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Probiotic Biotherapy: Discussing its Influential Role in Oral Health

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Abstract

Introduction: Probiotics are widely known for their health promoting benefits, especially with its association to gastrointestinal health. For many years, most research has primarily focused on the prevention or treatment of gastrointestinal infections or diseases. However, recently probiotics has been a subject of investigation with its association to oral health. This review of literature is designed to analyze the effectiveness of using probiotics as a preventative and therapeutic method for oral infections/diseases such as periodontitis, dental caries, and the like.

Methods/materials: Databases such as the PubMed, and Google Scholar were utilized to find current and relevant findings for this reviewed literature. Mendeley was utilized to gather and store findings. The following keyword search items include probiotics, oral health, periodontal diseases, periodontitis, dental caries, gingivitis, halitosis, *Lactobacilli*, and biotherapy. Articles were narrowed down to include studies published within the last 5 years.

Results: Short-term clinical studies suggest that the oral intake of probiotics show a strong inhibitory effect on the growth and biofilm formation of pathogenic strains, stimulate the immune function, and regulate the inflammatory response. Their repeated success in inhibiting harmful oral bacteria suggests that the use of probiotics holds a promising future in dentistry.

Conclusion: Although still in its initial stages, the use of probiotics is being established as a living biotherapeutic designed to restore normal oral microflora in the oral cavity. More long-term clinical trials are needed to fully understand their functioning and substantiate their role in oral health.

Introduction

Probiotics has been defined by the World Health Organization (WHO) as “live microorganisms which when administered in adequate amounts, confer benefits to the health of the host.” Their use has been increasingly substantiated due to the slow process of antibiotic production and the growing spread of antibiotic resistance plaguing healthcare systems and communities worldwide [6].

The use of living therapeutics has made its mark in the field of general medicine, where research of bacterial strains such as *Bifidobacteria* and *Lactobacilli* has progressed to biotherapeutic treatments managing conditions such as inflammatory bowel disease, vaginal infections, and respiratory tract infections [1,7,8]. Research suggests that these same bacterial strains show considerable promise in inhibiting the growth of *Streptococci* and *Candida* species [9].

The oral cavity contains an intricate population of diverse bacterial species with a healthy adult harboring a vast network of 700 predominant bacterial strains covering the tongue, cheeks, gingiva, palate and surrounding tissues where *Streptococci*, viruses and fungi form about 20% of this population[1]. These microbial communities are built upon synergistic and antagonistic interactions, cell to cell signaling, and gene transfer giving biofilms their structurally sound resistance to chemical interactions mediated by the neutralizing effect of saliva, contributing to our oral health [2,3].

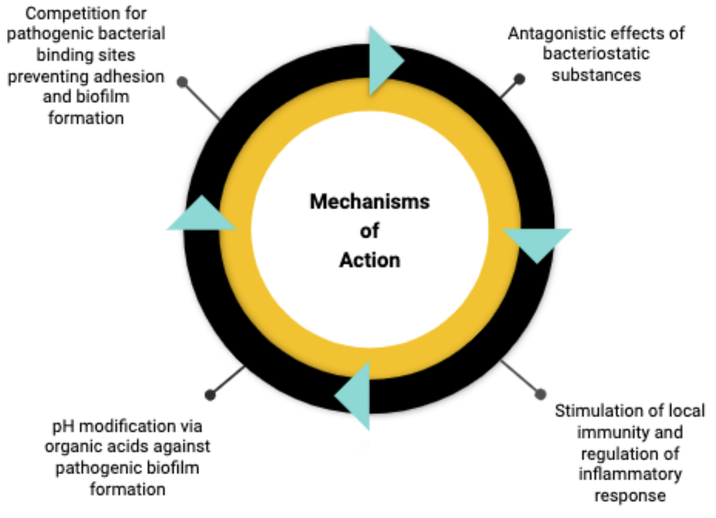
The triggering of homeostasis to dysbiosis is influenced by the pH fluctuations that create environmental conditions favoring acid tolerant bacterial species such as *S. mutans, P. gingivalis*, and *P. intermedia* giving way to several oral diseases including periodontitis and gingivitis [3,4]. In recent years, the use of antimicrobial capabilities of probiotics are being explored to combat the effects of pathogenic bacteria residing in the oral cavity, relying on the hypothesis that commensal bacteria can re-establish a neutral environment [5]. This review of literature underlines the possible therapeutic and preventative role of probiotics against the development of oral diseases.

Results

Results From the Studies Reviewed

Author/Year	Patients	Treatment	Probiotic strain/Vehicle	Conclusions
Morales *et al.* 2016	28 periodontal patients w/ 4 smokers (2 placebos)	Periodontitis - 1x daily for 3 months and monitored 3,6,9,12 months after deadline after SRP	*Lactobacillus rhamnosus* via sachet	Reduction in pocket depths after 1 year follow up
Iwasaki *et al.* 2016	19 (unspecified) test group (17 placebos)	Periodontitis - 1x daily for 12 weeks	*L. plantarum* via capsule	Reduction in pocket depths
Alok *et al.* 2017	Periodontal patients	Periodontitis - Absorb probiotic through lozenge for 4 days	*L. brevis* via lozenges	Reduction in inflammation, BOP, plaque and GI indices
Grusovin *et al.* 2019	20 patients (10 placebos)	Periodontitis - Administrations of L. reuteri lozenges 2x daily monitored 3,6,9,12 months	*L. reuteri* via lozenges	Pocket depth reduction and attachment gain within 6 months, BOP reduction at 6 to 9 months
Montero et al. 2017	59 patients (30 placebos)	Gingivitis - 2 oral tablets daily	*L. plantarum, L. brevis, Pediococcus acidilactici* via tablets	Reduction in pathogenic bacterial strain T. forsythia, no significant changes in GI indices
Alkaya et al. 2016	40 healthy patients, 18-31 years, (20 placebos)	Gingivitis - Probiotic toothpaste and mouth rinse for a period of 8 weeks along with adult prophylaxis treatments for 3 weeks	*Bacillus subtilis, bacillus megaterium, bacillus pumilus* via toothpaste and mouth rinse	Plaque and gingival indices reduced, no significant differences between test and placebo groups
Lin et al. 2017	18 patients (7-11 years)	Gingivitis - Consume Yakult, probiotic beverage, for 7 days	*Lactobacillus casei* via probiotic beverage	Increase in pH, significant cariostatic effects on oral biofilm acidogenicity
Benic et al. 2019	Orthodontic patients	Halitosis - 2 lozenges for 1 month with a 3-month follow-up	*Streptococcus salivarus* via lozenges	Reduced levels of halitosis with no significant changes in plaque and gingival indices and dental biofilm microflora.

Graph 1. Results from the published studies discussed in this review investigate the impact of probiotics on oral health utilizing various bacterial strains through multiple vehicles of administration [12-18]. The studies focus on the interaction between pathogenic bacterial strains and probiotics as a form of biotherapeutic treatment targeting periodontitis, gingivitis, halitosis, and dental caries. Most of the studies in this review demonstrate a reduction in pocket depths, plaque and GI indices, BOP, CAL, and inflammation.



Discussion

Though the mechanism of probiotics is not clearly established, probiotic strains such as *Lactobacilli* may play an important role in the maintenance and balance of indigenous microflora in the oral cavity. It has been suggested that the mode of action through which probiotics stimulate the immune function and regulate the inflammatory response involves:

- pH modification via organic acids
- Antagonistic effects on pathogens through production of bacteriostatic substances such as bacteriocins and proteins
- Impediment of adhesion and subsequent biofilm formation of pathogenic bacterial strains.

In addition, studies also emphasize that probiotic bacterial strains can exert differing effects based upon various environmental factors such as the type of pathogen present, or salivary components[17-19].These findings potentially reveal the immunological effects directed by the probiotic bacterial strains on the oral mucosa which includes [21]:

- Increased IgA antibody production
- Increased microphage activity
- Rise in phagocytotic activity

It is important to highlight that several of the studies utilized pretreatment procedures such as SRP or adult prophylaxis before the administration of probiotics[10-14]. Probiotic use in concurrence with nonsurgical periodontal therapy and preventative care may ensure a more consistent response in respect to long-term probiotic use to treat chronic diseases such as periodontitis and gingivitis. The effectiveness of probiotic use depends on a variety of factors including the type of probiotic bacterial strains, and the conditions present within the host [9]. Current findings on the potential benefits gained from the use of probiotics in the oral cavity is encouraging, but more consistent clinical research is needed to establish probiotics as a prevention and treatment method towards oral infections.

Conclusions & Future Research

Research is currently being conducted to target these obstacles, expanding towards the potential use of probiotics tailor-made to deliver treatment to the oral microflora of the individual, maximizing precision and site specificity [22].These studies need to be conducted in larger populations including long observation periods. With an increasing dilemma of antibiotic resistance contributing to the ineffective treatment of microbial diseases, probiotics as a means of biotherapy appears to be a new approach for the prevention of oral infections.

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Abstract

Introduction: Probiotics are widely known for their health promoting benefits, especially with its association to gastrointestinal health. For many years, most research has been focused on the prevention or treatment of gastrointestinal infections or diseases. However, recently probiotics has been a subject of investigation with its association to oral health. This review of literature is designed to analyze the effectiveness of using probiotics as a preventative and therapeutic method for oral infections/diseases such as periodontitis, dental caries, and the like.

Methods: Databases such as the PubMed, and Google Scholar were utilized to find current and relevant findings for this reviewed literature. Mendeley was utilized to gather and store findings. Following keyword search items include probiotics, oral health, periodontal diseases, periodontitis, dental caries, gingivitis, halitosis, *Lactobacilli*, and biotherapy. Articles were narrowed down to include studies published within the last 5 years.

Results: Short-term clinical studies suggest that the oral intake of probiotics show a strong inhibitory effect on the growth and biofilm formation of pathogenic strains, stimulate the immune function, and regulate the inflammatory response. Their repeated success in inhibiting harmful oral bacteria suggests that the use of probiotics holds a promising future in dentistry.

Conclusion: Although still in its initial stages, the use of probiotics is being established as a living biotherapeutic designed to restore normal oral microflora in the oral cavity. More long-term clinical trials are needed to fully understand their functioning and substantiate their role in oral health.

Introduction

The human microbiome is an incredibly complex structure encompassing a diverse community of microorganisms including bacteria, fungi, and viruses. The oral cavity alone contains an intricate population of diverse bacterial species with a healthy adult harboring as many as 100 billion bacteria (1). This vast network of bacterial composition has been estimated to be comprised of 700 predominant bacterial species covering the tongue, cheeks, gingiva, palate and surrounding tissues where Streptococci, viruses and fungi form about 20% of this population (2). These microbial communities are built upon synergistic and antagonistic interactions, cell to cell signaling, and gene transfer giving biofilms their structurally sound resistance to chemical interactions mediated by the neutralizing effect of saliva, contributing to our oral health (3,4).

The triggering of homeostasis to dysbiosis is influenced by the pH fluctuations that create environmental conditions favoring acid tolerant bacterial species such as *S. mutans, P. gingivalis*, and *P. intermedia* giving way to several oral diseases including periodontitis and gingivitis (4,5). In recent years, the use of antimicrobial capabilities of probiotics are being explored to combat the effects of pathogenic bacteria residing in the oral cavity, relying on the hypothesis that commensal bacteria can re-establish a neutral environment (6).

For the past 20 years, there has been a surge in interest focused on the promising effects of probiotics. Probiotics has been defined by the World Health Organization as “live microorganisms which when administered in adequate amounts, confer benefits to the health of the host.” Their use has been increasingly substantiated due to the slow process of antibiotic production and the growing spread of antibiotic resistance plaguing healthcare systems and communities worldwide (7).

The use of living biotherapeutics has made its mark in the field of general medicine, where research of bacterial strains such as *Bifidobacteria* and *Lactobacillus* has progressed to treatments managing conditions such as inflammatory bowel disease, vaginal infections, and respiratory tract infections (2,8,9). Research has suggested that these same bacterial strains have shown considerable promise in inhibiting the growth of *Streptococci* and *Candida* species (10). This review of literature underlines the possible therapeutic and preventative role of probiotics against the development of oral infections.

Methods

Databases such as the PubMed and Google Scholar were utilized to find current and relevant findings for this reviewed literature. Mendeley was utilized to gather and store findings. The following keyword search items include probiotics, oral health, periodontal diseases, periodontitis, dental caries, gingivitis, halitosis, *Lactobacilli,* and biotherapy. Articles were narrowed down to include studies published within the last 5 years.

Results

Periodontitis

Chronic periodontitis is an inflammation of the tissue surrounding the teeth, causing gum recession and destruction of the alveolar bone (10). Main bacteria associated with this disease are *P. gingivalis, T. denticola, and T. forsythia* (11).

Morales *et al.* conducted a study where they evaluate the clinical effects of the probiotic strain, *Lactobacillus rhamnosus*, in a non-surgical treatment of chronic periodontitis patients. (12). Twenty-eight volunteers with an equal proportion of male and female participated in this study. Four smokers were in the test group, while two were in the control group. Individuals received non-surgical therapy, such as scaling and root planing (SRP), and were randomly

assigned to a test (probiotic sachet containing *L.rhamnosus* SP1) or control (placebo) group. The test group administered the probiotic sachet once a day for 3 months, starting after the last session of SRP. Volunteers were monitored clinically at baseline and 3, 6, 9, 12 months after therapy. Main clinical parameters measured included BOP, plaque accumulation, probing depths (PDs), and clinical attachment loss (CAL). Upon evaluation, both test and control groups showed improvements in the clinical parameters measured. However, the test group showed significant reductions of PD than the control group. Also, after following up for 1 year, the results revealed a significant reduction in the number of individuals with pocket depths (PD) that were > 6mm, in contrast to the placebo group. Researchers concluded that administering *L.rhamnosus* SP1 resulted in similar clinical improvements compared with SRP alone (12).

Another similar study was conducted where an oral administration of heat-activated *L. plantarum* L-137 (HK L-137) was evaluated with respect to periodontal therapy outcome (13). This randomized, double-blind, placebo-controlled clinical trial included 39 patients (24 women and 15 men, mean age 66.2 years) divided into two groups (19 in the test group and 17 patients control groups). Test group received the HK L-137 capsule (10 mg) or the placebo capsule daily for 12 weeks. Clinical parameters include plaque and gingival indices, bleeding on probing (BOP), probing depth (PD) which were scored at baseline and 4, 8, and 12 weeks prior to prophylaxis together with supportive periodontal therapy visits. The study results indicate that daily intake of HK L-137 can reduce periodontal pocket depths in patients undergoing supportive periodontal therapy (13).

Another study evaluated the effect of *L. brevis* in the treatment of patients suffering from chronic periodontitis (10). Participants who have chronic periodontitis were asked to suck *L.brevis*-contained lozenges for a period of 4 days. The following results showed improved

clinical parameters (i.e. plaque and gingival indices and bleeding on probing) for all patients with significant salivary level reduction of prostaglandin E2 (PGE2) and matrix metalloproteinases (MMPs). The authors in this study emphasized that the anti-inflammatory effect of *L.brevis* could be due to a number of specific enzymes, aiding the prevention of producing nitric oxide; eventually stopping the release of PGE2 and MMP activation (10).

Grusovin et al. conducted a double-blind, randomized placebo-controlled pilot study to evaluate the efficacy of *L.reuteri* probiotic therapy with generalized stage III and IV periodontitis patients (14). This trial was conducted for 12 months with measurements being obtained every 3 months. A total of twenty patients were randomly divided into two equal groups. The test group received administrations of *L.reuteri*-contained lozenges twice a day, while the control one received a placebo. Outcome measures were probing periodontal pockets (PPD), probing attachment level (PAL), and bleeding on probing (BOP). Complications, tooth loss, and tooth survival were recorded as well. At the end of the 1-year period, the results showed some improved clinical outcomes. The test group (probiotic group) were found to have PPD reduction and PAL gain in 6 months and more BOP reduction at 6 and 9 months. The authors in this study suggested oral administration of *L.reuteri* probiotic lozenges could be considered as a useful supplement in the maintenance therapy of GPIII-IV patients (14).

Gingivitis

Montero et al. conducted a study to evaluate the effectiveness of a probiotic combination in treating gingivitis and assessing its effect on the subgingival microbiota. This placebo-controlled clinical trial was conducted for a period of 6 weeks involving a total of 59 patients (29 tests and 30 placebos). The test treatment consisted of taking two oral tablets per day containing

the combination of probiotic strains—*Lactobacillus plantarum, Lactobacillus brevis*, and *Pediococcus acidilactici*. The control group received the same tablets, but without the live bacterial strain. Outcome measures were changes in GI, collection and analysis of subgingival samples by quantitative polymerase chain reaction (qPCR) for five presumed periodontal pathogens. Both groups experienced statistical improvement in mean GI ($p < .0001$). However, no difference was found between treatment groups for any clinical index. Results revealed in the test group, a reduction in the number of sites with higher GI scores (GI=3 at baseline) were significant. In subgingival samples, a remarkable reduction of T. forsythia was significant only in the test group ($p < 0.008$). The authors concluded that the use of probiotic tablets did not lead to significant changes in the mean GI, although there is a significant decrease in the number of sites with severe inflammation (15).

A 2016 randomized, double-blind, placebo-controlled clinical trial was conducted to evaluate the effectiveness of bacilli-containing probiotic treatment in patients with gingivitis (16). Forty healthy patients (20 females and 20 males, aged 18-31 years) with generalized gingivitis were randomly assigned. The patients received a pretreatment, such as supragingival scaling and/or regular adult prophylaxis, for 3 weeks. Then, each participant in the test group (20 patients) received one toothbrush, probiotic toothpaste, two probiotic mouth rinses, and a box of probiotic toothbrush cleaner to be used for a span of 8 weeks, while the control group received similar placebo products. Probiotic products contained $5x10^7$ CFU of *Bacillus subtilis, Bacillus megaterium*, and *Bacillus pumulus* spores. Results showed that the plaque and gingival indices were significantly reduced after the 8-week treatment, yet it did not show any statistically significant differences between the probiotic and placebo group in terms of using the probiotic-contained products (16).

Caries

Streptococcus mutans is notably recognized as the primary causative microorganism and significant contributor in the etiology of dental caries. Several studies were conducted to evaluate the effect of different strains of probiotics in controlling the development of dental caries (11).

Lin et al. conducted a study to investigate the effects of Yakult intake, which is a well-known probiotic beverage consisting of *Lactobacillus casei Shirota*, on oral biofilm acidogenicity, cariogenic bacterial counts, and caries risk in children (17). Eighteen children (7-11 years) participated. They were instructed to consume standard Yakult daily for 7 days. Outcome measurements include oral biofilm acidogenicity distinguished by the Stephan curve, caries risk, and the *Lactobacillus* and *Streptococcus mutans* counts, were evaluated and calculated. The results after intervention showed significant increase in minimum pH value and significant cariostatic effects on oral biofilm acidogenicity. The authors concluded that short-term Yakult intake is beneficial in promoting oral health in children with certain oral biofilm and caries risk; however, further studies may be needed to justify or validate the efficacy of this probiotic. At present, it did not cause adverse effects or increase risks of caries (17).

Halitosis

Halitosis, or oral malodor, is a result of Gram-negative anaerobic bacteria that breaks down salivary and food proteins, generating amino acids, which eventually produces volatile malodorous compounds (VCS), causing bad breath. One of the earliest probiotic strains, *S. salivarius*, which helped reduce VCSs concentrations in individuals, represented as the predominant microorganism in the tongue microbiota (11).

A 2019 randomized, triple-blind, placebo-controlled study was conducted to evaluate the efficacy of the oral probiotic *Streptococcus salivarius* M18 on oral hygiene markers and halitosis in patients wearing orthodontic braces (18). Patients who underwent fixed orthodontic treatment were randomly assigned to a probiotic group (n=32) and a placebo group (n=32). They consumed 2 lozenges for one month. At the end of the intervention, assessments were taken at baseline, with a 3-month follow-up. Outcome measures include PI, GI, and halitosis-causing VCS levels. After analyzing the dental biofilms before and after intervention, it was found that the plaque and gingival indices were not significantly influenced by the probiotic intervention ($p > 0.05$). Yet the VCS levels had significant reduction in both the probiotic group (VCS reduction= -8.5%, 95% CI = -7.4% to -9.1%, p=0.015) and the placebo group (-6.5%, 95% CI= -6.0% to -7.4%, p=0.039) after the 1-month intervention. However, the VCS levels in the placebo group started back up to baseline levels compared to those in the probiotic test group, which decreased further (-10.8%, 95% CI= -10.5% to -12.9%, p=0.005). The authors of the study concluded that oral probiotic strain *S. salivarius* M18 reduced levels of halitosis in patients with orthodontic treatment, especially those wearing braces, but had minimal effects on PI, GI, and dental biofilm microflora (18).

Discussion

Though the mechanism of probiotics is not clearly established, probiotic strains such as *Lactobacilli* may play an important role in the maintenance and balance of indigenous microflora in the oral cavity. Results from the published studies discussed in this review investigate the impact of probiotics on oral health utilizing various bacterial strains through multiple vehicles of administration. The studies focus on the interaction between probiotic strains and pathogenic bacterial strains such as *S. mutans*, *P. intermedia, P. gingivalis* and *T. forsythia* , which are some

of the major etiologic agents that contribute to periodontitis, gingivitis, halitosis, and dental caries.

It has been suggested that the mode of action through which probiotics stimulate the immune function and regulate the inflammatory response involves pH modification via organic acids, antagonistic effects on pathogens through production of bacteriostatic substances such as bacteriocins and proteins, and impediment of adhesion and subsequent biofilm formation of pathogenic bacterial strains. In addition, studies also emphasize that probiotic bacterial strains can exert differing effects based upon various environmental factors such as the type of pathogen present, or salivary components (19–21). Most of the studies in this review demonstrate a reduction in pocket depths, plaque and GI indices, BOP, CAL, and inflammation (10,12,16,22). These findings potentially reveal the immunological effects directed by the probiotic bacterial strains on the oral mucosa which includes increased IgA antibody production, increased microphage activity, and a rise in phagocytotic activity (23). It is important to highlight that several of the studies utilized pretreatment procedures such as SRP or adult prophylaxis before the administration of probiotics (12,16). Probiotic use in concurrence with nonsurgical periodontal therapy and preventative care may ensure a more consistent response in respect to long-term probiotic use to treat chronic diseases such as periodontitis and gingivitis.

Although none of the studies in this review presented with adverse effects, there are major concerns that any probiotic-mediated treatment could induce immune function that could potentially lead to acute inflammatory responses, harmful metabolic activities, and an overly stimulated immune response. The effectiveness of probiotic use depends on a variety of factors including the type of probiotic bacterial strains, and the conditions present within the host (10). Research is currently being conducted to target these obstacles, expanding towards the potential

use of probiotics tailor-made to deliver treatment to the oral microflora of the individual, maximizing precision and site specificity (24). The use of probiotics is still in its initial stages. Current findings on the potential benefits gained from the use of probiotics in the oral cavity is encouraging, but more consistent clinical research is needed to establish probiotics as a prevention and treatment method towards oral infections.

Conclusion

Although studies have shown positive results, few limitations must be considered of these present studies. First, there was a significant heterogeneity in the studies such as using different strains of bacteria, and inconsistencies in the dosage and frequent intake of the probiotic per day. Second, the number of participants or volunteers were rather small, and the observation period was only short-term. Therefore, these studies need to be conducted in larger populations with longer observation periods. Nevertheless, the results in these studies support the effectiveness of oral probiotics in the oral cavity and the resulting clinical improvements, even though it was done on a short-term period. If these preliminary results are evaluated in a larger study scale, the practical application of probiotics could possibly be proposed in the future as a new mechanism in preventing oral diseases. With an increasing dilemma of antibiotic resistance contributing to the ineffective treatment of microbial diseases, probiotic as a means of biotherapy appears to be a new approach for the prevention of oral infections.

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