Title: Probiotics against periodontal disease: a systematic review. *:* Are probiotics a posible treatment of periodontitis?

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INTRODUCTION

Periodontal disease is one of the most prevalent oral diseases. It follows a chronic course, leading to bone destruction and dental loss. This disease can arise when balance in the oral microbiome is lost.[1] [i]The species *P. gingivalis* and *T. forsythia* are considered the most pathogenic, presenting in a similar prevalence in different forms of periodontitis. However, *A. actinomycetemcomitans* also plays an important role in the development of this disease.[2,3]

Until now, periodontal disease treatments have been classified as non-surgical and surgical, which in certain cases were completed with the administration of systemic antibiotics.[2,4] The preferred treatment is scaling and root planing (SRP); it offers good results, but periodontal pockets can be immediately recolonized by pathogenic bacteria.[5,6] The limitations of these treatments and the resistance created by the indiscriminate prescription of antibiotics have led to the need to find more effective alternatives that reinforce SRP and that have fewer side effects.[2,3,7]

In addition, it has been observed that the chronic inflammation derived from periodontal disease influences systemic diseases such as diabetes, cardiovascular and respiratory diseases, among others. That is why its treatment is so important at a systemic level.[1,3]

So far, probiotics have been used to treat gastrointestinal disorders, among other systemic conditions. But the results are also being studied orally, providing benefits against caries. In the periodontal field, clinical, microbiological and inflammatory improvements are observed.[2,3,]

Periodontal disease

Etiology and risk factors. Bacterial plaque or biofilm is the main etiological factor in periodontal disease and the host response will determine its progression. In the development of this disease, there are three factors to consider: the susceptibility of the host, the presence of periodontopathogenic species and the presence of beneficial bacteria.[1,8] The microorganisms present in the oral cavity in balance (symbiosis) maintain a healthy state, through pro and anti-inflammatory activities in homeostasis. On the contrary, the disruption of such balance (dysbiosis) is associated with disease and alteration of the proportions of microbiota species.[1] Also, local and systemic risk factors capable of making the host more likely to develop periodontal disease have been described, which affects maintaining short and long term periodontal treatments.[1]

Microbiology of periodontitis. The literature suggests that specific levels of gram-negative bacteria in the subgingival plaque increase the risk of onset and progression of periodontitis.[8] Likewise, higher levels of anaerobic bacteria are found in patients with periodontal disease, especially in deeper pockets. *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* are the most frequently detected species in deep pockets (>5 mm) and these are associated to a high risk of periodontitis progression.[8] Also, *Aggregatibacter actinomycetemcomitans* is related to the severity of chronic periodontitis.[2,8] This pool of periodontal pathogens in the subgingival area can activate a cascade of defense mechanisms with the production of factors that cause inflammation and destruction.[8]

Prebiotics

Prebiotics are selectively fermentable ingredients that allow specific changes in the composition and/or activity of the gastrointestinal microbiome, providing benefits in the host. These prebiotics have enormous potential to modify the intestinal microbiome.[9, 10] They are non-digestible carbohydrates, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), transgalactooligosaccharides (TOS), inulin, short chain fatty acids (SCFA), among others.[9, 10] Prebiotics come mainly from plants. Like probiotics, to ensure their continued effect, prebiotics must be consumed daily, at doses between 4 and 20 g.[11] According to various hypotheses, probiotics could use these substrates as energy input or the presence of such oligosaccharides could encourage the growth of those bacteria.[11]

Probiotics

According to the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) in 2001, probiotics are live microorganisms whose consumption in adequate quantities can bring health benefits.[2,11,12] The concept that probiotic bacteria could influence health was established in Russia by bacteriologist Elie Metchnikoff, noting that life expectancy in Bulgaria was higher, supposedly due to the consumption of fermented milk, rich in *Lactobacillus bulgaricus*.[11] The benefits of probiotics have been extensively studied systemically and their positive effect has been demonstrated for different disorders, such as certain gastrointestinal diseases, urogenital infections, etc.[12,13] By definition, probiotics must be safe in animals, resist bile acids and have the ability to adhere to and colonize the intestine.[10]

Oral effects. Several clinical studies show that regular consumption of probiotics of *Lactobacillus paracasei SD1* decreases the number of cariogenic *Streptococci* in the salivary and dental plaque and therefore reduces the risk of caries.[14,15] Also, there are some studies showing that the

probiotic *Lactobacillus* can reduce gingival inflammation, improving periodontal health and decreasing the concentration of *P. gingivalis* in saliva and subgingival plaque.[16,17,18] Several trials on peri-implantitis treated with *Lactobacillus reuteri* show a significant improvement of all the clinical parameters of this disease, with reduction of pro-inflammatory cytokine levels.[19, 20, 21] Studies in which halitosis was treated with *Lactobacillus salivarius* indicate a positive effect on its decrease and on the improvement of the patient's own perception.[22,23] Likewise, in the treatment of candidiasis, *Lactobacillus* and *Bifidobacterium* species significantly reduce candida levels; therefore, they could be used to prevent candidiasis, although there is no clear clinical improvement of stomatitis in all studies.[24, 25, 26, 27] However, these effects do not last long in the host. In order to reduce resistance to probiotics and improve oral benefits, new research, such as prebiotics or nitrate-reducing bacteria, are needed.

Probiotics mechanisms of action. Note: 1) Direct interaction with dental plaque (resistance to colonization). This mechanism could include the disruption of biofilm formation (acquired layer) with competition for tissue binding sites and for the same nutrients. The production of antimicrobial compounds could also inhibit other oral bacteria. Lactic acid-producing bacteria produce antimicrobial agents such as organic acids, oxygen peroxide, peptides, bacteriocins and anti-adhesion molecules[28] **(figure 1).** 2) The indirect actions of probiotics are in the modulation of innate and adaptive immune function. It has been described that lactic acid-producing bacteria could possibly interact with immunocompetent cells, such as macrophages and T cells, altering cytokine production and the effects of immunity in general. In addition to modulating immune responses, some species of probiotics are able to improve mucin production and barrier function, regulating defense peptides, promoting angiogenesis and wound healing[28] **(figure 1)**.

Symbiotics

The term "symbiotic" refers to the product of combining one or more probiotics with a prebiotic fiber, which favors positive interaction between them. This hunting strategy tends to be the most important, since it offers excellent potential to increase the effectiveness of this type of products, improving its stability and duration.[9, 10] Each prebiotic selectively favors a type of probiotic. The selection of the probiotic, prebiotic and their respective doses is crucial to obtain the therapeutic effect. Therefore, specific clinical trials are necessary to confirm health benefits.[9]

Work approach

Periodontal disease, as a chronic disease with a high prevalence worldwide, and with important local and systemic implications, requires seeking for more effective therapeutic alternatives with fewer side effects. The limitations of current treatments, such as recolonization after root scaling, the risk of surgical complications and the increasing development of bacterial resistance due to the use of antibiotics, lead to the need to look for other strategies. The results obtained with probiotics as adjuvants to periodontal treatment allow us to establish a new therapeutic approach. The objectives include: evaluating the effect of probiotics as an adjuvant to SRP, analyzing the effect of probiotics on the composition of the subgingival microbiota, and assessing possible short or long term side effects for the patient.

MATERIAL AND METHODS

This article adheres to the PRISMA (Preferred Reporting Items of Systematic Reviews and Meta-analyses) statement.[29]

Study selection criteria. Inclusion criteria considered randomized clinical trials, published in the last 10 years that evaluated the results of periodontal probiotic treatments with concentrations 10^7-10^8 CFU in terms of plaque index, bleeding at probing, gingival index, depth of probing and clinical attachment loss. The plaque index (PI) and the gingival index (GI) were evaluated according to Silness and Löe (1964). Probing depth (PD), bleeding at probing (BOP) and clinical attachment loss (CAL) were measured at 6 points on each tooth using the WHO 621 periodontal scale with a Hu-Friedy probe. The selected trials included patients without any systemic disease and with periodontal disease that was treated with scaling and root planing with adjuvant placebo/probiotic administration **(table 1)**. The exclusion criteria included patients treated during the past 6 months, who had systemic conditions that could influence the progression of periodontitis (HIV, Diabetes Mellitus, Down Syndrome, etc.), patients who were pregnant or breastfeeding, patients who were allergic to fermented products, smokers or patients in need of prophylactic antibiotic therapy, or who had been medicated with anti-inflammatories for a long time.

Search strategy. An electronic search in the MEDLINE database (via PubMed) to select randomized clinical trials assessing the effects of the use of probiotics in periodontal disease published in the last 10 years and with full text was performed. The terms searched were: *((“Probiotics” [Majr]) AND (“Periodontal Disease” [Majr] OR “Periodontitis” [Majr]))*.

Screening and selection of papers

Titles and abstracts of selected publications were scanned by two blinded reviewers independently (N.C and M.L.G) and categorized as suitable or not for inclusion. Full reports were obtained and reviewed independently for studies appearing to meet the inclusion criteria or for which there was insufficient information in the title and abstract to allow clear decision.

Study selection. Studies were selected according to the inclusion and exclusion criteria. Duplicates and irrelevant publications (based on title and summary) were also deleted. Then, a full text reading was carried out to select the articles that met the inclusion criteria (figure 2). All these trials followed the ethical principles as per the World Medical Association Declaration of Helsinki.

Risk and quality assessment of bias. In the trial selection process, the risk of bias in randomized clinical trials was evaluated using the Cochrane Handbook for Systematic Reviews of Interventions (version 5.1.0). The evaluated items were: 1) generation of the randomization sequence, 2) allocation concealment 3) blinding of the patients 4) end of blinding, 5) incomplete final results and 6) selective contributions (table 2).

RESULTS

Study selection. 66 items were found in the search. After reading the titles and abstracts, 52 were excluded since they were not randomized clinical trials, they were performed on animals, they were published before 10 years or they treated other diseases other than periodontitis. Fourteen articles were evaluated in full text and 4 trials were eliminated because they did not meet the inclusion and exclusion criteria (figure 2). Finally, a total of 10 clinical trials were included in this review (table 3).

General characteristics of the included studies. The general characteristics are shown in (table 3). All of them were randomized, parallel, double-blind clinical trials, except for the Vivekananda et al. trial, which was performed in cleft palate subjects, and 2 quadrants were treated with scaling and root planing (SRP) and with probiotic/placebo, and the other two without SRP treatment, but with the effect of probiotic/placebo.[30] The number of patients included in the sample is between 20 and 51 and they are between 25 and 68 years old. In all studies, the control and test groups were compared. The test groups received probiotics as an adjuvant to SRP, while the control groups received SRP plus placebo. The most widely used species was *Lactobacillus reuteri*, used in a total of 6 trials,[7, 12, 30, 31, 32, 33] but in one of these it was combined with *Lactobacillus salivarius*.[31] In two trials *Lactobacillus salivarius* was used.[13, 34] For only one trial different species of

Streptococcus[35] and one *Bifidobacterium animalis lactis*[4] were used **(table 3)**. Unlike the other trials, the study conducted by Shimauchi et al. compared the use of probiotics adjuvant to SRP with the control group in non-smoking patients, and also in smokers.[34] The dosage was in most studies twice a day; with 10 mg tablets after the usual manual brushing,[4, 7, 32, 35, 33] and in toothpaste.[31, 13, 30] For one study, taking tablets once a day [12] was prescribed and only in one trial a regimen of tablets 3 times a day was prescribed [34] **(tables 3 and 4)**. The follow-up period for all studies was between 1 month and 12 months. None of the participants in the included studies reported side effects with the use of probiotics **(table 4)**.

Bias risk analysis. None of the studies reported included smokers, patients with systemic diseases, or pregnant women, or patients who had received antibiotics during the previous 6 months. However, Shimauchi et al. compared the non-smoking test and placebo groups with a separate group of smokers, divided also into placebo and test groups[34]. After analyzing the quality of the trials using the Cochrane Handbook for Systematic Reviews of Interventions (version 5.1.0), all trials analyzed were considered to be at low risk of bias, except for the Szkaradkiewicz et al. trial, which has a low/unclear risk for not reporting how the random sequence, allocation concealment and blinding of patients were performed [7] **(table 2).**

Results of individual studies

In 7 trials, the clinical parameters evaluated were significantly improved in the test group compared with placebo.[4, 7, 12, 13, 33, 30, 34] However, in 3 studies no significant differences were reported between the two groups in the clinical parameters evaluated,[31, 32, 35] but in one there was a significant improvement in PI and GI [32] **(table 3)**. On the other hand, a representative reduction of the main periodontal pathogens was obtained in 4 clinical trials [4, 32, 33, 30] and in 2 studies there was a significant reduction in proinflammatory cytokines.[4, 7]

Depth at probing (PD). In 7 randomized clinical trials, it was reported that the test group had obtained a significant reduction in depth at probing in both moderate (4-6 mm) and deep (> 7 mm) pockets.[4, 7, 12, 13, 33, 30, 34] In addition, in the Vicario et al. trial, this improvement in the reduction of the pockets was reported 1 month after stopping treatment with probiotics [12] and in the Tekce et al. trial, this improvement was reported up to 3 months later.[33] In only 3 articles a significant difference between the study groups has not been reported.[31, 32, 35] However, a constant tendency to improve with the probiotic has been determined, as in the Teughels et al. trial, in which there was no significant decrease until 12 weeks.[32] Some trials also report a significant decrease in the risk of progression of periodontal disease in the groups treated with probiotic adjuvant to SRP and therefore the reduction in the number of patients needing

surgery.[4, 33] For example, this was evidenced in the Invernici et al. trial, in which the test group obtained 55% of patients with a low risk, compared with the 30% low risk of the placebo group.[4]

Plaque and gingival index (PI and GI). In 8 clinical trials, the PI and GI were significantly reduced.[7, 12, 30, 31, 32, 33, 34, 35] Thus, in the Vivekananda et al. split mouth trial, a significant improvement was reported with probiotic intake, compared to placebo, in quadrants not treated with SRP.[30] Only in 2 trials no significant improvement was observed in PI and GI.[4,13]

Bleeding on probing (BOP). In bleeding on probing a significant decrease was obtained in 6 trials,[7, 12, 13, 30, 33, 34] but, in the other 4, this was not the case.[4, 31, 32, 35] Shimauchi et al. reported a significant improvement in the test group, also in smokers.[34]

Clinical attachment loss (CAL). Significant improvement was obtained in 6 studies,[4, 7, 12, 13, 30, 34] and in 3 trial [31, 32, 35] it was not evidenced, although in the Teughels et al. trial, a tendency to have fewer recessions was reported.[32] On the other hand, the study by Shimauchi et al. showed that the reduction in CAL was also significant in the probiotics group in smokers.[34] The Tekce et al. trial did not examine this parameter.[33]

Microbiological effects. Of the clinical trials evaluated, 5 examined the microbiological impact of the main periodontal pathogens, using the PCR test.[4, 13, 30, 32, 35] In the vast majority, a significant reduction of *P. gingivalis* and *P. intermedia* was reported in the groups treated with probiotics, compared with placebo.[4, 13, 30, 32] Likewise, 2 trials determined a significant decrease of *T. denticola* and *F. nucleatum* [4, 35] and in 2 more a significant reduction of *A. actinomycetemcomitans* was observed.[13, 30]

Immunological effects. In only 2 trials the effect on the immune system of this treatment was evaluated with the ELISA test.[4, 7] In both trials, a significant decrease in IL-1B was reported with a reduction of up to three times.[7] A reduction in other factors involved in such inflammation was also obtained, such as IL-8, TNF and IL-17.[4, 7]

DISCUSSION

Summary of the evidence. The results of this evaluation show that there is significant efficacy in patients treated with probiotics adjuvant to SRP compared to those treated with placebo adjuvant to SRP in 7 of the clinical trials evaluated.[4, 7, 12, 13, 30, 33, 34] In the other 3 trials, no significant improvement was observed.[31, 32, 35] These results are comparable to those of other studies, such as Morales et al.,[36] which compare the effect of probiotics with that of antibiotics, as adjuvants to SRP, to treat periodontitis. However, probiotics would not cause risks, compared with antibiotics, which are possible causes of side effects or microbial resistance.[2, 37] The most

commonly used probiotic in these studies was *L. reuteri*,[7, 12, 30, 31, 32, 33] which in *in vitro* studies produces an antimicrobial compound, called reuterine, with an inhibitory effect against the main periodontal pathogens.[38] Until now, *L. reuteri, L. salivarius* and *Bifidobacterium animalis lactis* have been shown to be effective, having used various dosages and routes of administration **(table 4)**. However, *Streptococcus* [35] species and the combination of *L. reuteri* and *L. salivarius* [31] have not reported significant improvements. Therefore, it can be stated that there is no clearly better route of administration or dosage, but the species that would provide significant improvements are *L. reuteri, L. salivarius* and *B. animalis lactis* [4, 7, 12, 13, 30, 33, 34] In the clinical trials studied evaluating the microbiological repercussions, a significant reduction of the main periodontal pathogens was observed [7, 13, 30, 32, 35] *P. gingivalis* and *P. intermedia,*[7, 13, 30, 35] *T. denticola* and *F. nucleatum* [4, 35] and *A. actinomycetemcomitans*.[13, 30] These results are supported by other studies conducted by Imran et al.[39] and Mayanagi et al.,[40] who observed a significant reduction of the main periodontal pathogens: *P. gingivalis, P. intermedia, A. actinomycetemcomitans* and *T. forsythia* with the administration of *Lactobacillus*.[39, 40]

On the other hand, in 2 trials a reduction of other factors involved in inflammation was obtained, such as IL-8, TNF and IL-17 [4, 7] and IL-1B.[7] In the trials that evaluated the patient's adherence to treatment, all reported excellent compliance.[4, 32, 12, 35, 33, 30] In none of the included trials were side effects perceived by the patient or the researcher, administered orally, capsules and toothpaste found. According to the time of administration, no side effects were found either in the short term [4, 7, 12, 13, 30, 31, 32, 34, 35] or in the long term. [33, 35]

Limitations. Included in the limitations of this study are the different types of probiotics used, the different dosages and routes of administration. It would be convenient to unify these parameters and conduct comparative studies to determine which strategy is most beneficial for the treatment of periodontal disease. On the other hand, it is convenient to conduct long-term randomized clinical trials, which assess whether the effect of the probiotic endures after its consumption. This is demonstrated in the Tekce et al. trial, in which the probiotic was administered for 3 months and its benefits were reported up to 3 months after stopping treatment, when the onset of recolonization was detected.[33] Finally, it would be convenient to conduct clinical randomized trials that assess the effect of prebiotics with probiotics as adjuvants to periodontal treatment, since it has been seen in various studies that these could enhance the beneficial effects of probiotics and provide better long-term results.[9, 10, 11]

To conclude, according to the results of the clinical trials evaluated, the administration of probiotics as an adjuvant treatment to the mechanical treatment of periodontal disease, can: 1)

Improve clinical parameters at a general level (plaque index, gingival index, probing depth, bleeding on probing and clinical attachment loss). 2) Produce significant changes in the composition of the subgingival microbiota, significantly decreasing the concentration of the main periodontal pathogens. 3) Cause no short or long term side effects perceived by the patient or researcher.

This journal adheres to the ethical guidelines

Conflicts of interest: none

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