Chapter 5

Probiotics and Periodontal Diseases

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Additional information is available at the end of the chapter

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Abstract

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Probiotics have been used to directly modify the resident oral microbiome and proposed to modulate immune responses. In dentistry, probiotics have been employed as useful adjuncts for the reduction of caries development, suppressing oral Candida infection and controlling halitosis. Plaque-induced gingivitis is a gingival inflammation caused by the adherent bacterial biofilm around teeth. Gingivitis and periodontitis are considered to be a continuum of the same inflammatory process, although many gingivitis lesions do not progress to periodontitis. Periodontitis is an inflammatory process that affects the attachment structures of teeth. It constitutes a second cause of tooth loss worldwide. Conventional treatment modalities of periodontal disease include non-surgical and/or surgical management, with an emphasis on mechanical debridement. However, mechanical debridement as a sole therapy is not always effective to improve clinical parameters. A growing number of studies support probiotic therapy to prevent or treat gingivitis and periodontitis. Oral administration of probiotics is an effective adjunct in reducing pathogenic bacteria and improving clinical signs of disease. Probiotics may serve as adjunct or replacement therapy substitute antibiotics in managing human periodontal infections in future.

Keywords: probiotics, periodontitis, gingivitis, periodontal diseases, dental scaling

1. Introduction

In order to speak about probiotics, we have to go back to the twentieth century when the Russian scientist Elie Metchnikoff postulated the theory about the influence of gastrointestinal micro biota (gut flora) over ageing. In 1908, this Nobel Prize winner attached the longevity of some Balkans towns to the frequent consumption of fermented dairy, containing Lactobacillus, which reduced the toxins produced by intestinal bacteria, promoting health and prolonging life [1].

The discovery of Lactic Acid Bacteria (LAB) in the middle of the nineteenth century confirmed the interest in microorganism and since then many people have concluded that dairy fermented by lactobacillales provide numerous benefits to our health [2].

For a long time, microorganisms have been responsible for the production of numerous foods and drinks and also have had an important impact on human health. The discovery of a symbiotic relationship between bacteria and humans awoke the curiosity of seeing the bacteria as potentially beneficial rather than pathogenic [2].

Lilly and Stillwell coined the term "probiotics" first definition in 1965 as: "Living microorganisms that confer a benefit to the host's health when given in adequate amounts" [3]. The term "prebiotic" was defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" by Gibson and Roberfroid [4].

Symbiotic is the relationship between probiotics and prebiotics, which benefits the host by increasing the survival and implantation of live microorganisms from dietary supplements in the gastrointestinal system [5]. This has not been deeply studied, but it could increase bacteria potential to develop their function in the colon because symbiotic products could increase the survival of probiotic in their intestinal transit phase. Also, a synergic effect has been described. Prebiotics contribute to the installation of a specific bacterial flora with beneficial effects on health because they stimulate the growth of specific strains [6].

Nowadays the term probiotic has evolved and is now described as "living microorganisms, mostly bacteria, non-pathogenic, used as nutritional supplement, which after being ingested in the right amount, improve the intestinal microbial balance and cause beneficial effects on the health of those who ingest them" [7]. They are considered safe for human health [8]. Since the 1980s scientific investigation about healthy properties of ingesting probiotics has increased considerably, which boosted their use and led to their appearance in clinical practice as treatment for diseases such as chronic diarrhea, immune regulation, allergies, inflammatory bowel disease, constipation, lactose intolerance and lipid intolerance [9]. Lately there is big concern regarding the use of probiotics to treat oral infections like dental cavities (caries) and periodontal diseases. However, available information about the effects of probiotics on periodontal health is still minimal [10].

Chronic oral infections in soft tissues cause inflammatory alterations releasing pro-inflammatory substances such as cytokines, which through the circulatory system, access any area of the body, increasing the risk of muscular, digestive and cardiovascular problems, premature birth, diabetes and sports injuries. Hence, the treatment of chronic oral diseases and the maintenance of oral health should be considered as an asset in the prevention of systemic problems for general health [11].

Periodontal diseases and dental cavities have high prevalence [12], and according to the World Health Organization, the majority of children have signs of gingivitis and among adults the initial stages of periodontal disease are highly prevalent [13]. Bacterial biofilm that forms in the hard and soft tissues of the oral cavity is considered the main etiological factor in most pathological conditions

of the oral cavity. The accumulation of bacteria inside the biofilm, provided by a poor oral health, influences changes in the microbial community, leading to periodontal inflammation [14].

Several studies such as Gorbach and Goldin (1985) [15], Näse et al. (2001) [16], Grudianov et al. (2002) [17], Wei et al. [18], Von Bultzingslowen et al. [19], Hatakka et al. (2007) [20] have spoken about the relation between bacterial strains like *Lactobacillus rhamnosus, Bifidobacterium* spp, and *Lactobacillus plantarum*, which have a positive effect on tooth adhesion and their action against diseases such as dental cavities (caries) and yeast infection. In recent years, treatments of periodontal diseases have changed to an antibiotic or antimicrobial kind. Nonetheless, with the increased incidence of antibiotic resistance, probiotics may be a promising area of research in periodontal therapy [21]. Currently, there is a probiotic that can be used for oral hygiene, as it combats dental plaque, gingivitis and cariogenic bacteria through the patented combination of two strains of *Lactobacillus reuteri*. This is 100% natural due to its residence in the human gastrointestinal tract and production of an antibiotic substance of broad-spectrum called "reuterina", which administrated in the right amount, causes the desired antimicrobial effect to keep the intestinal microbiota intact [11].

Increasingly, antibiotics become complex elements to manage in medical therapies due to the increment of bacterial resistance to them. However, inversely proportional, clinical studies have shown the positive effects in human health associated with the use of probiotics. This is the reason why the World Health Organization supports the use of probiotics as microbial interference therapy. Consequently, the use of probiotics could be postulated as a useful alternative in the control of periodontal diseases, since it improves the conditions of the host, reducing periodontal pocket depth, inflammation, bleeding and halitosis.

2. Local mechanisms of probiotic action

Several mechanisms have been suggested to contribute to the probiotic action. Their effects at local level are mentioned as follows:

1. Production of lactic acid (a short chain fatty acid) can penetrate the bacterial membrane and acidify the cytoplasm by inhibiting the proliferation of *Porphyromonas gingivalis, Streptococcus mutans* [22] and *Prevotella intermedia* [23]. *Lactobacillus gasseri* is a homofermentative bacteria (metabolism via glycolysis) that is able to produce large quantities of lactic acid.
2. Production of hydrogen peroxide can inhibit the growth of pathogenic bacteria [24–26]. *Streptococcus sanguinis* reduces the levels of *Aggregatibacter actinomycetemcomitans* (in about 45 times) and *S. mutans*.
3. Protein modification on the site of attachment, removal of aglutinina gp 340, which is necessary for the attachment of *S. mutans* [16].
4. Production of biosurfactants that prevent bacterial adhesion. *Streptococcus mitis* produces a biosurfactant that prevents adhesion of *S. mutans* and some periodontopathogenic bacteria to the tooth surface.

5. Production of bacteriocins (cationic peptides synthesized on ribosomes with antimicrobial activity that have a narrow spectrum of action) [27–29].
    (a) Salivaricin A and B: Bacteriocins produced by *Streptococcus salivarius*. Salivaricin decreases the proliferation of *S. mutans* and *Streptococcus sobrinus* in carious lesions. Salivaricin B inhibits the growth of *Prevotella* spp. and *Micromonas micros* in halitosis.
    (b) Reuterin: Bacteriocin produced by *L. reuteri*, has antibacterial activity on bacterial Gram (+) and Gram (−), fungi (*Candida albicans*) and protozoa. Among them *S. mutans* and *P. intermedia*.
6. Production of inhibitory substances like bacteriocins: peptides synthesized on ribosomes with antimicrobial activity and broad spectrum of action.
7. Production of vitamins and other substances.
    (a) *Lactobacillus acidophilus* can participate in the production of niacin, folic acid and vitamin B6.
    (b) *Bifidobacterium dentium* increases the absorption of iron, zinc, calcium and magnesium.
    (c) *Streptococcus thermophilus* synthesizes polysaccharides such as hyaluronic acid and produces urease.
8. Changes in the cellular envelope: *Lactobacillus paracasei* HL32 inhibits *P. gingivalis* to induce a change in the cellular envelope [30].
9. Glucosiltransferasa enzyme inhibition. *L. rhamnosus* inhibits the glycosyl-transferase enzyme by reducing the synthesis of glucans in the formation of the biofilm.
10. Anti-oxidant effect.
    (a) *Bifidobacterium longum* has anti-oxidant effect by inhibiting the formation of linolenic acid in the form of hydrogen peroxide.
    (b) *Lactobacillus brevis* decreases the levels of nitric oxide synthetases (NOS).
11. Ingested probiotics can impact resident communities through trophic interactions, a direct alteration in fitness or an indirect alteration in fitness through altered production of host-derived molecules [31]. The major changes of gastrointestinal microbiome occur in stomach and small intestine. These are important not only quantitatively; they may also alter the relative abundance of major phyla [32–35].

3. Systemic mechanisms of probiotic action

The systemic mechanisms of probiotic action are associated with its effect on immune response. In past years, there have been an increasing number of studies linking gut health

with several chronic diseases. In order to understand these mechanisms, it is necessary to review this literature.

3.1. Use of probiotics in medicine

Clinical studies have demonstrated the clinical potential of probiotic against many diseases. However, generalizations concerning the potential health benefits of probiotic should not be made because its effects are strain-specific [36]. Probiotics have been used in some conditions, such as:

3.1.1. Atopic dermatitis

Atopic dermatitis is a chronically relapsing skin disease that occurs most commonly during early infancy and childhood. It is associated with allergen sensitization, recurrent skin infections and abnormalities in skin barriers function [37]. Foolad et al. published a meta-analysis study aimed to find evidence about the effect of probiotics in children with atopic dermatitis [38]. They concluded that the use of probiotics, specifically *L. rhamnosus* GG [39], showed to be effective in mothers and infants in preventing the development and reducing severity of atopic dermatitis [38]. In adult patients, the use of *L. salivarius* LS01 [40] and a combination of *L. salivarius* LS01 with *Bifidobacterium breve* BR03 [41] were associated with significant improvement of clinical manifestations of atopic dermatitis.

3.1.2. Antibiotic-associated diarrhea

Diarrhea is a common side effect of antibiotic use. It can be classified as *Clostridium*-associated antibiotic diarrhea or non-*Clostridium difficile*-associated antibiotic diarrhea. The first one is benign. In contrast, the second one refers to a wide spectrum of diarrhea illnesses caused by the toxins produced by *C. difficile*, including cases of severe colitis with or without the presence of pseudomembranes [42]. A series of meta-analysis concluded that probiotics significantly reduce the risk of antibiotic-associated diarrhea in children [43] and adult patients [44]. It was associated with the use of *L. rhamnosus* GG, *B. lactis* and *S. thermophiles* [43]. *S. boulardii* was reported to be effective in *C. difficile* disease [45, 46].

3.1.3. Irritable bowel syndrome (IBS)

IBS is defined as an abdominal discomfort or pain associated with altered bowel habits for at least 3 days in the previous 3 months, with the absence of organic disease [42]. A meta-analysis demonstrated that, compared with placebo, the use *L. rhamnosus* GG was associated with a significantly higher rate of treatment responders in the overall population with abdominal pain-related functional gastrointestinal disorders and in the irritable bowel syndrome patients [47].

3.1.4. Inflammatory bowel disease (IBD)

IBD consists of two disorders: ulcerative colitis (UC) and Crohn's disease (CD). The gut mucosa suffers a chronic, uncontrolled inflammation. CD is characterized by focal transmural

inflammatory lesions and ulcerations that can be present anywhere in the gastrointestinal tract. UC is more superficial and limited to colon.

A meta-analysis concluded that remission rates in patients with active UC were significantly higher in patients who were treated with probiotics, specifically, VSL#3 (a combination of probiotics containing *B. breve, B. longum, Bifidobacterium infantis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus* and *S. thermophiles*) [48].

3.1.5. Helicobacter pylori

Probiotics do not eradicate *H. pylori*, but they can diminish the levels of this bacterium in the stomach. In association with antibiotic treatments, some probiotics increased eradication rates and/or decreased adverse effects due to the antibiotics [49].

3.1.6. Necrotizing enterocolitis

It is a severe condition occurring especially in preterm infants. A Cochrane review concluded in 2011 that enteral supplementation with probiotics prevents sever necrotizing enterocolitis in preterm infants [50].

3.1.7. Hypocholesterolemic treatment

The combinations of prebiotics and probiotics such as bifidobacteria and FOS, lactobacilli and lactitol, and bifidobacteria and galactooligosacharides were used in trials and have shown promising results in hypocholesterolemic effect [51].

3.1.8. Radiation enteritis

Pelvic malignancies are commonly treated with radiation therapy. Chronic gastrointestinal side effects occur in over 30% of patients [42]. A meta-analysis concluded that probiotic treatment with *Lactobacillus* spp could prevent chemotherapy and radiation enteritis-induced diarrhea in patients with pelvic malignancies [52].

3.2. Probiotic effect on immune responses

Probiotics help maintain gut immune homeostasis by modulating immune response, enhancing epithelial barrier function and inhibiting pathogen growth. Probiotic interaction with mucosal immune system is through the same pathways as commensal bacteria. Its effect appears to be more immune regulating than immune activating [37].

Probiotics modulate epithelial barrier function through interactions with Toll-like receptor (TLR)-2 [53]. The initiation of TLRs signalling regulates synthesis of pro-inflammatory cytokines, chemokines and antimicrobial peptides, recruitment of B cells and production of IgA [54]. Probiotics have been shown to suppress systemic inflammatory response, modulating epithelial signal transduction pathways and cytokine production [55]. For example, *Lactobacillus johnsonni* N6.2 up-regulated type-1 interferon and IFN regulators Stat1 and IRF7 in a TLR-9 dependent way, in rats [56].

Several strains of lactic acid bacteria induce *in vitro* release of pro-inflammatory cytokines TNF-α and IL-6, reflecting stimulation of nonspecific immunity [57]. *L. acidophilus* Lal enhances phagocytosis in humans [58]. *L. casei* Shirota can enhance natural killer cell activity *in vivo* and *in vitro* in humans [59]. However, there are some bacteria that can decrease pro-inflammatory molecules. For example, *L. brevis* CD2 decreases inflammatory markers in saliva from patients with periodontal disease, including prostaglandin E2 (PGE2) [60]. Also, it was reported that probiotics, specifically, *L. reuteri* ATCC PTA 5289 decreased in CGF levels of TNF-α and IL-1β in patients with chronic periodontitis [61]. *Streptococcus cristatus, S. salivarius, S. mitis* and *S. sanguinis* may decrease the release of IL-8 by the epithelial cells stimulated with *Fusobacterium nucleatum* and *A. actinomycetemcomitans* [62–64].

Some specific strains generate an effect on maturation of dendritic cells (DC). DC has a central role in directing the T cell response. They can change to T helper cell (Th) 1, Th2, Th17 and T regulatory cells (Treg) [65]. Certain bacterial strains induce the production of Th polarizing key cytokines by DCs, such as IL-10, IL-12 and IL-23 [66, 67]. Also, some *Lactobacillus* strains have been shown to stimulate Th1 cytokine production while others have increased Th2 responses or induced a mixed Th1/Th2 response [37]. An example of this is the use of a combination of *L. salivarius* LS01 and *Bifidobacterium breve* BR03 in treatment of atopic dermatitis. This probiotic mix generated a significant reduction in microbial translocation, immune activation, improved Th17/Treg cell and Th1/Th2 [41]. Intervention with *B. bifidum, L. acidophilus, L. casei* and *L. salivarius* effectively reduced signs of atopic dermatitis and serum cytokines interleukin (IL-5, IL-6, IFN-γ and serum IgE) [68]. *L. rhamnosus* GG could up-regulate IFN-γ and IL-10 in infants with cow's milk allergy or with IgE-associated dermatitis [69]. The use of *Lactobacillus delbruekii* and *Lactobacillus fermentum* significantly reduced IL-6 concentration and expression of TNF-α and NFκB in colon of patients with ulcerative colitis [70]. *L. brevis* CD2 decreased IFN-γ levels in saliva [60]. *L. reuteri* ATCC PTA 5289 decreased in CGF levels of IL-17 in patients with chronic periodontitis [61].

Anti-inflammatory effect of probiotics has been associated with Treg. For example, oral administration of *L. casei* alleviated colitis and increased the suppressive function of Treg of colon lamina propia. Consumption of *B. infantis* drives the generation of Treg cells, which attenuate nuclear factor kappa B (NFκB) activation induced by LPS of *Salmonella typhimurium* [71].

There is evidence of the effect of some probiotics on matrix metalloproteinases (MMP). They represent a family of human zinc-dependent endopeptidases [65]. *L. brevis* CD2 decreased MMP in saliva [60]. *L. reuteri* DSM 17938 + ATCC PTA 5289 decreased GCF MMP-8 and increased tissue inhibitors of matrix metalloproteinase (TIMP)-1 levels in patients with periodontitis [72].

Current studies also mentioned the role of probiotics in modulation of cell signal transduction pathways, specifically NFκB, which monitors the inflammatory response in the host [73]. *L. reuteri* inhibited inhibitory κB (IκB) degradation and IL-8 expression in TNF-α induced T84 cells and NFκB translocation to the nucleus in HeLa cells [74]. In the same way, *L. rhamnosus* GG diminished nucleus translocation of NFκB, restored decreased cytoplasmic IκB and limited IL-8 secretion in Caco-2 cell model [75]. These actions impede the stimulation of transcription of a series of pro-inflammatory genes such as the encode cytokines, chemokines and grow factors that modulate the proliferation of immune cells [76]. In bronchial epithelial cells cocultured

with *S. salivarius* K12, an immunosuppression was observed, coincident with the inhibition of activation of the NF-κB pathway.

Certain strains of *Bifidobacteria, Lactobacilli, Escherichia coli, Propionibacterium, Bacillus* and *Saccharomyces* influence gene expression of TLRs, NFκB and interleukins. *In vitro*, the interaction of probiotics with antigen presenting cells results in downregulation of pro-inflammatory genes and upregulation of anti-inflammatory genes [36].

4. Probiotics and gingivitis

The adherent bacterial biofilm around the teeth produces a gingival inflammation called plaque-induced gingivitis [77]. It is the most common form of periodontal disease worldwide and plenty of data exist, from different countries and age groups, about the prevalence, extent and severity of gingivitis. Studies on population have shown that regardless of age, gender and race, gingivitis is always associated with the level of oral hygiene [78]. Large-scale population studies on children, adolescents, adults and elderly people have reported very high prevalence of gingivitis, ranging from 50 to 100% [79–82]. Gingivitis and periodontitis are regarded as a continuum of a same inflammatory process. However, it is necessary to point out that in many cases gingivitis does not progress to periodontitis [83, 84].

Once the teeth erupt, a bacterial biofilm immediately begins to form at their surfaces exposed to the oral cavity and in intimate contact with the gingival margin. The level of biofilm accumulation, the virulence of the biofilm bacteria and the humoral and cellular immune responses to the biofilm microbiome are the factors that determine the severity of the periodontal disease [85]. Normally gingivitis in young subjects remains chronic for an extended period of time and does not cause any damage to the periodontal ligament or bone. Nevertheless, an alteration of the balance between biofilm and host can originate a loss of periodontal attachment. Chronic and aggressive periodontitis start as gingivitis. However, the biological processes involved in the progression from gingivitis to periodontitis have been difficult to determine [86]. It is probable that the following elements are implicated in the disease progression to periodontitis: microbial dysbiosis, overgrowth of pathogenic bacteria, herpes virus reactivation, immune-system disruption and acquired and/or genetic susceptibility factors [87–89].

Mechanical removal of supragingival plaque is the most effective tool to prevent gingivitis [90] but most individuals do not adequately control plaque accumulation and gingivitis continues to be prevalent. To overcome this hindrance, antimicrobial products in the form of dentifrices or mouthwashes have been tested for their adjunctive efficacy in reducing plaque and gingivitis.

Probiotic technology represents a breakthrough approach to maintain oral health by utilizing natural beneficial bacteria commonly found in healthy mouths to provide a natural defence against the bacteria thought to be harmful to teeth and gums [15]. Within dentistry, the previous studies with *lactobacilli* strains such as *L. rhamnosus, L. casei, L. reuteri*, or a *Lactobacilli* mix have revealed mixed results on oral microorganisms.

Krasse et al. conducted one of the first clinical trial randomized double-blind placebo controlled. The principal aim of the study was to assess if the probiotic *L. reuteri* could be effective in the management of gingivitis and then to evaluate the influence of the probiotic on plaque and the lactobacilli population in the saliva. Fifty-nine patients with moderate to severe gingivitis were included and given either/or specific *L. reuteri* formulations (LR-1 or LR-2) at a dose of 2 × 108 CFU/day, or a corresponding placebo. At baseline (day 0), they collected saliva to determine the lactobacilli and measured gingival index and plaque on two surfaces. They taught patients to brush and to floss their teeth carefully and the treatment began. The patients returned on day 14 for final assessment of gingivitis and saliva and plaque were collected. Twenty patients were randomly given LR-1, 21 took LR-2 and 18 received inactive substances. The gingival index decreased evenly in all three groups ($p < 0.0001$). LR-1 only (not LR-2) improved more than placebo ($p < 0.0001$). Plaque index fell evenly in LR-1 ($p < 0.05$) and in LR-2 ($p < 0.01$) between day 0 and day 14 but without significant change in the inactive substance. On day 14, 65% of the patients in the LR-1 group were colonized with *L. reuteri* and 95% in the LR-2. *L. reuteri* reduce both gingivitis and plaque in patients with moderate to severe gingivitis [91].

Twetman et al. conducted a clinical trial randomized double-blind placebo controlled in patients with gingivitis (n = 42). The subjects were randomly assigned to one of three comparable arms: Group A/P (n = 15) was given one active and one test substance gum daily, Group A/A (n = 14) received two active chewing gums and Group P/P (n = 13) two placebo gums. They used chewing gum (2 times a day for 10 min in the morning and evening) with *L. reuteri* (ATCC 55730 and ATCC PTA 5289, 1×10^8 CFU). They taught the patients to chew the gums 10 min for 2 weeks and conducted bleeding on probing and GCF sampling at baseline and after 1, 2 and 4 weeks. The levels of IL-1β, TNFα IL-6, IL-8 and IL-10 were measured using luminex technology and multiplex immunoassay kits. Bleeding on probing improved and GCF volume decreased in all groups during the chewing period. Still, the results were statistically different ($p < 0.05$) only in Groups A/P and A/A. TNFα and IL-8 levels decreased significantly ($p < 0.05$) in Group A/A compared with baseline after 1 and 2 weeks, respectively. They also observed a non-significant tendency to decrease in IL-1β during the chewing period. The levels of IL-6 and IL-10 remained unchanged in all groups after 1 and 3 weeks. The elemental basis of the probiotic approach to confront inflammation in the oral cavity could be the decrease of pro-inflammatory cytokines in GCF [92].

Staab et al. conducted a parallel-designed non-blinded study. Fifty volunteer students took part in this study. The test group took a probiotic drink (*L. casei*, 100 billion per 100 ml everyday); the control group did not drink any product. After 8 weeks, individual mechanical plaque control was delayed for 96 h. Papilla bleeding index, interproximal plaque and Turesky plaque index were measured at baseline, after 8 weeks and again 96 h later. At the coincidence points, we collected GCF for evaluation of polymorphonuclear elastase, myeloperoxidase (MPO) and MMP-3. There was no difference in the interproximal plaque index and papillary bleeding between the groups. In the test group, the elastase activity and the amount of MMP-3 were significantly lower after the intake of the probiotic drink ($p < 0.001$ and 0.016). A significant increase of MPO activity was noted in the control group; both groups had differences at the end of the survey ($p = 0.014$). According to the data, it is suggested that the probiotic milk drink has a beneficial effect on the gingival inflammation [93].

Ierardo et al. conducted a clinical trial in patients with gingivitis: Test group (n = 21) consumed chewing gum (3 times per day for 60 days) containing probiotic *L. brevis*. Control group was used for the laboratory variables (n = 16). Measurements were taken at baseline, 30 and 60 days. It was concluded that *L. brevis* has anti-inflammatory effects showing clinical improvement. In addition, it allows to reduce the levels of immunoglobulin (Ig)-A [94].

Iniesta et al. conducted a clinical trial randomized double-blind in patients with gingivitis: Test Group (n = 20) obtained lozenges with *L. reuteri* (DSM-17938 and ATCC PTA 5289, 2×10^8 CFU) for two periods of 12 weeks (with an intermediate period of 4 weeks without measures of hygiene). Microbiological and clinical differences and the pattern of colonization of *L. reuteri* were determined again. In conclusion, no significant changes occurred between and within the groups in the clinical variables. Total anaerobic counts in saliva after 4 weeks ($p = 0.021$) and counts of *P. intermedia* after 8 weeks ($p = 0.030$) decreased in the test group. In subgingival samples, *P. gingivalis* counts reduced significantly from baseline to 4 weeks ($p = 0.008$). With PCR, the presence of *L. reuteri* ATCC-PTA-5289 was higher than *L. reuteri* DSM-17938. The administration of *L. reuteri* in tablets reduced the number of selected periodontal pathogens in the subgingival microbiota, with no associated clinical impact [95].

Hallstrom et al. conducted a clinical trial randomized double-blind controlled in patients with gingivitis: Group test (n = 18) accepted lozenges of *L. reuteri* (ATCC 55730 and PTA TM9061, 1×10^8 CFU), two times a day for 3 weeks (with a period of 2 weeks of experimental gingivitis). During the intervention periods, all the patients presented local plaque accumulation together with manifest gingivitis at the test sites. Both groups had an increase in the volume of GCF but it was statistically significant only in the placebo group ($p < 0.05$). The concentrations of IL1-β and IL-18 ($p < 0.05$) increased significantly, while IL-8 and macrophage inflammatory protein (MIP)1-β decreased ($p < 0.05$). No differences were found between test and inactive substance. Similarly, the microbial composition was not different between the groups. The plaque accumulation, inflammatory reaction or composition of the biofilm did not seem to be significantly affected by the daily intake of probiotic lozenges during experimental gingivitis [96].

Karuppaia et al. conducted a randomized double-blind clinical trial in patients with gingivitis (aged 14–17 years): Test group (n = 108) used curd (clump of milk) 4 weeks. The control group (n = 108) excluded curd in their diet for 30 days. Clinical differences were found, the probiotic was efficacious in reducing the plaque index and gingival index [97].

Purunaik et al. conducted a clinical trial randomized double-blind placebo controlled in patients (aged 15–16 years) (n = 90) with gingivitis: Group A (n = 30) used chlorhexidine 0.2%, Group B (n = 30) mouthwash of probiotic (*L. acidophilus, L. rhamnosus, B. longum* and *Saccharomyces boulardii*) (dose: 1.25 million mix, 2 times a day for 14 days), and Group C (n = 30) placebo (20 mL per day for 60 s.). It was found that both the chlorhexidine and the probiotic group can significantly reduce the plaque index (best chlorhexidine) and the gingival index (best probiotic) [98].

Lee et al. conducted a clinical trial randomized double-blind placebo controlled in patients (n = 34) with gingivitis: Group Test (n = 17) used lozenges of *L. brevis* (CD2, 1×10^9 CFU), three times per day × 2 weeks and Control group (n = 17) took placebo for the same period of time. It was found that probiotic reduced the bleeding on probing. There were no differences with

respect to the gingival index. The levels of NO (nitric oxid) increased in proportional form in the placebo group. The levels of MMP-8 and PGE-2 did not change [99].

Nadkerny et al. conducted a clinical trial randomized double-blind placebo controlled in three groups of patients (n = 45) with gingivitis: Group A (Test) used a mouthwash of probiotic (*L. acidophilus, L. rhamnosus, Lactibacillus sporogenes, B. longum* and *S. boulardii*; n = 15), Group B (positive control) with chlorhexidine 0.2% (n = 15), and Group C (placebo: saline solution; n = 15) for 4 weeks. The mouthwash of probiotic and chlorhexidine was efficacious in reducing the plaque index and gingival index [100].

5. Probiotics and periodontitis

Periodontitis is an inflammatory process caused by an infection, and it implicates the interaction of biofilm and immuno-inflammatory response of host [101]. Its consequence is the destruction of attachment structures of teeth, or periodontium. There are three signs of disease: clinical attachment loss, alveolar bone resorption and presence of periodontal pocket [102].

Periodontitis constitutes the second cause of tooth loss worldwide [103–105]. Moreover, studies [106, 107] performed in South and Central America have shown that the prevalence of severe disease is high (>30%) in these populations.

Periodontitis is caused by complex subgingival microbial communities, which are in a dysbiotic state [108]. However, a few bacteria in the subgingival biofilm have been associated with disease. Strong evidence concluded that *P. gingivalis, A. actinomycetemcomitans* and *T. forsythia* are periodontal pathogens [65]. Although the tooth-associated biofilm plays a role in the development of periodontitis, it is primarily the host inflammatory response that inflicts the irreversible damage on the periodontium [108]. T helper 1 and Th17 lymphocyte have been described in the pathogenesis of disease [65].

The aim of periodontal treatment is mechanical debridement of root surface. When periodontal pathogens are effectively reduced after therapy and higher proportions of host-compatible microorganisms is established, improvements in clinical parameters are achieved [109]. However, mechanical debridement as a sole therapy is not always effective to improve clinical parameters [110]. Therefore, systemic antibiotics were introduced as an adjunct to mechanical treatment [111]. This treatment modality eliminates the entire microbiota, irrespective of its pathogenicity. Also, it could generate antibiotic resistance and recolonization of treated sites with pathogenic bacteria is frequent [112, 113]. Hence, there is a need of new treatment paradigms in periodontal disease management.

Several clinical trials were conducted in order to study the effect of administration of probiotics in initial treatment of periodontitis [114–117]. The bacteria most frequently used as probiotic are *L. reuteri* (DSM 17938 + ATCC PTA 5289) [72, 118–120], *Lactibacillus salivarius* WB21 [121, 122], *L. reuteri* (ATCC 55730 + ATCC PTA 5289) [123], *L. reuteri* (ATCC PTA 5289) [61], *Streptoccus oralis* KJ3 + *Streptococcus uberis* KJ2 + *Streptoccus rattus* JH145 [124] and *L. rhamnosus* SP1 [125].

Vivekananda et al. conducted a randomized, placebo-controlled, double blind, split-mouth designed clinical study to evaluate the effect of *L. reuteri* (DSM 17938 + ATCC PTA 5289) lozenges with and without scaling and root planning (SRP) on clinical and microbiological parameters of chronic periodontitis patients. The study period was 42 days. The lozenges were used two times a day for 21 days, from day 21 to day 42. Thirty systemically healthy subjects were recruited. On day 42, plaque index, gingival index and bleeding on probing decreased for all treatments. However, the level of this reduction was higher in SRP + probiotic, and lower, in placebo (p <0.05). Probiotic, with or without SRP, reduced significantly *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* [118].

Teughels et al. conducted a randomized, parallel, controlled and double blinded clinical, whose aim was to evaluate the effect of *L. reuteri* (DSM 17938 + ATCC PTA 5289, 1×10^8 CFU)-containing lozenges as an adjunct to full mouth disinfection protocol (FMD). Thirty systemically healthy patients were recruited (n = 15 in each group). Clinical measurements and microbiological samples were collected at baseline and 3, 6, 9 and 12 months after initial therapy with FMD. The lozenges were used two times a day for 12 weeks. At the end of intervention, i.e., 12 weeks after FMD, all clinical parameters were significantly reduced in both groups. In probiotic group, there was more pocket depth reduction and attachment gain (p < 0.05). Also, there was more *P. gingivalis* reduction (p < 0.05) [119].

Tekce et al. and Ince et al. conducted a randomized, parallel, controlled and double blinded clinical trial in order to evaluate the effect of lozenges containing *L. reuteri* (DSM 17938 + ATCC PTA 5289, 1×10^8 CFU) as an adjuvant to full mouth scaling and root planning treatment for chronic periodontitis. The lozenges were used two times a day for 3 weeks. Forty systemically healthy subjects were recruited (n = 20 in test group). Clinical measurements, microbiological and GCF samples were obtained at baseline and on days 21, 90, 180 and 360. After treatment, plaque index, gingival index, bleeding on probing and probing pocket depth were lower in test group at all times points (p <0.05) [120]. Attachment gain was significantly higher in the test group on days 90, 180 and 360 (p <0.05) [72]. Total viable cell counts and the proportions of obligate anaerobes in subgingival plaque were lower in test group at all time points (p <0.05), with the exception of day 360 [120]. Also, decreased GCF MMP-8 and increased TIMP-1 levels were found to be significant up to day 180 in test group (p <0.05) [72].

Shimauchi et al. and Mayanagi et al. conducted a randomized placebo-controlled clinical trial, whose objective was to evaluate the effect of *L. salivarius* (WB21, 6.7×10^8 CFU)-containing tablet or a placebo in treatment of mild and moderate periodontitis. The dose was three tablets taken orally every day during 8 weeks. Periodontal treatment was not performed. Sixty-six systemically healthy volunteers were recruited (n = 34 in test group). Periodontal clinical parameters, whole saliva samples and supra and subgingival plaque samples were obtained at baseline, 4 weeks, and at the end of the interventional period (8 weeks). Current smokers in the test group showed a significantly greater improvement of plaque index and probing pocket depth when compared with placebo group. Salivary lactoferrin level was also significantly decreased in the test group smokers [121]. The numerical sum of five selected periodontopathic bacteria and *T. forsythia* in subgingival plaque decreased significantly in test group (p <0.05) at 4 weeks of treatment [122].

Vicario et al. conducted a randomized placebo-controlled, parallel design, double-blind clinical trial. The aim was to evaluate the effect of *L. reuteri* (ATCC 55730 + ATCC PTA 5289, 2×10^8 CFU)-containing lozenges in treatment of chronic periodontitis. Twenty systemically healthy subjects were recruited. Periodontal treatment was not performed. Subjects were advised to use a lozenge every day for 30 days. Clinical measurements were performed at baseline and at the end of interventional period. Only test group demonstrated a significant reduction in plaque index, bleeding on probing and pocket depth at the end of interventional period ($p < 0.05$) [123].

Szkaradkiewicz et al. conducted a clinical trial aimed to evaluate the pro-inflammatory cytokine response in patients with chronic periodontitis administered with probiotic *L. reuteri* (ATCC PTA 5289, 1×10^8 CFU)-containing lozenges. Control group did not receive lozenges. All patients were treated with SRP. They recruited 38 systemically healthy subjects (n = 24 in test group). The dose was two lozenges per day (authors did not mention the duration of intervention). Test group experimented a significant improvement of clinical measurements and a decrease in CGF levels of TNF-α, IL-1β and IL-17 ($p < 0.05$) [61].

Laleman et al. conducted a double-blind, placebo-controlled, randomized clinical trial with two parallel arms in chronic periodontitis. The aim was to evaluate the adjunctive effect of probiotic (*Streptoccus oralis* KJ3 + *Streptococcus uberis* KJ2 + *Streptoccus rattus* JH145, 1×10^8 CFU)-containing lozenges after FMD. The dose was two lozenges per day during 3 months. Forty-eight subjects were recruited. Clinical parameters and microbiological samples were collected at baseline, 4, 8, 12 and 24 weeks follow-up visit. In test group, plaque index was significantly lower at the 24-week evaluation ($p < 0.05$) Also, salivary *P. intermedia* counts were significantly lower at the 12-week visit in probiotic group ($p < 0.05$) [124].

Morales et al. conducted a double-blind placebo controlled parallel-arm randomized clinical trial whose objective was to evaluate the clinical effect of *L. rhamnosus* (SP1, 2×10^7 CFU)-containing sachet as an adjunct to non-surgical therapy (SRP) of chronic periodontitis. Twenty-eight systemically healthy subject were recruited (n = 14 in test group). The dose was one sachet of probiotic or placebo taken orally every day during 3 months. Treatment involved SRP per quadrant performed with 1-week intervals in four to six sessions. A periodontal supportive therapy was performed every 3 months. Clinical parameters were measured at baseline, 3, 6, 9 and 12 months follow-up visit. Both groups showed improvements in clinical parameters at all time points evaluated. However, there were no differences between groups in any visit [125, 126].

There are some systematic reviews whose aim was to explore the available clinical evidence on the efficacy of probiotic therapy in initial treatment of chronic periodontitis. There was a significant reduction in probing pocket depth, bleeding on probing, plaque index and attachment gain in probiotic group [116]. In the studies where *L. reuteri* was selected as probiotic, the reduction of probing pocket depth was 1.31–1.74 mm in probiotic group and 0.49–1.39 mm in placebo group. Also, the attachment gain was 0.99–1.39 mm in probiotic group and 0.29–0.76 mm in placebo group [117]. Finally, the authors concluded that oral administration of probiotics is a safe and effective adjunct to scaling and root planning in the treatment of chronic periodontitis. Their adjunctive use is likely to improve diseases indices and reduce the need for antibiotics [116].

The use of probiotics in supportive periodontal therapy (SPT) was reported in a clinical trial conducted by Iwasaki et al. The aim of this randomised, double-blind, placebo controlled clinical trial was to evaluate the effect of heat-killed *L. plantarum* L-137 on the outcome of SPT. Thirty-nine SPT subjects (n = 20 in test group) who completed active treatment for chronic periodontitis followed by SPT every 4 weeks were recruited. Subjects consumed a hard gelatine capsule of 50 mg of probiotic or placebo per day during 12 weeks. The SPT programmes and clinical examinations were performed at baseline 4, 8, and 12 weeks after start intervention. Bleeding on probing and sites with pocket probing depth ≥4 mm were significantly reduced in both groups. In test group, there was a significantly greater probing pocket depth (PPD) reduction in teeth with sites with PPD ≥ 4 mm at week 12 (p <0.05). This result indicates that daily intake of probiotic may be useful in SPT [127].

6. Conclusions

Oral diseases are a recognized public health problem worldwide [128, 129]. Dental caries, periodontitis and oral cancer, among other oral diseases, currently occupy the health agenda, seeking to establish policies that, when integrated with other health intervention programs, will impact the oral health of our population. Along with the economic impact on governments and individuals [130], a higher cost is added in terms of pain, discomfort, social and functional limitations, and time lost and absenteeism in schools and workplaces [131, 132].

The goals of periodontal therapy are to reduce probing pocket depth, to gain attachment level and to reduce bleeding on probing suppuration. A new microbial community is needed in order to achieve the clinical results [109]. Therefore, with the aim of potentiating the effects of periodontal treatment, other protocols, such as the association of mechanical debridement with systemic antibiotics, have been used successfully in the treatment of periodontal diseases. The problems of antibiotics are in association with the elimination of the entire microflora, irrespective of their pathogenicity, and the emergence of antibiotic resistance; the shift towards a less pathogenic microbiota is only temporary with a frequent recolonization of treated sites with pathogenic bacteria; and the temporary use of antibiotics locally or systemically, does not really improve the long-term effect of periodontal therapy.

Considering the beneficial effects of probiotics, this therapy could serve as a useful adjunct or alternative to periodontal treatment. The use of probiotics in oral care applications is gaining momentum [118]. There is increasing evidence that the use of existing probiotic strains can deliver oral health benefits. Therefore, proposing a treatment involving the non-surgical treatment plus probiotic intake may result in better regulation of bacterial plaque and thus contribute to a successful periodontal treatment, and they can also exert effects on modulating immunological parameters.

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