‘PROBIOTICS’ A NEW GENERATION FUNCTIONAL FOOD-A REVIEW

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ABSTRACT

Demand of probiotic bacteria has increased and they have become popular, as a result of the researches done to the beneficial effects of probiotics on human health, during last two decades. The probiotics are actively used in the food industry and have been incorporated in various food products such as in fermented dairy foods, non dairy products, plant based products, functional foods etc. Lactic acid bacteria, such as Lactobacillus and Bifidobacterium species exert their beneficial effects as probiotics through various mechanisms. In this review article, effects of probiotics on health, selection criteria , mechanisms of action of probiotics, advances of the probiotics in possible beneficial properties in the literature and emphasis of probiotics in food is discussed.

Keywords: Probiotics, Fermented foods, Lactic acid bacteria, prebiotics, synbiotics.

INTRODUCTION

The word ‘probiotic’ comes from Greek language ‘pro bios’ which means ‘for life’ opposed to ‘antibiotics’ which means ‘against life’. These microorganisms are helpful in maintaining intestinal microbial balance and play a beneficial role in health. The probiotic microorganisms are mostly the strains of the genera *Lactobacillus* and *Bifidobacterium*, but strains of *Bacillus, Pediococcus* and some yeasts have also been found as suitable candidates. Different enzymes, vitamins, capsules or tablets and some fermented foods are probiotic products which contains microorganism which beneficially affects host health. They can contain one or several species of probiotic bacteria. Most of the products of human consumption are produced in fermented milk or given in powders or tablets. Human intestinal tract is an enormously complex ecosystem that includes both facultative anaerobic and aerobic microorganisms Naidu *et al*. (1999). It is estimated that about 300-400 different cultivable species belonging to more than 190 genera are present in the colon of healthy adults. Among the known colonic microbial flora only a few major groups dominate at levels around 10^{10}-10^{11}/g, all of which are strict anaerobes such as *Bacteroids, Eubacterium, Bifidobacterium* and *Peptostreptococcus*. Facultative aerobes are considered to the subdominant flora, constituting *Enterobacteriaceae, lactobacilli* and *streptococci* Gedek *et al*. (1993). Minor groups of pathogenic and opportunistic organisms, the so called 'residual flora' according to Gedek, (1993) are always present in low numbers. There are lots of factors that may disturbs the balance of the gut microflora by some factors such as; age, diet, environment, stress and medication (Albertcllasic, 2007). The lifestyle is changing and it is difficult to have a healthy intestine and the balance of the bacteria must be maintained. Several factors make the host more susceptible to the illnesses. That's why Probiotics are suggested as food to provide for the balance of intestinal flora (Holzapfel *et al*., 1998).

Definition of probiotics

The explanation of probiotics has been growing over time, which was used for the first time by Lilly and Stillwell (1965) to describe compounds produced by organisms that stimulated the growth of another. However, Parker (1974) used this term to the substances that applied to the animals feed as supplements for health improvement by contributing to its intestinal microbial balance. This term probiotics' was taken by Roy Fuller (1989) who referred these substances as life microbes and substances supplements and give his definition as - a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance.

Applying probiotics to the human studies show more new definitions and the essential requirements have been moderated to suit the future researches, for example Food and Agriculture Organization/World Health Organization Working Group (FAO/WHO, 2002) recognize probiotics as - live microorganisms which when administered in adequate amounts confer a health benefit on the host. However, the Joint International Scientific Association for Probiotics and Prebiotics recently adopted the definition as probiotic bacteria are live food supplements which benefit the health of the consumer (Reid *et al.*, 2003). Probiotics are preparation of live micro organisms which beneficially affect the host by improving the properties of the indigenous microbes. Probiotics are used to improve intestinal health and stimulate the immune system. The microbes commonly used as probiotics for humans are the lactic acid bacteria. In general, there are
56 species of *lactobacillus* and 29 species of *Bifidobacteria*, which are used worldwide in dairy products (Shah, 2001). However, the main species of *lactobacillus* and *bifidobacteria* reported in literature are *L. lactis, L. casei, L. paracasei, B. longum, B. infantis, B. breve, B. lactis* and *B. bifidu*m (Shah, 2001). Consuming fermented products containing these organisms allows the continuous passage of these organisms through the gut (Modler *et al*., 1990).

In addition to probiotics, the terms *prebiotics* and *synbiotics* are often used. A definition for prebiotic was developed by Gibson and Roberfroid (1995), however in the light of published research, the authors redefined this in 2004 as *"A probiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health"* (Gibson *et al.*, 2004). Most commercial prebiotics are carbohydrates, predominantly oligosaccharides and some polysaccharides. The non-digestible character of prebiotics is a feature shared with dietary fibre, but their physiological functions are often different (Macfarlane *et al*., 2006). The prebiotics are selective in their growth stimulation and at the same time they are reported to suppress pathogenic bacteria present in the GIT because they can only use the prebiotic ingredient for growth to a limited extend or not at all (Bengmark, 2003; Holzapfel and Schillinger, 2002; Macfarlane *et al*., 2006). Hence, the prebiotic principle is based on selective stimulation of microorganisms able to hydrolyse the prebiotics to carbohydrate monomers and use these for growth in the GIT (Bielecka *et al*., 2002; Bomba *et al*., 2002; Macfarlane *et al*., 2006). Other beneficial effects of prebiotics are improvement of calcium bioavailability and reduction in the risk for cardiovascular diseases, non-insulin dependant diabetes, obesity, osteoporosis, colon cancer as well as reduction of traveller's diarrhea, however evidence to support these effects are still preliminary (Macfarlane *et al*., 2006).

A synbiotic refers to a food product with health benefits, which contains both a probiotic and a prebiotic (Roberfroid, 1998), and are beyond the benefits of the pro- and prebiotic on their own. If the prebiotic carbohydrate is utilised by a probiotic strain, its growth and proliferation in the gut will be selectively promoted (Holzapfel and Schillinger, 2002; Bengmark, 2003).

The health benefits and the growing consciousness about probiotics have caught attention of the food industries (Salminen and Gueimonde, 2004). Food companies are increasingly manufacturing foods with incorporated probiotic bacteria, which fall under the new category of foods called functional/ nutraceutical foods. Probiotic dairy products such as yogurts containing *L. Acidophilus* and *Bifidobacterium* spp. constitute a significant amount among the commercially available probiotic foods (Reid *et al*., 2003).

History of fermented foods

The first man to consume a fermented food must have lived in Africa, probably east Africa (Dirar, 1993). The first phase was agriculture, which involved gathering of wild seeds, grains and fruits, followed by cultivation of these plants, and finally domestication, involving a selective interference by man (El Mardi, 1988). The analogous stage for the development of food fermentation could be the gathering stage of fermented fruits and meat, the homofermentation of fruits and meat by simple storage at home and finally the domestication stage in which man interfered by manipulating various factors affecting the process of fermentation, such as the use of salt, spices and other additives to augment the flavor or even to direct the course of fermentation (Carr, 1982). Centuries ago, man learned that milk became sour soon after it was drawn from animal. It was also learned that sour milk does not readily undergo proteolysis and other desirable changes. Therefore, the milk was handled in a manner so as to encourage souring and long preservation. Thus, there originated a large number of fermented milks, which are known by different names, all of which being soured by lactic acid bacteria and in addition, some undergo an alcoholic fermentation (Foster *et al*., 1957). Much of the interest in fermented milks has stemmed from the ideas and writings of Metchnikoff, who worked in France during the early 20th century; he proposed the theory that the life span of man is shortened by the absorption of certain products of anaerobic protein degradation from the intestines.

Classification of fermented foods

Yokotsuka (1982) classified fermented foods into different categories such as (1) alcoholic

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beverages fermented by yeasts (2) vinegars fermented by acetobacter (3) milks fermented by *lactobacilli* (4) pickles fermented with *lactobacilli* (5) fish or meat fermented with *lactobacilli* (6) plant proteins fermented with molds with or without *lactobacilli* and yeasts. Later, Campbell-Platt (1994) classified them into the different classes: (1) beverages (2) cereal products (3) dairy products (4) fish products (5) fruit and vegetable products (6) legumes and (7) meat products. Odunfa (1988) classified the commodity as: (1) fermented starchy roots (2) fermented cereals (3) alcoholic beverages (4) fermented vegetable proteins and (5) fermented animal proteins. Some of common fermentations are detailed below in Table 1.

Table 1: Classification of fermented foods.

Yokotsuka (1982)	**Compbell-platt (1994)**	**Odunfa (1988)**	**Kuboye (1985)**	**Dirar (1993)**
Alcoholic beverages (yeast)	Beverages	Starchy roots	Cassava based	Kissar-staples
Vinegars (*Acetobacter*)	Cereal Products	Cereals	Cereals	Milhat-sauces and relishes for the staples
Milk products (*Lactobacilli*)	Dairy products	Alchoholic beverages	Legumes	Marayiss- beers and other alcoholic drinks
Pickles (*Lactobacilli*)	Fish products	Vegetable proteins	Beverages	Akil-munasabat- foods for special occasions
Fish or meat (enzymes and *Lactobacilli*)	Fruit and vegetable products	Animal proteins		
Plant protein (molds, with or without *Lactobacilli*)	Legumes			

(Peter Sahlin,1999)

Lactic acid Fermented Products

(a) Rabadi

Rabadi is a tradition fermented beverage consumed on regular basis. It is an indigenous natural lactic acid fermented beverage popular in North-Western semi arid regions of India. At homes, *rabadi,* in summer days is prepared by mixing and fermenting the flour of wheat, barley, pearl millet, sorghum or maize with home butter milk in earthen or metallic vessel in hot summer days in open sun or at room temperature (35-45ºC) for 4-6 h, followed by boiling, salting to taste and cooling before consumption (Dhankher, 1987). It is commonly used by low and average income rural populations in the millet producing regions. It is important staple food for millions of Indians. This traditional method of *rabadi* carries with it certain nutritional advantages. The fermentation of pearl millet brings about partial removal of the anti-nutrients, phytic acid, polyphenols and enhances the bioavailability of minerals. Thus, the fermentation process improves digestibility of proteins and carbohydrates, and the blending of cereals and milk proteins gives about all the essential amino acids (Dhanker and Chauhan,1987).

(b) Pearl millet rabadi

Modha and Pal (2011) developed a technology for manufacture of pearl millet based *rabadi* (like fermented milk beverage). In this process, skim milk and flour of 24 hour germinated pearl millet grains were used as source of solids. The blend was heated to 90°C for 5 min, cooled to 37°C, inoculated with *dahi* culture (NCDC 167) at 3 % and incubated at 37°C for 12 h. Set curd was mixed properly followed by the addition of pectin and other the spices such as cumin (0.285%), black pepper (0.057%) and salt (0.85%) with continuous agitation. The mixture was pasteurized at 75°C for 30 sec, cooled to 30°C and blended with the curd obtained previously, filled in bottles, crown corked and stored at refrigeration temperature. The final product contained 8.743% total solids, 0.65 % fat, 2.24
% protein and 1.28 % ash, with shelf life of 7 days at refrigeration temperature. After 7 days of storage, there was an increase in sedimentation, acidity and wheying- off and decrease in flavor and viscosity. Methods for manufacture of *rabadi* from wheat and sorghum based fermented milk beverage were also developed by Modha and Pal, (2011).

(c) Trahanas

Trahanas is prepared from wheat flour and curd (1:1) by lactic acid fermentation of cow, sheep and goat milk. *Str. lactis, Leuc. cremoris, L. lactis, L. casei, L. bulgaricus and L. acidophilus* are the major acid and aroma producing organisms involved in fermentation.

The whole fermentation lasts for about 50 h at a temperature of range of 30-40ºC. In addition to wheat and milk flour, salt, tomato juice or puree, red pepper, onion, sesame, olive oil etc. may also be added in various percentages and combination to improve the product acceptability (Blandino *et al*., 2002). The nutrient content and sensory attributes of *trahanas* depends upon *curd* and wheat flour ratio as well as other added materials. The fermented matter is dried and stored in the form of biscuits (Campbell-Platt, 1994). *Trahanas* has an acidic and sour taste with a strong yeasty flavor, and is a good source of protein and vitamins. While *trahanas* soup can be used as a part of any meal, it is often eaten for breakfast. The practical nutritional importance of *trahanas* is the improvement of the basic cereal protein diet by adding dairy protein in a highly acceptable form. The low pH (3.8-4.2) and low moisture content (6-9%) make *tarhanas* a poor medium for pathogens and spoilage organisms. In addition, *trahanas* powder is not hygroscopic and it can be stored for 1-2 years without any sign of deterioration (Haard *et al*., 1999).

Sensory properties of LAB fermented products Customers get the most important image of a commercial product from its sensory properties including color, consistency (texture and mouth feel), flavor, viscosity and so on. Flavor is considered to be the most important sensory property for a food product. The concept flavor, often involves a perception of taste (detection of nonvolatile compounds,) and odor (detection of volatile compounds). In a sensory profile of fermented milk products, sweetness and sourness corresponded respectively to sugar and organic acid. Aroma was affected by flavoring compounds divided in four groups including non-volatile acids, volatile acids, carbonyl compounds and miscellaneous compounds (Robinson and Tamime, 2002). Fructose, glucose and sucrose are the main sources for sweetness. However, in a plain fermented milk product, fructose and glucose are largely consumed by LAB. Hence, sweetness can be hardly detected in such products. Probiotic natural yoghurt is sweeter than traditional natural yoghurt because extra sugar, such as sucrose in TINE probiotic yoghurt, is added during processing (Tamime *et al*., 2005). The sourness of fermented milk products is affected by organic acids on different levels. Different acids give sourness in different degrees. For example, acetic acid is intensely sourer than lactic acid (Hartwig *et al*., 1995). The sourness of a fermented milk product comes mainly from lactic acid because of its high level in the product. Production of acetic acid by *L. acidophilus and Bifidobacterium spp*. is the main reason for the sharp sour taste of AB milk ('Cultura') stored under suboptimal conditions (Walstra *et al*., 2006).

Organic acids can be instrumentally detected by High pressure liquid chromatography (HPLC). Volatile compounds contribute mainly to the odor of a fermented milk product and hundreds of volatile compounds have been identified in plain yoghurt. A large number of them exist natively in cow's milk and their level is affected by processing, fermentation, storage and raw material others are produced by starter cultures during fermentation and are affected by type of bacteria strains and their enzymatic and chemical transformation ability of sugar, lipid and protein. Despite the large number of volatile compounds in yoghurt, some of them exist only in trace amount and not all of them are of sensory importance. Several studies showed that flavor compounds that contribute to the desired flavor of a fermented milk product are only few, such as acetaldehyde, ethanol, acetone, diacetyl and 2-butanol (Badings and Neeter, 1980; Tamime and Deeth, 1980; Marshall *et al*., 1984; Ulberth, 1991; Kneifel *et al*., 1992; Ulberth and Kneifel, 1992; Marshall, 1993).

HEALTH BENEFITS OF LAB FERMENTED PRODUCTS

(a) Probiotic effect

One of the reasons for the increasing interest in fermented foods is its ability to promote the function of the human digestive system in a number of positive ways. This particular contribution is called probiotic effect. Already, early in 1900, Metchinkoff pointed out the use of fermented milks in the diet for prevention of certain diseases of the gastrointestinal tract and promotion of health related benefits. Since then, a number of studies have now shown that the fermented food products do have a positive effect on health status in many ways. The human intestinal microbial flora is estimated to weigh about 1000 grams and may contain 10^{16}-10^{17} colony forming units representing more than

500 strains. For physiological purposes, it can be considered to a specialized organ of the body with a wide variety of functions in nutrition, immunology and metabolism (Gustafsson, 1983). The anaerobic organisms which outnumber the gram negative enteric bacteria by about 10000:1 are associated with the intestinal epethilium limiting adherence of potential pathogens by effective colonization (Nord and Kager, 1984). The stability of the intestinal microflora is affected by many factors including dietary habits. Decrease in the number of anaerobic bacteria is associated with increase in the number of gram negative pathogens in the intestinal tract and their translocation to extra-intestinal tissue. Under normal conditions, the intestinal wall prevents translocation of organisms both dead and living as well as microbial products like toxins from the gut to the blood.

A fermented food product or live microbial food supplement which has beneficial effects on the host by improving intestinal microbial balance is generally understood to have probiotic effect (Fuller, 1989). Studies on mice have shown that the indigenous microorganisms in the stomach are *Lactobacillus, Strptococcus* and *Torulpsis,* while in the small intestine; ceacum and colon of several different species (*Bacteriodes, Fusobacterium, Eubacterium, Clostridium*, etc.) coexist (Savage, 1983). The gastrointestinal microflora in humans are also known to contain hundreds of species. There is wide variation among individuals, the number of species and size of the population are usually kept stable in normal healthy subjects. There is a constant struggle in maintaining the desirable balance and a dynamic equilibrium between microbial populations within the intestinal flora (Robinson and Samona, 1992).

(b)Flatulence reducing effect

During fermentation of the beans for preparation of *tempe,* the trypsin inhibitor is inactivated and the amount of several oligosaccharides which usually cause flatulence are significantly reduced (Hesseltine, 1983). Bean flour inoculated with *Lactobacillus* and fermented with 20% moisture content, showed a reduction of the stachyose content (Duszkiewicz-Reinhard *et al.*, 1994).

(c)Anticholesterolemic effect

Hepner *et al*. (1979) reported hypercholesterolemic effect of yoghurt in human subjects with one week dietary supplement. Studies on supplementation of infant formula with *L.acidophilus* showed that the serum cholesterol in infants was reduced from 147 mg/ml to 119 mg/100 ml (Harrison and Peat, 1975). In an *in vitro* study, the ability of 23 strains of lactic acid bacteria isolated from various fermented milk products showed that the bacterial cells bind to cholesterol and no cholesterol was found inside the cells (Taranto *et al.*, 1997). Studies have also reported the ability of yoghurt to lower the cholesterol in serum by controlled human trials. Possible role of lactic acid bacteria in lowering cholesterol concentration was found inside the cells (Taranto *et al*., 1997; Haberer *et al.,* 1997).

(d)Effect on transit time, bowel function and glycemic index

The transit time for 50 % (t50) of the gastric content was significantly reduced for regular unfermented milk in comparison with a fermented milk product indigenous to Sweden called *langfil* or ropy milk. Another study reports increase in transport time and improved bowel function in patients with habitual constipation. The number of defecations per week increased from three during control period to seven using conventional fermented milk and fifteen when acidophilous milk was served. Regular unfermented milk also gave significantly higher increase in glycemic index curve than *langfil*. Studies have shown that presence of acid especially acetic or lactic acid which would lower the glycemic index in breads to a significant level. *Koki,* prepared from *Aspergillus oryzae* and *beni koji* made from *Monascus pilosus* were found to express rise in blood pressure (Helena *et al*., 1995).

(e)Anticarcinogenic effect

Apart from this, there are interesting data on anticarcinogenic effect of fermented foods showing potential role of *lactobacilli* in reducing or eliminating procarcinogens and carcinogens in the alimentary canal (Reddy *et al*., 1983; Mital and Garg, 1995). The enzymes b-glucurodinase, azoreductase and nitroreductase which are present in the intestinal canal are known to convert procarcinogen to carcinogens (Goldin and Gorbach, 1984). Oral administration of *L. rhamnosus* GG was shown to lower the faecal concentration of b-glcorinidase in humans (Salminen *et al*., 1998) implying a decrease in conversion of procarcinogens to carcinogens (Sabikhi and Mathur, 2004) fed probiotic. Fermented milk containing *L.acidophilus* given together with fried meat patties significantly lowered the excretion of mutagenic substances compared to ordinary fermented milk with *Lactococcus* fed together with fried meat patties (Lidbeck *et al.*, 1992). The process of fermentation of foods is also reported to reduce the mutagenecity by degrading the mutagenic substances. Lactic acid bacteria isolated from *dadih*, a traditional Indonesian fermented milk were found to be able to bind mutagens and inhibit mutagenic nitrosamines. Milk fermented with *L.acidophilus* LA-2 was demonstrated to suppress faecal mutagenicity in the human intestine. Studies on antimutagenic activity of milk fermented with mixed cultures of various lactic acid bacteria and yeast showed that the fermented milk produced with mixed cultures of lactic acid bacteria had a wider range of activity against mutagens than those produced with a single strain of lactic acid bacteria (Tamai *et al*., 1995). However, a review (Mcintosh, 1996) concludes that there is only limited data to support the hypothesis that probiotic bacteria are effective in cancer prevention. On the other hand, a study by Hosono and Hisamatsu, (1995) on the ability of the probiotic bacteria to bind carcinogenic substances have reported that *Enterococcus feacalis* was able to bind aflatoxin B1, G1 and G2 as well as some pyrolytic products of tryptophan.

(f)Immuno active effects

Some lactic acid bacteria which are present in fermented milk products are found to play an important role in the immune system of the host after colonization in the gut (De Simone, 1986). Oral administration of *L.casei* improved the function of the peritoneal macrophages and increased the production of IgA (Sato *et al.*, 1988). The mechanism of this effect is not clearly known, but it is speculated that the *lactobacillus*, their enzymes or the metabolic products present in the fermented food product may act as antigens, activating production of antibodies. Marin *et al*. (1997) studied the influence of *lactobacillus* used in fermented dairy products on the production of cytokines by macrophages. The results indicated that for most strains, direct interaction with macrophages caused a concentration dependent increase in tumour necrosis factor interleukin. A study showed that *L.casei* could prevent enteric infections and stimulate secretors IgA malnourished animals and also translocate bacteria, while yoghurt could inhibit growth of intestinal growth of intestinal carcinoma through increased activity of IgA, T cells and macrophages. Marteau and Rambaud (1993) concluded that there is a potential of using lactic acid bacteria for therapy and immunomodulation in mucosal diseases, especially in the gastrointestinal tract. Isolauri (1995) presented a study suggesting that *Lactobacillus sp* strain GG could be used in the prevention of food allergy. It is suggested that dietary antigens induce immune-inflammatory response that impairs the intestine's barrier function and that probiotic organisms could be a means of introducing a tool to reinforce the barrier effect of the gut. Kapila *et al*. (2008) evaluated the immune regulatory potential of *L. helveticus* fermented milk and its cell free supernatant in mice and upon feeding fermented milk and cell free supernatant, the production of total IgE and ovalbumin-specific IgE in serum declined significantly in comparison to allergic mice as well as in milk control group. The decline in IgE production was associated with increase in production of Th1(IFN- gamma,IL-2) cytokines and decrease in Th2(IL-4,IL-6) cytokines.

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LACTIC ACID BACTERIA AS PROBIOTICS

Lactic acid bacteria

Lactic acid bacteria (LAB) are a large group of bacteria that produce mainly lactic acid as a result of anaerobic carbohydrate fermentation. They have similar properties such as being gram-positive, non-motile, non-spore forming and in the form of cocci, coccobacilli or rods. They are non-respiratory and cannot produce certain chemical compounds such as catalase and cytochromes. They grow well in anaerobic conditions, but unlike many other anaerobic bacteria, some LAB species can grow in the presence of oxygen (Walstra *et al*., 2006). The most important members of LAB are the genera *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus and Vagococcus* (Walstra *et al*., 2006).

(a) Lactobacillus

This group belongs to the family *Lactobacillaceae* and contains about thirty five species and are gram positive non-spore forming rods that are catalase negative, and often occur in long chains (Salle, 1973, Banwart, 1989 and Jay, 1986). Most of them are microaerophilic or anaerobic, while both homofermentative and heterofermentative species exist among them and are widely distributed among plants and dairy fermented milks such as acidophilus milk and some of them are important in cheese making (Jay, 1986). Several species grow at relatively high temperature (50-65°C) and surface growth may be poor because species are generally anaerobic. *Lactobacilli* are widely distributed in nature, the important species are homofermentative and are found in dairy products that includes *L. casei,*
L. acidophilus, L. bulgaricus, L. helveticus and L. plantarum (Salle, 1967). In food microbiology, the *lactobacilli* are useful in fermentations where lactic acid production is desirable but can also cause spoilage (Banwart, 1989). *Lactobacilli* are able to live in a highly acidic environment with pH 4-5 and are responsible for the final stages of fermentation in products. They have complex nutritional requirements and need rich media to grow. *Lactobacilli* comprise about 25% of all intestinal micro-flora and more than 100 species have been described (Felis and Dellaglio, 2007).
L. delbrueckii subsp. *bulgaricus* is gram- positive, and has long rods. It has an optimum growth rate at 42°C and grows best under anaerobic and acidic (pH 4.6-5.4) conditions. *L. delbrueckii* subsp. *bulgaricus* is responsible for the production of acetaldehyde, which is a main contributor of the characteristic flavor in yoghurt. It dominates the final stage of yoghurt fermentation and its metabolic activities under low pH are considered to be the reason for post acidification of yoghurt (Walstra *et al*., 2006).
L. acidophilus is a component of the normal intestinal flora of healthy humans. They are gram-positive rod- shaped, non-motile, non-spore forming bacteria with rounded ends. Typically, the cells are 0.6-0.9 μm in width and 1.5-6.0μm in length. It grows in or without presence of oxygen,

but its growth is enhanced by anaerobic conditions. *L. acidophilus* exists either as single cell, in pairs or in short chains.

Its optimum growth temperature is between 35- 40°C and its optimum pH is between 5.5-6.0 (Shah, 2000). Henneberg from Kiel, Germany was the first who proposed the use of a combination of *L. acidophilus* and yoghurt culture to produce a so-called Acidophilus – Milch in the early 1980s'. This product finally became a big success in the German market under the name of 'yoghurt mild' (Heller, 2010). *L. rhamnosus* GG was also isolated from a healthy person by Gorbach *et al*.1987 and is the most clinically studied probiotic bacterium. The strain grows best under anaerobic conditions, but can also grow in the presence of CO_2 and does not ferment lactose or sucrose (Goldin *et al*., 1992; Ouwehand *et al*., 2002). *L. rhamnosus* GG survives but does not grow in fermented milk stored at 4°C (Tamime *et al*., 2005).

(b) Streptococcus

The *streptococci* are facultative anaerobes and catalase negative, the nutrients required for growth vary with species and strains, with some strain requiring certain amino acids, vitamins, purines, primidines, fatty acids and elevated levels of CO_2 (Banwart, 1989). Spherical cells occur in pairs or chains and with the exception of some strains, they are not motile, fermenting glucose primarily by the hexose diphosphate pathway and producing mainly lactic acid, thus are homofermentative. The genus is widely used in manufacturing fermented milk products and includes mesophillic bacteria such as *Lactococcus lactis spp cremoris, Lactococcus lactis spp. diacetilactis and L. Lactis spp.lactis* (Jay, 1986). The *streptococci* are widely distributed, being found in air, water or plants, in intestinal tract of man and animals and various food products (Abdallah, 1992). Some *Streptococci* are involved with bovine mastitis and are found in raw milk and some species of strains are rather heat resistant surviving 60°C for 30 minutes (Jay, 1986). This genus is responsible for the normal souring of milk as it is of wide spread occurrence in dairy products (Rasic and Kurmann, 1978). Several varieties of organisms are recognized which show difference in flavour produced, character of fermented milk, rate of acid formation and rate of litmus reduction (Salle, 1967). The fermentation of carbohydrates to lactic acid is a desirable process in fermented food products, some strains utilize citric acid and form acetone and diacetyl (Banwart, 1989).

(c) Leuconostoc

This genus belongs to the family *Streptococcaceae*. It contains at least six species, which are Gram-positive and spherical to oval, catalase-negative, hetrofermentative (Jay, 1986). They are widely distributed among plants, from which they find their way into milk and dairy product. Some cause problems in sugar refineries and some species are employed in dairy starter cultures, while others are often found in cured-meat products (Jay, 1986). It was also reported that *Leuconostoca spp* are found on green vegetable, roots, butter, sour cream and milk. *Leuconostoc dextranicum* is found in the later two products, but *Leuconostoc cirovorum* is recovered only from commercial mixed culture

(Foster *et al.*, 1957).

CHARACTERISTICS OF LACTIC ACID BACTERIA

(a) Sugar fermentation

All fermented milk products are produced utilizing the souring activity of lactic acid bacteria (Walstra *et al.*, 2006). Lactose is the main source of carbon and energy for the microorganisms in milk and it can be converted into lactic acid and other products by most of lactic acid bacteria under fermentation. Other kinds of sugar, such as sucrose can also be converted by certain lactic acid bacteria species depending on the enzymes available in the bacteria. Both lactose and sucrose are disaccharides and their common empirical formula is $C_{12}H_{22}O_{11}$. Lactose is also called milk sugar and is the main source for lactic acid fermentation. One molecule of lactose consists of one molecule of glucose and one molecule of galactose. Sucrose is obtained from sugar cane or sugar beets commercially and is widely used as sweetening agent in food industry. One molecule of sucrose consists of one molecule of glucose and one molecule of fructose (Coultate, 2009). Unlike respiration, fermentation process does not require the presence of oxygen and therefore less energy is produced. A fermentation pathway always starts with sugar and finishes with various end products. In addition, there are intermediate compounds produced along the metabolic pathway as one compound is converted into another. Pyruvate is considered to be the most important intermediate compound. In the metabolism of pyruvate, different chemical compounds, such as lactic acid, formic acid, acetic acid, ethanol and acetoin, are produced depending on the metabolic path way utilized by the bacteria and the kind of sugars available. Depending on the metabolic pathway that is utilized in sugar fermentation, lactic acid bacteria can be divided into three different groups. The first step in the metabolism of lactose is the transportation of lactose into the bacteria cell either via the phosphoenol pyruvate phosphotransferase system (PEP-PTS) (typical for *lactococci*) or lactose permease system. During sucrose fermentation, it is transported into bacteria cell by a permease system and cleaved by sucrose hydrolase to glucose and fructose. In some *lactococci*, sucrose is transported by PTS forming sucrose-6-phosphate. Sucrose-6-phosphate is then cleaved by sucrose-6- phosphate hydrolase to glucose-6-phosphate and fructose.

This enzyme will be induced when sucrose is present in the medium (Thompson and Chassy, 1981). For further metabolism, Homo-fermentative LAB (such as *lactococci*) produces only lactic acid as the end product of carbohydrate fermentation. When there is excess glucose and limited oxygen in the environment, glucose is metabolized via the glycolytic or Embden-Meyerhof (EM) pathway and galactose-6-phosphate is metabolized via the tagatose pathway. Enzyme aldolase is characteristic in this process. Some thermophilic LAB cannot metabolize galactose and they excrete galactose out of cell as a metabolic shunt for uptake of lactose. The mechanism of homo-fermentative pathway can be simply explained as that a hexose is split into two identical 3-carbon molecules, which are transformed into lactic acid molecules in the following reaction sequences. Hetero-fermentative lactic acid bacteria, such as *Leuconostoc* species and some of the *Lactobacillus* species also produce lactic acid as the major end product, but in addition they also produce ethanol, acetic acid and CO_2 as end products. Glucose is metabolized via the phosphoketolase pathway. Galactose is first transformed into glucose-1-phosphate via the Leloir rote, which then enters the phosphoketolase pathway. One carbon is released in the form of CO_2 from hexose leaving a pentose. Pentose is then split into one 3-carbon units which are converted into lactic acid and one 2-carbon units, and then converted into ethanol or acetic acid. Important enzymes involved in the hetero-fermentative metabolism of *Leuconostoc* species are glucose-6-P dehydrogenase and phosphoketolase. In addition to homo- and hetero- fermentative pathways, there are certain lactic acid bacteria (often lactobacilli) which perform a so-called facultative hetero-fermentative pathway. These bacteria are not restricted to the two pathways and the pathway used depends on the sugar available and condition in the environment (and Robinson, 1999; Walstra *et al*., 2006).

(b) Citrate metabolism

The major activity of LAB under fermentation is their catabolism of sugar, but some species also have the ability to metabolize citrate which originates naturally in milk. The citrate is firstly transported into the cell membrane through a specific membrane protein and converted subsequently into acetate and oxaloacetate by enzyme citrate lyase. Oxaloacetate can be converted into pyruvate, which can be later metabolized into various compounds. The major end products of citrate metabolism are 4-carbon compounds, mainly diacetyl, acetoin and butanediol depending on bacteria strains and growth conditions (Quintans *et al*., 2008). The three stages involved in citrate metabolism are: 1) transportation of citrate by pemease. 2) conversion into oxaloacetate by citrate lyase and 3) further conversion to pyruvate and CO_2. Metabolism of pyruvate will produce more different end products (Fox and McSweeney, 1998; McSweeney and Sousa, 2000).

HEALTH BENEFITS OF PROBIOTICS

Since 1980s, the awareness of the healthier food and drink market has been increased in all over the world which is named as functional foods (Roberfroid, 2002). The uses of insoluble fiber ingredients (Gibson, 2004), such as bran, have been used in products such as breakfast cereals, bread and pasta, but the acceptability of these materials is limited in different systems, which decreased their addition into foods. Soluble fiber ingredients such as oligosaccharides are currently of more interest in formulating healthy foods as they are more acceptable. Moreover, some of them can be used as thickners in the food system, add viscosity or gel (Dreher, 1999). The main reason of prebiotics supplementation to human diet is to beneficially enhance the gut microflora (Kolida *et al*., 2002), which is *Bifidobacterium spp*. the most dominant and important flora in the breastfed and healthy infants. The beneficial effects of the presence of *bifidobacteria* in the gastrointestinal tract are dependent on their viability and metabolic activity. Their growth is dependent on the presence of complex carbohydrates known as oligosaccharides. Some oligosaccharides, because of their chemical structure, are resistant to digestive enzymes and therefore pass into the large intestine, these considered as prebiotics which are defined as - non-digestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Therefore, prebiotics are used as bifidogenic factors in diet applications, especially because of their ability not to degrade in the stomach and small intestine (Crociani *et al*., 1994). Gibson (2004) stated that for a dietary substrate to be classified as a prebiotic, it has to meet at least three requirements; (1) the substrate must not be hydrolysed or absorbed in the stomach or small intestine, (2) it must be selective for beneficial bacteria in the colon such as the *bifidobacteria* and (3) fermentation of the substrate should induce beneficial luminal/systemic effects within the host. There are lot of studies on the health benefits of probiotics. However, in most of these studies, researchers did not use sufficient test subjects or they use microorganisms which were not identified definitely (Çakır, 2003). The main targets of probiotic intervention have been (Crittenden *et al*., 2005):

(i) Increasing natural resistance to infectious disease in the gastrointestinal tract and a first line of defense against disease.

(ii) Prevention of dangerous fungal overgrowth and some allergic reactions.

(iii) Reducing putrefactive/toxic microbial metabolism in the gut.

(iv) Promoting optimized digestive processes, allowing maximum nutritional benefit from food.

(v) Improved resistance to toxic bowel problems and diarrhea. (vi) Stimulation of the immune system.

(vii) Production of needed nutrients, like vitamin K, one form of which the body itself cannot make.

(viii) Improving lactose intolerance conditions.

(ix) Reduction of cholesterol levels and act as antioxidants.

(x) Controlling diseases where components of the intestinal microbiota have been implicated in aetiology.

Probiotic bacteria have many health benefits to the host which include prevention of infectious diseases, reduction in serum cholesterol, anti carcinogenic activity, combating lactose intolerance etc. Besides these, probiotic bacteria have also the ability to modulate the host immune response.

COMMERCIAL PROBIOTIC STRAINS AND PRODUCTS

There are already more than 90 probiotic products containing one or several probiotic organisms available worldwide (Tharmaraj and Shah, 2003). Table 2 shows the different probiotic fermented milk products dominating in Europe market.

Table 2: Different probiotic products along with the bacterial strains.

Type of product and trade name	Probiotic microorganisms
Non-drinkable fermented milks Bifosoft, Bifidus, Bioghurt, Biofit, Biofarde Plus, Biola, Biologic Bifidus, Cultura Dofilus, Dujat Bio Aktiv, Ekollogis Jordgubbs Youghure, Fit & Aktiv, Fjallyoughurt, fysiq, Gaio dofilus, Gefilac, Gefilus, L1, Probiotisches joghurt, proViva, RELA, Verum,Vifit Vitamel, Vitality, Weight Watchers, yogosan Milbona.	*L. acidophilus, L. acidophilus La5, L. rhamnosus GG, LB21 and* 271, *L. casei* (also strain F19), *L. johnsonii, L. plantarum 299v, L. reuteri, Lactococcus. lactis subsp. lactis L1A B. bifidum, B. aminalis subsp. lactis BB-12, B. animalis subsp. Animalis*
Drinkable fermented milks (including cultured buttermilk, yoghurt drink, dairy drink) A-fil, Actimel, Aktifit, AB-piim, Bella Vita, Bifidus, Biofit, Biola, Casilus, Cultura, Emmifit, Everybody, Fit & Aktiv,Fundo, Gaio, Gefilac, Gefilus, Kaiku Actif, LC1 go, LGG+, Onaka, Oresundsfil, Philura, Probiotic drink, Proviva, Pro.x, Verum, Vikt Vaktarna, Vitality, Vive+, Yakult, Yoco acti-vit	*L. acidophilus, L. acidophilus La5, L. casei (F19, 431, Imunitass, Shirota). L. rhamnosus GG (271 and LB21), L. johnsonii, L. plantarum 299v, L. reuteri, L. fortis Lac. lactis subsp. lactis L1AB. aminalis subsp. lactis BB-12, B. bifidum, B. animalis subsp. animalis, B. longum BB536*
Non-fermented dairy products (milk, ice-cream) Gefilus, god Hals, RELA, Vivi Vivo	*L. rhamnosus GG, L. johnsonii, L. plantarum 299v, L. reuteri*

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(Tamime *et al*., 2005)

The probiotic bacteria strains used in these products vary from country to country. The possible combinations of bacterial strains are many, however, in order to obtain unique and specific aroma and flavor in fermented milk product, it is important to choose appropriate bacterial strain according to their technologic and sensory properties during fermentation and after fermentation. Several factors have to be considered with selection of bacterial strains for use in a starter culture (Stenby, 1998):

1) **Acidity:** Bacterial strains determine the acidity of the end product depending on their ability to produce acid. Certain bacterial strains, such as *L. delbrueckii* subsp. *bulgaricus,* are able to produce acid at low temperature resulting in post-acidification during storage, which was not desirable.

2) **Flavor:** Ability to produce flavor compounds determines the flavor of the end products. For example,

L. delbrueckii subsp. *bulgaricus* produces acetaldehyde which is indispensable for formation of yoghurt taste. *L. acidophilus* and *Bifidobacterium* spp. produce acetic acid, which in high concentration could result in flavor defect.

3) **Viscosity:** Proteolytic activity and ability to produce EPS contribute to gel formation and gel stability during storage.

4) **Fermentation:** The time required for the fermentation affects the production process of products. Fermented milk with single LAB strain is not common in market. Practically, in order to achieve desired properties of product, bacterial strains are mixed in different combinations. A culture with *L. acidophilus* and *Bifidobacterium* spp. are known as AB culture. A culture with *L. acidophilus* and *Bifidobacterium spp*. and *L. casei/ L. rhamnosus* GG is known as ABC culture. Since time for fermenting milk with only AB and ABC culture is relatively long, yoghurt culture is often added to shorten the time of fermentation (Schlichtherle-Cerny *et al*., 2008; Tamime, 2005). Probiotics have been used for centuries in fermented dairy products.

However, the potential applications of probiotics in nondairy food products and agriculture have not received formal recognition. Recently, interest to food and agricultural applications of probiotics has increased and the selection of new probiotic strains and the development of new application have gained much importance. The uses of probiotics have been very beneficial to the human health and to play a key role in normal digestive processes and in maintaining the animal’s health. However, a number of uncertainties concerning technological, microbiological, and regulatory aspects exist (Saarela *et al*. 2000). The presence of probiotics in commercial food products has been claimed for certain health benefits. This has led to industries focusing on different applications of probiotics in food products and creating a new generation of ‘probiotic health’ foods. Among probiotics carrier food products, dairy drinks were the first commercialized products that are still consumed in larger quantities than other probiotic beverages. Growing awareness among people and newer advances in the medical field has brought them into the limelight (Gupta and Sharma, 2016).

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