROB, the mandibular first molars of the animals received a ligature; orally administered PROB with 10 mL/d of 10^9 CFU of *B lactis* HN019 for 15 days	**Microbiological effects** of *B lactis* on biofilm; **histomorphometric, microtomographic,** and **immunohistochemical analyses**	Proinflammatory (IL-1β and cytokine-induced neutrophil chemoattractant) and anti-inflammatory (IL-10 and TGF-β1) cytokines; the number of osteoclasts, attachments, and alveolar bone losses

IL, interleukin; OPG, osteoprotegerin; PE/EP, experimental periodontitis; RANKL, receptor activator of nuclear factor kappa-B ligand; TNF, tumour necrosis factor.

Table 4 – Results of the included studies.

Study	Measured outcome using probiotics Clinical outcomes	Microbiological outcomes	Immunologic outcomes
		RCTs	
Invernici et al (2020)[12]	Compared to the control group, the test group had a lower BOMP at 90 days and a lower PI at 30 days	–	Between the test and control groups at 30 and 90 days in the immunoglobulin A levels, no significant differences; higher βD-3, TLR4, and CD-4 expressions were observed in gingival tissues in the test group than in the control group
Invernici et al (2018)[13]	The test group had larger clinical attachment gain and lower PPD than the control group at 90 days	For deep periodontal pockets: the test group exhibited a larger count of *Actinomyces naeslundii* and *Streptococcus mitis* and a more pronounced reduction in the count of *P gingivalis*, *Treponema denticola*, *Fusobacterium nucleatum vincentii*, *Campylobacter showae*, and *Eubacterium nodatum* than the control group	The test group had higher levels of IL-10 than those at baseline at 30 days; the control group had a higher ratio of IL-1β (at 30 and 90 days) and of IL-8 (at 30 days)
Kuru et al (2017)[18]	After abstinence from oral hygiene practices, *B animalis* positively affected plaque accumulation and gingival inflammatory parameters, lower PI and GI, less BOP, and a minor increase in GCF volume	–	Total amount and concentration of IL-1β in GCF were lowered
		In vitro studies	
Argandoña Valdez et al (2021)[19]	–	*B infantis* and *B lactis*, as single species, have antagonist effects on *F nucleatum* and *P gingivalis* biofilms; double combinations of bifidobacteria tested to have an inhibitory effect on *F nucleatum* and *P gingivalis* biofilms; single and double combinations of bifidobacteria did not affect *S oralis* counts	–
Invernici et al (2020)[12]	–	**In vitro evaluation of the adhesion of *B lactis* HN019 and *P gingivalis* to BEC;** lower mean adhesion of *P gingivalis* combined with *B lactis* HN019 when compared to the mean adhesion of *P gingivalis* alone to BEC; **in vitro evaluation of *B lactis* HN019 antimicrobial activity;** growth inhibition of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*	–
Jasberg et al (2016)[20]	–	The growth of *Porphyromonas gingivalis* was reduced significantly in biofilms assays and *P. gingivalis* growth was completely inhibited in agar-overlay tests with *Bifidobacterium*	–
		Animal model studies	
Silva et al (2022)[21]	The PEP and MSPEP groups showed lower levels of alveolar bone loss when compared with the PE and MSPE groups	–	*B lactis* HN019 reduced the severity of periodontitis significantly in rats with metabolic syndrome; **immunoenzymatic analysis** showed higher levels of IL-1β and a higher RANKL/OPG ratio in the MSPE group when compared with the MSPEP group; the PEP group showed lower levels of TNF-α and IL-6 when compared with the PE group
Oliveira et al (2017)[22]	Topical use of *B lactis* HN019 promotes a protective effect against alveolar bone loss and connective tissue attachment loss	**EP+PROB group vs EP:** more significant proportions of *Actinomyces*- and *Streptococcus*-like species and lower proportions of *Veillonella parvula*, *Capnocytophaga sputigena*, *Eikenella corrodens*, and *Prevotella intermedia*-like species than group EP	**EP+PROB group vs EP:** group EP-HN019 presented more significant expressions of OPG and βDs than group EP ($P < .05$); group EP presented greater levels of IL-1β and RANKL than group EP+PROB
Ricoldi et al (2017)[23]	Group EP-SRP-PROB presented reduced alveolar bone resorption and attachment loss when compared with group EP-SRP; *B lactis* HN019 potentiates the effects of SRP in the treatment of EP in rats	*B lactis* promoted a higher ratio between aerobic and anaerobic bacteria in biofilm samples	**Group EP-SRP-PROB vs group EP-SRP:** the PROB group showed significantly fewer osteoclasts, increased expression of anti-inflammatory cytokines, and reduced expression of proinflammatory cytokines compared with the comparator

βD, β-defensin; BEC, buccal epithelial cells; BOMP, bleeding on marginal probing; BOP, bleeding on probing; CD, cluster of differentiation; GCF, gingival crevicular fluid; GI, gingival index; IL, interleukin; MSPE, periodontitis associated with metabolic syndrome; MSPEP, probiotic in group with experimental periodontitis with metabolic syndrome; OPG, osteoprotegerin; PE/EP, experimental periodontitis; PEP, probiotic in group with experimental periodontitis; PI, plaque index; PPD, probing pocket depth; PROB, probiotic; RANKL, receptor activator of nuclear factor kappa-B ligand; RCT, randomised controlled trial; SRP, scaling and root planning; TLR4, toll-like receptor 4; TNF, tumour necrosis factor.

was discontinued, significantly elevated PI, GI, and BOP were seen in both groups, but these indices were significantly lower in the probiotic yoghurt group. In addition, a lower volume of GCF was collected from the probiotic group.

Invernici et al[12,13] conducted a similar probiotic intervention protocol in 2 successive RCTs. They evaluated 2 groups of patients with chronic periodontitis treated with SRP alone or in combination with *B animalis* HN019. In their first study, they noted a lower BOP and PPD and larger clinical attachment gain at day 90 (30 days after probiotic cessation) in the probiotic group.[13] In the subsequent study,[12] PI improved immediately after the probiotic therapy, but the difference was not significant on day 90. Furthermore, the BOP was significantly lower only after day 90 ($P < .05$).

The 3 animal studies included in this review used the identical strain of *B animalis* HN019 to treat ligature-induced periodontitis.[21-23] Thus, periodontitis was induced in rats with and without metabolic syndrome induced by a high-fat diet (HFD).[19] Eight groups were created based on rat diet, presence of periodontitis, and probiotic therapy (*B lactis* HN019-laced water for 8 weeks). The groups with periodontitis that received *B lactis* orally showed lower levels of alveolar bone loss compared to control groups. Moreover, rats with metabolic syndrome had favourable bone volume and reduced porosity after probiotic therapy compared to controls.

Subgingival application of probiotics *B lactis* has also been evaluated in rats with periodontitis.[22] Greater bone porosity, trabecular separation, connective tissue attachment loss, and reduced bone volume were observed in the animals with no probiotic therapy. In another study,[23] rats with ligature-induced periodontitis were fed with *B lactis* HN019–supplemented milk for 15 days after SRP. This led to a significant reduction in the eventual alveolar bone resorption and attachment loss.

Discussion

Bifidobacterial probiotics and their effect on periodontopathogens

Several recent reports indicate that bifidobacteria competitively inhibits key periodontopathogens.[12,20,26] One reason for this effect in biofilms and agar-overlay assays might be the bifidobacterial competition for nutrients and growth factors.[20] It is known that salivary bifidobacteria depletes vitamin K concentration in the immediate environment, thereby inhibiting the growth of *P gingivalis* possibly by competing for this necessary growth factor.[24] Another plausible contributory factor could be the acidic conditions generated by bifidobacteria that are inimical to key periodontopathogens.[20] However, this is unlikely to be the primary mode of their antimicrobial action, as it appears to be strain-specific against many pathogens. In addition, the production of antimicrobial agents, such as lactic acid, hydrogen peroxide, and bacteriocins by probiotic organisms are also considered important factors that deter the growth of periodontopathogens.[22] This postulate is validated by the fact that live suspensions of probiotic bifidobacteria are more potent against periodontopathogens than the heat-treated strains.[27]

Interspecies bacterial coaggregation is another important mechanism underpinning the development of complex plaque biofilms. As proteinaceous elements on the cell surfaces of bifidobacteria seem to facilitate bacterial coaggregation,[28] competitive reduction in the number of these binding sites on biofilm is likely to reduce the level of periodontopathogens and plaque mass.

The competition between bifidobacteria and periodontopathogens seems to be affected by the sequence of colonisation.[24] For instance, Zhu et al[27] noted that when bifidobacterial probiotics were inoculated first, they inhibited *P gingivalis, F nucleatum, A actinomycetemcomitans, Porphyromonas circumdentaria*, and *Prevotella nigrescens* colonisation, but this antagonistic effect was minimal when both the probiotic and pathogens were co-inoculated.

It is noteworthy that bifidobacteria do not seem to affect the composition of key components of supragingival biofilms, mainly Gram-positive organisms, such as *Streptococcus mutans*,[20] *S sanguinis*,[27] and *S oralis* that confer protection against invading periodontopathogens.[19]

In summary, bifidobacteria may have the potential to enhance gingival health through competitive inhibition of periodontopathogens and support the health-associated bacteria in subgingival biofilms. This beneficial effect appears to be strain-specific and highly variable, possibly due to the complex probiotic–pathogens and probiotic–host interactions, which include competition for adhesion sites and nutrients, modulation of the immune system, production of antimicrobial substances, and modulation of pH and oxidation–reduction potential of the biofilm.[10] Also, the outcome of probiotic therapy is likely to be impacted by their dosage, frequency, and mode of administration.[13]

Bifidobacterial probiotics and immune modulation

A dysbiotic microbial community induces local inflammation, overactivation of the host immune response, osteoclastic activity, and alveolar bone loss, eventually leading to the development of periodontitis.[29] During this process, pro-inflammatory cytokines (eg, IL-1β, TNF-α) enhance osteoclastogenesis by upregulating RANKL expression in periodontal tissues. The latter is an essential regulator of osteoclast differentiation and activation, and a higher level of RANKL mRNA has been reported in advanced periodontitis.[30] Additionally, human periodontal ligament cells, epithelial cells, and gingival fibroblasts possess OPGs, which inhibit differentiation and activation of osteoclasts. Intriguingly, OPG acts as an antagonist of the Receptor activator of nuclear factor-kappa B (RANK), thus hindering the differentiation of osteoclasts and osteoclastic activity.[31]

Pathogenesis of periodontitis entails the interaction of RANKL and OPG proteins with bacterial by-products, whilst inflammatory mediators initiate and regulate the degenerative process of periodontal tissue destruction.[32] Hence, impeding periodontal bone resorption via RANKL expression by modifying lymphocyte activity or modulating cytokine production could be a potential approach for periodontal therapy.

It is evident from the reviewed studies that systemic or topical use of bifidobacterial probiotics favours the expression of anti-inflammatory cytokines IL-10[13,22,23] and curbed pro-inflammatory cytokine production.[12,13,18,21-23] Therefore, bifidobacteria appear to play a critical role in the inflammogenic processes of chronic periodontal diseases by modifying this critical host response.

The gingival epithelium has defences against pathogens, including the release of antimicrobial peptides βDs[33,34] that function as antibacterial, chemotactic, and anti-inflammatory agents.[35] Invernici et al[12] noted increased βD-3 expression in gingival biopsies of the diseased sites in probiotic groups. Their observation concurs with an animal study,[22] in which increased βD-3 expression in periodontal tissues and reduced periodontal inflammation were seen in a rat litter administered *B lactis* HN019.

The foregoing clearly indicates that bifidobacteria initiate beneficial immunomodulatory effects through the regulation of inflammogenic mediators and bone remodelling, eventually favouring periodontal health.

Bifidobacterial probiotics and tooth-supporting tissues

Our review demonstrates that the topical application of bifidobacterial probiotics as an adjunct to SRP is safe and effective in managing periodontitis[10]; in particular, *B lactis* administration significantly improves gingival inflammation in chronic periodontitis.[12,18] Both these phenomena imply that bifidobacterial probiotics, in general, support the conservation of a healthy periodontium. In clinical terms, a significant reduction in deep pockets and a gain in clinical attachment when probiotics are used[20] equates with the results obtained by conventional antibiotics and SRP.[36] These beneficial outcomes are sustained for a few weeks beyond the period of probiotic consumption, implying the likely assimilation of bifidobacteria into the subgingival biofilm ecosystem.

Two different protocols in animal models were identified for ligature-induced periodontitis, namely maintaining the ligature during the entire experimental period[22,21] and removal of the ligature once the lesion is initiated.[23] The latter, rather than the former approach, mimics closely the natural history of periodontal disease in humans; hence, it is likely to be the preferred approach for future workers.

Limitations in the available evidence

The existing literature provides limited evidence on the impact of *Bifidobacterium* species on periodontopathogens and the immune system, especially in patients with comorbidities.[37] One major reason for this is that periodontal disease is naturally a relatively chronic insidious infection that may take weeks or months for development or recovery. Thus, mimicking the clinical situation in animal studies over prolonged periods is intrinsically difficult and costly. Moreover, appropriate clinical investigations applying the full scope of clinical microbiology are needed to confirm a permanent or transient integration of probiotics into the periodontal microflora.

Conclusions

The short duration of microbiological and clinical assessments, poorly standardised probiotic therapies, and less-than-ideal animal models for periodontal healing assessment are limitations in the available literature. Despite these limitations, bifidobacterial probiotics, especially *Bifidobacterium animalis* subspecies *lactis*, were found to promote the development of a healthy plaque microbiome through microbiological and immunomodulatory pathways. In particular, the probiotic appears to reduce pro-inflammatory biomarkers associated with periodontal disease and regulate mediators of bone remodelling. However, further clinical and molecular studies are essential to confirm and unveil the mechanisms underpinning these actions of bifidobacterial probiotics.

Author contributions

VHM and KSF contributed substantially to the conception and design of the work and acquisition, analysis, and interpretation of data. LPS worked on the acquisition, analysis, and interpretation of data. VHM, KSF, HN, and LPS drafted and critically revised the work for important intellectual content. All authors gave final approval of the version to be published.

Conflict of interest

None disclosed.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2022.11.018.

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