

Probiotics, Prebiotics and Synbiotics - A Review

T. MANIGANDAN¹, S.P. MANGAIYARKARASI², R. HEMALATHA²,
V.T. HEMALATHA¹ and N.P. MURALI³

¹Department of Oral Medicine and Radiology, Sree Balaji Dental College and Hospital, Pallikaranai, Chennai, India.

²Department of Pedodontics and Preventive Dentistry, SRM Dental College and Hospital, Ramapuram, Chennai, India.

³Department of Pharmacology, Sree Balaji Dental College and Hospital, Pallikaranai, Chennai, India.

*Corresponding author E-mail: manident@yahoo.com

(Received: October 13, 2012; Accepted: November 23, 2012)

ABSTRACT

Probiotics, prebiotics and synbiotics are emerging as a fascinating field in medicine and dentistry. Probiotics are dietary supplements containing beneficial bacteria and yeast which play an important role to strengthen the immunity. Probiotics play a major role to strengthen the immunity and an important role in dentistry by creating a biofilm and helps in prevention of dental caries. Prebiotics are designed in a way to stimulate the number and activities of probiotics to improve health. Synbiotics refer to nutritional supplements combining pro and prebiotics that acts together in a synergistic manner.

Key words: Probiotics, Prebiotics, Synbiotics, Microorganisms, Oral health.

INTRODUCTION

The benefits of Probiotics have been recognized and explored for over a century.¹ Probiotic approach can be effective in selectively inhibiting oral pathogens or modulate the microbial composition of dental plaque.² Prebiotics are nondigestible dietary supplements.³ Prebiotics are designed to improve health by stimulating numbers and/or activities of probiotics like Bifidobacteria and Lactobacilli.¹ Prebiotics have been proved to be an aid to complement probiotics in the treatment of oral diseases.³ Their function is to enhance the growth and activity of beneficial organisms and simultaneously suppress the growth and activity of potentially deleterious bacteria.⁴ Synbiotics refer to nutritional supplements combining probiotics and prebiotics that are thought to act together; i.e. synergism. The potential benefits of synbiotic therapy are obvious, however, the great challenge, as is the case with probiotics and prebiotics alone, is to determine the best combination for each

disease setting and each individual.¹ Probiotics and prebiotics that are thought to act together; i.e. synergism. It has been suggested that a combination of a probiotic and a prebiotic, i.e. Synbiotics might be more active than either a probiotic or prebiotic alone.¹

Probiotics

Probiotic is derived from Latin word "pro"- for and Greek word "biotic"- life.⁵ The role of fermented milk in human diet was known even in Vedic times. But, the scientific interest in this area boosted after the publication of the book entitled 'The Prolongation of Life' by Ellie Metchinkoff in 1908. He suggested that people should consume fermented milk containing lactobacilli to prolong their lives.⁴

At this time Henry Tissier, a French paediatrician, observed that children with diarrhoea had in their stools a low number of bacteria characterized by a peculiar, Y shaped morphology.

These bacteria were, on the contrary, abundant in healthy children (Tissier, 1906). He suggested that these bacteria could be administered to patients with diarrhoea to help restore a healthy gut flora. The works of Metchnikoff and Tissier were the first to make scientific suggestions about the probiotic use of bacteria, even if the word "probiotic" was not coined until 1960.⁶

The term Probiotic was first introduced by Lily and Stillwell (1965).⁷ The first probiotic bacteria

studied were lactic acid bacteria (Fuller, 1991).⁸ Lactobacillae & Bifidobacterium are the main probiotics. Other probiotics are Escherichia, Enterococcus, Bacillus, Saccharomyces, Streptococcus and Propionibacteria. In 1984 Hull identified the first probiotic species, the lactobacillus acidophilus. Later in 1991, Holcomb identified bifidobacterium bifidum. WHO in 1994 described the probiotics as next most important in immune defense system following antibiotic resistance.^{9,10,11}

Definition of probiotics

Author	Definition
Lilly & Stillwell, 1965 ⁷	Substances produced by microorganisms that promote the growth of other microorganisms
Parker, 1974 ¹²	Organisms and substances that contribute to intestinal microbial balance
Fuller, 1989 ¹³	A live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance
Havenaar & Huis Int Veld, 1992 ¹⁴	A viable monoculture or mixed-culture of microorganisms that, when applied to animal or human, beneficially affects the host by improving the properties of the indigenous microflora
Schaafsma, 1996 ¹⁵	Living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition
Naidu et al, 1999 ¹⁶	A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as by improving nutritional and microbial balance in the intestinal tract.
Schreuzemir & De Vrese, 2001 ¹⁷	A preparation of, or a product containing, viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and as such exert beneficial health effects in this host
FAO/WHO Report, 2001	Live microorganisms that, when administered in adequate amounts, confer a health benefit to the host

Composition of probiotics¹⁸

Probiotics, which are regulated as dietary supplements and foods, consist of yeast or bacteria. They are available as capsules, gels, pastes, tablets, packets, liquids, or powders, and are contained in various fermented foods, most commonly yogurt or dairy drinks. Probiotic products may contain a single microorganism or a mixture of several species. Probiotics can be bacteria, moulds, yeast. But most probiotics are bacteria. Among bacteria, lactic acid bacteria are more popular.¹⁸

Criteria for probiotics^{13,19}

Fuller in 1989 listed the following as features of a good probiotic.

It should be

- 1) A strain, which is capable of exerting a beneficial effect on the host animal, e.g. Increased growth or resistance to disease.
- 2) Non-pathogenic and non-toxic.
- 3) Present as viable cells, preferably in large numbers.
- 4) Stable and capable of remaining viable for

long periods under storage and field conditions

Currently used probiotics²⁰

Bacteria

- ✓ Lactobacillus: acidophilus, sporogenes, plantarum, rhamnosum, delbrueck, reuteri, fermentum, lactus, cellobiosus, brevis
- ✓ Bifidobacterium: bifidum, infantis, longum, thermophilum, animalis
- ✓ Streptococcus: lactis, cremoris, alivarius, intermedius
- ✓ Leuconostoc
- ✓ Pediococcus
- ✓ Propionibacterium
- ✓ Bacillus
- ✓ Enterococcus
- ✓ E. Faecium

Yeast and moulds

- ✓ cerevisiae
- ✓ niger
- ✓ oryzae
- ✓ Pintolopesii
- ✓ Sacharomyces boulardii

Therapeutic actions of probiotics^{19,21,22}

1. Reduces progression of AIDS.
2. Enhancement of calcium absorption.
3. Competition against harmful microorganisms including Candida, preventing colonisation of pathogens through the production of inhibitory substances including acids and hydrogen peroxide and natural antibiotics
4. Reduction in liver toxicity
5. Enhancement of peristalsis, digestion, regularity and re-absorption of nutrients, In infants, promotion of healthy digestive tract colonisation
6. Enhancement and balance of oestrogen levels, prevention of osteoporosis through increased calcium uptake
7. Enhancement of vitamin status (B, K), digestion of proteins, fats, carbohydrates.
8. Increased resistance to infectious diseases (Perdigon *et al.*, 1995; Arunachalam *et al.*, 2000)
9. Alleviate lactose intolerance (McDonough *et al.*, 1987)

10. Prevention from gut (Naidu *et al.*, 1999), diarrhoea (Vanderhoof *et al.*, 1999), gastritis (Elmer *et al.*, 1996), vaginal and urogenital infections (Hilton *et al.*, 1992)
11. Reduction in blood pressure and regulation of hypertension, serum cholesterol concentration (Fuller, 1997)
12. Reduction in allergy (Bengmark, 2000), respiratory infections (Hatakka *et al.*, 2001)
13. Resistance to cancer chemotherapy and decreasing risk of colon cancer (Von Bultzingslowen *et al.*, 2003)

Probiotic products

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).
4. As concentrated and dried cells packaged as dietary supplements (non-dairy products).

Mechanism of action of probiotics²⁸

Possible modes of action of probiotic

Suppression of viable count by

- (a) Production of antibacterial compounds
- (b) Competition for nutrients
- (c) Competition for adhesion sites

Alteration of microbial metabolism

- (a) Increased enzyme activity
- (b) Decreased enzyme activity

Stimulation of immunity

- (a) Increased antibody levels
- (b) Increased macrophage activity

Role of prebiotics in dentistry⁴²

Most probiotics are in dairy forms containing high calcium, possibly reducing demineralization of teeth. It is possible that these act at bio-film to keep pathogens away and occupy a space that might otherwise be occupied by a pathogen. Probiotics should adhere to dental tissues to establish a cariostatic effect and thus should be a part of the bio-film to fight the cariogenic bacteria. The duration of their stay locally also is important for beneficial effect. Ideal vehicles of probiotics installation are yogurt, milk and cheese.

Different means of probiotic administration for oral health purposes

Author	Vehicle	Strain	Result
Caglar E <i>et al.</i> , 2005 ²²	Straw, tablet	L. reuteri ATCC 55730	S.mutans level reduction
J.P. Burton <i>et al.</i> , 2006 ²³	Lozenge	S. salivarius	Reduces oral VSC (Volatile sulphur compounds) levels
K. Hatakka <i>et al.</i> , 2007 ²⁴	Cheese	L. rhamnosus GG; Prorionibacterium JS	Reduced risk of high yeast counts and Hyposalivation
Kang <i>et al.</i> , 2005 ²⁵ Montalto M <i>et al.</i> , 2004 ²⁶	Rinse solution Capsule, liquid	W. cibaria L. sporogenes, L.bifidum,L.bulgaricus, L. thermophilus, L.acidophilus, L. casei,	Reduction of VSC Increased salivary counts of lactobacilli without significant decrease in S. mutans counts
Yli-Knuuttila H <i>et al.</i> , 2006 ²⁷	Yogurt drink	L. rhamnosus L. rhamnosus GG	Temporary oral cavity colonization

Some of the hypothetical mechanism of probiotics action in the oral cavity is by:

- ✓ Direct interaction in dental plaque
- ✓ Involvement in binding of oral micro-organisms to proteins
- ✓ Action on plaque formation and on its complex ecosystem by competing and intervening with bacterial attachments.
- ✓ Involvement in metabolism of substrate and production of chemicals that inhibit oral bacteria

Side effects and safety aspects of probiotics

Probiotics may theoretically be responsible for four types of side effects: systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, and gene transfer.²⁹ Cases of infection due to lactobacilli and bifidobacterium are extremely rare and are estimated to represent 0.05 - 0.4% of cases of infective endocarditis and bacteraemia.³⁰

In general gas & bloating is one of the side effects of eating probiotic supplement. Probiotic can heighten & stimulate the immune system. So there is a great chance that people with underlying health

issues can catch infections caused by probiotics.² Till date no pathogenic or virulence properties have been found for Lactobacilli, Bifidobacterium or Lactococci (Aguirre & Collins 1993).

How to assess the safety of probiotic products?²⁹

Three approaches can be used to assess the safety:

- 1) Studies on the intrinsic properties of the strain.
- 2) Studies on the pharmacokinetics of the strain.
- 3) Studies searching for interactions between the strain and the host.

Disadvantages of probiotics³¹

Liquid preparations like yogurt have some major disadvantages

- i) short shelf- life,
- ii) bacteria damaged by pasteurization and/or centrifugation
- iii) use of additives and preservatives
- iv) difficult transport and storage because of its bulky nature
- v) use of normally only one or more strains of bacteria (multiple strains probiotics are more potent)
- vi) damage by stomach acidity
- vii) refrigeration requirement.

Freeze dried powder probiotics have some disadvantages such as i) bacteria damage by freeze drying ii) short powder shelf life iii) upon absorption of water by powder, bacteria become activated and die iv) poor adherence, colonization and survival in the gut due to damage caused by freeze drying v) Probiotics may become weakened due to addition of stabilizers and preservatives.

Prebiotics

The bacterial population of the human gastrointestinal tract constitutes an enormously complex ecosystem. Most of these organisms are beneficial (e.g. bifidobacterium and lactobacillus) but some are harmful (e.g. Salmonella species, Helicobacter pylori, Clostridium perfringens). Some dietary substances, the so-called prebiotics can favor the growth of these beneficial bacteria over that of harmful ones.²²

Prebiotics are defined as non-digestible or low-digestible food ingredients that benefit the host organism by selectively stimulating the growth or activity of one or a limited number of probiotic bacteria in the colon (Crittenden & Playne, 1996; Dimer & Gibson, 1998; Zimmer & Gibson, 1998; Manning & Gibson, 2004).³²

Classification of prebiotics

Based on the number of monomers linked together, prebiotics can be classified:

- ' Disaccharides
- ' Oligosaccharides (3-10 monomers)
- ' Polysaccharides

Commonly used prebiotics

Lactulose, galacto-oligosaccharides, fructo-oligosaccharides, inulin and its hydrolysates, malto-oligosaccharides, and transgalacto-oligosaccharides. The main end products of carbohydrate metabolism are short-chained fatty acids, namely acetate, butyrate and propionate, which are further used by the host organism as an energy source³²

Prebiotic products³²

Prebiotic oligosaccharides can be produced in three different ways: by extraction from plant materials, microbiological synthesis or enzymatic synthesis, and enzymatic hydrolysis of

polysaccharides (Crittenden & Playne, 1996; Gulewicz *et al.*, 2003).³ Prebiotics are naturally found plenty in certain fruits like bananas, asparagus, garlic, tomato and onion wheat.

Criteria for prebiotics³³

- 1) Neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract
- 2) A selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated
- 3) Consequently, be able to alter the colonic flora in favour of a healthier composition
- 4) Induce luminal or systemic effects that are beneficial to the host health.

Therapeutic actions of prebiotics³⁵

The positive effects of prebiotics include antimicrobial, anticarcinogenic, hypolipidemic, glucosemodulatory and anti-osteoporotic activities. They may be used for the treatment of constipation, hepatic encephalopathy and inflammatory bowel disease. They can protect against some intestinal pathogens and may exert favourable lipid effects as well as have some benefit in diabetes mellitus. Besides, prebiotics also have a very important role in improving mineral absorption and balance, for instance, they may enhance the colonic absorption of some minerals.

Benefits of prebiotic supplemented formulas³

Clearly, breast milk is the "gold standard" for neonatal and infant nutrition and is recommended by the American Academy of Pediatrics as the nutrition. Use of prebiotic-supplemented infant formulas may have benefits for the infant who does not receive mother's milk. The benefits are,

- 1) Higher counts of Bifidobacteria by 4 weeks of age. An increased number of Bifidobacteria is associated with lower numbers of intestinal pathogens.
- 2) The pattern of bifidobacterial sub-species is similar to the pattern of the breast-fed infant.
- 3) Prebiotic formulas result in stool pH and short-chain fatty acid patterns similar to the breast-fed infant.
- 4) Stool frequency and consistency is more like the breast-fed infant.

- 5) Reduced allergic reaction (atopic dermatitis) and reduced URI in the first year of life.
- 6) Fewer episodes of acute diarrhoea.
- 7) Prebiotic-supplemented formula is easily tolerated, with no difference in growth patterns.

Studies using prebiotic-supplemented formulas³

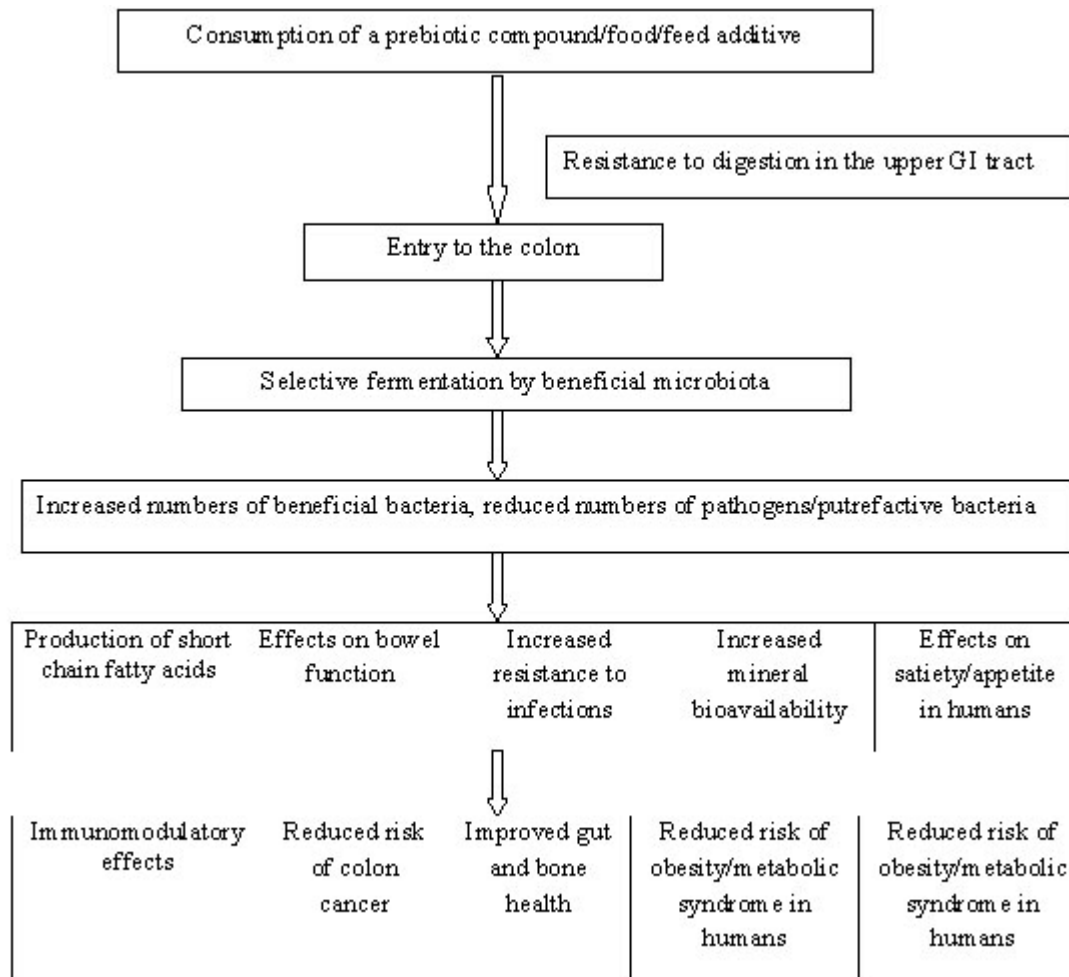
Study	Prebiotic & quantity	Outcomes
Bruzzese et al 2006 ³⁶	GOS/FOS or control	Infants on prebiotic formula had fewer episodes of acute diarrhea, fewer upper respiratory infections
Moro et al 2006 ³⁷	0.8 g/dL of GOS, FOS and control hydrolysed protein formula	Incidence of atopic dermatitis significantly reduced in the infant fed prebiotic formula
Costalos et al 2007 ³⁸	0.4 g/dL of GOS and FOS and control	Prebiotic formula well tolerated, normal growth trend toward higher percentage of <i>Bifidobacterium</i> and lower percentage of <i>E. coli</i> in stool, suppresses <i>Clostridium</i> in stool
Ziegler et al 2007 ³⁹	0.4 g/dL PDX,GOS or 0.8 g/dL PDX,GOS and LOS or control	Looser stools on either prebiotic formula.more adverse events: diarrhoea, eczema, in supplemented groups
Scholtens et al 2008 ⁴⁰	0.6 g/dL FOS and GOS	At 27 weeks the concentration of secretory IgA was higher in prebiotic group than control, also <i>Bifidobacterium</i> percentage higher than control and <i>Clostridium</i> lower
Arslonaglu et al 2008 ⁴¹	0.8 g/dL GOS/FOS	Formula fed for first six months; follow up for 2 years. Prebiotic group had significantly lower allergic symptoms – atopic dermatitis, wheezing, urticaria, fewer upper respiratory infections than controls during the first 2 years

FAGA—full-term appropriate for gestational age; GOS—galacto-oligosaccharides; FOS—fructo-oligosaccharides; PDX—Polydextrose; LOS—Lactulose.

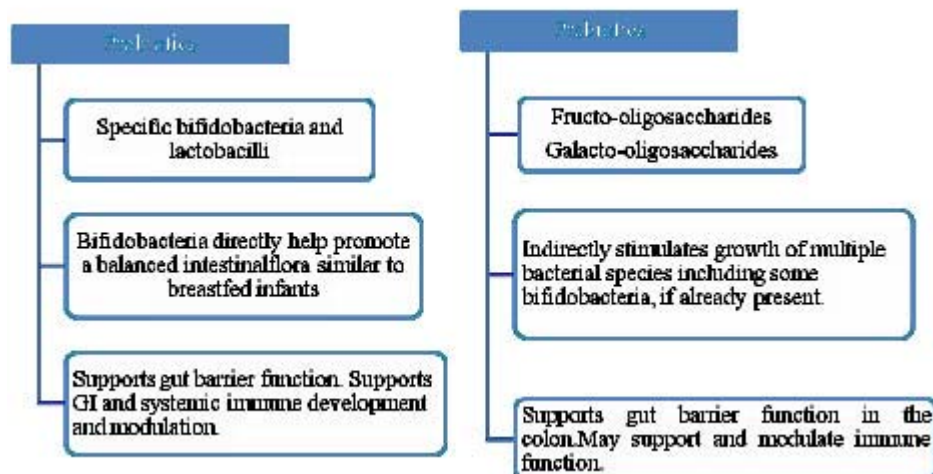
Relationship between prebiotics and probiotics

Prebiotics when combined with probiotics have many advantages Basically, prebiotics selectively stimulate the growth of probiotics, which is dose and strain dependent. Prebiotics serve as a selective growth substrate for the probiotics strain during fermentation, during the period of storage, or during its passage through the gut. These two combinations implant live microbial dietary supplements and create a congenial environment for their survival in gut flora. Thereby, this

environment in gut flora improves healthy microbial balance. So, the combination of prebiotics and probiotics may have additive and synergistic effect in providing better oral health conditions.⁴An 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.⁴ Probiotics and



Mode of action of prebiotics and health benefits in humans and animals³⁴



Comparison of Probiotics Vs Prebiotics

prebiotics could affect the host in combination by synergistic action.⁴²

Synbiotics

A synbiotic has been defined as 'a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the GI tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare' (Gibson & Roberfroid, 1995).²⁸

The main reason for using a synbiotic is that a true probiotic, without its prebiotic food, does not survive well in the digestive system. Without the necessary food source for the probiotic, it will have a greater intolerance for oxygen, low pH, and temperature. As prebiotics provides a great place for probiotics to thrive, the population of these good bacteria is known to preserve. Studies have shown that by harnessing both the benefits of these prebiotics and probiotics into synergy, the number of good bacteria in the digestive systems increased many folds for the betterment of our health.³¹

Synbiotics work in two ways i) by improving the viability of probiotics and ii) by delivering specific health benefits.³¹ The intake of a synbiotic food leads to a modulation of the gut metabolic activities with a maintenance of the gut

biostructure. In particular, the significant increase of short chain fatty acids, ketones, carbon disulfide and methyl acetate following the feeding period suggested potential health promoting effects of the synbiotic food.⁴³

Therapeutic actions of synbiotics

For therapeutic efficacy, the desirable characteristics of synbiotics include antimicrobial and anticarcinogenic qualities, antidiarrheal aspects, antiallergenic qualities, osteoporosis prevention, reduction in serum fats and blood sugars, regulation of the immune system, and treating liver-related brain dysfunction.⁴

CONCLUSION

The use of probiotics for use in oral care applications is gaining momentum. There is increasing evidence that the use of existing probiotic strains can deliver oral health benefits. Further work will be needed to fully optimise and quantify the extent of this benefit. In parallel, the potential of prebiotics to maintain and enhance the benefits provided by the resident oral microbiota will be investigated. However, whether considering probiotics or prebiotics, it will be essential to develop an understanding of the broad ecological changes induced in the mouth by their ingestion and the long-term consequences of their use on oral health and disease.⁴⁴

REFERENCES

1. Sweta V. Chauhan and Mehul R. Chorawala. Probiotics, Prebiotics And Synbiotics. *IJPSR* **3**(3): 711 -726 (2012).
2. Shabnam Zahir, Surajit Bose and Utpal Roychaudhury. *Probiotics and its Role in Dental Caries. Sci. & Cult.* **77**(11-12): 507-510 (2011).
3. Jacqueline J Wessel. Prebiotics, Probiotics, and Synbiotics: Functional Foods. Abbott Nutrition Health Institute.org
4. Sudhakar Reddy R ,Swapna L.A ,Ramesh T, Rajesh Singh T , Vijayalaxmi N , Lavanya R. Bacteria in Oral Health – Probiotics and Prebiotics A Review. *Int J Biol Med Res.*, **2**(4): 1226 -1233 (2011).
5. Kamlesh Singh, Basavaraj Kallali, Ajay Kumar, Vidhi Thaker. Probiotics: A review. *Asian Pacific Journal of Tropical Biomedicine* S287-S290 (2011).
6. Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria, (2001).
7. DM Lily, R.H Stillwell. *Science*, **147**: 747-748 (1965).

8. R. Fuller. *J Appl Bacteriol*, **66**: 365-378 (1989).
9. Patil MB, Reddy N. Bacteriotherapy and probiotics in dentistry. *KSDJ* **2**: 98-102 (2006).
10. Elisa KB, Scott BS. Regulatory t cells in IBD, *current opinion of gastroenterology* **24**: 733-41 (2008).
11. Manisha N, Ashar, Prajapathi JB. Role of probiotic cultures and fermented milk in combating blood cholestrol. *Indian J Microbial* **41**: 75-86 (2001).
12. Parker R B. Probiotics, the other half of the antibiotic story. *Anim Nutr Health*.**29**: 4-8 (1974).
13. Fuller R . Probiotics in man and animals: A review. *Journal of Applied Bacteriology*, **66**: 365-378 (1989).
14. Havenaar R, Huis Int Veld MJH. Probiotics: a general view. In: Lactic acid bacteria in health and disease. Vol.1 Amsterdam: Elsevier Applied Science Publishers (1992).
15. Schaafsma G. State of art concerning probiotic strains in milk products. *IDF Nutr News Lett*. **5**: 23-24 (1996).
16. Naidu AS, Bidlack WR, Clemens RA. Probiotic spectra of lactic acid bacteria. *Crit Rev Food Sci Nutr*. **39**(1): 13-126 (1999).
17. Schrezenmeir J, DeVrese M. Probiotics, Prebiotics, and Synbiotics—Approaching a definition. *Am J Clin Nutr.*, **73**(2 Suppl): 361S-364S (2001).
18. Suvarna V.C and Bobby V.U. Probiotics in human health: A current Assessment. *Current Science* **88**(11): 1744-1748 (2005).
19. Mohit Sareen, Sayak Roy, Siddharth Kumar Singh, Anjali Gupta. A Review on Probiotics and their Implications in Dentistry. *Journal of Dentofacial Sciences*, **1**(2): 7-10 (2012).
20. Anuradha S, Rajeshwari K. Probiotics in Health and Disease. *JACM* **6**(1): 67-72 (2005).
21. Biradar S.S, Bahagvati S.T, Baburao Shegunshi. Probiotics And Antibiotics: A Brief Overview. *The International Journal Of Nutrition and Wellness*. **2**(1) (2005).
22. Aglar EC, Kargul B, Tanboga I. Bacteriotherapy and probiotics role on oral health. *Oral Diseases* **11**: 131-137 (2005).
23. Burton J.P, Chilcott C.N, Moore C.J, Speiser G and Tagg J.R .A preliminary study of the effect of probiotic Streptococcus salivarius K12 on oral malodour parameters. *Journal of Applied Microbiology.*, 754-764 (2006).
24. Hatakka K, Ahola A.J, Yli-Knuuttila H, Richardson M, T. Poussa. T, Meurman J.H, and Korpela R . Probiotics Reduce the Prevalence of Oral Candida in the Elderly-A Randomized *Controlled Trial*. *J Dent Res* **86**(2): 125-130 (2007).
25. Mi-Sun Kang, Hee-Sam Na, Jong-Suk Oh. Coaggregation ability of Weissella cibaria isolates with Fusobacterium nucleatum and their adhesiveness to epithelial cells. *FEMS Microbiology Letters*, **253**: 323–329 (2005).
26. Montalto M, Vastola M, Marigo L, Covino M, Graziosetto R, Curigliano V, Santoro L, Cuoco L, Manna R, Gasbarrini G. Probiotic Treatment Increases Salivary Counts of Lactobacilli: A Double-Blind, Randomized, Controlled Study. *Digestion*, **69**: 53-56 (2004).
27. Yli-Knuuttila H, Snall J. Kari K, Meurman J.H. Colonization of Lactobacillus rhamnosus GG in the oral cavity. *Oral Microbiology Immunology*. **21**: 129-131 (2006).
28. Helena Parracho, Anne L. McCartney and Glenn R. Gibson. Probiotics and prebiotics in infant nutrition. *Proceedings of the Nutrition Society* **66**: 405-411 (2007).
29. Philippe Marteau. Safety aspects of probiotic products. *Scandinavian Journal of Nutrition* **45**: 22-24 (2001).
30. Gasser F. Safety of lactic acid bacteria and their occurrence in human clinical infections. *Bulletin de L' Institut Pasteur* **92**: 45-67 (1994).
31. Bhupinder Singh Sekhon and Saloni Jairath. Prebiotics, probiotics and synbiotics: an overview. *J Pharm Educ Res* **1**(2):13-36 (2010).
32. Wodzimierz Grajek, Anna Olejnik and Anna Sip. Probiotics, prebiotics and antioxidants as functional foods. *Acta biochimica polonica* **52**(3): 665-671 (2005).
33. Glenn R. Gibson and Marcel B. Roberfroid. Dietary Modulation of the Human Colonie Microbiota: Introducing the Concept of Prebiotics. *J. Nutr.* **25**: 1401-1412 (1995).
34. Laurie O Sullivan, Brian Murphy, Peter McLoughlin, Patrick Duggan, Peadar G. Lawlor, Helen Hughes, and Gillian E.

- Gardiner. Prebiotics from Marine Macroalgae for Human and Animal Health Applications. *Mar. Drugs* **8**: 2038-2064 (2010).
35. Raquel Guine, Mario Joao Lima, Mario Joao Barroca. Role and health benefits of different functional food components.
36. Bruzzese E, Volpicelli M, Salvini F, Bisceglia M, Lionetti P, Cinquetti M, Iacono G, Guarino A. Early Administration of GOS/FOS Prevents Intestinal and Respiratory Infections in Infants. *Journal of Pediatric Gastroenterology & Nutrition*, **42**(5): E95 (2006).
37. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child*, **91**: 814–819 (2006).
38. Costalos C, Kapiki A, Apostolou M, Papatoma E. The effect of a prebiotic supplemented formula on growth and stool microbiology of term infants. *Early Human Development* **84**: 45-49 (2008).
39. Ziegler E, Vanderhoof JA, Petschow B, Mitmesser SH, Stolz SI, Harris CL, Berseth CL. Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. *Pediatr Gastroenterol Nutr.* **44**(3): 359-64 (2007).
40. Scholtens PA, Alliet P, Raes M, Alles MS, Kroes H, Boehm G, Knippels LM, Knol J, Vandenplas Y. Fecal secretory immunoglobulin A is increased in healthy infants who receive a formula with short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides. *J Nutr.* **138**(6):1141-7 (2008).
41. Arslanoglu S, Moro GE, Boehm G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *J Nutr.*, **137**(11): 2420-4 (2007).
42. Kedar Saraf, Shashikanth MC, Tulasi Priya, Nishat Sultana, Nallan CSK Chaitanya. Probiotics - Do they have a Role in Medicine and Dentistry? *JAPI* **58**: 488-492 (2010).
43. Beatrice Vitali, Maurice Ndagijimana, Federica Cruciani, Paola Carnevali, Marco Candela, Maria Elisabetta Guerzoni and Patrizia Brigidi. Impact of a synbiotic food on the gut microbial ecology and metabolic profiles. *BMC Microbiology*, **10**: 4:1-13 (2010).
44. Deirdre A. Devine¹ and Philip D. Marsh. Prospects for the development of probiotics and prebiotics for oral applications. *Journal of Oral Microbiology* 1-11 (2009).