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Probiotics: A Comprehensive Approach towards Health Foods

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Probiotics: A Comprehensive Approach towards Health Foods

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ABSTRACT

Food products containing probiotics and prebiotics are an important development in Health foods, which enhance health promoting microbial flora in the intestine. Probiotic refers to viable microorganism that promotes or support a beneficial balance of the autochthonous microbial population of the gastro-intestinal tract. A number of genera of bacteria (and yeast) are used as probiotics, including Lactobacillus, Leuconostoc, Pediococcus, Bifidobacterium, Saccharomyces and Enterococcus, but the main species believed to have probiotic characteristics are L. acidophilus, Bifidobacterium spp., and L. casei. Probiotics can alleviate reduce diarrhoeal incidence, lactose intolerance, lower serum cholesterol, stimulate the immune system, control infections, act as antibiotics, suppress tumors and protect against colon or bladder cancer by maintaining a healthy intestinal microflora balance. Lactic acid bacteria produce bio-preservatives such as lactic acid, hydrogen peroxide, and bacteriocins that are used to retard both spoilage and the growth of pathogenic bacteria. Food, particularly dairy products are considered as an ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract. Cereals being rich source of prebiotics such as β-glucan and arabinoxylan, galacto- and fructooligosaccharides, are considered for development of probiotic foods. Good manufacturing

practices must be applied in the manufacture of probiotic foods with quality assurance, and shelf-life conditions established.

Key words probiotics, prebiotics, health benefits, probiotic strains

INTRODUCTION

Tremendous changes in lifestyle, eating habits and shifting rural habitations are causing an irreversible change that is leading to manifold multiplication of health problems. Due to growing universal control over nutrition and personal health, consumers worldwide are trying to take a proactive position concerning health. With millions of rupees being spent on health care each year, consumers' desire for food products with desired health benefits continues to grow (Singh, 2007). Consumers are interested in foods that boost the immune system, reduce the risk of disease and enhance health, which consumers self-prescribe for themselves and their families. Nowadays functional foods are gaining public acceptance in many developed and developing countries and the recent market surveys show that, in India there is great scope for value-added as well as health promoting food products (Singh, 2007). It is estimated that the sale of functional and fortified foods in U.S. will reach $ 59.87 billion in 2009 (Sloan, 2006). The growing consumer demand and industry interest for functional foods clearly state that the market of functional foods has huge potential.

Functional foods are defined as 'foods that contain some health-promoting component(s) beyond traditional nutrients' (Shah, 2001). Functional foods are also known as designer foods, medicinal foods, nutraceuticals, therapeutic foods, superfoods, foodiceuticals, and medifoods

(Shah, 2001). Within the functional foods, probiotics are the small but rapidly expanding area. At the beginning of this century, Nobel Laureate Elie Metchnikoff, at the Pasteur Institute, linked health and longevity to ingestion of bacteria present in yoghurt (monograph reprinted in: Metchnikoff, 2004). Due to their perceived health benefits, probiotic bacteria have been increasingly included in yoghurts and fermented milks during the past two decades. Most commonly they have been lactobacilli such as *Lacto-bacillus acidophilus*, and *Bifidobacteria* often referred to as 'bifidus' (Daly and Davis, 1998).

A major development in functional foods pertains to foods containing probiotics and prebiotics which enhance health promoting microbial flora in the intestine. There is growing scientific evidence to support the concept that the maintenance of healthy gut microflora may provide protection against gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases, and even cancer (Haenel and Bendig, 1975).

The word "Probiotic" was initially used as an antonym of the word "antibiotic". It is derived from Greek words pro and biotos and translated as "for life" (Hamilton-Miller et al., 2003). It was probably Vergio (1954) who first introduced the term ''probiotic'', when he compared in his manuscript ''Anti- und Probiotika'' the detrimental effects of antibiotics and other antimicrobial substances on the gut microbial population, with factors (''Probiotika'') favourable to the gut microflora (Holzapfel and, Schillinger, 2001). Presently, there is general agreement that a ''probiotic'' refers to viable microorganism that promote or support a beneficial balance of the autochthonous microbial population of the GIT (Holzapfel, *et al.*, 2001; Holzapfel, *et al.*, 1998). Food and Agriculture Organization (FAO) and World Health

Organization (WHO) defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/ WHO, 2001).

Although it was recognized that dead cells may have health benefits, the consultants suggested that a different term be used for dead cells to reflect to the consumer that probiotics are living microbes (Tannis, 2008). Fuller, (1992) defined probiotic as “a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance”. Some selected strains of Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus and Saccharomyces have been promoted in food products because of their reputed health benefits (Dimer & Gibson 1998; Fuller, 1991; Ouwehand *et al*., 1999; Puupponen-Pimia *et al*., 2002; Sanders, 1998).

CRITERION FOR CONSIDERATION AS PROBIOTIC

Probiotics must have good technological properties so that it can be manufactured and incorporated into food products without losing viability and functionality or creating unpleasant flavours or textures; it must survive passage through the upper gastrointestinal (GI) tract and arrive alive at its site of action; and it must be able to function in the gut environment. To study the probiotic strain in the GI-tract, molecular techniques must be established for distinguishing the ingested probiotic strain from the potentially thousands of other bacterial strains that make up the gastrointestinal ecosystem. Additionally, techniques are required to establish the effect of the probiotic strain on other members of the intestinal microbiota and importantly on the host. This includes not only positive health benefits, but also demonstration that probiotic strains do not have any deleterious effects. Armed with this knowledge, the probiotics can then enter human

pilot studies that attempt to assess their health benefits to consumers (Mattila-Sandholm *et al*., 1999).

GASTROINTESTINAL TRACT MICROFLORA

The gastrointestinal tract represents an ecosystem of the highest complexity. The mucosal surface provides a large area for the adherence to and microbial colonisation of the small intestine. When compared to cross sectional area of skin surface of our body, the area of our GI system, calculated to be 150–200 m^2, is huge (Waldeck, 1990). A three-fold increase in the surface area is accomplished by circular folds, 7–10-fold by folding of the epithelium (intestinal villi) and 15–40-fold by the formation of microvilli in the enterocyte resorptive luminal membrane. Thereby the necessary space for interactions during the digestive process and for adhesion to the mucosal wall and concomitant colonisation is provided.

Many factors affect the composition of the large-intestinal microbiota in humans. These include the age, susceptibility to infections, nutritional requirements, and immunological status of the host and the pH, transit time, interactions between flora components, and presence and availability of fermentable material in the gut. Of these, it is probably the amount and type of growth substrate that has the most influential role. Dietary residues that are undigested in the upper gastrointestinal tract, as well as endogenous materials like mucin, sloughed epithelial cells, and bacterial lysis products, contribute to the pool of metabolizable substrates. Diet may exert a major influence on gut bacterial populations and their development. In this context, possible differences between the microbiota of breast-fed and bottle-fed infants are notable (Drasar and Roberts, 1990).

The major factors influencing the composition of the microflora are summarized in Table 1. These factors may be related to changes in physiological conditions of the host, composition of the diet and environmental circumstances. Implicit interactions of typical intestinal bacteria may also contribute to stabilisation or destabilisation, e.g. by the production of H_2O_2, acids and bacteriocins. The main fermentable dietary substrates in the adult gut are carbohydrate-based materials such as dietary fibers, resistant starches, oligosaccharides, food sweeteners, and other non absorbed sugars. There is a lesser contribution from nitrogen-based materials like proteins and amino acids and some dietary lipids may also reach the colon in a metabolizable form. In the infant gut, the form of the milk substrate can have important effects on the composition of gut flora (Collins and Gibson, 1999).

Microbial colonization of the intestine begins immediately after birth. The maternal intestinal microbiota is a source of bacteria colonizing the intestine of a newborn. Colonization is also determined by contact with the surroundings. At this stage, the most common bacterial strains are facultative anaerobes such as, enterobacteria, coliforms and lactobacilli (Benno and Mitsuoka, 1986). Normally, the first microbes colonizing the intestinal tract of a newborn will be derived from the mother, and these microbes have a strong stimulatory effect for both the normal development of the micro-biota and the maturation of the gut-associated lymphoid tissue. These effects are less apparent in caesarian born infants who are colonized by microbes from the hospital environment (Gronlund *et al*., 1999, 2000). Diet can have a major effect on the gut microbiota activities. In infants it is thought that those who are breast-fed have a natural predominance of bifidobacteria, and also specific strains of bifidobacteria (Saarela *et al*., 1995). After weaning, the composition of the microbiota resembles that of the adult (Edwards, 1993).

The GIT of the average human adult is colonised by approximately 10^{14} microbial cells (Luckey & Floch, 1972), about 10 times more than all tissue cells of the body taken together. This immense metabolic potential suggests strong regulatory effects on body functions, especially in the colon where the largest concentration of upto 5×10^{11} bacterial cells per g is found. Representing more than 400 species, these ‘‘autochthonous’’ microorganisms include diverse bacterial genera, of which the Gram-positive, anaerobic genera Bacteroides, Eubacterium and Bifidobacterium, predominate in the densely populated large intestine. Other groups such as the clostridia, peptostreptococci, ‘‘streptococci’’ and lactobacilli also seem to play an important role, e.g. in the maintenance of a stable gut mucosa, and in the generation of short chain fatty acids (SCFA) in a beneficial ratio (Holzapfel and Schillinger, 2002).

TYPES OF PROBIOTIC MICROBES

The basic notion behind probiotics is pretty straightforward i.e. to restore the depleted ecology of the intestine with new, healthful bacteria. Many different microorganisms are added to dairy products for their probiotic potential (Fuller, 1997; Fuller and Gibson, 1997). A number of genera of bacteria (and yeast) are used as probiotics, including Lactobacillus, Leuconostoc, Pediococcus, Bifidobacterium, and Enterococcus, but the main species believed to have probiotic characteristics are L. acidophilus, Bifidobacterium spp., and L. casei. Members of the genera Lactobacillus and Bifidobacterium have a long and safe history in the manufacture of dairy products and are also found as a part of gastrointestinal microflora. Probiotic bacteria with desirable properties and well-documented clinical effects include *L. johnsonii* La1, *L. rhamnosus* GG (ATCC 53103), *L. casei Shirota, L. acidophilus* NCFB 1478, *B. animalis* Bb12 and *L. reuteri* (Shah, 2004).

Lactobacillus acidophilus bacteria (LAB) are usually described as Gram-positive microorganisms, devoid of cytochromes and preferring anaerobic conditions but are aerotolerant, fastidious, acid-tolerant, and strictly fermentative, producing lactic acid as a main product (Stiles & Holzapfel, 1997). Members of the LAB are usually subdivided into two distinct groups based on their carbohydrate metabolism. The homofermentative group consisting of Lactococcus, Pediococcus, Enterococcus, Streptococcus and some lactobacilli utilize the Embden– Meyerhof–Parnas (glycolytic) pathway to transform a carbon source chiefly into lactic acid. As opposed to homofermentors, heterofermentative bacteria produce equimolar amounts of lactate, CO2, ethanol or acetate from glucose exploiting phosphoketolase pathway. Members of this group include Leuconostoc, Weissella and some lactobacilli.

Bifidobacterium are normal inhabitants of the human gastrointestinal tract. Recent *in vivo* scientific studies using animals or human volunteers have shown that consumption of live Bifidobacterium has an effect on the gut microflora. Selected strains survive stomach and intestinal transit and reach the colon in abundant numbers. Newborns are colonized with Bifidobacterium within days after birth and the population appears to be relatively stable until advanced age, when a decline in their numbers occurs. However, diet, antibiotics, and stress are reported to influence the population of Bifidobacterium in the intestines (Shah, 2007).

Presently, there are 29 species in the genus Bifidobacterium (Table 3), 14 of which are isolated from human sources (i.e., dental caries, faeces and vagina), 12 from animal intestinal tracts or rumen, and 3 from honeybees. Bifidobacterium species found in humans are: B. adolescentis, B. angulatum, B. bifidum, B. breve, B. catenulatum, B. dentium, B. infantis, B. longum, and B. pseudocatenula-tum. B. breve, B. infantis, and B. longum are found in human

infants; B. adolescentis, and B. longum are found in human adults (Shah & Lankaputhra, 2002). The optimum pH for the growth of Bifidobacterium is 6.0–7.0, with virtually no growth at pH 4.5–5.0 and below or at pH 8.0–8.5 and above. Optimum growth occurs at a temperature of 37–41ºC, the minimum and maximum growth temperatures are 25–28 and 43–45ºC, respectively (Shah, 2007).

Nonpathogenic microorganisms that occupy important niches in the host gut or tissues, such as yeasts, enterococci and Enterobacteriaceae, are used as human and animal probiotics. Though, Lactobacillus and Bifidobacterium are the most common species of bacteria used as probiotics for the production of fermented milks and other dairy products (Fuller, 1992). Saccharomyces boulardii is the only probiotic fungus which has been successfully used for curing the intestinal infections, especially diarrhoea (Surawicz *et al*., 1989; Mombelli and Gismondo, 2000).

Spore probiotics being heat-stable have a number of advantages over other non-spore formers such as *Lactobacillus* spp., namely, that the product can be stored at room temperature in a desiccated form without any deleterious effect on viability. A second advantage is that the spore is capable of surviving the low pH of the gastric barrier (Barbosa *et al*., 2005; Spinosa *et al*., 2000) which is not the case for all species of Lactobacillus (Tuohy *et al*., 2007) so in principle a specified dose of spores can be stored indefinitely without refrigeration and the entire dose of ingested bacteria will reach the small intestine intact.

Bacillus species have been used as probiotics for at least 50 years with the Italian product known as Enterogermina® registered 1958 in Italy as an OTC medicinal supplement. Of the species that have been most extensively examined these are *Bacillus subtilis, Bacillus clausii,*

Bacillus cereus, *Bacillus coagulans* and *Bacillus licheniformis.* Spore probiotics are being used extensively in humans as dietary supplements, in animals as growth promoters and competitive exclusion agents and lastly in aquaculture for enhancing the growth and disease-resistance of cultured shrimps. The single and most important advantage of these products is that they can be produced easily and the stability of the finished product can be assured, further they can be incorporated into everyday foods (Cutting, 2010).

A healthy microbiota has been considered to be one that is predominantly saccharolytic and comprises significant numbers of bifidobacteria and lactobacilli (Gibson and Roberfroid, 2008). Today, probiotic food vehicles around the world include live yoghurts, cereals, infant formulas, fermented dairy drinks, freeze-dried supplements (capsules, pills, liquid suspensions, sprays), cheese, fromage frais and fruit juices. Both single and multiple strain products are available (Gibson and Drakoularakou, 2009).

Health benefits of probiotics

Health benefits imparted by probiotic bacteria are very strain specific; therefore, there is no universal strain that would provide all proposed benefits, not even strains of the same species. Moreover, not all the strains of the same species are effective against defined health conditions. The role of the gut flora as a barrier against pathogenic and opportunistic microorganisms is surprisingly effective, considering the large amounts of allochthonous ('non-resident') bacteria entering the GIT. Most of them have no chance to establish within the system. The strains L. Rhamnosus GG (Valio), Saccharomyces cerevisiae Boulardii (Biocodex), L. Casei Shirota (Yakult), and B. Animalis Bb-12 (Chr.Hansen) are certainly the most investigated probiotic cultures with the established human health efficacy data against management of lactose

malabsorption, rotaviral diarrhoea, antibiotic associated diarrhoea, and Clostridium difficile diarrhoea.

Enhance immune response

Modulation of host immunity is one of the most commonly purported benefits of the consumption of probiotics. Increasingly growing, but still limited, clinical evidence exists to support this concept. Presumably, to modulate immunity, probiotic organisms must ‘‘talk’’ to immune cells that are endowed with recognition receptors or that are otherwise sensitive to probiotic-derived products (e.g., metabolites, cell wall components, DNA). Given the diversity of inflammatory or immune responses that can be mounted by the intestinal epithelium, association of probiotics with epithelial cells might be sufficient to trigger signaling cascades that ultimately activate underlying immune cells in the lamina propria (Menard, et al., 2004). Alternatively, probiotics may also release soluble factors that themselves trigger signaling cascades at the level of the epithelium or associated immune system.

Research on immune system stimulation by probiotics has focused on (a) response of mammalian cell cultures to exposure to LAB or their cellular products; (b) response of mice to intraperitoneal injection of LAB or their cellular products; (c) response of mice to oral administration of LAB or fermented milks; and (d) human feeding studies. Studies involving (a) or (b) have shown effects, but these do not involve oral consumption and their significance is unclear. Feeding lactobacilli or yogurt to mice stimulated macrophages and increased secretory IgA concentrations (Perdigon *et al.*, 1986, Scheinbach, 1998). Mice were also somewhat

protected against infection with *Salmonella thyphimurium* by prefeeding *L. casei* or *S. thermophilus*, but not *L. acidophilus* or *L. bulgaricus* (Perdigon *et al*., 1990).

Probiotics in Diarrhoea

Despite the multiple advances in the management of acute diarrhoea, it continues to cause some three million deaths in children. Furthermore, it is one of the main causes of deterioration of their nutritional status. Probiotics may have preventive or therapeutic effects on diarrhoea of various etiologies. However, not all probiotics are effective and physicians must select preparations with proven efficacy (Guarino, *et al*., 2009).

Antibiotic-associated diarrhoea is a common problem occurring in 5% to 25% patients receiving antibiotics, with rates varying depending upon the population studied and the antibiotic used (Bartlett, 2002). Diarrhoea related to antibiotic use can begin after a single dose of antibiotics or persist as long as 6 weeks after antibiotics have been stopped. The most commonly diagnosed and potentially severe form of antibiotic-associated diarrhoea is caused by *Clostridium difficile* accounting for 15% to 25% of antibiotic associated diarrhoea (Kelly *et al*., 1994). Antibiotics alter the normal fecal flora and this disturbance of gastrointestinal microecology leads to a loss of colonization resistance predisposing the gut to colonization by potentially pathogenic microbes, such as *C. difficile* (Katz, 2006). The possible advantages of probiotics as biotherapeutic agents are that they can lower the dependence on antibiotic use, they are relatively inexpensive, they can have multiple mechanisms of action, and they are generally safe and well tolerated (Elmer and, McFarland, 2001).

The effect of *S. boulardii* is associated with its capacity to neutralize the cytotoxins produced by *C. difficile*, through the release of a protease capable of degrading the toxin and its receptor in the mucosa. This yeast exerts trophic effects on the intestinal mucosa by increasing its concentrations of polyamines. In addition, *S. boulardii* secretes a small peptide, the *S. boulardii* anti-inflammatory factor that inhibits signaling pathways activated by the toxins produced by *C. difficile* (Brunser and Gotteland, 2010). Lactinex, a commercial preparation containing *L. acidophilus* and *L. bulgaricus* at a concentration of 2×10^8 CFU, showed no protective effect in the prevention of amoxicillin induced diarrhoea in children (Tankanow *et al*., 1990). However, controlled trials of B. lactis and *S. thermophilus*-supplemented infant formula (Correa *et al*., 2005), L. acidophilus combined with B. infantis (Hoyos, 1993), *Clostridium butyricum* MIYARI (Seki *et al*., 2003) and *L. sporogenes* with fructooligosaccharides (LaRosa *et al*., 2003) showed positive effects in prevention of antibiotic-associated diarrhoea in infants and children.

Traveller's Diarrhoea: Travel is a risk factor for infectious gastroenteritis. A recent meta-analysis revealed evidence of a protective effect by *S. boulardi* and by mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (McFarland, 2007). However, evidence of the efficacy of probiotics in the prevention of traveller's diarrhoea is preliminary.

Probiotics for treating persistent diarrhea: WHO defines persistent diarrhoea as an illness of proven or presumed infectious aetiology that lasts 14 days or more (Anonymous 1988). Persistent diarrhoea accounts for 3% to 20% of all diarrhoeal episodes in children aged less than five years (IWGPD 1996). It is also directly responsible for between 36% and 54% of all diarrhoea-related deaths according to two large, community-based studies (Schorling *et. al.*,

1990; Fauveau *et. al.,* 1992). Thus, the main consequences of persistent diarrhoea aremorbidity (with an increased risk of hospital admission) are death and malnutrition.

Bernaola, *et al*. (2010), carried out a study to evaluate the efficacy and adverse effects of probiotics for the treatment of persistent diarrhoea in children. Four trials were included in the study, with a total number of 464 participants; one trial had a low risk of bias. Meta-analysis showed that probiotics reduced the duration of persistent diarrhoea (mean difference 4.02 days, 95% CI 4.61 to 3.43 days, n=324, 2 trials). Stool frequency was reduced with probiotics in two trials. One trial reported a shorter hospital stay, which was significant, but numbers were small. No adverse events were reported.

Use of probiotics in combating IBD

Inflammatory bowel disease (IBD) refers to a group of disorders of unknown aetiology that are characterized by chronic or recurrent mucosal inflammation. An immunological reaction to some members of the gut microbiota is thought to play a role in disease onset or maintenance. Ulcerative colitis (UC) and Crohn's disease (CD) are the two most frequent causes of this chronic inflammation of the gastrointestinal tract; these are lifelong diseases characterized by recurrent episodes of diarrhoea, frequently with blood in the feces, abdominal pain, fever, malaise and weight loss.

Probiotic administration, either through regulation of the inflammatory response or modulation of gut microbiota composition and/or activity might bring about relief in IBD symptoms or maintain remission from symptoms. The well-defined, nonpathogenic strain *E. coli* Nissle 1917 has proven more effective in preventing relapse in Crohn's disease patients

compared with a placebo (Table 4). *S. boulardii* has shown some success in relieving the symptoms of active Crohn's disease and in reducing the risk of relapse (Guslandi, *et al.* 2000).

VSL#3 is a mixture of four lactobacilli (*L. acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei* and *Lactobacillus plantarum*), three bifidobacteria (*Bifidobacterium breve*, *Bifidobacterium infantis* and *Bifidobacterium longum*) and *S. thermophilus*. The mixture has proven effective in reducing the recurrence of chronic relapsing pouchitis. VSL#3 (at 6 g/day) significantly reduced relapse recurrence (15%) compared with placebo (100%) over a 9 month period (Gionchetti, *et al.*, 2000) and was also effective in preventing the occurrence of pouchitis in patients with ileo-pouch anal anastomosis for ulcerative colitis (UC).

Probiotics in relief of lactose intolerance

Most of the world's population (60-90% of non-Caucasians and 6-12% of Caucasians) become lactose intolerant after weaning (Bayless, *et al.*, 1971, Kretchmer, 1972). This stems from a 90-95% decline in the production of lactase or B-galactosidase (Gilat, *et al*., 1972). This lactase is essential to assimilate the disaccharide in milk and needs to be split into glucose and galactose. Lactose intolerance is a physiological state in human beings where they lack the ability to produce an enzyme named lactase. Any lactose reaching the large intestine is metabolized by the colonic microflora to yield CO_2, methane and hydrogen and the latter is typically noted as an increase in breath hydrogen. The presence of lactose also alters the osmotic balance in the colonic lumen. Thus, symptoms including abdominal bloating, cramping, flatulence and diarrhoea ensue (Suarez and Savaiano, 1997).

Alleviation of lactose intolerance is probably the best established health claim for probiotics. Yogurt contains less lactose than milk and delays gastric emptying, which partly explains why lactose- intolerant individuals tolerate yogurt. However, yogurt tolerance is mainly due to the supply of lactase activity from the lactic acid bacteria present in the yogurt itself. Evidence shows that bacteria must be live and present in sufficient quantity to be of benefit; yogurts containing 10^8 bacteria/ ml are required (Pelletier *et al*., 2001). Fermented milk products like yogurt are less likely to cause gastric upsets in two ways. First, they usually contain less lactose (4% *vs* 6%) due to microbial digestion during fermentation. Second, indigenous lactase, present in the ingested cells can make up for the host's deficiency.

Milk is the richest source of calcium and Ca requirement of the body is met only through milk. Hence, a person consuming non-milk diet will naturally develop Ca deficiency, leading to osteoporosis. Birge *et al*. (1967) confirmed that lactose deficiency leads to calcium malabsorption and thereby to osteoporosis. Calcium malabsorption may be due to deletion of diets with milk to avoid the complications of lactose intolerance. Calcium absorption is better and more in acidic conditions; hence, if lactose is converted to lactic acid, pH of the gut decreases, i.e. it becomes acidic favouring enhanced absorption of calcium. So, if probiotics are fed to lactose intolerance patients, then milk lactose is hydrolysed by probiotic strains and lactose is assimilated and calcium absorption is also favoured (Suvarna and Boby, 2005).

Use of probiotics in less well defined gut disorders

Irritable bowel syndrome (IBS) affects 8-22% of the population, with women being most affected. Irritable bowel syndrome (IBS) is the name for a variety of functional bowel disorders without (known) organic cause. Characteristic symptoms are chronic abdominal pain, cramping

and discomfort, flatulence, meteorisms and a change in bowel habits (either diarrhoea or constipation, or both). IBS is a common disorder and women are more likely to contract IBS and to suffer from more severe symptoms. The causes are diverse, but are often related to a depletion of beneficial gut bacteria. To date, human feeding studies in IBS patients have yielded mixed results. O'Sullivan and O'Morain (2000) found that *L. rhamnosus* GG had little effect on IBS symptoms whereas *L. plantarum* 299V had a measurably beneficial effect. Hamilton-Miller (2001) reviewed the results of 12 clinical trials, involving a total of 1371 patients treated with probiotics. Although in 10 trials (of which five were randomized, double blind and placebo controlled) the use of probiotics was beneficial, the probiotic agents, dosages and duration of treatment varied too widely between the studies. Furthermore, the number of patients included was too small to allow any definite conclusions other than the assumption that probiotics might be more effective in preventing IBS than in its treatment.

Gastroenteritis is commonly associated with autistic spectrum disorders and there is some evidence that an altered gut microbiota might even play a role in autistic pathology (Finegold, *et al.* 2002). Probiotic therapies could hold promise, not only in the relief of gastrointestinal symptoms associated with autism but also in normalizing the autistic gut microbiota in terms of its composition and the profile of microbial metabolites produced, some of which are thought to play a psychoactive role in autism (www.drugdiscoverytoday.com). Appropriate intervention studies using probiotics are needed to establish any impact on the disease.

Inhibition of Helicobacter pylori and intestinal pathogens

The gastrointestinal environment contains a wide range of contents ranging from harmless beneficial dietary and microbial flora to harmful pathogenic bacteria. The mammalian

organism fights against these pathogenic bacteria through various ways. A wide range of antibacterial compounds including organic acids (e.g., lactic acid and acetic acid), hydrogen peroxide, bacteriocins, various low-molecular mass peptides, and antifungal peptides/proteins, fatty acids, phenyllactic acid, and OH-phenyllactic acid are being produced by probiotic cultures. Lactic and acetic acids are the main organic acids produced during the growth of probiotics and their pH lowering effect in the gastrointestinal tract has a bacteriocidal or bacteriostatic effect. Low-molecular mass compounds such as lactic acid have been reported to be inhibitory towards gram-negative pathogenic bacteria (Alakomi *et al*., 2000).

Helicobacter pylori is an intestinal pathogen, long-term infection by which leads to chronic gastritis, peptic ulcer and increases the risk of gastric malignancies (Plummer, Franceschi, & Munoz, 2004). Currently *H. pylori* infection is treated by a combined therapy consisting of two antibiotics and a proton pump inhibitor, which, although in many cases appeared very effective, presents a very expensive treatment with many side effects including antibiotic-associated diarrhoea and likelihood of induction of the antibiotic resistance in intestinal pathogens (Malfertheiner *et al*., 2002). Clinical studies and experimental animal models have shown that *L. acidophillus* can affect growth and development of *Helicobacter pylori* both in vitro and in vivo (Felley *et al.*, 2003). However to date there is insufficient data to suggest the use of probiotics in the absence of antibiotics to prevent infection with *H. pylori*.

Probiotics in the Management of Allergies

A change in the proper functioning of the immune system can present itself as an allergy. Large-scale studies have indicated an alteration in the composition of the gut microflora, such as decrease in the numbers of lactobacilli, preceding the development of an allergy. Probiotics have

been shown to reduce the incidence of childhood eczema by half, compared to placebo, when administered during pregnancy and up to 6 months postnatally. The incidence of asthma or rhinitis was not altered. A follow-up study demonstrated a two-fold increase in transforming growth factor β2, an anti-inflammatory cytokine, in the breast milk of mothers receiving probiotics compared to placebo (Kalliomaki, *et al*. 2001).

A number of studies suggest that there exists a relationship between allergic conditions and the composition of the gut microbiota. The fecal counts of bifidobacteria in allergic infants, particularly those with atopic eczema, are significantly lower than in healthy peers (Sepp *et al*. 2005). Probiotics are involved in controlling the inflammation of the intestinal system by promoting defense mechanisms and preventing bad microbes from growing there. To date, the research, both in laboratory and clinical settings, suggests that the prevention of atopic eczema in high-risk infants is possible by changing the infant's intestinal microflora with probiotics and prebiotics. Probiotics may exert a beneficial effect on allergic reaction by improving mucosal barrier function (Suvarna and Boby, 2005). In addition, probiotics consumption by young children may beneficially affect immune system development. Probiotics such as *Lactobacillus* GG may be helpful in alleviating some of the symptoms of food allergies such as those associated with milk protein (Majamaa and Isolauri, 1997).

Food Allergies are estimated to affect 3.5% of adults and 8 to 10% of children; the food allergens most frequently involved are egg, peanuts, milk, fish, nuts, shellfish, wheat, kiwi and mustard. Children with a food allergy have an increased intestinal permeability. There are three ways in which probiotics are hypothesized to offer beneficial effects in the individuals with food allergies (Tannis, 2008). These are (a) probiotics reduce the permeability of the intestines,

preventing or treating food allergies; (b) probiotics increase IgA in the intestines; and (c) changes in number and type of intestinal microbes are common in allergic individuals. Probiotics have the ability to promote normal intestinal microflora, thus reducing the development of allergies.

Hypocholesterolemic effect of probiotics

High levels of plasmatic triglycerides and cholesterol, in addition to abdominal obesity, arterial hypertension and insulin resistance, are important components of the metabolic syndrome, a pathological condition associated with an increased risk of cardiovascular diseases. Probiotic strains, especially lactic acid bacteria have a major role to play in the cholesterol lowering mechanism. One of the possible mechanisms for microbially-induced lowering of serum cholesterol have been suggested, that, since cholesterol is used in the production of bile acids, enhanced catabolism and excretion of bile acids might reduce serum cholesterol (Chikai *et al*., 1987) by deconjugating the cholesterol to bile acids, thereby reducing the total body pool. Deconjugation by different lactic acid bacterial cultures was also tried using two forms of bile salts, viz. taurocholates and glycocholates. Most of the strains could deconjugate glycocholates.

Cholesterol absorption is reduced by three ways – assimilating, binding or by degradation. Probiotic strains assimilate the cholesterol for their own metabolism. Probiotic strains can get bound to the cholesterol molecule, and they are capable of degrading cholesterol to its catabolic products. The potential hypocholesterolemic effect of probiotics sparked much interest based on evidence from animal work and from human studies, when 0.5–5 l yogurt/day was consumed. However, the ingestion of realistic quantities of yogurt or probiotics in man has not been shown to reduce cholesterol levels significantly (Lin *et al*., 1989 and, Roos *et al*., 1999). Two double-blind, placebo-controlled studies looked at the hypocholesterolemic effects of a Danish milk

product (Gaio) fermented by *Enterococcus faecium* (about 2 x 10^8cfu/ml) and two strains of S. *thermophilus* (about 7xl0^8cfu/ml). Agerbaek, *et al*., 1995 tested 29 normocholesterolemic men, all aged 44 years. The unrandomized test group exhibited a 10% drop in serum LDL-cholesterol following six weeks of diet supplemented daily with 200 ml of the product as compared to a placebo group of 28 subjects who drank the unfermented low-fat milk that was chemically acidified. Unrandomization resulted in a significantly higher LDL-cholesterol mean for the test group at the start of the trial. Thus, at least a portion of the observed decrease could have resulted from regression to the "true" mean. According to a study, conducted by Gilliland *et al.* (1985) on pigs, fed with high cholesterol diet for ten days followed by feeding with probiotic strains of *L. acidophilus* P-47 and RP-32., found significant difference in treatments and also, *L. acidophilus* RP-32 performed better than *L. acidophilus* P-47 (Table 5).

Conflicting and variable results among studies may occur from the use of different doses, strains and even subjects. For example, Richelsen, *et al*. (1996), found that three months into their study they could divide subjects into responders, who exhibited a decrease in LDL cholesterol, and non-responders who did not. The biological basis for this is unknown. Since there are no convincing double-blind trials in humans, a hypocholesterolemic effect of feeding LAB to humans has yet to be scientifically proven.

Anticarcinogenic effect of probiotic microbes

Diet makes an important contribution to cancer, e.g., up to 75% of colorectal cancer cases are thought to be associated with diet, implying that risks of cancer are potentially reducible. Evidence from a wide range of sources supports the view that the colonic microflora is involved in the etiology of cancer. This has led to intense interest in factors such as probiotics that can

modulate gut microflora and its metabolism. Probiotics have been given credit for numerous health-promoting effects; one of which is their anticarcinogenic properties.

Consumption of large quantities of dairy products such as yogurt and fermented milk containing *Lactobacillus* or *Bifidobacterium* may be related to a lower incidence of colon cancer (Shahani and, Ayebo, 1980). An epidemiological study demonstrated that, despite the high fat intake, colon cancer incidence was lower than in other countries because of the high consumption of milk, yogurt, and other dairy products (Malhotra, 1977). When *Lactobacillus acidophilus* was given to healthy volunteers on a fried meat diet known to increase fecal mutagenicity, a lower fecal mutagenic activity was noted on day 3 with *L. acidophilus* compared to day 3 without *L. acidophilus* when fried meat and ordinary fermented milk were given (Lidbeck *et al*., 1992). A number of mechanisms for the anti-tumor action of probiotics have been proposed which are presented in Table 6. There are many reports about the anticarcinogenic effect of probiotics strains and probiotic foods (Shahani and Ayebo, 1980; Malhotra, 1977; Lidbeck *et al*., 1992). The strongest evidence for anticancer effects of probiotics comes from animal studies, and evidence from human studies (epidemiology and experimental) is still limited. Despite the encouraging results from current studies, there are some discrepancies in our knowledge, the prime one being the mechanisms involved in anticancer effects of probiotics. It is possible that different strains target different mechanisms.

Probiotics and AIDS Treatment

Even with the development in HIV medical treatment research, many social and clinical factors can affect functional status and quality of life for HIV-infected children. HIV is recognized to alter the structure and the function of human small intestines. Since intestinal

dysfunction is common in HIV infection—that is, carbohydrate malabsorption, frequent diarrhoea, steatorrhea—the intestinal permeability is increased, and might cause enteric pathogens translocation, propitiated by bacterial gastrointestinal overgrowth (Miller, 2002, Miller, *et al*. 1997 and, Johnson *et al*. 2000). It is quite common among HIV-infected children to have a depression of immune function caused by malnutrition, which is potentially reversible by restoring gut microflora and nutritional rehabilitation (Wittenberg, *et al.*, 2004). As the probiotics, defined as live microbial feed supplements, can improve intestinal microbial balance and promote health benefits, we can give them a goal of enhanced mucosal immune defense (Trois, 2010). This mechanism can happen through enhancing macrophage activity, elevating the numbers of killer cells, T cells, and interferon; and their action against pathogenic microbial colonization and translocation. Consequently, probiotic therapy may help the immune system and restore intestinal digestive-absorptive function (Fuller and Gibson, 1997, Vanderhoof, 2001, Tuomola, *et al*., 2001, Duggan, *et al*., 2002, Kalliomäki and Isolauri, 2003).

Trois *et al*. 2008, studied 77 HIV-infected children (2–12 years old), divided into two groups. In one, 38 children received probiotics (formula containing *Bifidobacterium bifidum* with *Streptococcus thermophilus*—2.5×10^{10} cfu/g) and in the other, 39 children received a standard formula (control group), for 2 months this study showed that probiotics might have immune stimulatory properties and can be helpful in the treatment of HIV-infected children, since the antiretroviral therapy had not changed during the study. According to a 3-week draw-study performed by Schifrin *et al*. (1997), using *Bifidobacterium bifidum* and *Streptococcus thermophilus*, presented an increase in global phagocytic activity of blood phagocytes (granulocytes and monocytes) and in a further study by Meyer *et al*., (2006) with healthy

women, which showed a stimulator effect of LAB in activating T cells, through an increase in CD8+ and CD4+. CD4 stands for cluster of differentiation 4. Like many cell surface receptors/markers, CD4 is a member of the immunoglobulin superfamily. CD4 is a primary receptor used by HIV-1 to gain entry into host T cells. It must be emphasized that not all probiotics have immunomodulatory properties capable of increasing the CD4 count. However, the proof of probiotics' direct involvement needs to be studied further.

Urogenital Health

Similar to the intestinal tract, the urogenital tract in women is highly colonized and susceptible to infection upon colonization disruption (Reid *et al.*, 1998). Bacteria originating from the colon are often linked to both urinary and genital tract infections. Several studies have correlated vaginal health (absence of infections) with the presence of lactobacilli, and specifically hydrogen peroxide-producing lactobacilli (Hillier *et al.*, 1992). Most of these studies were conducted with intravaginal instillations of probiotic bacteria. Oral consumption of certain probiotic-containing products, however, was found to mediate decreased recurrence of *Candida* infections and bacterial vaginosis (Shalev et al., 1996).

Other Health benefits

Probiotic influence on a variety of other clinical targets has been evaluated. Probiotic-mediated reduction in the severity of reaction to exposure to radioactive isotopes has been shown in mice (Dong *et al.*, 1987) and humans (Henriksson *et al.*, 1995; Korschunov *et al.*, 1996; Salminen *et al.*, 1988). The effects of endotoxemia associated with alcoholic liver disease were reduced by probiotics (Nanji *et al.*, 1994). Probiotics have been used in enteral feeding to

provide nutritional support of surgery patients (Bengmark and Gianotti, 1996). In addition to proposed direct effects on humans, probiotics may also have implications for human health through their use in animal agriculture.

Mechanism of action of probiotics

The mode of action of probiotic strains is likely to be multifactorial and, from existing evidence, appears to be strain specific**.** The mechanisms of probiotic action in the human and animal intestinal tract pertaining to various ailments and infections have been summarized in Table 7.

Probiotic Bacteria in Food Safety Applications

In addition to health benefits, researchers have demonstrated that probiotic bacteria produce bacteriocins and organic acids that act as antimicrobials to inhibit the growth of pathogens as well as can be used to extend the shelf life of food products (Goktepe, 2006). Today's consumers expect and demand a safer food supply than ever before. Yet, despite the improvements in food safety, food-borne illnesses continue to rise. Lactic acid bacteria produce bio-preservatives such as lactic acid, hydrogen peroxide, and bacteriocins that are used to retard both spoilage and the growth of pathogenic bacteria (*B Ray, 1992*). One of the most studied bacteriocins is nisin, which is used as a food additive in more than 48 countries to preserve the quality of processed cheese, dairy products, and canned foods.

A number of bacteriocins from *Lactobacillus* species have been identified (Table 8). One of the most studied types of *Lactobacillus* bacteriocins used in food products is pediocin AcH. The activity spectrum of pediocin AcH is relatively wide, and its action leads to cell lysis

(Loessner *et al*., 2003). Sakacin A, produced by *L. sake*, was found to be highly effective in inhibiting the growth of *L. monocytogenes* in minced meat and raw pork (Schillinger, *et al*., *1991*).

The potential of bifidobacteria and their by-products to control pathogens and spoilage microorganisms in food systems has been evaluated by several researchers. Gagnon *et al*. (2004) tested the ability of five bifidobacterial strains to inhibit *E. coli* O157:H7 *in vitro*. Two of the isolates were found to be significantly effective in inhibiting *E. coli* O157:H7 on nalidix acid lithium chloride agar. O'Riordan and Fitzgerald (*1998*) screened 22 *Bifidobacterium* strains for antimicrobial activity and found that 12 of the strains exhibited antagonistic activity against Gram-positive and Gram-negative bacteria, including *Pseuedomonas* spp., *Salmonella* spp, *E. coli*, *Lactobacillus* spp., *Strep tococcus spp., and Listeria innocua. Kim et al. (1995) used a combination of B. infantis ATCC 15697, B. longum ATCC 15707, B. adolescentis 9H Martin, and sodium acetate to increase the shelf life of catfish fillets.* The shelf life of fresh camel meat was also extended by 12 d after treatment with 10% sodium acetate and *B. breve* NCFB 2258 (*Sheddy, et al., 1999*).

Selection of probiotic strains

Several aspects, including safety, functional and technological characteristics, have to be taken into consideration in the selection process of probiotic microorganisms. Careful screening of probiotic strains for their technological suitability can also allow selection of strains with the best manufacturing and food technology characteristics. The theoretical basis for the selection of probiotic micro-organisms including safety, functional and technological aspects is illustrated in Table 9.

Factors related to the technological and sensory aspects of probiotic food production are of utmost importance since only by satisfying the demands of consumers; the food industry can achieve success in promoting the consumption of functional probiotic products in the future. Good viability and activity of probiotics are considered prerequisites for optimal functionality. Probiotic foods should include specific probiotic strains at a suitable level throughout their shelf life. However, several studies have shown that non-viable probiotics can have beneficial effects such as immune modulation and carcinogen binding in the host.

Thus, for certain probiotic strains it might be sufficient that they grow well during initial production steps (to obtain high enough cell numbers in the product) but they do not necessarily need to retain good viability during storage (Saarela, *et al.* 2000).

Production of probiotic foods

Food substrate/diet is considered as one of the major factors in regulating colonization of micro-organisms in gastrointestinal tract. Food helps to buffer the bacteria through the stomach and may contain other functional ingredients that could interact with probiotics to alter their functionality. Colonic foods, which encourage the growth of favourable bacteria, are referred to as prebiotics. By increasing the amount of prebiotics in the diet, it is possible to increase and maintain healthy bacterial gut flora in the host (Gibson, *et al*. 2003; Sanders, 1998). Ingredients in certain food products may naturally contain prebiotics which help to improve the functional efficacy of probiotics. Foods can be fortified with prebiotics during manufacturing process to increase probiotic efficacy (Ranadheera, *et al*. 2010). When both prebiotics and probiotics are present in a food then, those functional foods are referred to as synbiotic. This is represented in the Table 10 given below.

Better growth and survivability during food manufacturing and storage as well as in gastrointestinal tract, protection against acid, bile and gastrointestinal enzymes, adhesion to intestinal epithelium, antimicrobial properties and antibiotic resistance could be considered as factors that might be important in maintaining probiotic efficacy.

Foods used for dissemination of probiotics are usually fermented foods even if probiotics also could be present in infant formula, fruit drinks, whey drinks and sweet milk. Food, particularly dairy products are considered as an ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract (Ross, *et al*. 2002). Different products have demonstrated different viability levels of probiotics over shelf lives. Limitations of dairy products such as the presence of allergens and the requirement for cold storage facilities, as well as an increasing demand for new foods and tastes have initiated a trend in non-dairy probiotic product development (Lavermicocca, 2006). Furthermore, lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products. Nowadays there is an increasing consumer demand for non-dairy-based probiotic products and these organisms are being incorporated into drinks as well as marketed as supplements in the form of tablets, capsules and freeze dried preparations (e.g. Multibionta, Enterogermina, Reuterina, UltraLevure, Florastor) (Yadira and, Yoja, 2010).

Adding probiotics to the juices is more complex than formulating in the diary products because the bacteria need protection from the acidic conditions in the fruit juice. However, with microencapsulation technologies, the probiotics can become an important ingredient in the functional foods, expanding the probiotic application outside the pharmaceutical and supplement

industries. Microencapsulation technologies have been developed and successfully applied using various matrices to protect the bacterial cells from the damage caused by the external environment (Del Piano *et al*., 2006). There is a genuine interest in the development of fruit juice based functional beverages with probiotics because they have taste profiles that are appealing to all age groups and because they are perceived as healthy and refreshing foods (Sheehan *et al.*, 2007). Although LAB has been considered a difficult microorganism that demands various essential amino acids and vitamins for growing (Salminen and Von Wrigh, 1993), it has been found that some probiotic strains have the capability to grow in fruit matrices. Researchers have reported that cell viability depends of the strains used, the characteristics of the substrate, the oxygen content and the final acidity of the product (Shah, 2001).

In recent years, cereals have also been investigated regarding their potential use in developing functional foods. Lactic acid fermentation of cereals is a long-established processing method and is being used in Asia and Africa for the production of foods in various forms such as beverages, gruels, and porridge. Cereals contain water-soluble fiber (such as β-glucan and arabinoxylan), oligosaccharides (such as galacto- and fructooligosaccharides) and resistant starch, and thus have been suggested to fulfill the prebiotic concept (Shah, 2001; Andersson *et al*., 2001). Whole grains are also sources of many phytochemicals, including phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols (Katina *et al*., 2007). In Western countries, cereals, like wheat and rye, are used for sourdough production, which is traditionally prepared by adding a pre-fermented sourdough of good quality to the dough. The population of lactobacilli in fully fermented sourdoughs is more than 10^9cfu/g, while the LAB/yeast ratio is generally 100:1 (Salovaara, 1998).

Probiotic products are usually standardised based on the presumption that culture viability is a reasonable measure of probiotic activity, thus the ability of the strain to attain high cell population is of primary importance. A concentration of approximately 10^7 cells/ ml at the time of consumption is considered functional (Gomes and Malcata, 1999; Shortt, 1999). Malt, wheat and barley extracts have a positive influence in increasing bile tolerance of L. acidophilus, L. reuteri and L. plantarum. However, addition of malt demonstrated a greater positive influence while barley and wheat extracts demonstrated similar levels of influence in retaining viability (Michida et al., 2006; Patel, Pandiella, Wang, & Webb, 2004). Immobilization of L. plantarum within malt and barley fiber seems to play a major role on the gastrointestinal tolerance (Michida *et al.*, 2006). Furthermore, malt medium has demonstrated better support for the growth of L. acidophilus, L. fermentum, L. reuteri and L. plantarum than wheat or barley mediums due to its favourable chemical composition and availability of considerable amount of maltose, sucrose, glucose, fructose and free amino nitrogen (Charalampopoulos, *et al*., 2002).

Lactic acid fermentation improves usually the nutritional value and digestibility of cereals (Charalampopoulos, *et al*., 2002). Lactic acid fermentation of different cereals, such as maize, sorghum, finger millet, has been found effectively to reduce the amount of phytic acid, tannins and improve protein availability (Chavan *et al*., 1988; Lorri and Svanberg, 1993). Increased amounts of riboflavin, thiamine, niacin, and lysine due to the action of LAB in fermented blends of cereals were also reported (Hamad and Fields, 1979; Sanni *et al*., 1999). Khetarpaul and Chauhan (1990) reported improved minerals availability of pearl millet fermented with pure cultures of lactobacilli and yeasts.

Safety considerations for probiotics

An enormous amount of money and energy can be spent in assessing the risk of each probiotic strain. A low risk may be accepted, but the risk to benefit ratio needs to be clearly established. This requires relevant information on the efficacy and safety of the products. Knowledge on survival of the probiotics within the GI-tract, their translocation and colonization properties, and the fate of probiotic-derived active components is important for the evaluation of possible positive and negative effects of probiotic consumption (*Saarela et al*., 2000).

There are two instances where harmful effects of these bacteria have been observed by some researchers which include a *L. rhamnosus* strain indistinguishable from *L. rhamnosus* GG isolated from a liver abscess from an elderly lady with a history of hypertension and diabetes mellitus (Rautio *et al*., 1999). In another case a probiotic *L. rhamnosus* strain (strain or product specifications were not given) was suggested to have caused endocarditis in an elderly male (Mackay *et al*., 1999).

The minimum requirements needed for probiotic status include the assessment of strain identity, in vitro tests to screen potential probiotics, assessment of safety above all, and in vivo studies for substantiation of effects. In recognition of the importance of assuring safety, even among a group of bacteria that is Generally Recognized as Safe (GRAS), the Working Group recommends that probiotic strains be characterized at a minimum with the tests (FAO/WHO, 2002), mentioned in Table 11. Assessment of lack of infectivity by a probiotic strain in immunocompromized animals would add a measure of confidence in the safety of the probiotic. Good manufacturing practices must be applied in the manufacture of probiotic foods with quality assurance, and shelf-life conditions established.

Conclusion

The use of probiotic bacterial cultures stimulates the growth of preferred micro-organisms, crowds out potentially harmful bacteria, and reinforces the body's natural defence mechanisms. Today, plenty of evidence exists on the positive effects of probiotics on human health. However, to be effective, probiotics must be capable of being prepared in a viable manner and on large scale (e.g. for industrial purposes), whilst during use and under storage the probiotic should remain viable and stable. India's probiotics market has been highlighted by researcher Frost & Sullivan as a "*major growth market of the future*", with annual growth of 22.6 per cent until 2015. With India's urban middle class growing rapidly it is easy to see why there is so much interest in a country with a population approaching 1.2bn (Starling, 2010). When considered together, the above mentioned evidences reinforce the fact that probiotics can influence human health in a positive manner. Thus, it can be concluded that, the field of probiotics is developing rapidly as evident from expansion of research and increased familiarity of probiotics to the general public.

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Table 1 Factors affecting the microflora of the gastro-intestinal tract

I. Host mediated factors	pH, secretions such as immunoglobulins, bile, salts, enzymes
	Motility, e.g. speed, peristalsis
	Physiology, e.g. compartmentalisation
	Exfoliated cells, mucin, tissue exudate
II. Microbial factors	Adhesion
	Motility
	Nutritional flexibility
	Spores, capsules, enzymes, antimicrobial components
	Generation time
III. Microbial interactions	
i. Synergy	Metabolic cooperation
	Growth factors and vitamin excretion
	Changes to *E* , pH, O tension
ii. Antagonism/ stimulation	Short-chain fatty acids, amines
	Changes to *E* , pH, O tension
	Antimicrobial components, siderophores
	Nutritional requirements, etc.
IV. Diet	Composition, non-digestible fibres, drugs, etc.

Source: *Holzapfel et al*., 1998

Table 2 Lactobacilli used as probiotic cultures[a]

Species	Strains
L. acidophilus	LA-1/LA-5 (Chr. Hansen)
L. acidophilus	NCFM (Rhodia)
L. acidophilus Johsonii	La1 (Nestle)

L. acidophilus	DDS-1 (Nebraska Cultures)
L. acidophilus	SBT-2062 (Snow Brand Milk Products)
L. bulgaricus	Lb12
L. lactis	L1A (Essum AB)
L. casei Immunitas	(Danone)
L. plantarum	299v, Lp01
L. rhamnosus	LB21 (Essum AB)
L. reuteri	SD2112/MM2 (Biogaia)
L. rhamnosus	271 (Probi AB)
L. plantarum	(Probi AB)
L. reuteri	SD2112 (also known as MM2)
L. casei	Shirota (Yakult)
L. paracasei	CRL 431 (Chr. Hnasen)
L. fermentum	RC-14 (Urex Biotech)
L. helveticus	B02

[a]source: Krishnakumar and Gordon (2001), Holm (2003), Playne *et al*. (2003); Shah (2004).

Table 3 Bifidobacterium cultures used as probiotic cultures[a]

Species	Strains
B. adolescentis	ATCC 15703, 94-BIM
B. longum	BB536 (Morinaga Milk Industry)
B. longum	SBT-2928 (Snow Brand Milk Products)
B. breve	Yakult
B. bifidus	Bb-11
B. lactis (reclassified as B.	Bb-12 (Chr. Hansen)

animalis)	
B. essensis	Danone (Bioactivia)
B. lactis	Bb-02
B. infantis	Shirota
B. infantis	Immunitass
B. infantis	744
B. infantis	01
B. laterosporus	CRL 431
B. lactis	Lafti^{TM}, B94 (DSM)
B. longum	UCC 35624 (UCCork)
B. lactis DR10/HOWARU	Danisco

[a]source: Krishnakumar and Gordon (2001); Holm (2003); Playne *et al*. (2003); Shah (2004).

Table 4 Human studies with probiotics in treatment of inflammatory bowel disease (IBD)

Disease	Probiotic strain	Outcome of treatment
UC	*Escherichia coli* Nissle 1917	Maintenance of remission in UC (as effective as mesalazine, the standard treatment)
UC (not placebo controlled)	VSL#3	75% of patients given VSL#3 remained in remission for 12 months. No side effects
Pouchitis	VSL#3 pouchitis	Reduced risk of relapse recurring compared with placebo
Crohns' disease (active, moderate	*Streptococcus boulardii*	Reduction in bowel movements and decrease in disease activity compared with placebo

disease)		
Crohn's disease (in remission)	*S. boulardi*	Probiotic plus mesalamine reduced incidence of relapse compared to mesalamine alone
Crohn's disease	*E. coli* Nissle 1917	Significant reduction in rate of relapse compared with placebo
Crohn's disease	VSL#3	Reduced risk of relapse in postoperative Crohn's disease patients compared with patients given mesalazine
Pouchitis	VSL#3	More effective than placebo in preventing pouchitis

Source: Tuohy *et al*., 2003

Table 5 Influence of feeding *L. acidophilus* on serum cholesterol levels in pigs

Cholesterol (mg/dl)			
Days / Test Group	0^{th} Day	5^{th} Day	10^{th} Day
Control	52.23	69.10	74.44
L. acidophilus RP 32	52.84	61.48	62.29
L. acidophilus P 47	55.58	72.01	73.48

Table 6 Mechanisms for the anti-tumor action of probiotics

1. Suppressing the carcinogen/procarcinogen by binding, blocking or removing it;
2. Curbing the growth of bacteria with enzyme activities such as β-glucuronidase, azoreductase, nitroreductase, that directly or indirectly convert procarcinogens to carcinogens;
3. Reducing the intestinal pH, thereby altering microflora activity and bile acid solubility;

4. Altering colonic transit time to remove fecal mutagens more rapidly;
5. Stimulating the immune system.
6. Production of short-chain fatty acids such as butyrate and propionate, is another mechanism by which probiotics may help in the treatment for colorectal cancer (Geier *et al*. 2006).
7. It has also been suggested that LAB or compounds produced by these microorganisms may directly interact with tumor cells in culture and inhibit their growth, supporting the idea that they can directly produce anti-tumorigenic or antimutagenic compounds (Reddy *et al*. 1983).

Source: Mclntosh, 1996

Table 7 Potential and established effects of probiotic bacteria

Target Health Benefit	Postulated Mechanism
Aid in lactose digestion	Bacterial lactase hydrolyses lactose
Resistance to enteric pathogens	Secretory immune effect Colonization resistance Alteration of intestinal conditions to be less favorable for pathogenicity (pH, short chain fatty acids, bacteriocins) Alteration of toxin binding sites Influence on gut flora populations Adherence to intestinal mucosa, interfering with pathogen adherence g. Upregulation of intestinal mucin production, interfering with pathogen attachment to intestinal epithelial cells
Anti-colon cancer effect	Mutagen binding Carcinogen deactivation Inhibition of carcinogen-producing enzymes of colonic microbes Immune response Influence on secondary bile salt concentration

Small bowel bacterial overgrowth	Influence on activity of overgrowth flora, decreasing toxic metabolite production Alteration of intestinal conditions to be less favorable to overgrowth flora activities or populations
Immune system modulation	Strengthening of non-specific defense against infection and tumors Adjuvant effect in antigen-specific immune responses Enhancement of secretory IgA production
Allergy	Prevention of antigen translocation into blood stream
Blood lipids, heart disease	Assimilation of cholesterol within bacterial cell Increased excretion of bile salts due to deconjugation by bile salt hydrolase Antioxidative effect
Antihypertensive effect	Peptidase action on milk protein yields tripeptides which inhibit angiotensin 1 converting enzyme Cell wall components act as angiotensin converting enzyme inhibitors
Hepatic encephalopathy	Inhibition of urease producing gut flora
Urogenital infections	Colonization resistance Adhesion to urinary and vaginal tract cells
Helicobacter pylori infections	Production of inhibitors of *H. pylori* (lactic acid and others)

Source: Sanders and Huisin't Veld, 1999

Table 8 Application of *Lactobacillus* Bacteriocins as Food Bio-preservatives

Bacteriocin	Example of Food Application	Active Against
Pediocin AcH	Muenster cheese, cooked sausage	*L. monocytogenes*
Sakacin A	Meat, pork	*L. monocytogenes*
Sakacin K	Dry fermented sausage	*L. monocytogenes*
Sakacin P	Cold smoked salmon, chicken cold-cuts	*L. monocytogenes*
Reuterin	Beef sausages	*L. monocytogenes*

Curvacin	Pork steaks and ground beef	*L. monocytogenes*
Plantaricin D	Ready-to-eat salad	*L. monocytogenes*

Source: Goktepe, 2006

Table 9 Selection criteria for probiotics

Particulars	Selection criteria
General aspects	Origin/ Definition/Characteristics
	Strain & genus safety properties
Safety and Stability	Activity and viability in products, adherence, invasive potential
	Resistance to low pH, gastric juice, bile juice, pancreatic juice, colonization/ survival in vivo
Functional and physiological aspects	Adherence to intestinal epithelium/ tissue/virulence and/or
	Antagonism to pathogens antimicrobial activity and/or
	Stimulation/suppression of beneficial bacteria and suppression of harmful bacteria and/or
	Clinical side effects
	Volunteers/patients

Source: Gasser, 1994; Donohue and Salminen, 1996

Table 10 Development of Synbiotic Food

Probiotics	***Synbiotics***	***Integrated synbiotics***

*Exogenous strains *Known health properties *Immunomodulation potential *Specificity to individual hosts *Colonization ***Prebiotics*** *Proliferation of endogenous strains *Effects on the microbiota's metabolic activity as well as population dynamics *Specificity *Oligosaccharides- relatively rapid fermentation in the proximal colon	*Synergy between probiotic and prebiotic effects in the GI tract *Limited specificity of prebiotic for added probiotic strain	*Specific synergy between probiotic and prebiotic ingredients in the GI tract *Prebiotics that additionally protect probiotics during manufacture, storage, formulation and, intestinal transit (carriers/encapsulation) *Controlled, site specific release of probiotics in the GI tract *Larger, more slowly fermentable prebiotics with SCFA production more distally in the colon

Source: Mattila-Sandholm *et al*. 2002

Table 11 Important tests to characterize probiotic strains for GRAS

1. Determination of antibiotic resistance patterns
2. Assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation)
3. Assessment of side-effects during human studies
4. Epidemiological surveillance of adverse incidents in consumers (post-market)
5. If the strain under evaluation belongs to a species that is a known mammalian toxin Producer, it must be tested for toxin production. One possible scheme for testing toxin

production has been recommended by the EU Scientific Committee on Animal Nutrition (SCAN, 2000)

6. If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required.

