Review :Probiotic Bacteria In Dairy Products

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Definition of probiotics: In recent years, research into the benefits of gut bacteria has exploded. Scientists across the globe are examining how these microbes can help improve health and prevent disease.

Probiotics are defined as living microorganisms, which when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora. In addition to improving gut health, probiotics may play a beneficial role in several medical conditions, including lactose intolerance, cancer, allergies, hepatic disease, Helicobacter pylori infections, urinary tract infections, hyperlipidemia and assimilation of cholesterol **(Ejtahed et al., 2011)**. Probiotic microorganisms that are known to be beneficial to human health can be ingested through fermented dairy products, enrichment of various foods with these bacteria and consumption of pharmaceutical products that are obtained by using viable cells (lyophiled preparations and tablets). Also, Probiotics are defined as viable microorganisms which can be consumed separately or with foods, which assist dietary and microbial balance by regulating the mucosal and systemical immunity and beneficially affect the consumer's health. A great variety of dairy products contain probiotic bacteria **(Ziemer & Gibson, 1998; Moayednia et al., 1999; Salminen et al., 1999; Gibson & Fuller, 2000; Granato et al., 2010; Guldas & Irkin, 2010; Kanmani et al., 2013; Lollo et al., 2013).**

The word probiotic derived from the Greek meaning “for life”. The term “probotics” was introduced by **Lilly and Stillwell (1965)** for defining probiotics as microorganisms promoting the growth of other microorganisms. **Fuller (1989)** defined “probotics” as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance. However, according to this definition probiotics were restricted to feeding supplements, animals and the intestinal tract, and the term “probiotic” thus could not be used for living microorganisms administered in any other way than in food or feed, or for locations other than the gastrointestinal tract. Consequently, **Havenaar and Veld (1992)** have defined probiotics as “mono- or mixed cultures of live microorganisms which, when applied to animal or human, beneficially affect the host by improving the properties of the indigenous microflora”. When these probiotic bacteria are present in yogurt and other fermented foods, they may beneficially alter the normal gut flora **(Metchnikoff, 1907)**. Probiotics have also been defined by the European Union (EU) Expert Group on Functional Foods in Europe (FUFOSE) to be “viable preparations in foods or dietary supplements to improve the health of humans and animals” **(FUFOSE working group, 1999)**. The current widely accepted definition of probiotics is that they are “live microorganisms which when administered in adequate amounts confer a health benefit on the host” beyond inherent general nutrition **(FAO/WHO, 2001)**.

Probiotic bacteria species:

Lactobacillus and Bifidobacterium species are the most widely used probiotics **(Salminen2001 ,Fooks2oo2,Ouuuweh 2003)**. Emiley 2015 reported that one of the most well-known strain of these is *Lactobacillus rhamnosus* GG (LGG).

***Bifidobacterium* ssp.**

In 8th Bergey's Manual, *Bifidobacterium* ssp. was defined as an independent genus by taxonomists and named *Bifidobacterium* and was included in Actinomycetaceae family. Some of the defined 24 species in 9th Bergey's Manual are *B. bifidum*, *B. breve, B. infantis, B. thermophilum, B. adolescentis, B. longum, B. pseudolongum, B. coryneforme, B. indicum* and *B. dentim* **(Scardovi, 1986).**

The use of *Bifidobacterium* species in fermented and cultured milk and growing knowledge of their taxonomy and ecology resulted in an increase in their popularity in the late 1970s. They grew in popularity considering low acid formation during their shelf life and higher consumption of L(+) lactic acid in comparison with D(-) lactic acid. Among the many probiotic traits that have been attributed to bifidobacteria are a) the induction of immunoglobulin production, b) improvement of food nutritional value by assimilation of substrates not metabolized by the host, c) anti-carcinogenic activity and d) folic acid synthesis **(Martinez et al., 2013)**. Within various probiotic bacteria, *Bifidobacterium lactis* has been studied intensively and its beneficial roles for host health has been described. *B. lactis* is preferred for industrial production because of the oxygen and acid tolerance compared with other bifidobacteria species **(Janer et al., 2004; Elizaquível et al., 2011; Akalin et al., 2012)**.

Lactobacillus rhamnosus Due to ***Lactobacillus rhamnosus*** probiotic traits, *Lactobacillus rhamnosus* GG or *Lactobacillus* GG is the most common microorganism used in dairy products marketed for infant's and children's consumption. *Lactobacillus rhamnosus* GG, was isolated from human faeces in 1983 and was patented in 1985. *Lactobacillus rhamnosus* GG is one of the most studied strains and is one of the most common bacteria that is used in probiotic preparations and foods. It has the suffix "GG" because it was discovered in Tufts University by Sherwood Gorbach and Barry Goldin. *Lactobacillus rhamnosus* GG was first used in the studies at 1990 and was found beneficial to children's health. Due to it's favourable impact on children's health it is widely used with products for infants and children. Some of the main traits of *Lactobacillus rhamnosus* GG are being indigenous to human intestinal flora, resistance to low pH values and adherence to gastrointestinal track **(Canbulat & Ozcan, 2007)**.

Enterococcus faecium/Enterococcus faecalis

Enterococci are singular, double or short chained gram positive cocci. *Streptococcus faecalis* was defined by Andrewea and Horder in 1906 and *Streptococcus faecium* was defined by Orla-Jensen in 1919. In 1984 Schleifer and Kilpper-Balz suggested that *S. faecalis* and *S. faecium* should be distinguished from *Streptococcus* genus and considered in *Enterococcus* genus.

These bacteria can be found with high amounts in dairy products and other foods and although having extensive biotechnological properties such as; capability to produce bacteriocin, having probiotic traits and usage in dairy industry, there isn't a consensus on to consider them foodborne pathogens. However recent studies have shown that *E. faecalis* and some lactic acid bacteria species can cause clinical infections, especially infective endocarditis. *E. faecalis* can be found not only in human and animal faeces but also on plants and this largely reduces it's usage as a sanitation indicator **(Foulquié Moreno et al., 2006; Bhardwaj et al., 2008).**

Among *Enterococcus* genus, *Enterococcus faecium* and *Enterococcus faecalis* are stated to have probiotic traits. *Enterococcus faecium*'s usage on diarrhea treatment is considered to be an alternative for antibiotic use. The probiotic effect of *Enterococcus faecium* on humans arise out of reducing the absorbtion of cholesterol from digestive system **(Erginkaya et al., 2007).**

Lactobacillus gasseri:

Lactobacillus gasseri is a rod shaped, non spore forming lactic acid bacteria. The niche-related phenotypes involved incolonization of the human mucosa, including the oral cavity, GIT, and vagina are exhibited by LAB such as *L. gasseri* and may contribute to or potentiate probiotic activity **(Selle & Klaenhammer, 2013).** *L. gasseri* shows to be beneficiary to gastrointestinal system and is stated to have the capability to reduce fecal mutagenic enzyms due to it's probiotic acitivity. It has the ability to adhere to intestines and has a role in bacteriocin formation and macrophage stimulation. In the view of it's probiotic traits, it can be used in the production of fermented diary products and in commercial preparations **(Uzuner, 2012).**

Saccharomyces cerevisiae boulardii:

Saccharomyces boulardii was discovered by French researcher Boulard in 1923 and is a Gram positive yeast which is a member of Saccharomycetaceae family. It is elliptical or spherical shaped and has a size of 4-8 µm. Preclinical and experimental studies has shown that *Saccharomycess boulardii* has anti inflammatory, antimicrobial, enzymatic, metabolic and antitoxic activities **(Ertor, 2003; Billoo et al., 2006; Szajewska, 2012)**.

Table (1): Species of probiotic bacteria[1].

Lactobacillus species	Bifidobacterium species	Other lactic acid bacteria	Non lactic acid bacteria
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis*[2]	*Bacillus cereus var. toyoi*[2,3]
L. amylovorus	*B. animalis*	*Enterococcus faecium*	*Escherichia coli strain nissle*
L. casei	*B. bifidum*	*Lactococcus lactis*[4]	*Propionibacterium freudenreichii*[2,3]
L. crispatus	*B. breve*	*Leuconstoc mesenteroides*	*Saccharomyces cerevisiae*[3]
L. delbrueckii	*B. infantis*	*Pediococcus acidilactici*[4]	*Saccharomyces boulardii*[3]

subsp. bulgaricus[4]	*B. lactis*[5]	*Sporolactobacillus inulinus*[2]	
L. gallinarum[2]	*B. longum*	*Streptococcus thermophilus*[4]	
L. gasseri			
L. johnsonii			
L. paracasei			
L. plantarum			
L. reuteri			
L. rhamnosus			

[1] Data from reference **Holzapfel *et al.* (2001)**.

[2] Main application for animals.

[3] Applied mainly as pharmaceutical preparations.

[4] There is either little known about the probiotic properties or the microorganism is non probiotic.

[5] Probably synonymous with *B. animalis*.

Health benefits associated with probiotics:

Over recent years, there has been significant interest in the development of innovative food products conferring customized benefits to the consumer, that is, improving physical and mental well-being, prevention of diet-associated health complications in addition to fulfilling basic dietary function (hunger satisfaction and fulfillment of the daily nutritional need of consumers). The increased awareness of the consumer regarding health and nutrition related issues as well as the role of several food regulatory bodies to promote the production and consumption of minimally processed, healthier and more nutritious food products, appear to be steering a transformation within the food industry. Moreover, cultural, educational, and economic effects together with food quality and safety criteria have also been highlighted as drivers of consumer demand for healthy and safe food products **(Fogliano and Vitaglione 2005).**Several beneficial functions have suggested for probiotic bacteria and illustrated in Figure 1.

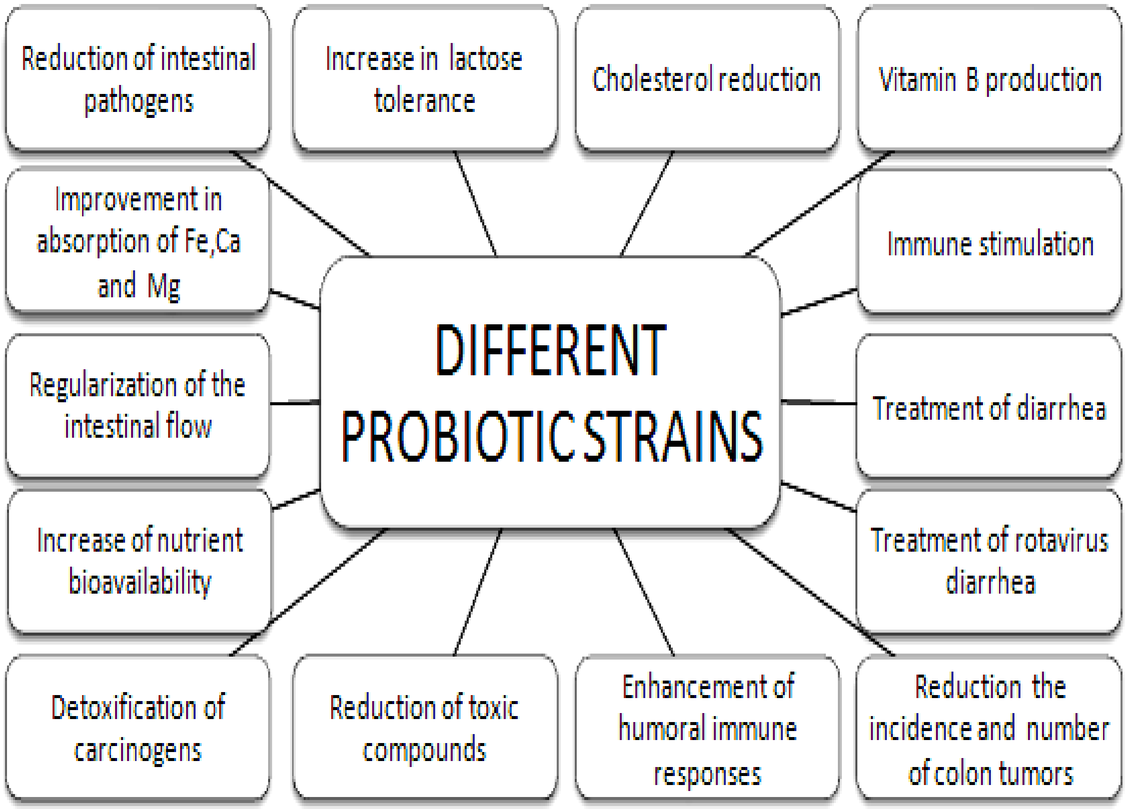

Figure (1). Some documented physiological benefits of functional foods containing probiotic bacteria.

Probiotics generally do not colonies the gut permanent because the exogenous bacteria are outcompeted by the endogenous micro biota, which is better adapted to the prevailing conditions in the gastrointestinal tract. Prebiotics (typicallynon-digestible carbohydrates) may give a competitive advantageto the live-fed probiotic bacteria in the gastrointestinal tract and may also have direct effects on the resident microbial community in the large intestine **(Gibson and Roberfroid, 1995)** Their potential influence on the mechanisms underlying stress-related disorders such as irritable bowel syndrome (IBS), anxiety and depression is also beginning to be elucidated (**Dinan et al. 2006; Rhee et al. 2009; Mayer 2011; Bravo et al. 2012)**. A particular focus has been put on their ability to influence neural and endocrine systems and behavioural phenotypes **(Cryan and O'Mahony 2011; Dinan and Cryan 2012).** Probiotic strains, which have the ability to confer beneficial effects upon the host, have received renewed attention in recent years (e.g. **Forsythe and Kunze 2013**).

Przemyslaw Jan Tomasik and Piotr Tomasik 2003 found that probiotics, bacteria from the genera *Bifidobacterium* and *Lactobacillus*, and yeast, *Saccharomyces*, as well as prebiotics belonging to the group of dietary fiber (inulin with low degree of polymerization, fructose-derived oligosaccharides, and resistant starch) are natural factors useful in prophylaxis and therapy of several common diseases including some types of cancer. They are available commercially and can be introduced to produce so-called functional food. Probiotics and prebiotics can be utilized either separately or jointly (as synbiotics or eubiotics) increases gastrointestinal symptoms such Probiotic dairy products have favorable effects on human health such as, reducing lactose intolerance, prevention of diarrhea and constipation, increase in the effectiveness against *Helicobacter pylori* infection, preservation of oral health, partial prevention of cancer, cholesterol lowering, enhancement of mineral absorption. Along with their extensive effects on human health, they have the ability to form low molecular weight components such as conjugated linoleic acid (CLA), gamma aminobutyric acid (GABA) and bacteriocin **(Gobbetti et al., 2010; Šušković et al., 2010; Divya et al., 2012; Kanmani et al., 2013).**

Seema Patel[1] and Arun Goyal2012 found that the introduction of functional compounds like prebiotics in the diet seems to be an attractive alternative to ameliorate the quality of life ridden with obesity, cancer, hypersensitivity, vascular diseases and degenerative ailments. The enormous functional metagenomic data provided by the Human Microbiome Project is expected to revolutionize the prebiotic research by rational production of desired prebiotic molecules with specific functional properties. There are claims that prebiotics are capable of preventing weight gain in adolescents and improving immunity in geriatrics and infants. Prebiotics are expected to enter the dermatological sector and boost skin health. Also, it is being mooted that prebiotics will eventually replace the antibiotics used as growth stimulants in apiary, fishery, poultry and animal husbandry. The clinical significance of the prebiotics remains to be clarified, the claims of efficacy proved and underlying mechanism decoded. Owing to its wide range of preventative and therapeutic possibilities prebiotics research is certainly catching momentum. *Lactobacillus rhamnosus* GG (LGG). This strain of bacteria, which is part of many popular probiotic products, has a reputation as a helpful microbe. Researchers have found evidence that it can help with intestinal problems, respiratory infections and some skin disorders. Some research suggests that it may even help with weight loss. But a key question has remained unanswered: How does LGG actually produce benefits? found that ingesting LGG led to increases in several genes that foster several species of gut bacteria, including Bacteroides, Eubacterium, Faecalibacterium, Bifidobacterium and Streptococcus. These microbes have been shown to have a range of benefits in humans, including the promotion of a healthy immune system. LGG may also have direct effects, in addition to its ability to modify the overall ecosystem **(Emiley2015).**

Probiotic cultures in fermented dairy products: Probiotic dairy products have favourable effects on human health such as, reducing lactose intolerance, prevention of diarrhea and constipation, increase in the effectiveness against *Helicobacter pylori* infection, preservation of oral health, partial prevention of cancer, cholesterol lowering, enhancement of mineral absorption. Along with their extensive effects on human health, they have the ability to form low molecular weight components such as conjugated linoleic acid (CLA), gamma aminobutyric acid (GABA) and bacteriocin **(Gobbetti et al., 2010; Šušković et al., 2010; Divya et al., 2012; Kanmani et al., 2013).**

Dairy products that contain probiotic bacteria are those that are produced with various fermentation methods, especially lactic acid fermentation, by using starter cultures and those that have various textures and aromas. Fermented dairy products are popular due to their differences in taste and their favorable physiological effects. Today, fermented dairy beverages in general are produced locally by using traditional methods. Recently, due to the increased demand for natural nutrients and probiotic products, fermented dairy beverages have reached a different position and are considered to have an important impact on human health and nutrition**.**

Probiotic microorganisms that are known to be beneficial to human health can be ingested through fermented dairy products, enrichment of various foods with these bacteria and consumption of pharmaceutical products that are obtained by using viable cells (lyophiled preparations and tablets). Probiotics are defined as viable microorganisms which can be consumed separately or with foods, which assist dietary and microbial balance by regulating the mucosal and systemical immunity and beneficially affect the consumer's health. A great variety of dairy products contain probiotic bacteria **(Ziemer & Gibson, 1998; Moayednia et al., 1999; Salminen et al., 1999; Gibson & Fuller, 2000; Granato et al., 2010; Guldas & Irkin, 2010; Kanmani et al., 2013; Lollo et al., 2013).**

Strains of lactic acid bacteria (LAB), such as *Lactobacillus, Bifidobacterium, Eubacterium* and *Streptococcus*, have traditionally been used in the manufacture of fermented dairy products and are generally regarded as safe (GRAS) **(O'Sullivan *et al.,* 1992)**. In addition, these bacteria are desirable members of the intestinal microflora **(Berg, 1998)**. Table 1 shows a list of microorganisms including both LAB and non-lactics, which generally considered as probiotics. Lack of pathogenicity, tolerance to gastrointestinal conditions (acid and bile), ability to adhere to the gastrointestinal mucosa and competitive exclusion of pathogens **(Collins *et al.,* 1998 and Ouwehand *et al.,* 2002)** are some of the general criteria that used for the selection of robotics. Dairy products considered as ideal vehicles for delivering probiotics in the human gut. Yoghurt considered as the most important, followed by cultured buttermilk, kefir, cheeses and ice-cream **(Shah, 2007 and Soccol, 2010)**. A minimum viable LAB count of 10^6 CFU/g in fermented dairy food is recommended for the claimed health benefits **(Tamime *et al.,* 2005)**.

Fermented milks have long been used as the main vehicles for probiotic strains. Less frequently, cheeses have been used for incorporation of probiotic microorganisms, but they may offer a number of advantages compared with fermented milks (**Gomes et al., 2011; Minervini et al., 2012).** Cheese has higher pH, more solid consistency, and relatively higher fat content compared with fermented milks such as yoghurt (**Karimi et al., 2012).** probiotic bacteria and functional dairy products that are produced by using probiotic bacteria are discussed by **Oktay Yerlikaya2014.**

The majority of studies investigating survival of probiotic bacteria (in real or model ice cream systems) have revealed that *Bifidobacteria* are more freeze-resistant than *Lactobacilli* or *Saccharomyces* **(Hekmat and McMahon 1992; Başyiğit et al 2006; Akin et al 2007; Akalin and Erişir 2008; Homayouni et al 2008b). Homayouni et al (2008b)** reported that storing probiotic ice cream at −20 °C for 6 mo was associated with higher inactivation rates of *L. casei* compared to *Bifidobacterium lactis*. **Magariños et al (2007)** demonstrated that freezing (scraped-surface freezer, −10 °C draw temperature) resulted in lower viability of *L. casei* compared to *B. animalis*, whereas no significant changes on the inactivation rates of both species upon frozen storage were observed.

Increasing sugar or fat content in order to enhance texture or structural features has been reported to moderately impact probiotic cell viability. **Alamprese et al (2002)** did not observe any significant effect of sugar/fat content on the viability of *L. johnsonii* La1 in ice cream stored for 180 d at −28 °C. Similarly, the presence of sucrose or aspartame in acidified ice cream did not affect viable counts of a symbiotic/probiotic blend composed of *L. acidophilus* and *L. rhamnosus* during 180 d at −20 °C (**Başyiğit et al 2006)**. On the other hand, **Akin et al (2007)** found that 18% w/w of sucrose was required to ensure maximal survival of mixed strains of LAB and bifidobacteria, regardless of the species/strain. In model ice cream systems, **Homayouni et al (2008b**) demonstrated that probiotic osmotolerance in the presence of sucrose up to 25% w/w is strain- and type-dependent, with *Lactobacilli (L. casei, L. acidophilus*-La5) exhibiting a higher sugar tolerance than *Bifidobacteria* (*B. bifidum* and *B. longum*). However, including a very strong osmolytic agent (2% w/w glycerol) has been reported to have a negligible effect on the cryopreservation of mixed probiotic bacteria (*L. acidophilus* and *B. bifidum*) during freezing and storage (−20 °C, 52 wk), while remarkably higher amounts of the cryoprotectant were required to achieve a significant cryoprotective effect (**Hagen and Narvhus 1999**).

Definition of synbiotic:

The concept of "synbiotic" associate with products where the simultaneous presence of probiotic strains and prebiotic non-digestible ingredients, usually oligosaccharides have synergistically beneficial effect on the host by improving the survival and/or implantation of the probiotic in the intestinal tract **(Steed *et al.*, 2008)**. Most functional foods containing synbiotics are currently in dairy matrices; these most frequently are cheese, yoghurt, ice cream and other dairy products, during the period of storage and throughout the process of consumption **(Madureira *et al.*, 2005)**.

Schmidt et al (2015) found that the salivary cortisol awakening response was significantly lower after galacto-oligosaccharides, B-GOS intake compared with placebo. Participants also showed decreased attentional vigilance to negative versus positive information in a dot-probe task after B-GOS compared to placebo intake.

Convincing evidence now exists for a role of the gut microbiota composition in the regulation of the stress hormone corticosterone (cortisol in humans). Raised levels of circulating corticosterone in germ-free rodents (**Crumeyrolle-Arias et al. 2014**) are reduced following the administration of probiotics (**Sudo et al. 2004**), an effect replicated in mice subjected to a stress-inducing behavioural paradigm designed to elevate corticosterone levels **(Bravo et al. 2011).** Additionally, there is now preliminary evidence for reduced subjective feelings of anxiety and improved aspects of well-being after probiotic intake **(Rao et al. 2009; Messaoudi et al. 2011**). More recently, a functional MRI investigation found that healthy subjects who received a fermented milk product with probiotics showed decreased BOLD activity to an emotional attention task using facial expressions in the insula and somatosensory regions (**Tillisch et al. 2013**). These areas play a crucial role in the integration of visceral inputs and the processing of emotional and interoceptive information (**Craig 2009**). The study demonstrates that manipulations of the gut microbiota can result in measurable changes in emotional processing in the healthy brain.

Joanne Slavin 2013 mentioned that the health benefits of dietary fiber have long been appreciated. Higher intakes of dietary fiber are linked to less cardiovascular disease and fiber plays a role in gut health, with many effective laxatives actually isolated fiber sources. Higher intakes of fiber are linked to lower body weights. Only polysaccharides were included in dietary fiber originally, but more recent definitions have included oligosaccharides as dietary fiber, not based on their chemical measurement as dietary fiber by the accepted total dietary fiber (TDF) method, but on their physiological effects. Inulin, fructo-oligosaccharides, and other oligosaccharides are included as fiber in food labels in the US. Additionally, oligosaccharides are the best known "prebiotics", "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-bring and health." To date, all known and suspected prebiotics are carbohydrate compounds, primarily oligosaccharides, known to resist digestion in the human small intestine and reach the colon where they are fermented by the gut microflora. Studies have provided evidence that inulin and oligofructose (OF), lactulose, and resistant starch (RS) meet all aspects of the definition, including the stimulation of *Bifidobacterium*, a beneficial bacterial genus. Other isolated carbohydrates and carbohydrate-containing foods, including galactooligosaccharides (GOS), transgalactooligosaccharides (TOS), polydextrose, wheat dextrin, acacia gum, psyllium, banana, whole grain wheat, and whole grain corn also have prebiotic effects.

Cereals as prebiotics:

Apart from having a nutritive value comparable to wheat, barley is unique among cereals containing high concentrations of β-glucan which is known to have the effect of cholesterol lowering effect **(Newman *et al.,* 1989 and McIntosh *et al.,* 1991)**, regulating blood glucose levels and insulin response in diabetics **(Cavallero *et al.,* 2002)** and even reducing the risk of cancer **(Jacobs *et al.,* 1998)**.

Cereals are good sources of dietary fiber (DF). Grains consist of endosperm, germ, and bran. The endosperm comprises the main part of the whole grain. Bran consists of the outer parts of the grain, and depending on the milling process **(Weipert, 1997)**. The main components of cereals are a starch 57.66%, DF 15.17% and protein 7.13% **(Nilsson *et al.,* 1997)**. The DF content of wheat is 12.14 g / 100 g **(Graham *et al.,* 1988 and Bach Knudsen, 1997)**, whereas wheat bran contains c. 45 g of DF /100 g (**Bach Knudsen 1997**). The large variation in composition of cereals depends on both the cultivar and the growing conditions.

The effect of cereal polysaccharides on microbial population dynamics in the intestinal tract has not intensively studied, but a few reports have indicated that they may have potential to act as prebiotics. Cereal β-glucans are not fermented by most lactobacilli and bifidobacteria **(Crittenden *et al.,* 2002)**, but β-glucooligosaccharides produced by controlling hydrolysis of cereal β-glucans can be utilized by some bifidobacteria and lactobacilli **(Van Laere *et al.,* 2000 and Kontula *et al.,* 1998)**, although studies of their selectivity and prebiotic effect in vivo have not yet been reported.

Cereals are considered one of the most important sources of dietary carbohydrates, proteins, vitamins, minerals and fibers for people all over the world Nowadays, cereals alone or mixed with other ingredients are used for the production of traditional fermented food and beverages as well as for the development of new foods with enhanced healthy properties **(Blandino *et al.,* 2003)**.

Barley is an excellent source of many valuable nutrients because it contains bioactive compounds like dietary fiber, B complex vitamins, β-glucan, phenolic compounds **(Škrbić *et al*., 2009)**. Barley has been classified as hull-less and hulled, where hull-less Barley has better nutritional value, i.e., more proteins, lipids and soluble dietary fibers (**Soares *et al*., 2007**). The nutritional value of hullless barley can be further increased with biological activation, i.e., increased content of dietary fiber, vitamins B2, E and niacin **(Rakcejeva and Skudra, 2007).**

Barley and malt have been considered as ingredients for Production of functional foods due to their concentration antioxidant compounds furthermore malt should be considered as a new source of natural antioxidant for dietary needs **(Qingming *et al.*, 2010)**. Therefore, both valuable products-biologically activated hull-less barley grain and malt extract were added to fermented milk in order to increase the nutritional value of the end products.

Microencapsulation probiotic bacteria:

Encapsulation is a technique that can be used for protecting probiotics from acidic environments, such as those in fermented dairy products or fruit juices **(Krasaekoopt *et al.,* 2003 and Ding and Shah, 2007).**

Encapsulation methods have also been applied to lactobacilli and probotics **(Kailasapathy, 2002)** as a means of delivery of bacteria, as well as a way to increase bacterial survival, as this method creates a barrier and separates the bacterial cell from the adverse and stressful conditions of hostile environment like digestive extremes of gastric acid and bile salts.

Among the various encapsulation materials, alginate is one of the most used polymers for the encapsulation of microorganisms. It can be found in the cell walls and intercellular spaces of brown algae. It is a linear co-polymer with homo-polymeric blocs, covalently linked in different sequences, depending on the source of algae Figure (2). Alginic acid, the free acid from alginate is the intermediate product in the commercial alginates and has limited stability. In order to make stable water-soluble products alginic acid is transformed into a range of salts i.e., Ca-alginate; Na-alginate; K-alginate; Mg-alginate; NH4-alginate. The ratio of mannuronic acid to gluconic acid and the structure of the polymer determine the properties of alginate in solution. Alginates may be prepared with a wide range of molecular weights. Alginate capsules are formed by dripping an aqueous alginate solution into a solution containing a multivalent cation, usually a calcium salt. The calcium ion attaches to two polymer strands by replacing the salt bond, and can thus form a very fine network **(Thu *et al.,* 1996)**.

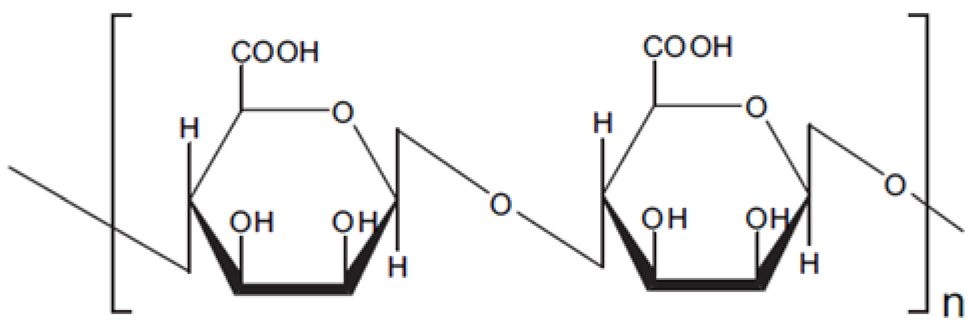

Figure (2). Structure of alginate.

Kamaly (1998) reported that *Bif. bifidum* immobilized in double layer alginate beads exhibited the highest survival rate between 61 – 26 and 92.45% in the absence and presence of a protective medium, whereas free cells were dramatically affected by freeze drying and had the lowest survival rate ranging from 18.31 to 24.08%. Furthermore survival of *Bif. Bifidum* immobilized in double layer alginate beads was stable, whereas the survival rate of free cells dropped to 7.5% after 3 h incubation time in simulated gastric. Also, in simulated intestinal juice (pH 7.43), survival of free and immobilized cells was not significantly affected ($p < 0.05$). Sequential incubation in simulated gastric pH 1.33 and intestinal (pH 7.43) juices resulted in significant ($p > 0.05$) decrease in survivability by 6.43, 5.79 and 0.22 orders of magnitude for free, single and double layer of alginate beads, respectively.

Hussein and Kebary (1999) entrapped cells of *Bif. bifidum* and *Bif infantis* were able to produce antimicrobial agents which inhibited *E. coli* and *Staph. aureus* used as test organisms. Viable counts of unentrapped bifidobacteria decreased sharply, while entrapped cells of bifidobacteria were quite stable during refrigerated storage of stirred yoghurt. *Bif. infantis* was more tolerant to storage conditions than *Bif. bifidum*. Microentrapment of bifidobacteria improved their survival during storage of stirred yoghurt, especially *Bif. bifidum*, whose viability was not significantly ($p > 0.05$) different from entrapped *Bif. infantis*.

Badawi (2004) microentrapped bifidobacteria were more tolerant to Nisin and could withstand its concentrated up to 200 IU/ml. Development of acidity was more evident by free cells bifidobacteria than those of encapsulated ones. The growth of free cells *Bif. bifidum* decreased significantly by adding nisin and this effect was more pronounced than those of *Bif. infantis*, which was tolerant to 100 IU/ml nisin. Encapsulated bifidobacteria were more resistant to nisin and their counts were higher than those of free cells bifidobacteria at any time of incubation period and any nisin concentration.

Paul de Vos *et al.* (2010) encapsulation promotes not only viable but more importantly also protects the functionality, and may facilitate targeted release in specific parts of the gut. Different encapsulation approaches the quality for the protection of bioactive food components. When a targeted release is desired in combination with adequate protection in the product. It is essential to realize which processes in the human gut can be applied to facilitate targeted release. The majority of systems that have been used in the past were either sensitive to mechanical stress, pH or transport time variations in the gut. More recent systems take advantage of the different enzyme concentrations associated with variations in the composition of the microbiota in different parts of the gut. The latter system should receive more attention in the food industry as it allows for precise release of bioactive food components. The principle of target release by the enzymatic activity of the microbiota is compatible with many carbohydrates that are generally regarded as safe (GRAS).

Kebary et al 2014 prepared symbiotic kishk from buffalo's skim milk and crushed barley (2:1) with adding free cells and immobilized (single and double layer) alginate beads from *Bif.bifidum* ATCC 15696 and *Bif. Infantis* ATCC 15697. Encapsulation of bifidobacteria improved their survival during storage of symbiotic kishk. Viability of Bif.bifidum was not significantly ($p > 0.05$) different from Bif. Infantis . Adding of free and immobilized bifidobacteria inhibited the growth of moulds , yeasts and spore forming bacteria.

Synbiotic kishk containing double layer alginate beads of bifidobacteria attained the highest organoleptic scores , while synbiotic kishk containing free cells gained the lowest score.

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