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Probiotics in dental domain: A review

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Abstract
Probiotics represent an emerging field with enormous potential. Probiotic organisms being identical to the natural microflora of human body are safe, easily acceptable by the body, devoid of side-effects. Probiotics are of great interest to the researchers and its application as therapeutic agent is a topic of extensive research. Probiotics have been analyzed for treatment and prevention of various diseases and disorders of human body and the results obtained are very encouraging. Probiotics have turned out to be very promising in ensuring oral health and wellbeing.

Keywords: Probiotics, Antibiotic resistance, Mutans streptococci, volatile sulphur compounds, prebiotics

Introduction
Probiotic consumption is reported to exert a myriad of beneficial effects including: enhanced immune response, balancing of colonic microbiota, vaccine adjuvant effects, reduction of enzymes implicated in cancer initiation, treatment of diarrhea associated with travel and antibiotic therapy, reduction of serum cholesterol, the antagonism against food-borne pathogens and tooth decay organisms, the amelioration of lactose malabsorption symptoms as well as candidiasis and urinary tract infections, control of rotavirus and Clostridium difficile induced colitis and prevention of ulcers related to Helicobacter pylori [1]. Probiotics can be defined as living microbes, or as food ingredients containing living microbes, that beneficially influence the health of the host when used in adequate numbers [2]. WHO describes probiotics as "live microorganisms which, when administered in adequate amounts in food or as dietary supplement confer a health benefit on the host" [3]. Probiotics increase the number of beneficial microflora, competes with the pathogenic species to inhibit its growth and to prevent the occurrence of a disease [4]. To be called a probiotic, a bacterial strain must be fully characterized. The genus and species of the microorganism must be identified according to internationally accepted methods, and its nomenclature corroborated by reference to the Approved Lists of Bacterial Names. The FAO and the WHO have recommended that probiotic bacterial strains be characterized by their spectrum of resistance to antibiotics, their metabolic and hemolytic activities, their capacity to produce toxins, their infectious power in immunosuppressed animal models and their side effects in humans [5]. The term prebiotic was introduced by Gibson and Roberfroid who exchanged "pro" for "pre" which means "before" or "for". They defined prebiotics as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon [6]. Antibiotic resistance, with the emergence of multiple resistant strains, is an increasingly important global problem. Probiotics play an important role in combating issues concerned with overuse of antibiotics and antimicrobial resistance [7].

History
The use of microorganisms to promote health is very ancient and can even be traced back to the classical Roman literature where food fermented with microorganisms was used as a therapeutic agent [8]. The concept of probiotics was brought forward in the first decade of 1900 by a Ukrainian bacteriologist and Nobel Laureate, Elie Metchnikoff [9]. who observed that bacteria in the fermented milk competed with the microorganisms that are injurious to health.

While studying the flora of the human intestine, he developed a concept that senility is caused by poisoning of the body by the products of some of the harmful bacteria of the gut. He proposed a diet containing fermented milk products rich in live lactic acid bacteria to neutralize deleterious effects of these gut organisms. He credited these fermented products for extending the longevity of some populations of Bulgaria, Turkey and Armenia. He discovered Lactobacillus bulgaricus and claimed that cholera could be controlled by the presence of antagonistic organisms in the intestine. The term Probiotic, as an antonym to the term antibiotic, was first used by Lilly and Stillwell in 1965 to describe substances secreted by one microorganism which stimulates the growth of another [10]. Fuller [11] described probiotics as a live microbial food supplement, which beneficially affects the host animal by improving its microbial balance.

Ideal Requirement of a Probiotic [12]

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.

Therapeutic Actions of Probiotics [13].

1. Prevention of diarrhoea caused by clostridium difficile.
2. Prevention of colon cancer.
3. Reduces progression of AIDS.
4. Enhancement of calcium absorption.
5. Regulation of Immunity.
6. Compete against harmful microorganisms, preventing colonization of pathogens.
7. Reduction of blood cholesterol levels.
8. Reduction of liver toxicities.
9. Enhancement of vitamin status (B, K).
10. Increases the lifetime of voice prosthesis.

Probiotic Products

Probiotics are provided in products in one of the four basic ways. [14].

1. A culture concentrate added to a beverage or food (such as a fruit juice).
2. Inoculated into prebiotic fibers.
3. Inoculants into a milk-based food (dairy products such as milk, milk drink, yoghurt, yoghurt drink, cheese, kefir, and bio-drink).
4. As concentrated and dried cells packaged as dietary supplements (non-dairy products) such as powder, capsule, gelatin tablets.

Probiotic Species for Oral Health

Probiotics can be varied. They can be yeast, bacteria or moulds. But most commonly, bacterial species are predominant. Some of the species are [14].

1. Lactic acid producing bacteria (LAB): Lactobacillus, bifidobacterium, streptococcus.
2. Non lactic acid producing bacterial species: Bacillus, propionibacterium.
3. Non pathogenic yeasts: Saccharomyces.
4. Non spore forming and non flagellated rod or coccobacilli.

Mechanism of action in oral cavity [15].

1. Prevention of adhesion of pathogens to host tissues.
2. Stimulation and modulation of the mucosal immune system, e.g. by reducing production of proinflammatory cytokines through actions on NFkB pathways, increasing production of anti-inflammatory cytokines such as IL-10 and host defence peptides such as b-defensin 2, enhancing IgA defences and influencing dendritic cell maturation.
3. Modulation of cell proliferation and apoptosis through cell responses to, for example, microbially produced short chain fatty acids.
4. Improvement of intestinal barrier integrity and upregulation of mucin production.
5. Killing or inhibition of growth of pathogens through production of bacteriocins or other products, such as acid or peroxide, which are antagonistic towards pathogenic bacteria.
6. Involvement in binding of oral microorganisms to proteins (biofilm formation).
7. Action on plaque formation and on its complex ecosystem by competing and intervening with bacteria-to-bacteria attachments.
8. Involvement in metabolism of substrates (competing with oral microorganisms of substrates available).

Applications in dentistry

Role of probiotics in dental caries

Probiotics show possibility in reducing the incidence of dental caries [16]. Heng *et al* reported that S. salivarius M18 (formerly strain Mia) exhibited broad-spectrum inhibitory activity against several streptococcal pathogens, notably the caries-causing Streptococcus mutans [17]. The introduction of mutated gtfC gene affects the ability of S. mutans to produce extracellular glucans result in a decrease in extracellular matrix component of mixed oral biofilms from 51 to 33% of the biofilm volume [18]. A study aimed at showing the benefit of cheese containing Lactobacillus rhamnosus showed that probiotic intervention reduced the highest level of Streptococcus mutans [19]. Long-term consumption of milk containing Lactobacillus rhamnosus GG strain can reduce initial caries in kindergarten children. Ingestion of Lactobacillus reuteri ATCC 55739 or Bifidobacterium DN-173 010 can induce significant reduction of cariogenic S. mutans in saliva [20] Recombinant DNA technology was used to delete the gene encoding lactate dehydrogenase in BCS3-L1 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 that gives it a strong selective advantage over most other strains of S. mutans [21].

Probiotics in periodontal health

P. gingivalis, A.actinomycetemcomitans, T. denticola and T.forsythia are the main periopathogens. Probiotics decrease the pH of the oral cavity so that plaque bacteria cannot form dental plaque and calculus that causes the periodontal disease [22]. Probiotic mouth wash has been demonstrated to reduce the incidence of plaque formation and gingivitis in 6-8 year old children [16]. *In vitro* study of L. salivarius TI 2711 behaviour isolated from a healthy human volunteer showed inhibitory action on P. gingivalis, Prevotella intermedia and Prevotella nigrescens after 6-12 hrs coculturing [23]. Dave and colleagues [24]. claimed that Acilact (a probiotic complex of five live lyophilized lactic acid bacteria) improve both clinical

and microbiologic parameters in gingivitis and mild periodontitis patients. Krasse *et al*. [25] evaluated L. reuteri in patients with recurrent gingivitis. Patients having moderate to severe gingivitis were selected. L. reuteri strains were administered along with scaling and root planing surfaces of tooth. After 2 weeks, the clinical parameters were improved in the group consuming probiotic chewing gums.

Role of Probiotics in Halitosis

Halitosis is caused by a number of volatiles and most of its etiologic factors are present in the orpharynx (gingivitis, periodontitis, tongue coating, tonsillitis). F. nucleatum, P. intermedia, P. gingivalis, and T. denticola are produce "Volatile Sulphur Compounds" (VSC's) are responsible for halitosis. Co-aggregation of F. nucleatum with other periopathogens results in secondary colonization of biofilm and contributes to VSC production in oral cavity [26] A study on the patients of halitosis reported reduced levels of volatile sulphur compounds after consumption of gum or lozenges containing S. salivarius K12 [27] When a probiotic solution containing W. cibaria was used for gargling, there was a marked reduction in the production of hydrogen sulphide and methanethiol and hence, reduction in foul smell [28].

Probiotics in orthodontic treatment

The complex design of orthodontic bands and brackets creates an ecological environment that facilitates the establishment and growth of cariogenic mutans streptococci strains [29]. Cildir *et al*. [30] in 2009 conducted a clinical study with probiotics and found out that daily consumption of fruit yogurt with Bifidobacterium animalis subsp. Lactis DN - 173010 could reduce the salivary levels of mutans streptococci in orthodontic patients with fixed appliances.

Probiotics and Candida species

Candida species specially, C. albicans is a leading cause of fungal infection in oral cavity; it is particularly common in the elderly and in immunocompromised patients. The intake of probiotics in cheese containing L. rhamnosus GG and Propionibacterium freudenreichii resulted in reduced risk of C. albicans infections [31].

Probiotic Dosage

No consensus exists regarding the minimum number of microorganisms that must be ingested to obtain a beneficial effect [32]. Milk and milk products are the most popular carriers of probiotics. Milk contains calcium, calcium lactate and other organic and inorganic compounds with known anti-cariogenic properties [33]. Probiotics are supplied along with prebiotic in form of powder sachet, gelatin capsules, or suspension. Combination of pre and pro-biotic has 0.48 billion spores of Lactobacillus bifidum, Streptococcus thermophilus, and 0.10 billion spores of Saccharomyces boulardi along with 300 mg of fructo-oligosaccharides, is given as single dose daily before meals in the morning [34].

Safety concerns

The biggest challenge in the development of probiotic products is to maintain the adequate number of viable cells during the shelf life of the product as well as during the gastrointestinal tract transit after consumption, so that the claimed health benefits can be delivered to the consumer [35]. Some probiotic organisms, such as Enterococcus, Bacillus, and other spore-forming bacteria, as well as streptococci, that are not generally regarded as safe but have been used as probiotics, their use is associated with increased risk of Bacteremia and endocarditis development [36]. Several approaches are possible in the assessment of the probiotic safety [37].

1. Studies on the intrinsic properties of the probiotic strain;
2. Studies on the pharmacokinetics of the probiotic strain;
3. Studies on interactions between the probiotic strain and the host.

The probiotics should be able to maintain genetic stability in oral microflora environment [38].

Conclusion

Biotechnological advancements in the field of probiotics, advocate golden opportunity to treat diseases in a natural and non-invasive way. Despite being effective in combating issues with overuse of antibiotics and antimicrobial resistance, further studies needed in order to determine appropriate parameters for strain selection, physiological characteristics, tolerance to conditions of the digestive tract, multiplication and operating capacity in the intestine, the effect on the immune system, antibacterial factors, the ability to colonize, resistance to industrial processing, efficacy and safety of Probiotics.

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