

Present scenario of clinical Significance of probiotic microorganisms

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Abstract - The review gives a glance of various roles of probiotics for the improvement of human health. Probiotics possess different beneficiary characteristics and have been extensively studied and explored commercially throughout the world. Benefits of probiotic micro organisms to human and animal health have been proven in many researches. *Lactobacillus* and *Bifidobacterium* are the prominent bacterial genera in Lactic acid bacteria (LAB); however, there are reports on the probiotic potential of other bacterial strains like *Pediococcus*, *Lactococcus*, *Bacillus* and yeasts. Probiotic strains are being widely used due to the reason that they exhibit powerful anti-inflammatory, anti-allergic and other important properties. Apart from that, the consumption of dairy and non-dairy products stimulates the immunity in different ways. Various food matrices have been used with probiotics, which are briefly documented. Researchers are going on to find new and better prebiotic as well as to develop effective symbiotic preparations (i.e. combination of probiotics and prebiotics) to maintain the beneficial microbiota of the human gastrointestinal tract. The present review aims at studying probiotics, prebiotics, application of probiotics in the health and food areas along with the clinical significances of probiotic.

Key words - probiotics, intestinal microflora, antimicrobial activity, *Lactobacillus*, immune stimulation, dairy products, non-dairy products

I. INTRODUCTION

Probiotics are usually defined as microbial food supplements with beneficial effects on the consumers. The history of probiotics began with the history of man. The earliest type of probiotic supplements were fermented milk and cheese. Probiotics is the fastest growing area in the development of new food product. The era of probiotics has introduced several beneficial physiological effects i.e. good health along with negligible adverse effects. In case of development of new products, the pre-existing strain which are capable of introducing probiotic action are been compared and tested against new strains for their efficacy (John O' Brien et al., 1999). Certain parameters have been made for probiotic selection which include, safety measures for the host, adhesion to epithelial cells, resistance against gastric acidity and pancreatic secretions, antimicrobial activity, restricting the adhesion of pathogenic bacteria, evaluation of antibiotics resistance, tolerance to food additives and stability in the food matrix (Havenaar et al.).

Probiotic bacteria are beneficial in balancing disturbed intestinal microflora and related dysfunction of the gastrointestinal tract. Regular consumption of food (400–500 g/week) containing 10^6 CFU/g of *Bifidobacterium* spp. and *L. acidophilus* (human origin), which are able to survive the upper regions of the gastrointestinal tract, is essential to achieve therapeutic benefits.

Lactic acid bacteria are a major group of probiotic micro organisms. LAB are usually obtained from dairy products, mainly yogurt, fermented milks or other fermented foods. Most commonly used probiotics in humans are, *Lactobacillus acidophilus*, *L. casei*, *Bifidobacterium bifidum*, *B. longum* and the yeast *Saccharomyces boulardii*. However, there are more common species of probiotic micro organisms such as: 1 – Bacteria: (i) *Lactobacillus*: *acidophilus*, *sporogenes*, *plantarum*, *rhamnosum*, *delbrueck*, *reuteri*, *fermentum*, *lactus*, *cellobiosus*, *brevis*, *casei*, *farciminis*, *paracasei*, *gasseri*, *crispatus*; (ii) *Bifidobacterium*: *bifidum*, *infantis*, *adolescentis*, *longum*, *thermophilum*, *breve*, *lactis*, *animalis*; (iii) *Streptococcus*: *lactis*, *cremoris*, *alivarius*, *intermedius*, *thermophilis*, *diacetylactis*; (iv) *Leuconostoc mesenteroides*; (v) *Pediococcus*; (vi) *Propionibacterium*; (vii) *Bacillus*; (viii) *Enterococcus*; (ix) *Enterococcus faecium*; 2 – Yeast and molds: *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Aspergillus niger*, *Aspergillus oryzae*, *Candida pintolopesii*, *Saccharomyces boulardii*.

General Characteristics of probiotics

The general features of probiotic are following as-

- They are mostly non-pathogenic and of human origin
- They are capable of outlasting and metabolizing in the gut environment e.g. resistance to low pH and organic acids.
- They are gram-positive, non-spore forming, rod shape, non-motile microorganisms.
- They show glycosidase activity.
- Their colonies are creamy white in color, transparent and circular in shape and convex elevated with flat and smooth rough textured.
- They release a variety of chemical compounds that are inhibitory for pathogenic microorganisms (Fuller, 1989).

II. FUNCTIONING AND HEALTH BENEFITS OF PROBIOTICS IN BODY

Probiotics microorganisms are extremely beneficial for the human health. They function in various ways to maintain the healthy environment of the body. Their functioning involves: inhibiting growth or reducing the activity of bad bacteria in intestine by colonizing the gut. They show antimicrobial activity and aid in increasing our immunity by making our body more resistant to diseases and infections. Probiotic micro organisms also improve the secretion of digestive enzymes and helps in proper digestion. They increase the production of lactic acid and regulate pH balance in intestine and other parts of body. They promote acidic pH which facilitates the absorption of protein and minerals like calcium, copper, magnesium, iron and manganese. They show anti-inflammatory properties and can ferment fructo-oligosaccharides which thereby results in reduced pH balance. This increases acidity in gut thereby enabling better absorption of calcium and allow it to get into blood stream.

Clinical evidences supporting health claims for probiotics

a) Lactose intolerance: This is a condition in which patients lack the enzyme lactase which is used for the breakdown of the sugar lactose. Consequently such patients are unable to digest lactose sugar present in dairy products. In this respect, yogurt and the non-dairy fermented products such as rice fermented beverages, coconut fermented water, etc. which are a rich source of probiotic and devoid of lactose, promise to be an alternative to milk fermented probiotic products (**Kolars et al. 1984**). In a study, researchers observed that lactose intolerant subjects consuming 18 gram of lactose in the form of yogurt could digest and absorb lactose efficiently than those who receive same amount of lactose from milk. Besides this, improved lactose tolerance in yogurt group also led to less diarrhea and other gastro intestinal symptoms. Through this study, researchers concluded that probiotics in yogurt helps in the release of β -galactosidase, an enzyme involved in lactose breakdown/digestion (**Rosado 1996**).

b) Aids in calcium absorption: Studies have shown that milk contains abundant amount of calcium apart from other dietary sources. The individuals suffering from lactose intolerance may develop osteoporosis due to decreased consumption of milk in diet. In such cases, if probiotics (curd, yogurt, fermented milk or non-dairy fermented foods) are fed to lactose intolerant individuals, milk lactose is hydrolyzed by probiotic strains and thereby favors calcium absorption (**Vandenplas and Benniga 2009**).

c) Antibiotic associated diarrhea: In one of the post by Nicole Cunningham (2014) in Puraforce Remedies, mentioned that there are two sides of the biotic coin, on one side antibiotics and then there are probiotics. If antibiotics are consumed for any reason then in order to counter act their effects, probiotics 'friendly bacteria' have to be consumed to fight illness and diseases so that chances to consume antibiotics is reduced to a certain extent. Antibiotics could not differentiate between good and bad bacteria. In turn both organisms are killed and due to the suppression of good bacteria, immune system as well as digestion suffers. It could be inferred that certain probiotic cultures have the greater ability to treat one disease compared to others present in the probiotic product.

d) Prevent urinary tract infections: In immune compromised as well as those with antibiotic treated hosts, the natural protective biofilm of bacteria and surface walls is lost through disruption. For a normal bacterial flora, host defense is ensured through a balance between non pathogenic commensals and pathogenic bacteria. In case of HIV+ patients colonization of lactobacillus in urogenital tract is diminished and this correlates with the shedding of HIV into the urogenital tract (**Sha et al. 2005**). Besides this, disruption of natural flora renders patients with many more severe infections.

e) Prevent anaemia: It has been reported that anaemic women had lesser number of *Lactobacillus* as compared to normal women. The main reason for this could be that *lactobacilli* increase the expression of iron transporters in the caecum due to production of propionic acid, a short chain fatty acid (**Balamurugan et al. 2010**).

f) Prevent type-2 diabetes: Musso and coworkers reported that the intestinal microbiota in type 2 diabetic patients have significantly reduced proportions of phylum Firmicutes and class Clostridia in the diabetic group compared to the control group (**Musso et al. 2010**).

g) Obesity associated problems: In early times it was said that yogurt had obesity fighting potential. Therefore, various researches have been conducted till date to find the cause of it. Johnson *et al.* while conducting their researches on mice studied the anti obesity effects linked to probiotic potential of yogurt. They observed that mice which fed on moderate fat diet was when supplemented with yogurt powder had significant weight loss compared to control (no yogurt fed). The reduction in weight was attributed to an increased level of lipid in faeces of yogurt supplemented mice group which further suggest that yogurt helps in reducing fat absorption in small intestine (**Johnson et al. 2007**).

III. PREBIOTICS

Prebiotics are a source of nutrients for the probiotic micro organisms to grow. These are the non-digestible food constituents like fibres which cannot be used by the body but are favorable for the growth and activity of probiotic bacteria in the colon and thus improving host health. As a result of the presence of fibres in prebiotic it reaches the large intestine in an intact form. Once these non-digestible carbohydrates pass into the intestines, they are used by the probiotic bacteria that live there for their survival and growth. Prebiotics of proven efficacy are able to modulate the gut microbiota by stimulating indigenous beneficial flora while inhibiting the growth of pathogenic bacteria therein (**Chow, 2002**).

IV. PROBIOTICS IN FOOD AND BEVERAGES

It is reported that food could serve as medicine was first conceived thousands of years ago by the Greek philosopher and father of medicine, Hippocrates, who once wrote: 'Let food be thy medicine, and let medicine be thy food'. However, during recent times, the concept of food having medicinal value has been reborn as 'functional foods'. A probiotic may also be a functional food (**S. Scheinbach, 1998**). Functional foods are defined as: 'foods that contain some health-promoting component(s) beyond traditional nutrients'. Functional foods are also known as designer foods, medicinal foods, nutraceuticals, therapeutic foods, super foods,

foodceuticals, and medifoods. In general, the term refers to a food that has been modified in some way to become 'functional'. One way in which foods can be modified to become functional is by the addition of probiotics (FAO/WHO, 2002).

Ever since the consumers became aware of the potential of probiotics in improving their health, more and more food products have come in market, which are supplemented with added probiotic cultures. A number of different types of food matrices are being used such as various types of cheese, ice creams, milk-based desserts, powdered milk for newborn infants, butter, mayonnaise, powder products or capsules and fermented food of vegetable origin (Tamime AY, 2005).

Dairy products

Dairy products are especially considered as ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract. The matrices used most frequently are cheese, yogurt, ice cream and other dairy products.

The most common means to incorporate probiotics to fermented milk include: (i) addition of probiotics together with the starter cultures (DVI culture); (ii) the production of two batches separately, one containing the probiotic microorganism in milk to achieve a high concentration of viable cells and another with starter cultures. When the fermentation stages are completed, the batches are mixed; (iii) the use of a probiotic microorganism as a starter culture. In this situation, the time of fermentation is generally higher than traditional processes using non-probiotic starter cultures (Tamime AY, 2005). In this respect, it is necessary to consider the supplementation of the culture medium and the production conditions (e.g. incubation temperatures), since metabolites produced by probiotics can lead to off-flavours (Saarela, 2000; Østlie, 2003). In addition to it, the probiotic strains must be compatible with starter cultures so that the starter cultures do not produce inhibitory substances that damage the probiotics (Vinderola, 2002).

Yogurts which had a high content of fat inhibited the growth of probiotic cultures, particularly *B. bifidum* (Vinderola, 2000). It is reported that vitamin supplements (e.g. ascorbic acid) improve the viability of *L. acidophilus* in yogurts (Dave, 1997). The addition of substances such as whey protein may also enhance the viability of some probiotics. Moreover, the introduction of prebiotics in yogurt formulations stimulates the growth and activity of probiotics. In this regard, fructo-oligosaccharides are reported to be most effective in maintaining the probiotic viability (Capela, 2006).

Briefly explained role of probiotic bacteria in dairy fermentations is to: (i) preserve the milk by the generation of lactic acid; (ii) produce essential compounds and other metabolites for commercial use that will provide a product with the organoleptic properties desired by the consumer; (iii) improve the nutritional value of food, like release of free amino acids or the synthesis of vitamins; and (iv) the probiotic also possess therapeutic or prophylactic properties against cancer (Reddy et al. 1973; Fernandes et al. 1987; Gilliland 1990; O'Sullivan et al. 2000) and control of serum cholesterol levels (Lin et al. 1991).

Non-dairy products

Dairy products are a great source of providing probiotics as they present the best matrix for probiotic growth. But there are also few demerits attached with consuming dairy products like people having lactose intolerance, fat content, or sensitivity to milk products. Therefore, there is increase in the population going for the better alternative i.e. non-dairy products. The non-dairy probiotic products are based on the fermentation of fruits, vegetables, legumes and cereals. Fruits and vegetables are considered as good matrices since they contain nutrients such as minerals, vitamins, dietary fibres, and antioxidants. The development of different probiotic fruit and vegetable beverages have been studied (Yoon, 2004; Soccol, 2007). A variety of non-dairy probiotic beverages are being formulated and produced (Prado et al., 2008). However, the incorporation of probiotics in fruit juices requires the protection against acid conditions. This can be achieved by microencapsulation technologies or lyophilization techniques for entrapping or drying of cells to increase the shelf life of the beneficial microbes. Gelatin and vegetable gum have been demonstrated to provide a good protection for acid-sensitive *Bifidobacterium* and *Lactobacillus* (Sultana et al., 2000; O'Riordan et al., 2001; Chandramouli et al., 2004).

Encapsulation processes in milk protein have also been studied (Heidebach et al., 2009). When *B. lactis* were microencapsulated, incorporated into African fermented beverages (amasi and mahewu) and assayed for physiological conditions of the stomach, they showed a high survival rate, i.e. the microencapsulation enhanced the viability in comparison with free cells (McMaster et al., 2005).

Probiotic strains usually found in vegetable materials are species belonging to *Lactobacillus* and *Leuconostoc* genera. *L. plantarum*, *L. casei* and *L. delbrueckii*, for example, were able to grow in cabbage juice without nutrient supplementation and reached 10^8 CFU/mL after 48 h of incubation at 30 °C (Yoon et al., 2006). In addition, it was found that these same bacteria grew in beet juice (Kyung et al., 2005).

Cereal based fermented foods

The cereal and legumes are the part of our diet since Indus valley civilization (Samanta et al., 2011). They are used as base materials for the production of probioticated functional food, as they are a good source non-digestible carbohydrates, which supports and triggers the growth of *Lactobacilli* and *Bifidobacteria*. Infant follow up formulas and young child formulas are designed so as to fulfill their health needs as these foods are cereal based so that they can easily accept homely foods. These foods are easily digestible and triggers the growth of healthy gut microbes. They contain water-soluble fibers such as beta glucan, Arabinoxylan, galacto-oligosaccharides and fructo-oligosaccharides, which are broken down into smaller molecules by selective groups of LAB (Swennen et al., 2006).

Weissella paramesenteroides, *Lactobacillus fermentum*, *L. plantarum*, *Enterococcus faecalis*, *Pediococcus acidilactici*, *P. cerevisiae* are found in the batter prepared for south Indian foods like dhokla, idli, kallappam, ambali and dosa. *L. plantarum* AS1 isolated Kallappam is been reported to restrict the multiplication of *Vibrio parahaemolyticus* (Satish et al., 2011) and colorectal cancer in male rats (Satish et al., 2012). Whereas LAB isolated from dosa can restrict the growth of causative agents such as *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *V. parahaemolyticus* and *Aeromonas hydrophila* (Pal et al., 2005).

Meat based fermented foods

Meat is highly prone to microbial spoilage therefore drying, smoking and fermentation of meat are required for its preservation (Oki *et al.*, 2011). Meat fermentation is a very easy and simple technique which give it a particular taste, flavor, color and softness. *L.lactis*, *L.plantarum*, *E.faecium*, *L.fructosus*, *L.amylophilus* and *L.corneiformis* are predominant LAB species reported in fermented fish (Thapa *et al.*, 2004). Salami is a type of meat which shows the presence of microbes not only in manufacturing section but also sustain when passes through intestinal tract even after fermenting and drying for many days. LAB strains isolated from fermented meat exhibited the inhabitation of proteolytic enzymes, absence of biogenic amines, adherence to epithelium walls and inhibitory estate opposing *Klebsiella pneumonia* (Rai *et al.*, 2010).

Vegetable and fruits based fermented foods

The lactic acid fermentation of vegetables exercised as a preservation method for the production of finished and semi-finished products, is weighed as an relevant technology because of its capability to improve the nutritive value, palatability, acceptability, microbial trait and shelf-life of the fermented product (Kingston *et al.*, 2010). Fermented vegetable based foods inspite of having surplus therapeutic properties, they are low in fat and cholesterol and higher in potassium, fibres, carbohydrates, photochemicals, minerals and water soluble vitamins B and C complex (Chaudhary *et al.*, 2011). *Pediococcus pentasaceus*, *L. cellubiosus*, *L. plantarum*, *L. fermentum*, *L. brevis*, *L. mesenteroides*, *L. lactis*, *E. faecium* and *P. acidilactici* are predominant LAB species found in fermented vegetables and have shown strong acidification and coagulation properties (Tammang *et al.*, 2009).

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