PROBIOTICS - FRIENDLY BACTERIAL SUPPLEMENT

‘A MODERN OPINION’

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Introduction

Chemotherapeutics are widely used to prevent and treat infections caused by indigenous and exogenous microbes. The availability of effective and cheap antibiotics in the latter half of the 20th century revolutionized the treatment of infectious diseases and reduced the death rate drastically. The Nobel laureate in immunology, Macfarlane Burnett, stated in 1962 that “By the late twentieth century, we can anticipate the virtual elimination of infectious diseases as a significant factor in social life”.1 But this happiness was short-lived as the development of resistance to a range of antibiotics by some important pathogens has raised the possibility of a return to the pre-antibiotic “dark-ages”. These developments have encouraged researchers in various fields of healthcare to develop alternative antimicrobial approaches. The application of ‘health-promoting’ bacteria for therapeutic purposes is one of the strongest emerging fields in this regard.1 It was at that time known that milk fermented with lactic-acid bacteria inhibits the growth of proteolytic bacteria because of the low pH produced by the fermentation of lactose. Metchnikoff had also observed that certain rural populations in Europe, for example in Bulgaria and the Russian steppes that lived largely on milk fermented by lactic-acid bacteria were exceptionally long lived. Based on these facts, Metchnikoff proposed that consumption of fermented milk would “seed” the intestine with harmless lactic-acid bacteria and decrease the intestinal pH and that this would suppress the growth of proteolytic bacteria. Metchnikoff himself introduced in his diet sour milk fermented with the bacteria he called ‘Bulgarian Bacillus’ and found his health benefited.2 In 1920, Rettger demonstrated that Metchnikoff’s ‘Bulgarian Bacillus’, later called Lactobacillus delbrueckii subsp. bulgaricus, could not live in the human intestine, and the fermented food phenomenon petered out.3 Metchnikoff’s theory was disputable at this stage, and people doubted his theory of longevity. The concept of beneficial-for-health microorganisms dates back to the ideas of Nobel Prize laureate Ilya Metchnikov in the early years of the 20th century. Working at the Pasteur Institute in Paris, the Ukrainian bacteriologist laid down the scientific foundations of probiotics.4 Each day, every human being ingests a large number of living microorganisms, predominantly bacteria. Although these organisms are naturally present in food and water, they can also be deliberately added during the processing of foods such as sausages, cheese, yoghurt and fermented milk products to render health benefits.5 These organisms are known as ‘Probiotics’. By definition Probiotics are ‘live microorganisms which when administered in adequate amounts confer health benefits’. Inactivated microorganisms or their cell components may also exert beneficial effects on the human health and such findings have broadened the spectrum of probiotics by abating the importance of
live microorganisms. It has also been recommended for probiotics to be combined with prebiotics, thus forming a symbiotic combination with proven health benefits. This concept of simultaneous use of prebiotics and probiotics is known as synbiotic which reduces the dose, frequency, or duration of each treatment. Prebiotics are defined as non-digestible carbohydrates used as a nutrient source by probiotic species in the gut that promote their establishment and extend their mode of action. The vast majority of probiotic bacteria belong to the genera Lactobacillus, Bifidobacterium, Propionibacterium and Streptococcus. Several clinical studies have already demonstrated the effectiveness of certain probiotics in the treatment of systemic and infectious diseases. Clinical symptoms that have been reportedly treated or have the potential to be treated with probiotics include diarrhoea, gastroenteritis, irritable bowel syndrome, and inflammatory bowel disease (Crohn’s disease and ulcerative colitis), cancer, depressed immune function, inadequate lactase digestion, infant allergies, failure-to-thrive, hyperlipidaemia, hepatic diseases, helicobacter pylori infections, HIV and others. Although the use of such probiotics specifically to improve oral health is still in its infancy, the potential application of probiotics for oral health has recently attracted the attention of several teams of researchers. Clinical studies conducted so far, suggest that probiotics could be useful in preventing and treating oral infections, including dental caries, periodontal disease and halitosis.

**Probiotics in Oral Health**

The oral cavity is a rather intricate habitat providing the establishment of a great diversity of microbial species. Each environment within the mouth supports distinct yet overlapping communities of hundreds of species. It has been recently estimated that over 1000 bacterial species are present in the oral cavity. Bacteria reside in the mouth either in planktonic state or are finely integrated in oral biofilm on various oral surfaces. Interaction between species in biofilms is characteristic as the species may depend on each other to provide favorable environment for colonization. Furthermore, bacteria in biofilms differ physiologically from their planktonic counterparts and tend to be much more resistant to environmental factors and antimicrobial agents. It has been established that distinct genes become active when planktonic bacteria bind to surfaces and grow in biofilms. This complexity of biofilm development and interspecies interactions require more thorough investigations in order to assert true probiotic candidates with activity in the oral cavity. It is essential for these beneficial microorganisms to resist the oral environmental conditions and defence mechanisms, to be able to adhere to saliva-coated surfaces, to colonize and grow in the mouth, and to inhibit oral pathogens. The term replacement therapy sometimes has been used interchangeably with
‘probiotics’. Although both approaches use live bacteria for prevention or treatment of infectious disease, the replacement therapy includes application of bacterial strain directly at the site of infection to form a colony, which is directed at displacing or preventing the colonization of the pathogen. It also involves dramatic and long-term change in the indigenous microbiota. The replacement therapy has shown minimal impact on the immune system.5

Probiotics in Gingivitis
L. reuteri and L. brevis are among the species able to affect gingivitis and plaque composition positively as well as being specific markers for periodontal disease.11 Krasse and colleagues (2006) assessed the beneficial effect of L. reuteri against gingivitis. After 14 days of ingesting the probiotic incorporated into chewing gum, the oral cavity of patients with a moderate to severe form of gingivitis had been colonized by L. reuteri and the plaque index had been reduced. Although the exact mechanisms of action of L. reuteri remain to be elucidated, previous studies have suggested at least three plausible possibilities: first, secretion of bacteriocins (reuterin and reutericyclin), that inhibit the growth of a wide variety of pathogens; second, adherence of L. reuteri to host tissues, thereby competing with pathogenic bacteria; and third, the recognized anti-inflammatory effects of L. reuteri, leading to inhibition of secretion of pro-inflammatory cytokines, could be the foundation for a direct or indirect beneficial effect of this bacterium.11

Probiotics in Periodontal Diseases
The current view on the etiology of plaque-related periodontal inflammation considers three factors that determine whether disease will develop in a subject: a susceptible host; the presence of pathogenic species; and the reduction or absence of so-called beneficial bacteria. Because it is difficult to influence the host response without the risk of serious side-effects (e.g. as a result of the use of cyclooxygenase-2 inhibitors), periodontal therapy especially envisages the reduction of the bacterial threat. The main pathogenic agents associated with periodontitis are Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia and Aggregatibacter actinomycetemcomitans. These bacteria have a variety of virulent characteristics allowing them to colonize the subgingival sites, escape the host’s defense system and cause tissue damage. The persistence of the host’s immune response also constitutes a determining factor in progression of the disease.12 The worldwide treatment strategy applied for periodontal disease is based on mechanical subgingival debridement eventually including periodontal surgery to reduce the depth of the periodontal pocket improving the oral hygiene. This shifts the sub gingival flora to a less pathogenic composition, characterized by high proportions of gram-positive aerobic species. Although reductions in the total sub gingival micro
biota of up to two-log values can easily be achieved, a re-colonization, primarily by less pathogenic bacteria, towards baseline numbers occurs within 1-2 weeks.\(^1\) The shift towards a less pathogenic microbiota is only temporary, with the re-establishment of a more aggressive microbiota within weeks to months. The dynamics of this re-colonization depends on the level of oral hygiene, the efficacy of the sub gingival debridement and the residual probing depth. The use of antibiotics or antiseptics, either locally or systemically, does not really improve the long-term effect of periodontal therapy. Therefore, some authors have started to focus on the third etiological factor for plaque-related periodontal inflammation, namely the reduction or absence of so-called beneficial bacteria.\(^1\) In one recent study, the prevalence of *lactobacilli*, particularly *L. gasseri* and *L. fermentum*, in the oral cavity was greater among healthy participants than among patients with chronic periodontitis. Studies have reported the capacity of *lactobacilli* to inhibit the growth of periodontopathogens, including *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*. Together, these observations suggest that *lactobacilli* residing in the oral cavity could play a role in the oral ecological balance.\(^13\) Riccia and colleagues recently studied the anti-inflammatory effects of *L. brevis* in a group of patients with chronic periodontitis. The treatment, which involved sucking on lozenges containing *L. brevis* over a period of 4 days, led to improvements in the targeted clinical parameters for all patients. The authors suggested that the beneficial anti-inflammatory effects of *L. brevis* could be attributed to its capacity to prevent the production of nitric oxide and, consequently, the release of PGE2 and the activation of MMPs induced by the nitric oxide and, consequently, the release of PGE2 and activation of MMPs induced by the nitric oxide.\(^14\) During the fermentation process in milk, *L. helveticus* produces short peptides that act on osteoblasts and increase their activity in bone formation.\(^14\) These bioactive peptides could thereby contribute to reducing the bone resorption associated with periodontitis.\(^9\) Shimazaki and colleagues used epidemiological data to assess the relationship between periodontal health and the consumption of dairy products such as cheese, milk and yoghurt and found that individuals, particularly non-smokers, who regularly consumed yoghurt or beverages containing lactic acid exhibited lower probing depths and less loss of clinical attachment than individuals who consumed few of these dairy products. By controlling the growth of the pathogens responsible for periodontitis, the lactic acid bacteria present in yoghurt would be in part responsible for the beneficial effects observed.\(^15\)

**Probiotics in Growth Inhibition of Causal Organisms of Periodontal Disease**

The first study linking probiotics to periodontics was initiated in the late 1970s by Socransky’s and found that subgingival plaque samples of healthy patients contained organisms that could inhibit the
growth of *A. actinomycetemcomitans* and other periodontopathogens. These microorganisms that inhibited the growth of periodontopathogens were almost invariably identified, at that time, as *S. sanguis* and *S. uberis*. The basis for their inhibition of *A. actinomycetemcomitans* lay in the production of hydrogen peroxide. These findings, together with the strong negative association between *A. actinomycetemcomitans* and *S. sanguinis* encouraged these researchers to proceed to in vivo studies using *S. sanguinis* as an effector strain.

**Probiotics in the halitosis**

Although halitosis is primarily of oropharyngeal origin, the first report on the treatment of halitosis via probiotics claims to treat a gut-caused halitosis. In a case report, Henker et al. describe the history of, in 2001, a 9.5-year old girl was treated with a suspension of live non-pathogenic bacteria (*E. coli* strain Nissle 1917), 2 ml daily for almost 3 months. The breath gas analysis showed a result comparable to that of the healthy test subjects. In a series of in vitro experiments, *Weisella cibaria* was shown to be the most effective at inhibiting *F. nucleatum* viability and its production of volatile sulfur compounds (VSC). The *W. cibaria* strains have, thus far, never been associated with any pathogenicity and they have the ability to co-aggregate with *F. nucleatum*, hence, are more easily removed from the oral environment by salivary flow. Kang et al showed that *W cibaria* isolates used as mouthrinse possess the ability to inhibit VSC production under both in vitro & in vivo conditions, demonstrating that they bear the potential for development into novel probiotics for use in the oral cavity. Burton et al also investigated the effect of *S. salivarius* on oral malodour parameters of 85% of the patients in the experimental group and 30% of the patients in the placebo group who had substantial (>100 parts per billion) reductions in volatile sulphur compound scores.

**Anticipated Mechanisms of Probiotic Activity**

Periodontal diseases are largely caused by specific gram-negative anaerobic bacterial infections, leading to the initial destruction of the soft connective tissue and, subsequently, to the disruption of the underlying alveolar bone and ligament supporting the teeth. The bacterial species *T. forsythia*, *P. gingivalis* and *T. denticola* have been the most commonly found pathogens in subgingival dental plaque. Several mechanisms have been proposed to explain how probiotics work. They exert antimicrobial activity by decreasing the pH locally, secreting antimicrobial peptides like human β defensins, inhibiting bacterial invasion and blocking bacterial adhesion to epithelial cells. They also enhance the barrier function by increasing mucus production and enhancing barrier integrity. Probiotic bacteria act through Pattern Recognition Molecules or Toll-Like Receptors, like TLR-2 and TLR-4, possibly on epithelial cells and induce the production of
protective cytokines that enhance epithelial cell regeneration and inhibit epithelial cell apoptosis. They have also shown distinct immunomodulatory effects on epithelial cells, dendritic cells, monocytes/macrophage, lymphocytes and NK cells. In the oral cavity a probiotic candidate should be able to co-aggregate and adhere to the microbes present in the oral biofilm to exert health benefits. Once the probiotics establishes itself in the oral biofilm, it affects the pathogenic potential of the species based on their antimicrobial activity. Sookhee and colleagues isolated 3,790 strains of lactic acid bacteria from 130 individuals and found that the isolates identified as L. paracasei and L. rhamnosus had a high capacity to antagonize important oral pathogens, including S. mutans and P. gingivalis. Weissella cibaria, a gram-positive facultative anaerobic is considered a potential probiotic agent as it secretes a significant quantity of hydrogen peroxide as well as bacteriocin that acts against gram positive bacteria. This bacterial species also has the capacity to co-aggregate with F. nucleatum and to adhere to epithelial cells. These properties could enable W. cibaria to effectively colonize the oral cavity and limit the proliferation of pathogenic bacteria. The probiotic species should also be able to prevent colonization of the periodontal pathogen. Studies have shown that although lactobacilli are not a common inhabitant of the gingival sulcus, they still can be effective against P. gingivalis.

Probiotics in Dental Caries

In caries, there is an increase in acidogenic and acid tolerating species such as mutans streptococci and lactobacilli, although other bacteria with similar properties can also be found like Bifidobacteria, non-mutans streptococci, Actinomyces spp., Propionibacterium spp., Veillonella spp. and Atopobium spp. Use of probiotics and molecular genetics to replace and displace cariogenic bacteria with non cariogenic bacteria has shown promising results. Naturally occurring oral lactobacilli have probiotic properties, isolated from saliva and plaque from children and adolescents, with or without caries lesions. Twenty three Lactobacillus spp. completely inhibited the growth of all mutans streptococci tested. Species with maximum interference capacity against mutans streptococci included Lactobacillus paracasei, Lactobacillus plantarum, and Lactobacillus rhamnosus. Few studies have reported reduction in mutans streptococci levels in saliva following use of probiotic containing yoghurts but it is not clear whether this decrease is due to the bactericidal activity of yoghurt or other mechanisms. In vitro, yoghurt with live bacteria showed selective anti-mutans activity, suggesting that the overall decrease in mutans streptococci in vivo could be due to a bactericidal effect on S. Mutans. Yogurt products containing L.reuteri showed a significant growth inhibitory effect against S. mutans, while yoghurts with lactobacilli other than L. reuteri did not show such inhibition. Calgar
et al. (2006) investigated the effect of probiotic bacterium *Lactobacillus reuteri* on levels of mutans streptococci and lactobacilli which was introduced by two different -straw containing *L. reuteri* and lozenges containing *L. reuteri* and concluded that short-term daily ingestion of lactobacilli-derived probiotics delivered by prepared straws or lozenges reduced the levels of salivary mutans streptococci in young adults. Calgar et al. (2007) evaluated the effect of xylitol and probiotic chewing gums on salivary *mutans streptococci* and *lactobacilli* and concluded that daily chewing on gums containing probiotic bacteria or xylitol reduced the levels of salivary *mutans streptococci* in a significant way. However, a combination of probiotic and xylitol gums did not seem to enhance this effect. *L. rhamnosus* is one of the most extensively studied probiotic and of particular interest in oral biology since it does not readily ferment sucrose and is safer for teeth than lactic acid producing bacteria. Controlled studies have shown the effectiveness of *L. rhamnosus* in reducing caries. *L. rhamnosus* was found to inhibit cariogenic *S. mutans* but colonization of oral cavity by by *L. Rhamnosus* seems improbable.

**Ideal Properties of Probiotics**

An effective probiotic should:

1. Exert a beneficial effect on the host
2. Be non-pathogenic and non-toxic
3. Contain a large number of viable cells
4. Be capable of surviving and metabolizing in the gut
5. Remain viable during storage and use
6. Have good sensory properties
7. Be isolated from the same species as its intended host.

**Risk Factors Associated with Probiotics**

Probiotics are often regulated as dietary supplements rather than as pharmaceuticals or biological products. Thus, there is usually no requirement to demonstrate safety, purity, or potency before marketing probiotics. This can lead to significant inconsistencies between the stated and actual contents of probiotic preparations, although most commercially available probiotic strains are widely regarded as safe, there are significant concerns with respect to safety in particular populations.

**Risk Factors for Probiotic Sepsis**

All cases of probiotic bacteremia or fungemia have occurred in patients with underlying immune compromise, chronic disease, or debilitation, and no reports have described sepsis related to probiotic use in otherwise healthy persons. Most cases of probiotic sepsis have resolved with appropriate antimicrobial therapy, but in some cases patients have developed septic
shock. In other cases the outcome has been fatal, but these fatalities were usually related to underlying disease rather than directly to probiotic sepsis. One exception is the report by Lestin et al of a 48 year old diabetic woman with diarrhoea attributable to *Clostridium difficile* who died from multiorgan failure and septic shock in association with a toxic megacolon and probiotic fungemia. The case is suggestive of fatal probiotic sepsis, but molecular methods were not used to confirm homology between the probiotic and pathogenic fungi. Many case reports of probiotic sepsis describe persons with pre-existing intestinal pathology, including diarrhoea and short intestine.

**Deleterious Metabolic Activities**

The intestinal microbiota plays an important role in many metabolic activities, including complex carbohydrate digestion, lipid metabolism and glucose homeostasis. There is complex carbohydrate digestion, lipid metabolism, and glucose homeostasis. There is therefore a theoretical risk of adverse metabolic effects from manipulation of the microbiota with the use of probiotics, since the likelihood of significant adverse effects in this regard seems low and probiotic studies to date have not shown significant adverse effects on growth or nutrition.

**Immune Deviation or Stimulation**

Experiments also showed that the intestinal microbiota is important in stimulating normal immune development, particularly the development of gut-associated lymphoid tissue. The presence of an intestinal microbiota is necessary for a range of immune functions, including antibody production, the development and persistence of oral tolerance to food antigens and the formation of germinal centers within lymphoid follicles. This crucial role of the intestinal microbiota in normal immune development suggests that manipulations designed to alter the microbiota may have significant immune-modulatory effects. The long-term effect of these manipulations on the host is difficult to predict, and adverse effects on immune development remain a possibility. This is particularly relevant in the field of neonatal probiotic supplementation, where medium to long-term alteration of the microbiota or life-long modification of the immune response might be achieved. A second group that may be at increased risk of adverse immune stimulation is pregnant women. During pregnancy there is a bias in T cell responses toward a Th2 phenotype, which is thought to be important in maintaining foetal viability because Th1 cytokines are associated with pregnancy loss. Probiotic *Lactobacillus species* have been shown to suppress Th2 cytokine responses in vitro, and in some human studies were found to increase production of the Th1 cytokine interferon γ55. These
effects may be detrimental to pregnancy viability. However, there is currently no direct evidence for this and such a risk remains theoretical. At present there is little support for the hypothesis that probiotics cause adverse immune development from empirical studies.

**Microbial Resistance**

In most circumstances the available data suggests that probiotics colonize the human intestine transiently. Nevertheless, concern exists regarding the possible transfer of antimicrobial resistance from probiotic strains to more pathogenic bacteria in the intestinal microbiota. Many *Lactobacillus* strains are naturally resistant to vancomycin, which raises concerns regarding the possible transfer of such resistance to more pathogenic organisms, particularly *enterococci* and *S. aureus*. However, the vancomycin-resistant genes of *Lactobacillus spp.* are chromosomal and, therefore, not readily transferable to other species. Conjugation studies have not found the vancomycin-resistant genes of *Lactobacilli* to be transferable to other genera. To prevent the undesirable transfer of resistance or conferment of resistance to endogenous bacteria, probiotics should not carry resistance other than that required. Although special-purpose probiotics for use in combination with antibiotics have been developed through the introduction of multiple resistances to the bacteria, probiotics generally should not be designed to carry more resistance than is required for a specific purpose.

**Administration of Probiotics**

Appropriate forms of administration of probiotic strains have been discussed in several articles. A particular concern when evaluating probiotic effects on periodontal disease relates to the means of administration of these bacteria. Generally, probiotics are delivered in dairy products, yoghurt, as food supplements in capsule & liquid form, straws & lozenges, chewing gum, tablets, rinse solution or in soft drinks. However, these routes of administration cannot provide prolonged contact with oral tissues, facilitating probiotic adhesion to saliva-coated surfaces. A lozenge form or a chewing tablet or gum might better serve the needs for periodontal health prophylaxis. Moreover, the frequency of intake needs further evaluation because of the inability of most probiotic species tested to reside permanently in the oral cavity.

**Conclusion**

Probiotics are gaining importance because of the innumerable benefits. Today probiotics are available in a variety of food products and supplements. With the current focus on disease prevention and the quest for optimal health at all ages, the probiotics market potential is enormous. Health professionals are in an ideal position to help and guide their patients toward appropriate prophylactic and therapeutic uses of probiotics that deliver the desired
beneficial health effects. In recent years, there has been an upsurge in research in probiotics as well as growing commercial interest in the probiotic food concept. This increased research has resulted in significant advances in our understanding and ability to characterize specific probiotic organisms, which has resulted in an increasing amount of evidence indicating health benefits by consumption of food containing probiotics.

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