

Probiotics: Healthy bugs and nourishing elements of diet

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Abstract

The use of probiotics is based on the concept that adding the right live microbes to the complex human system can result in physiological benefits. The benefit of fermented milk in human diet has been acknowledged since Vedic times; however, the scientific interest in this field was evoked by Ellie Metchnikoff who recommended that people should consume fermented milk containing lactobacilli to prolong their lives, as accelerated aging is due to autointoxication caused by the toxins produced by the gut microflora. They have been used to improve gastrointestinal health and their attractiveness has evinced interest to study their role in the promotion of oral health also. Studies have been widely carried out to establish the role of intestinal lactobacilli as probiotic to treat various gastrointestinal disorders, but only limited studies are available on the oral use of probiotics. The probiotic products usually contain lactobacilli and bifidobacteria, and their demand in the market is growing day by day. This paper provides an overview of various studies in the literature that emphasize on the role of probiotics to combat oral diseases and encourages more research in this field.

Key words: *Fermented products, Lactobacillus, probiotics*

INTRODUCTION

Probiotics are defined as live microorganisms which when administered in sufficient quantity provide a health benefit to the host.^[1] They have been used to improve gastrointestinal (GI) health and their popularity has generated interest to study their role in fostering oral health also.^[2] The word “probiotics” is the antonym of the term “antibiotics” that was introduced by Lilly and Stillwell in 1965 referring to the substances produced by microorganisms which encourage the

growth of other microorganisms. The word probiotic means “for life”, and nowadays, it is used in reference to bacteria associated with useful effects on humans and animals. The belief in the beneficial effects of probiotics is based on the knowledge that the intestinal flora can protect humans against infection and that disturbance of this flora can increase susceptibility to infection. The bacteria in yogurt and fermented milk products constitute the most important source of probiotics for humans.^[3] For the effect of probiotics, they must be alive in the food or supplement and should be able to survive the harsh conditions of the GI tract while still maintaining their activity.^[4]

Lactobacillus rhamnosus GG (LGG) was the first probiotic to gain attention in this field and has shown beneficial effects on intestinal immunity. It is known to increase the number of IgA and cells secreting other immunoglobulins in the intestinal mucosa, and thus stimulates the local release of interferons and

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also facilitates antigen transport to the underlying lymphoid cells, increasing the uptake of antigen in Peyer's patches.^[5] Lactobacilli are widely used for the manufacture of fermented foodstuffs and, as such, have been consumed for centuries.^[6] The probiotics could affect the host synergistically in combination with prebiotics. Prebiotics are engaged to promote both beneficial bacteria which are already established in the colon as well as externally administered probiotic bacteria. These are the food ingredients which are indigestible in the upper GI tract and reach the colon to beneficially influence the host by selectively promoting the growth and/or activity of certain bacteria in the colon.^[7] The term probiotics is related to functional food, whereas the term prebiotics is used to define non-digestible food ingredients that benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species that had already been established in the colon, and thus plays a role in improving the host health. These prebiotics include inulin, fructooligosaccharides, galactooligosaccharides, and lactulose. The theory of prebiotics essentially has the same aim as probiotics, which is to improve host health via intonation of the intestinal flora, however, by an altered mechanism.^[8]

Ingestion of probiotics in any form, such as in foodstuff (cheese, yogurt, fermented milk, fruit juice, or chewing gum) or as a constituent of tablets and capsules, has been shown to have a health gaining effect on the oral tissues, like reducing the incidence of dental caries, improved management of periodontitis, abridged halitosis and oral candidal infections.^[9]

THE MECHANISM OF ACTION OF PROBIOTICS

The mechanisms by which probiotics exert their effects may involve modification of gut pH, competition for pathogen binding and receptor sites as well as for the available nutrients and growth factors, antagonizing the pathogens through production of antimicrobial compounds that stimulate immunomodulatory cells, and lactase production. Probiotic bacteria have been shown to influence the immune system through several molecular mechanisms.^[1] The mechanisms of probiotic action in the oral cavity may be similar to those described for the intestine. Thus, oral colonization by probiotic bacteria seems to be essential for them to show their oral effects; however, the possibility of systemic effects cannot be excluded, although the total sIgA levels in saliva seem to be unaffected by the use of probiotics. Interestingly, maternal use of some probiotic

strains seems to exert influence on the composition of breast milk.^[10]

Some probiotic strains have been found to have significant anti-infective and immunomodulatory nature. In addition of possessing *in vivo* antagonistic properties against pathogenic bacteria, they also prevent the pathogenic bacteria from adhering to the gut mucosa, due to their strong affinity for enterocytes. Production of lactic acid and antimicrobial peptides is considered to be responsible for the complex bacteriostatic and bactericidal effects of probiotics.^[11]

STRAINS OF BACTERIA USED AS PROBIOTICS

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 *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus casei*, *L. rhamnosus*, *Lactobacillus gasseri*, and *Lactobacillus reuteri*. *Bifidobacterium* strains include *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Bifidobacterium infantis*. In dentistry, studies with *L. rhamnosus* GG, *L. reuteri* have established their potential in interacting with *Streptococcus mutans* by reducing the number of this caries pathogen, thus suggesting a role of probiotics in caries prophylaxis. A few studies also revealed that probiotic *Lactobacillus* strains were helpful in reducing gingival inflammation and the number of black pigmented rods including *Porphyromonas gingivalis* in the saliva and subgingival plaque.^[12] Lactobacilli and bifidobacteria are generally regarded as safe, and since the early writing of Metchnikoff, even more fermented food products have been associated with health benefits.^[10]

The criteria for a substance to act as a good probiotic agent include the following: It should be nonpathogenic, nontoxic, well-tolerate gastric acid, adhere to gut epithelial tissue, and produce antibacterial substances. It should persist and withstand for short periods in the GI tract, thus influencing metabolic activities like cholesterol assimilation, lactose activity, and vitamin production. The survival of probiotic organisms in the gut depends on the colonization factors that they possess, which are the organelles that enable them to resist the antibacterial mechanisms that operate in the gut. In addition to the antibacterial mechanisms, they need to avert the effects of peristalsis that tend to flush out bacteria along with food. This can be achieved either by immobilizing themselves or by

growing at a much faster rate than the rate of removal by peristalsis. The probiotic strain also needs to be resistant to the bile acid.^[13]

POTENTIAL BENEFITS OF PROBIOTICS

Probiotics have consistently been used for prevention of colon cancer, management of lactose intolerance, protecting against *Helicobacter pylori* infection, lowering cholesterol and blood pressure, improving the immune function and preventing infections, preventing antibiotic-associated diarrhea, reducing inflammation, improving mineral absorption, preventing harmful bacterial growth under stress, irritable bowel syndrome, and colitis, and managing urogenital health.^[14] *Lactobacillus* and *B. longum* were successfully used by Colombel for reducing the after-effects of antibiotic therapy.^[15] The main fields of research with reference to probiotics are allergic reaction, cancer, heart diseases, diarrhea, etc., Rettiger *et al.*^[16] documented the role of probiotics in alleviation of lactose intolerance due to increased concentration of β -galactosidase in the small intestine and in relieving constipation by increasing the bowel function, whereas Reddy *et al.*^[17] recognized the antitumor activities by inhibition of tumor cells and destruction of carcinogens. Ishizaka *et al.* carried out a randomized, double-blinded, placebo-controlled crossover study in which fermented probiotics were administered to 10 healthy adult geldings for 28 days. The product contained 5.6×10^8 colony-forming units (CFU)/g of *L. acidophilus* and 2.6×10^4 CFU/g of yeast species, including *Saccharomyces cerevisiae* and *Saccharomyces boulardii* and found gradual reduction in the levels of phenol and indole, as well as a dramatic decrease in 4-methylphenol levels. The relative amounts of *Escherichia coli* and *Clostridium perfringens* were also reduced in the feces of the probiotic-treated group as compared with the control group.^[18]

Probiotics as efficient immunopotentiators

Unbalanced diet, mental stress, infections and antibiotic use, imbalances in the equilibrium of gut microbiota, and impaired gut microbiota can become the causes of some diseases.^[19] Several dietary or biliary compounds can be converted by anaerobic gut bacteria to genotoxic substances. For example, heterocyclic amines, formed from pyrolysis of fried protein-containing foods, undergo bioconversion in the liver. These can be acted upon by eubacteria and clostridia present in the colon and can be reactivated by the bacterial β -glucuronidase. Chronic exposure to such substances may initiate the process of colorectal carcinogenesis

or enhance its progression. Protective factors from nutrition, such as prebiotic foods containing inulin-type fructans, may form other fecal compounds that play a significant part in cancer prevention. This product of gut flora-mediated fermentation leads to induction of apoptosis of tumor cells and protects the cells from genotoxic damage by increasing phase II detoxification.^[20]

A large number of abnormal cells with genetic mutations are produced every day in our body. The immune surveillance system plays an important role in the clearance of such cells and prevents the development of cancer. Gut bacteria play a role in providing defense against cancer by modulating the immune functions of the host.^[19] Takagi *et al.*^[21] evaluated the inhibitory effect of oral administration of *L. casei* (LC) on 3-methylcholanthrene (MC)-induced carcinogenesis in mice by intradermally injecting MC and monitoring the tumor incidence. LC was blended into the diet at a concentration of 0.05% (w/w) and the diet was fed from the day of MC injection throughout the study. Spleen cells were analyzed for the immune parameters and it was observed that oral feeding of mice with LC led to reduction of tumor incidence. The study suggested that oral feeding of mice with LC inhibits MC-induced tumorigenesis by modulating the disrupted host immune responses during MC carcinogenesis. Hori *et al.*^[22] investigated the effect of oral administration of *L. casei* strain Shirota on natural killer (NK) cell activity in splenocytes and NK cell activity in control, and observed that NK cell activity in the *L. casei* strain Shirota group was 1.5 times that in the control group. Ditu *et al.*^[23] assessed the serum levels of cytokine in holoxenic mice after oral administration of non-viable components (NVC) of *Enterococcus faecium* probiotic culture stimulated with heat-inactivated *Es. coli* and *Bacillus cereus* in comparison to NVC of unstimulated *En. faecium* culture, and demonstrated that the serum concentrations of interleukin (IL)-12 were maintained at a higher level in the samples collected post administration only. Thus, non-viable fractions of probiotic bacteria, stimulated by other bacterial species could induce immunostimulatory effects mediated by cytokines and act as immunological adjuvants.

The role of probiotic bacteria in promoting the gut microbiota with well-balanced composition and maintaining sufficient immunosurveillance could prove to be valuable for cancer prevention, and further research should be directed to uncover its role in oral cancer prevention.

Probiotics and oral health

Probiotics and dental caries

Dental caries or tooth decay is a disease where bacterial processes cause damage to the hard tooth structure, characterized by acid demineralization of the tooth enamel.^[24] This results in formation of cavities on the surface of the tooth. Changes to the microflora within the oral cavity result in an overgrowth of various bacteria including *Streptococcus sorbinus*, *S. mutans*, and *Po. gingivalis*, which are known to be the primary cause of the dental caries.^[25,26]

Koll *et al.*^[6] isolated 10 species of salivary and subgingival lactobacilli from healthy humans and carried out a study to evaluate the characteristics of oral lactobacilli. All strains were identified using amplified ribosomal DNA restriction analysis, tested for antimicrobial activity against oral pathogens, tolerance of low pH, and bile content, and it was concluded that the strains of *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus salivarius*, and *L. rhamnosus* showed both high antimicrobial activity and high tolerance to environmental stress. *L. rhamnosus* is one of the most widely studied probiotics and is of peculiar interest as it does not readily ferment sucrose and is thus safer for teeth than the lactic acid producing bacteria.^[1] *L. rhamnosus* was found to inhibit cariogenic *S. mutans*, but colonization of oral cavity by *L. rhamnosus* seems unlikely.^[27] Calgar *et al.*^[28] assessed the effect of probiotic chewing gums over a period of 3 weeks on salivary mutans streptococci and lactobacilli, and concluded that daily chewing gums containing probiotic bacteria reduces the salivary mutans streptococci in a significant manner.

Astekar *et al.*^[29] carried out a study by addition of probiotic in the form of curd over a period of 3 months to the daily diet of 15 children and showed decrease in *Streptococcus* count in 73% children and increase in *Lactobacillus* count in 67% children. In a similar study carried out by Ahola *et al.*,^[30] on administration of probiotic in the form of cheese containing *Lactobacillus* over a period of 3 weeks, the probiotic interference helped in reducing the level of *S. mutans* and all the samples in the probiotic group taken after intervention showed growth of some lactobacilli. Nase *et al.*^[31] carried out a study by complementing 1–6 year old children with *L. rhamnosus* for 7 months and found a significant reduction in the risk of dental caries. Nikawa *et al.*^[32] carried out a study to examine the effects of edible yogurt containing *L. rhamnosus* and found significant reduction of the oral carriage of

mutans streptococci and four periodontal pathogens, *Po. gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Fusobacterium* spp., thus suggesting that yogurt with *L. rhamnosus* could reduce the risk of periodontal disease as well as dental caries. Kang *et al.*^[33] carried out a study and found that *Weissella cibaria* isolates produced water-soluble polymers from sucrose that inhibited the formation of *S. mutans* biofilm. Jindal *et al.*^[34] carried out a study in which *Lactobacillus sporogenes* was administered for 14 days and this resulted in a significant reduction of salivary *S. mutans* colony counts. Nishihara *et al.*^[35] evaluated the effects of the lactic acid bacterium *L. salivarius* containing tablets and found significant reduction in mutans streptococci count. Thus, the studies carried out by various authors suggest that oral administration of probiotics increases the resistance to caries risk factors.

Probiotics and periodontal health

The role of probiotics in periodontitis has been documented in various studies. The probiotic tablets (Wakamate D) containing 6.7×10^8 CFU/tablet of *L. salivarius* WB21 and xylitol (280 mg/tablet) were prepared originally in order to balance the intestinal microbial flora by providing acid-tolerant *L. salivarius* WB21. However, oral administration of these tablets caused a significant reduction in the plaque index and probing pocket depth of the study subjects, and thus suggested clinical improvement of the periodontal condition by probiotic intervention. This study stresses that a probiotic intervention could be a useful tool for the treatment of inflammation and the clinical symptoms of periodontitis.^[36] Teughels *et al.*^[37] reported that the subgingival application of a mixture including *Streptococcus sanguis*, *Streptococcus salivarius*, and *Streptococcus mitis* after scaling and root planing significantly concealed the re-colonization of *Po. gingivalis* and *Pr. intermedia* in the beagle dog model. Al-Zahrani^[38] carried out a study on periodontitis patients and found an inverse relationship between the intake of dairy products and prevalence of periodontitis. Riccia and colleagues^[39] recently studied the anti-inflammatory effects of *Lactobacillus brevis* in a group of patients with chronic periodontitis and showed a significant reduction in the salivary levels of prostaglandin E2 (PGE2) and matrix metalloproteinases (MMPs). 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 nitric oxide. Krasse and colleagues^[40] assessed the beneficial effect of *L. reuteri* against gingivitis. After 14 days of

ingesting the probiotic incorporated into the chewing gum, the oral cavity of patients with a moderate to severe form of gingivitis was colonized with *L. reuteri* and the plaque index was found to be reduced. Thus, these studies suggest that lactobacilli residing in the oral cavity could play a role in the oral ecological balance. Nissen *et al.*^[41] investigated the effect of lactobacilli (*L. alivarius* and *L. gasseri*) in relation to two major virulence factors of *Aggregatibacter actinomycetemcomitans*, a Gram-negative species involved in the etiology of localized aggressive periodontitis. The gene expressions of leukotoxin and cytolethal distending toxin by *A. actinomycetemcomitans* were analyzed in response to lactobacilli cell-free supernatants and it was found that lactobacilli affected the growth as well as reduced the virulence expression of both leukotoxin and cytolethal distending toxin. Thus, these fundamental findings indicate that lactobacilli can reduce the virulence of opportunistic oral pathogens and may provide the basis of studies on the therapeutic ways for such diseases in future.

Probiotics and imbalanced oral ecosystem

Halitosis, or oral malodor, is a condition normally attributed to disturbed equilibrium of commensal microflora.^[42] This condition affects comparatively a large proportion of the population. In approximately 90% of the cases, the origin of halitosis can be found in the oral cavity, although in some cases, few systemic diseases may be the underlying cause. Bad breath in the oral cavity is mainly attributed to the production of volatile sulfur compounds (VSC), especially by Gram-negative anaerobes inhabiting the periodontal pockets and on the tongue dorsum.^[43] The oral microorganisms, particularly those present on the tongue, are regarded as the primary cause of halitosis. Hence, the current treatments focus on the use of chemical or physical antibacterial agents to reduce the number of these bacteria. However, most of these treatments exhibit undesirable side-effects when used over a long period of time and also show temporary therapeutic effects. It has been documented that regular administration of probiotics affects the growth of these anaerobes.^[42] Kang *et al.*^[33] showed a definite inhibitory effect on the production of VSC by *Fusobacterium nucleatum* after ingestion of *W. cibaria* in both *in vitro* and *in vivo* studies. 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 the species causing an increase in the levels of

VSC. Thus, the replacement of bacteria concerned in halitosis by colonization with probiotic bacterial strains may have potential relevance as an additional therapy for the prevention and treatment of halitosis. Staab *et al.*^[44] carried out a study to determine the effect of a probiotic milk drink on gingival health and suggested a beneficial effect of the probiotic milk drink on gingival inflammation. Cildir *et al.*^[45] examined whether short-term intervention with fruit yogurt containing probiotic bifidobacteria would affect the levels of salivary mutans streptococci and lactobacilli in patients with fixed orthodontic appliances and concluded that short-term daily consumption of fruit yogurt containing *Bifidobacterium animalis* subsp. *lactis* DN-173010 may reduce the levels of mutans streptococci in saliva during orthodontic treatment with fixed appliances.

The Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) developed Operating Standards in 2002, which gave guidelines for all companies producing probiotic products. These guidelines include the following:

- Implementation of guidelines for use of probiotics
- Phase I, II, and III clinical trials for approval of health benefits that are as good as or better than standard prevention or treatments for a particular condition or disease
- Good manufacturing practice and production of high-quality products
- Studies for identification of mechanism of action *in vivo*
- Informative/precise labeling
- Development of probiotic organisms that can carry vaccines to hosts and/or antiviral probiotics
- Expansion of proven strains to benefit the oral cavity, nasopharynx, respiratory tract, stomach, vagina, bladder, and skin, as well as for cancer, allergies, and recovery from surgery/injury.^[46,47]

OVER-THE-COUNTER PROBIOTIC PRODUCTS

In India, probiotics generally come in two forms, milk and fermented milk products, with the former occupying 62% of the market share and the latter having 38% market share.^[48] Worldwide, a diverse array of probiotic products is on the market. Yogurt is perhaps the most common probiotic-carrying food, but the market has expanded beyond yogurt. Cheese, fermented and unfermented milk, juices, smoothies, cereal, nutrition bars, and infant/toddler formula all are food vehicles for probiotic delivery. In addition to being sold as foods, probiotics are sold as dietary supplements,

medical foods, and drugs (although there are no probiotics currently sold as drugs in the United States). Often, these products are composed of concentrated, dried microbes packaged into capsules, tablets, or sachets. This format is convenient for the delivery of large numbers of microbes that, if manufactured and stored properly, can be quite stable even at room temperature.^[49] Indian probiotic products currently in use are Dahi (Indian yoghurt), flavored milk, and butter milk. Yakult is a probiotic drink made from lactobacillus, fermented milk, and some sugar. Yakult is fermented milk that consists of healthy bacteria *L. casei* strain Shirota. According to the data available at the brand site, a 65-ml Yakult bottle contains 6.5 billion probiotic bacteria. Mother dairy launched b-Activ Probiotic Dahi, b-Activ Probiotic Lassi Nutrifit (strawberry and mango), and b-Activ Curd into the market. Amul thrust into the category with its probiotic ice creams and then marketed lassi (sweetened curd) in some parts of the country. Nestle also introduced flavored milk varieties of probiotic nature.^[48] Sunstar (Etoy, Switzerland) recently began marketing the first probiotic specifically prepared to fight periodontal disease. Gum PerioBalance contains a patented combination of two strains of *L. reuteri*, selected for their synergistic effect in fighting cariogenic bacteria and periodontal pathogens. Each dosage of lozenge consists of at least 2×10^8 living cells of *L. reuteri*. Users are recommended to use a lozenge every day, either after a meal or in the evening after brushing their teeth, so that probiotics could spread throughout the oral cavity and can attach to the different dental surfaces.^[50] Gum PerioBalance lozenge and chewing gum with the active ingredient *L. reuteri* strains have shown reduction in the levels of mutans streptococci in the oral cavity and in the amount of dental plaque, which may be beneficial to health.^[51] Shetty *et al.* conducted a study to find the availability status of probiotic products in India and came out with a list which includes Actigel, Lactolus, I-lac, Lac-M, Abigut, Sporolac, Gutrit, Labale, Aglac, Eugi, Enterolac-Z, Yakut, Beezo, V-bac, Prozo, Ezora, Eubioz, Progu, Imm, Enbolac, Pro-sig, Hepolac, Flora-bc, Pro-biza, and Lact-Flora.^[47] Align and Cuturelle are the probiotic supplements available in the market for digestive care, and a product named Nature Made is available which contains a line of vitamins and supplements in an acidophilus tablet. Ganeden Sustenex is a dietary probiotic available to improve the immune system and digestive health.^[52]

CONCLUSION

Probiotics are emerging as an intriguing field in oral health. Efforts should be made to increase the awareness

and motivation among the general dental practitioners on this aspect of oral disease therapy and invigorate the implementation of the concept of “*food rather than medicine.*”

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