PROBIOTICS: A NOVEL STEP TOWARDS ORAL HEALTH

Esha Agarwal*, Pavan Bajaj*, C. N. Guruprasad*, Savitha Naik†, A. R. Pradeep*

ABSTRACT

The concept of microbial ecological change as a mechanism for preventing dental diseases is an important one while altered microbial ecology may also lead to dental diseases. New methods such as probiotic approaches to eliminate pathogenic members of the microbiota can be investigated. Probiotics have been extensively studied for their health promoting effects. The main field of research has been in the gastrointestinal tract. However, in the past few years probiotics have also been investigated in the oral health perspective. We suggest that probiotic treatment of diseases other than dental caries and periodontal disease should also be systematically investigated. In general, hardly any randomized controlled trials have been conducted in this area and the studies on probiotics and oral health are still in their cradle. Hence, much more investigations are called for before any evidence-based conclusions can be drawn: if or not probiotic therapy can be recommended for oral health purposes.


KEY WORDS: Probiotics; oral health; lactobacillus; bifidobacterium.

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INTRODUCTION:

Antibiotic resistance, with the emergence of multiresistant strains, is an increasingly important global problem.¹ This unfortunate development has led scientists to seek other means of combating infectious diseases.

Looking back through history, however, one forgotten concept of using bacteria beneficial to health has been resurrected and has now come under intensive research using modern study designs and methods. The term probiotics, the antonym of the term antibiotics, was introduced in 1965 by Lilly & Stillwell as Substances produced by microorganisms which promote the growth of other microorganisms.² The term ‘probiotics’ has undergone several definitions arriving at the final one, officially adopted by the International Scientific Association for Probiotics and Prebiotics term, outlining the breadth and scope of probiotics as they are known today: ‘Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host’.² The idea of probiotics dates back to the first decade of 1900 when the Ukrainian bacteriologist and Nobel Laureate Ilya Metchnikof studying the flora of the human intestine developed a theory that senility is caused by poisoning of the body by the products of some of these bacteria. To prevent the multiplication of these organisms he proposed a diet containing milk fermented by lactobacilli which produces a large amount of lactic acid and for a while this diet became widely popular. Table 1 presents some of the main fields of activity of probiotics in general medicine. Discussing these investigations in detail, however, is beyond the scope of the present review. This is a literature review and the database used for this review search was PubMed or MEDLINE.

There are a number of different organisms that can be classified as probiotics. The most common probiotic strains belong to the genera Lactobacillus and Bifidobacterium. Lactobacillus species from which probiotic strains have been isolated include L. acidophilus, L. johnsonii, L. casei, L. rhamnosus, L. gasseri, and L. reuteri. Bifidobacterium strains include B. bifidum, B. longum, and B. infantis. Within dentistry, studies with L. rhamnosus GG, L. reuteri have defined their potential in interacting with Streptococcus mutans (S. mutans) by reducing the number of this caries pathogen, thus suggesting a role of probiotics in caries prophylaxis.⁴,⁵,⁶ A few studies also revealed that probiotic Lactobacillus strains were useful in reducing gingival inflammation and the number of black-pigmented rods including Porphyromonas gingivalis (P. gingivalis) in the saliva and subgingival plaque.⁷,⁸,⁹ It has recently been observed that probiotic administration reduced oral Candida counts in the elderly – a finding that might offer a new strategy for controlling oral yeast infections.¹⁰ Table 2 presents the possible probiotic strains in the oral cavity.
In contrast, prebiotics are generally defined as not digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already established in the colon, and thus in effect improve host health. These prebiotics include inuline, fructo-oligosaccharides, galacto-oligosaccharides and lactulose. The concept of prebiotics essentially has the same aim as probiotics, which is to improve host health via modulation of the intestinal flora, although by a different mechanism. However, there are some cases in which probiotics may be beneficial for the probiotic, especially with regard to bifidobacteria. This is known as the symbiotic concept. Synbiotics are defined as mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host.

The term probiotics is often connected to the term functional foods. This term comprises the knowledge of the relationship between foods and health and the effect of food ingredients on physiological functions.

The term replacement therapy (also called bacteriotherapy or bacterial interference) is sometimes used interchangeably with probiotics. Although both approaches use live bacteria for the prevention or treatment of infectious disease, there are some slight differences (Table 3).

The replacement therapy involves the direct application of the effector strain on the site of infection and it is directed at displacing or preventing colonization of a pathogen. The concept of periodontal replacement therapy, consists of applying beneficial oral bacteria subgingivally to prevent re-colonization of periodontal pockets by pathogens after scaling and root planing. The mechanism behind the concept seems to be related to bacterial interference.

Yet, there is a paucity of information regarding the contributions of probiotics to oral health. The present review article aims at summarizing the literature published in the past few years with respect to the possible role of probiotics on oral and dental health.

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<th>TABLE 1: VARIOUS FIELDS WHERE PROBIOTICS HAVE BEEN INVESTIGATED</th>
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<td>Cancer risk reduction</td>
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<tr>
<td>Gastrointestinal health</td>
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<td>Urinary tract health</td>
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<td>Immune response induction</td>
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<td>Antimicrobial potential</td>
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<tr>
<td>S. salivarius</td>
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<tr>
<td>L. rhamnosus GG, L. acidophilus, L. casei</td>
</tr>
<tr>
<td>L. reuteri</td>
</tr>
<tr>
<td>Bifidobacterium DN-173 010</td>
</tr>
<tr>
<td>L. rhamnosus GG, Propionibacterium freudenreichii ssp. shermanii JS</td>
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</table>
TABLE 3: DIFFERENCES BETWEEN PROBIOTIC THERAPY AND REPLACEMENT THERAPY

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<tr>
<th></th>
<th>PROBIOTIC THERAPY</th>
<th>REPLACEMENT THERAPY</th>
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<tr>
<td>L. rhamnosus, L. paracasei, L. johnsonii</td>
<td>23 In vitro Adherence</td>
<td>Better adherence than bifidobacteria</td>
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<tr>
<td>W. cibaria</td>
<td>20 In vitro Adherence</td>
<td>S-protein positively affects adhesion</td>
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<tr>
<td>L. casei Shiroti, L. acidophilus</td>
<td>46 In vitro Adhesion</td>
<td>Different pattern of adhesion according to the test strain</td>
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<td>L. rhamnosus GG</td>
<td>19 In vivo Adherence</td>
<td>Only temporary colonization in oral cavity</td>
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PROBIOTIC STRAINS IN THE ORAL CAVITY:

An essential requirement for a microorganism to be an oral probiotic is its ability to adhere to and colonize surfaces in the oral cavity. Microorganisms generally considered as probiotics may not have oral cavity as their inherent habitat and, subsequently, their possibility to confer benefit on oral health is then questionable. Paster et al. in an attempt to determine bacterial diversity in the human subgingival plaque by using culture-independent molecular methods have estimated that the total species diversity in the oral cavity ranges between 500 and 600 species. This number was further extended by Kazor et al. who detected 200 additional unknown species on the dorsum of the tongue, making the number of species in the mouth to reach 700. Lactobacilli make approximately 1% of the cultivable oral microflora. The most common lactobacilli species recovered from saliva in a study by Teanpaisan and Dahlen were L. fermentum, L. rhamnosus, L. salivarius, L. casei, L. acidophilus and L. plantarum. Three of them are probiotic strains used in dairy products. A similar diversity in the oral lactobacilli flora was observed by Colloca et al who found L. fermentum, L. plantarum, L. salivarius and L. rhamnosus to be the predominant species in healthy human mouth. Koll-Klais et al found no differences in salivary lactobacilli counts between chronic periodontitis and healthy patients, L. gasseri and L. fermentum being the predominant species among other isolates: L. oris, L. plantarum, L. paracasei, L. rhamnosus, L. gasseri, L. acidophilus and L. cispatus. These findings indicate that lactobacilli as members of resident oral microflora could play an important role in the microecological balance in the oral cavity.

These studies further demonstrated that lactobacilli strains with probiotic properties may indeed be found in the oral cavity. Yet there is no evidence whether these lactobacilli strains were detected due to the frequent consumption of dairy products leading to temporary colonization only, or if the oral environment is their permanent habitat. There are no long-term follow-up studies published to answer this question.

PROBIOTIC ACTIVITY IN THE ORAL CAVITY:

The mechanism of adhesion to oral surfaces is an issue of importance for the long-term probiotic effect of the microorganisms. Among the different assays available to study the adhesion phenomenon, two model systems predominate: systems using saliva-coated hydroxylapatite, and hydroxylapatite coated with buffers, proteins, and other substances. The pattern of adhesion of different probiotic strains to oral epithelial cells has been tested as well. Most of the experiments on adhesion have been carried out with
strains broadly used as probiotics in dairy products such as yogurt and cheese (Table 4).

Yli-Knuuttila et al assessed colonization of *L. rhamnosus* GG (LGG) in the oral cavity of healthy students. After the 14-day trial period, the occurrence of LGG in the oral cavity decreased gradually, indicating that no permanent colonization had occurred and that the oral persistence of LGG was only temporary.19 However, further colonization studies with larger materials and in different patient groups are still needed. A relatively new strain and a potential candidate for a probiotic, *Weissella cibaria* (*W. Cibaria*), isolated from humans and animals worldwide, as well as from fermented foods, was tested for co-aggregation ability with *Fusobacterium nucleatum* (*F. nucleatum*) and their attachment to epithelial cells.20 *F. nucleatum* plays an important role as a bridge-organism that facilitates the colonization of other bacteria by co-aggregation.21 It has also been suggested that the co-aggregation abilities of lactobacilli species might enable them to form a barrier that prevents colonization of pathogenic bacteria, due to the production of a microenvironment around these pathogens in which inhibiting substances were generated by *Lactobacillus* species.22

**TABLE 4: DIFFERENT MEANS OF PROBIOTIC ADMINISTRATION FOR ORAL HEALTH PURPOSES**

<table>
<thead>
<tr>
<th>VEHICLE</th>
<th>STRAIN</th>
<th>OUTCOME</th>
<th>REFERENCE</th>
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<tr>
<td>Lozenge</td>
<td><em>S. salivarius</em></td>
<td>Reduces oral VSC levels</td>
<td>31</td>
</tr>
<tr>
<td>Straw, tablet</td>
<td><em>L. reuteri</em> ATCC 55 730</td>
<td><em>S. mutans</em> level reduction</td>
<td>25</td>
</tr>
<tr>
<td>Cheese</td>
<td><em>L. rhamnosus</em> GG; <em>Prorionibacterium JS</em></td>
<td>Reduced risk of high yeast counts and Hyposalivation</td>
<td>10</td>
</tr>
<tr>
<td>Rinse solution</td>
<td><em>W. cibaria</em></td>
<td>Reduction of VSC</td>
<td>20</td>
</tr>
<tr>
<td>Capsule, liquid</td>
<td><em>L. sporogenes, L. bifidum, L. bulgaricus, L. thermophilus, L. acidophilus, L. casei, L. rhamnosus</em></td>
<td>Increased salivary counts of lactobacilli without significant decrease in <em>S. mutans</em> counts</td>
<td>33</td>
</tr>
<tr>
<td>Yogurt drink</td>
<td><em>L. rhamnosus</em> GG</td>
<td>Temporary oral cavity colonization</td>
<td>19</td>
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</tbody>
</table>

Kang et al reported that *W. cibaria* efficiently co-aggregated with *F. nucleatum*. Pronase treatment led to additional reduction in co-aggregation between both species, thus indicating the proteinaceous character of the interspecies interaction.20 Heat-resistant components firmly attached to the cell surface of *W. cibaria* were responsible for the co-aggregation with *F. nucleatum*. The results of this study clearly showed that the S-layer proteins of the bacterial cell wall may play an important role in the adherence of *W. cibaria* to the epithelial cells. In a study addressing the survival of bacteria in saliva and their adherence to oral surfaces, Haukioja et al tested the colonization potential of different commercially available probiotics and *Lactobacillus* and *Bifidobacterium* strains obtained from the dairy industry.23 The results cast light on several controversial points reflecting mechanisms of colonization in the oral cavity. All test strains demonstrated 24-h survival rates in saliva but with great variations among the strains in their binding capacity to the saliva-coated surfaces. Lactobacilli showed better adherence than bifidobacteria. Thus, lactobacilli may compete for the same binding sites on saliva coated hydroxyapatite with *F. nucleatum* which explains their lower colonization capacity. This phenomenon indicates that probiotics might affect the formation of oral biofilms and modify resident microflora. Haukioja et al defined a novel mechanism whereby lactobacilli and *B. lactis* Bb12 affected the composition of salivary pellicle on hydroxyapatite and thereby inhibited *S. mutans* adherence in vitro.23

**PROBIOTICS AND DENTAL CARIES:**

The impact of oral administration of probiotics on dental caries has been studied in several experiments utilizing different test strains. *Lactobacillus rhamnosus* GG15 and *L. casei*24 have proved their potential to hamper growth of these oral streptococci. Caglar et al registered definite *S. mutans* count reduction after a 2-week consumption of yoghurt containing *L. reuteri*.25 A temporary reduction in *S. mutans* was observed during the period of yogurt intake and few days after cessation of consumption, indicating the necessity of continual administration of the probiotic in order to achieve an effect. Little information is available about the relationship between probiotic bifidobacteria and counts of *S. mutans*. The only study addressing this
study question tested *Bifidobacterium* DN-173 010. A statistically significant reduction in salivary mutants streptococci was observed. Due to the limitations of the study protocol with *bifidobacteria*, however, further investigations are needed for drawing final conclusions.

Considering the growing body of evidence about the role of probiotics on caries pathogens, however, it has been suggested that the operative approach in caries treatment might be challenged by probiotic implementation with subsequent less invasive intervention in clinical dentistry.26 However, more studies are definitely needed before this goal could be achieved. Most of the studies cited above do not meet the criteria of investigations for evidence-based medicine. Therefore, further long term Randomized, placebo controlled, double blinded, clinical trials with a longitudinal prospective study design and a large sample size are needed to confirm these findings.

**PROBIOTICS AND PERIODONTAL DISEASE:**

Chronic Periodontitis could also benefit from orally administered probiotics. The presence of periodontal pathogens could be regulated by means of antagonistic interactions. A decrease in gingival bleeding and reduced gingivitis has been observed by Krasse et al8 with the application of *L. reuteri*. Koll-Klais et al reported that resident lactobacilli flora inhibits the growth of *P. gingivalis* and *Prevotella intermedia* (*P. intermedia*) in 82% and 65%, respectively.17

Probiotic strains included in periodontal dressings at optimal concentration of 108 CFU/ml have been shown to diminish the number of most frequently isolated periodontal pathogens: *Bacteroides* sp., *Actinomyces* sp. and *S. intermedius*, and also *C. albicans.*27 These authors registered a 10- to 12-month remission period after periodontal treatment by application of the periodontal dressing that comprised collagen and *L. casei*. Grudianov et al reported that probiotics were effective in normalization of microbiota in periodontitis and gingivitis patients when compared with a control group.28

Recently, Teughels et al reported that the subgingival application of a bacterial mixture including *Streptococcus sanguinis*, *Streptococcus salivarius* (*S. salivarius*), and *Streptococcus mitis* after scaling and root planing significantly suppressed the re-colonization of *Porphyromonas gulae* (canine *P. gingivalis*) and *P. intermedia* in a beagle dog model.29 In a double-blind, randomized, placebo controlled clinical trial in healthy volunteers without severe periodontitis conducted to evaluate whether the oral administration of probiotic tablets containing *L. salivarius* WB21 could change the clinical parameters of periodontal tissues and the expression of salivary inflammatory markers, it was found that probiotics could be used in the improvement of oral health in subjects at risk of periodontal disease.30 However, further longitudinal studies are required to confirm these findings.

**PROBIOTICS AND IMBALANCED ORAL ECOSYSTEM:**

Halitosis, the oral malodor, is a condition normally ascribed to disturbed commensal microflora equilibrium. It has recently been positively affected by regular administration of probiotics. Kang et al have shown a definite inhibitory effect on the production of volatile sulfur compounds (VSC) by *F. nucleatum* after ingestion of *W. cibaria* both in vitro and in vivo.20 In children, a marked reduction in the levels of H2S and CH3SH by approximately 48.2% (P < 0.01) and 59.4% (P < 0.05), respectively, was registered after gargling with *W. cibaria* containing rinse. The possible mechanism in the VSC reduction is the hydrogen peroxide generated by *W. cibaria* that inhibits the proliferation of *F. nucleatum*, *S. salivarius*, also a possible candidate for an oral probiotic, has demonstrated inhibitory effect on VSC by competing for colonization sites with species causing an increase in levels of VSC.31 Burton et al further reported that *S. salivarius* strain K12 produced two antibiotic bacteriocins, compounds that are inhibitory to strains of several species of gram-positive bacteria implicated in halitosis.32

However, the few studies published on the role of probiotics in the treatment of halitosis do not entitle any evidence-based conclusions. Nevertheless, we think that this might be an area where probiotic therapy indeed could bring something new if the preliminary observations on the balancing effect of probiotics on VSC-generating microflora are confirmed. Randomized, blinded, and placebo-controlled studies with large enough patient materials are also needed in this area.

**PROBIOTICS AND YEASTS:**

*Candida albicans* (*C. albicans*) is among the most common infectious agents in the oral cavity. The incidence of yeast infections is higher at older age and under conditions of impaired immunity. Testing the pattern of colonization of *L. acidophilus* and *L. fermentum*, Elahi et al showed a rapid decline in *C. albicans* in mice after the intake of probiotic strains.32

Continuous consumption of probiotics led to almost undetectable numbers of fungi in the oral cavity, maintaining the protective effect for a prolonged period after cessation of application. The capacity of different *lactobacilli* to stimulate cellular and humoral factors of mucosal protection varies particularly in terms of salivary nitrous oxide and c-interferon levels. Elahi et al have observed a correlation between the highest peak of interleukin-4 secretion and complete eradication of *C. albicans*.33 The results obtained in animal studies, however, require further testing of the
effect of the strains on cases with clinically manifested C. albicans infection in humans.

A reduction in the prevalence of C. albicans in the elderly after consumption of probiotic cheese containing L. rhamnosus GG and Propionibacterium freudenreichii ssp. shermanii JS has been registered by Hatakka et al. which was as an interesting observation in this randomized placebo-controlled trial. It could be hypothesized that extending research on yeast infections, with respect to probiotics, and analyzing the molecular mechanisms of probiotic activity, might further broaden the field of their potential applications.

ADMINISTRATION OF PROBIOTICS:

Appropriate forms of administration of probiotic strains have been discussed in several articles. Dairy products supplemented with probiotics are a natural means of oral administration and can be easily adopted in dietary regime. However, for the purposes of prevention or treatment of oral diseases, specifically targeted applications, formulas, devices, or carriers with slow release of probiotics might be needed.

Montalto et al administered probiotic mix both in capsules and in liquid form without observing statistically significant difference, however, in the S. mutans counts between the two test groups. Table 4 summarizes the variety of vehicles used so far for the administration of probiotics for oral health purposes.

SAFETY ISSUES:

The issue of safety is of special concern during the past few years due to the increased probiotic supplementation of different food products. From the safety point of view, the putative probiotic microorganisms should not be pathogenic, should not have any growth-stimulating effects on bacteria causing diarrhea, and should not have an ability to transfer antibiotic resistance genes. The probiotics should rather be able to maintain genetic stability in oral microflora.

The increased probiotic consumption inevitably leads to increased concentrations of these species in the host organism. Lactobacillus bacteremia is a rare entity, and data on its clinical significance are mainly found through case reports. For the last 30 years there have been approximately 180 reported cases. Clinical characteristics of Lactobacillus bacteremia are highly variable, ranging from asymptomatic to septic shock-like symptoms. Any viable microorganism is capable of causing bacteremia, however, especially in patients with severe underlying diseases or in immunocompromised state. Nevertheless, the present literature supports the conclusion that the incidence of Lactobacillus bacteremia is unsubstantial and that all the cases where it has been registered are individuals with other systemic diseases such as diabetes, cardiovascular diseases, gastrointestinal disorders, malignancies, or organ transplant patients. However, it is evident that careful monitoring is needed in this regard in the future.

CONCLUSIONS AND FUTURE RESEARCH:

The concept of Elie Metchnikoff is now supported by increasing evidence that some members of the gut microflora indeed are beneficial for health. They are probably going to play an important role in combating problems arising from antimicrobial resistance. It remains to be seen, however, whether Metchnikoff’s ideas are applicable to promoting oral health, as the results of the first intervention trials seem to suggest. The concept casts new light on the connections between diet and health, including oral health. However, data on oral probiotics’ are yet insufficient, and it is not known whether the putative probiotic strains could modulate immune response in the oral cavity as has been suggested to take place in the gut mucosa. The epithelial structure and chemical composition of excretions in the gastrointestinal tract differ from those in the mouth mucosa and saliva.

The resident microbiota is also different in these anatomic sites. Consequently, results from studies conducted in patients with gastrointestinal diseases cannot be directly adopted in oral medicine and dentistry. The oral cavity with its diversity of microbial species has been shown to harbor strains also distinguished as such as probiotics. In this regard further studies identifying resident probiotics of the mouth, clarifying the mechanism of their colonization, and the eventual effect on the oral environment are needed. Of particular interest might be studies on the combined effect of different probiotics applied simultaneously, thus testing the possible additive, cumulative, or competitive modes of action in the oral environment. Randomized controlled trials are needed to assess the best means of administering probiotics and the dosages needed for different preventive or therapeutic purposes.

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