

Probiotics and oral health: an update

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ABSTRACT

Probiotics are micro-organisms, principally bacteria, which, when ingested, confer health benefits beyond basic nutrition. Probiotics have been extensively studied for their health promoting effects. The main field of research has been focussed on the gastro-intestinal tract. However, in the past few years probiotics have also been investigated from an oral health perspective and their use has shown promising results with respect to control of chronic conditions, such as dental caries, periodontitis, halitosis and candidial infections. Despite the immense potential of probiotics, hardly any randomised, controlled trials have been conducted on their action and application and studies on their effects on oral health are still in early stages. Hence, more research is needed before any evidence-based conclusions can be drawn. This paper considers some recent literature and insights on which further investigations could be based.

Key words: Probiotics, Oral diseases, *Streptococcus mutans*.

INTRODUCTION

Every human being ingests bacteria daily, either un-intentionally as contaminants of food, or intentionally, in processed foods such as yogurt, fermented milk or cheese. A review of available literature revealed reports of the beneficial effects of probiotics in treating systemic infections such as diarrhoea, urogenital infections and some systemic conditions such as liver disease, Crohn's disease and cancer. The vast majority of probiotics are bacilli e.g. *Lactobacillus acidophilus* or cocci e.g. *Streptococcus salivarius*, *Streptococcus lactis*, *Enterococcus faecium*, and yeasts e.g. *Saccharomyces cerevisiae*, *Aspergillus niger*, *Candida pintolopesii*. With the evolution of nutritional science, research is now being directed towards improving understanding of the specific physiologic effects of the diet beyond its nutritional

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ACRONYMS

FAO: Food and Agriculture Organisation of the United States
VSC: Volatile Sulphur Compounds

value.¹ In this respect, probiotics are the subject of intense and widespread research in food and nutritional science.

According to the currently adopted definition by FAO/WHO, **Probiotics are:** "living micro-organisms, principally bacteria, safe for human consumption and when ingested in sufficient quantities, have beneficial effects on human health, beyond basic nutrition".²

The term **prebiotic** was introduced by Gibson and Roberfroid.³ A Prebiotic is a non-digestible food ingredient conferring benefits on the host by selectively stimulating the growth and/or activity of one species of bacteria or group of bacteria in the colon, thus improving the host's health. In the oral cavity, probiotics can create a biofilm, acting as a protective lining for oral tissues against oral diseases. Such a biofilm keeps bacterial pathogens off oral tissues, by filling a space which pathogens would otherwise invade, and by competing with cariogenic bacteria and growth of periodontal pathogens. The term **synbiotic** is used when a product contains both probiotics and prebiotics. As the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favours the probiotic compound.⁴

The term **replacement therapy**, also called bacteriotherapy or bacterial interference, is sometimes used interchangeably with the term probiotics. Although both treatment modalities use live bacteria for the prevention or treatment of infectious diseases, there are some slight differences. Replacement therapy involves the direct application of the effector strain to the site of infection and it is directed at displacing or preventing colonisation by a pathogen. Probiotics are generally used as dietary supplements and act indirectly in preventing colonisation by pathogens.

The aim of this review is to present an update and to discuss the current status of using probiotics in the maintenance of oral health.

The ideal probiotic micro-organism should have the following characteristics:⁵

- To be called a probiotic, the micro-organism has to be microbiologically characterised and to have been subjected to randomised clinical trials;

- It should be safe for human consumption and beneficial physiological effects should be scientifically demonstrable.
- The probiotic should:
 - be stable in acidic and alkaline environments;
 - be able to adhere to the intestinal mucosa or the target tissue;
 - demonstrate high stability at room temperature, either separately or when mixed with other ingredients.
 - have no potential to develop diseases.

HISTORY

The use of micro-organisms to promote health is ancient and can be tracked as far back as classical Roman literature, where a food, fermented with micro-organisms, was used as a therapeutic agent. The concept of probiotics was conceptualised in 1908 when the Ukrainian bacteriologist and Nobel Laureate, Ilya Metchnikof (1908), proposed that lactic acid bacilli may have beneficial health effects. He attributed his own longevity to regular probiotic ingestion. He suggested that "the dependence of the intestinal microbes on food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes."⁶ The term 'probiotics', which is the antonym of the term 'antibiotics', was introduced in 1965 by Lilly & Stillwell and defined as "Substances produced by micro-organisms, which promote the growth of other microorganisms." They showed that several species of protozoa, during their logarithmic phases of growth, produce substances which prolong the logarithmic growth phase in other species.⁶

In 1984, Hull identified the first probiotic species, the *L. acidophilus*. Later, in 1991, Holcomb identified *Bifidobacterium bifidum*.⁷ The World Health Organisation, (WHO), in 1994, described probiotics as the next most important barrier in the immune defense system following the resistance offered by antibiotics. These discoveries paved the way for a new concept of probiotics in medicine and dentistry.⁷ Recently, there were reports of using the immunostimulatory properties of lactic acid bacteria in treatment of conditions such as diarrhoea, vaginal infection and colon cancer.⁷ These micro-organisms can inhabit the bio-film and actually protect oral tissue from disease. They also have cariostatic activity, aid in preventing candidial colonisation and act as antioxidants.

PROBIOTIC STRAINS FOR ORAL HEALTH

An essential requirement for a micro-organism to be classed as an oral probiotic is its ability to adhere to and colonise both hard and soft surfaces in the oral cavity. The most frequently used strains belong to the genera *Lactobacillus* and *Bifidobacterium*, commonly found in the oral cavity, including in carious lesions.⁸ These were the first probiotics to be introduced into research (*L. acidophilus* by Hull *et al.* in 1984 and *B. bifidum* by Holcomb *et al.*, 1991).⁷ *Lactobacillus rhamnosus* GG, ATCC 53103, produces a growth inhibitory substance against *Streptococcus sobrinus* and has been proposed as a probiotic to reduce the risk of caries.⁸

S. salivarius strains appear to be excellent candidates for an oral probiotic, because they are early colonisers of oral surfaces and are amongst the most numerically predominant members of the microbiota of the tongue of healthy individuals.⁹ Other strains considered as probiotics in the oral cavity include: *L. acidophilus*, *L. casei*, *L. casei shirota*, *L. paracasei*, *L. reuteri*, *L. johnsonii*, *Propionibacterium* and *Weissella cibaria*.¹⁰

Table 1: Examples of microorganisms considered to be probiotics¹²

<i>Lactobacillus spp.</i>	<i>Bifidobacterium pp.</i>	Others
<i>L. acidophilus</i>	<i>B. bifidum</i>	<i>Saccharomyces boulardii</i>
<i>L. casei</i>	<i>B. breve</i>	<i>Lactococcus lactis subsp. cremoris</i>
<i>L. crispatus</i>	<i>B. infantis</i>	<i>Enterococcus faecium</i>
<i>L. delbrueckii subsp. bulgaricus</i>	<i>B. longum</i>	<i>S. salivarius subsp. Thermophilus</i>
<i>L. fermentum</i>	<i>B. lactis</i>	<i>S. diaacetylactis</i>
<i>L. gasserii</i>	<i>B. adolescentis</i>	<i>S. intermedius</i>
<i>L. johnsonii</i>		
<i>L. paracasei</i>		
<i>L. plantarum</i>		
<i>L. reuteri</i>		
<i>L. rhamnosus</i>		

These early studies⁷ further demonstrated that probiotic effects are strain-specific; therefore, a beneficial effect attributed to one strain cannot be assumed to be provided by another, even when both belong to the same species.¹¹ A combination of strains can enhance adherence in a synergistic manner. Some examples of micro-organisms considered to be probiotics are listed in Table 1.

MECHANISM OF ACTION OF PROBIOTICS IN THE ORAL CAVITY

The mechanisms of probiotic action in the oral cavity could be similar to those described for intestine i.e.

- Normalisation of the intestinal (or oral) microbiota
- Modulation of immune responses and
- Metabolic effects.

In the oral cavity, in addition to the creation of a protective bio-film, probiotic micro-organisms can compete for substrates used by cariogenic bacteria and periodontal pathogens, thereby inhibiting their growth. Until now, oral colonisation by probiotic bacteria has often been considered essential for it to exert oral effects. However, the possibility that probiotic bacteria may influence oral health through the systemic route cannot be excluded. Contrary to this belief, however, the total salivary IgA levels, which indicate the systemic effects of probiotics in oral health, seem unaffected.¹³ Figure 1 summarises possible ways in which probiotics might affect oral health.¹⁴



Figure 1: Possible probiotic activities in the oral cavity.¹³

MODE OF SUPPLY OF PROBIOTICS

1. A culture concentrate added to a beverage or food (such as a fruit juice);
2. Inoculation into probiotic fibres;
3. Inoculants incorporated into a milk-based food. eg yoghurt, cheese, kefir, etc;
4. Concentrated and freeze-dried products packaged as dietary supplements (e.g. non-dairy products such as powders, capsules, gelatin tablets).

PROBIOTICS AND DENTAL CARIES

S. mutans is the main organism involved in the causation of dental caries. "Probiotic", as used here, means that mechanisms are employed to selectively remove only the pathogen, while leaving the remainder of the oral ecosystem intact.¹⁵ One of the replacement therapy options entails the application of a genetically engineered "effector strain" of *S. mutans* which will replace the cariogenic or "wild strain," to prevent or arrest caries and to promote optimal remineralisation of tooth surfaces which have been demineralised, but have not become cavitated. This effect is mainly by modulation of the caries process, when the probiotics replace cariogenic bacteria with non-cariogenic bacteria, thus favouring remineralisation. *S. mutans* strain BCS3-L1, is a genetically modified effector strain, designed for use in replacement therapy to prevent dental caries.¹⁶ Recombinant DNA technology was used to delete the gene encoding for lactate dehydrogenase in *S. mutans* BCS3-L1 strain, making it unable to produce lactic acid. This effector strain was also designed to produce elevated amounts of a novel peptide antibiotic called mutacin 1140, giving it a strong selective advantage over most other strains of *S. mutans*.¹⁷ Clinical trials have shown that the inclusion of *L. rhamnosus* GG in milk or processed cheese, was associated with a reduction of the incidence of dental caries in children. *B. lactis* in ice cream reduced oral *S. mutans* count.^{18,19,20} Nikawa *et al.* reported a reduction in *S. mutans* counts and subsequently, dental caries incidence in children fed with cow's milk, fermented with *L. reuteri*.²¹

Genetically modified bacteria can prevent the growth of cariogenic bacteria, e.g. modifications of two strains of plaque streptococci will create organisms producing ammonia from urea and arginine. These micro-organisms will reside in dental plaque and the alkaline ammonia, produced from salivary and dietary substrates, will prevent colonisation with cariogenic bacteria and ensure internal pH homeostasis. If the effector strain is better adapted than the pathogen, colonisation or outgrowth of the pathogen will be prevented by blocking the attachment sites, by competing for essential nutrients, or via other mechanisms.¹⁶ The main idea is to develop an inexpensive targeting molecule that will reliably attach to only the organism of interest, in this case *S. mutans*, *S. sobrinus*, or another chosen pathogen. Once the targeting molecule is perfected, then a "killer" molecule can be optimised and linked to the targeting molecule. According to Saavedra *et al.* once the bacterial ecosystem is free of *S. mutans*, it is difficult to reintroduce these organisms (another example of competitive inhibition).¹⁵

PROBIOTICS AND PERIODONTAL DISEASES

The primary aetiological factors for the development of periodontal diseases are bacteria in supra- and sub-gingival

biofilms. Mucosal immune responses may be invoked by probiotic immunisation. A proposed mechanism of action of probiotics is the strengthening of the mucosal barrier via topical effects on the epithelium, and the stimulation of both the innate and adaptive immune systems. A decrease in gingival bleeding and reduced gingivitis has been observed by Krasse *et al.* with the ingestion of *L. reuteri*.²² According to Koll – Klais *et al.* probiotics containing *Lactobacillus* effected an 82% and 65% inhibition in growth in, respectively, *Porphyromonas gingivalis* and *Prevotella intermedia*.²³ Twetman *et al.* used *L. reuteri*-containing chewing gum in 42 healthy patients and assessed the effects on crevicular fluid volume, cytokine (interleukin-1 β , interleukin-6, interleukin-10, and TNF- α) levels and bleeding on probing. He found crevicular fluid volume, the levels of TNF- α and interleukin-8 and bleeding, were all significantly reduced.²⁴ Riccia *et al.* used lozenges into which *L. brevis* was incorporated to study its anti-inflammatory effects in a group of patients with chronic periodontitis. The study showed significant improvement, not only in the plaque index, gingival index and bleeding on probing for all patients, but also a significant reduction in gingival inflammation.²³

Probiotic strains included in periodontal dressings at optimal concentrations of 108 CFU mL⁻¹ were shown to diminish the number of the most frequently isolated periodontal pathogens: *Bacteroides spp.*, *Actinomyces spp.* and *S. intermedius*, and also *C. Albicans*.²⁵ Probiotic bacteria may favour periodontal health and may even recolonise a gingival pocket after scaling and root planing if they are able to establish themselves in the oral biofilm. However, further longitudinal studies are required to confirm these findings.

PROBIOTICS AND HALITOSIS

Halitosis is a common problem with multiple local and systemic etiological factors. The main oral causes include periodontitis, poor plaque control, deep dental caries, tongue coating and faulty restorations, along with gastrointestinal and lung infections. Halitosis 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 sulphur compounds (VSC's) by *Fusobacterium nucleatum* after ingestion of *W. cibaria* both *in vitro* and *in vivo*.²⁶ *L. salivarius* T12711 bacteria have been shown to reduce the count of the oral black pigmented bacteroides, the bacteria strongly associated with production of the VSC's responsible for halitosis.²²

However, the few studies published on the role of probiotics in the treatment of halitosis do not confirm any evidence-based conclusions. It may be that this is where probiotic therapy could indeed bring something new, if there is confirmation of preliminary observations on the "balancing" effect of probiotics on the oral microflora which generate volatile sulphur compounds. Randomised, blinded, and placebo-controlled studies with large enough sample sizes are also needed in this endeavour.

PROBIOTICS AND ORAL CANDIDIASIS

Candida species constitute part of the commensal oral flora in about 50% of healthy subjects, but they are able to cause a clinically apparent lesion if the immune defenses are breached, either on the local or systemic level. A reduction has been demonstrated in the prevalence of *C. Albicans*

in the elderly, after their consumption of probiotic cheese, containing *L. Rhamnosus GG* and *Propionibacterium freudenreichii ssp.*²⁷ It is important to extend research on yeast infections with respect to probiotics. Analysing the molecular mechanisms of probiotic activity might further broaden the field of potential applications.

ADMINISTRATION OF PROBIOTICS

The most useful methods of administration of probiotic strains have been discussed in two recent articles.^{28,29} Milk products, supplemented with probiotics, are a natural means of oral administration and can be easily adopted in most dietary regimens. However, for the purposes of prevention or treatment of oral diseases, it is specifically targeted applications, formulas, devices, or carriers with slow release of probiotics, which will be needed. Montalto *et al.* administered probiotic mixtures, both in capsules and in liquid form, without observing statistically significant differences in the *S. mutans* counts between the two test groups.³⁰

The different vehicles used to administer probiotics are:

- Lozenges
- Tablets
- Cheeses
- Mouthwashes
- Capsules, liquids

SAFETY CONCERNS REGARDING PROBIOTICS

From a human safety point, the putative probiotic microorganisms should not be pathogenic, should not have any growth-stimulating effects on bacteria causing diarrhoea and should not have an ability to transfer antibiotic resistance genes. The probiotics should rather be able to maintain genetic stability in oral micro flora.³¹ Lactobacillus-induced bacteraemia is a relatively rare condition, with approximately 180 cases reported over the last 30 years.³² Clinical characteristics of Lactobacillus-induced bacteraemia are highly variable, ranging from asymptomatic to serious septic conditions. However, these symptoms are particularly seen in individuals who have severe underlying diseases, or who are immuno-compromised. Careful monitoring of patients is mandatory for this potentially serious condition.

FUTURE PROSPECTS

Genetically modified microbes bring a new dimension to the concept of probiotics. An ambitious and promising option is to enhance the properties of a beneficial strain. An example is the construction of an *L. paracasei* strain, with a functional single chain variable fragment (ScFv) anti-body which binds to the surface of *Porphyromonas gingivalis* in the prevention of periodontal diseases.^{22,33}

Early mucosal colonisation with *Escherichia coli* bacteria stimulates the mucosal immune system to produce specific antibodies as well as non-specific secretory immunoglobulins.⁹ Studies by Nezami *et al.* reveal that probiotics seem to improve the oral immune response.³² Dairy products containing probiotic lactobacilli in combination with prebiotics, are also currently being developed with the intention of enhancing immunity in humans and animals.³⁴ In the field of oncology, serious systemic infections may occur during cancer chemotherapy because of disturbances in the

oropharyngeal and gastrointestinal microflora, impaired mucosal barrier functions and immune-suppression. In this respect it has been shown that treatment with the probiotic *L. plantarum 299v* improves food intake and body weight in animals undergoing chemotherapy.³⁵

Probiotics combined with prebiotics appear to be an innovative and revolutionary method for the treatment of diseases of the hard and soft tissues of the oral cavity.

CONCLUDING REMARKS

The interest in oral probiotics has been growing over the last few decades. A literature review shows that probiotics use may result in improvement of oral health, which is mainly due to reduced levels of cariogenic, as well as periodontal pathogens. Probiotics can be best used as an adjunctive therapy for the prevention of oral diseases, reducing the cost of conventional treatment methods. A knowledge of the potential role of probiotics in the treatment of oral diseases is most pertinent for all practitioners.

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