Chapter 10

Highlights in Probiotic Research

Gülden Başyiğit Kılıç

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/50004

1. Introduction

For centuries, lactic acid bacteria (LAB) have been used for the preservation of food for human consumption. LAB are a large group of fermentative, anaerobe facultative, aerotolerant microorganisms which are usually present in the gut of humans and other animals, raw vegetables, meat and meat products, and cereals (Carr et al., 2002). In animals, their numbers may vary with the species, the age of the host, or the location within the gut (De Vries et al., 2006). In the food industry, lactic acid bacterial strains are widely employed either as starter cultures or as non-starter lactic acid bacteria. Furthermore, owing to their probiotic properties, several LAB strains are used as adjunctive cultures in foods and feed (Sanders, 2000; Leroy & de Vuyst, 2004).

The term "probiotic" originated from the Greek word "probios" meaning "for life" (as opposed to "antibiotic," which means "against life") (Longdet et al., 2011). Probiotics are microbial food supplements which, when administered in adequate amounts, confer health benefits to consumers by maintaining or improving their intestinal microbial flora (Salminen et al., 1998; Reid et al., 2003). The US Food and Drug Administration uses other terms for live microbes for regulatory purposes (Sanders, 2008); live microbes used in animal feeds are called "direct-fed microbials" (FDA, 1995), and, when intended for use as human drugs, they are classified as "live biotherapeutics" (Vaillancourt, 2006). Probiotics are mainly members of the genera *Lactobacillus* and *Bifidobacterium* and are normal residents of the complex ecosystem of the gastrointestinal tract (GIT) of humans.

The research of novel probiotic strains is important in order to satisfy the increasing request of the market and to obtain functional products in which the probiotic cultures are more active and with better probiotic characteristics than those already present on the market (Verdenelli, et al., 2009). According to a recent market research report 'Probiotics Market (2009-2014)', the global probiotics market generated US $15.9 billion in 2008 and is expected to be worth US $ 32.6 billion by 2014 with a compound annual growth rate of 12.6 percent from 2009 to 2014 (FB 1046, 2009).

Several aspects, including general, functional and technological characteristics, have to be taken into consideration while selecting probiotic strains (Sanders & Huis in't Veld 1999; Šušković et al., 2001). This chapter includes selection criteria of bacteria as probiotics, technological usage of probiotics, new approaches for enhancing the performance of probiotics, and health effects of probiotic bacteria.

2. Selection of probiotic bacteria

Probiotics are living, health-promoting microorganisms that are incorporated into various kinds of foods. Although there has been a growing interest in using LAB isolated both from naturally fermented products and humans for health benefits (Lim & Im, 2009), the strains should preferably be of human origin and possess a Generally-Recognized-As-Safe status (Rönkä et al., 2003).

In order to exhibit their beneficial effects, probiotic bacteria need to survive during the food-manufacturing process and in human ecosystem conditions; therefore it is important to investigate bacterial behavior under conditions which mimic the GIT (Zago et al., 2011; Lo Curto et al., 2011). Stresses to microorganisms begin in the mouth, with the lysozyme-containing saliva; continue in the stomach, which has a pH between 1.5 and 3.0; and go on to the upper intestine, which contains bile (Corzo & Gilliland, 1999). Acid and bile tolerances are two fundamental properties that indicate the ability of a probiotic microorganism to survive the passage through the GIT, resisting the acidic conditions in the stomach and the bile acids at the beginning of the small intestine (Prasad et al., 1998; Park et al., 2002). To evaluate the probiotic survival in the GIT, several *in vitro* static models of digestion have been developed (Kitazawa et al., 1991; Charteris et al., 1998). One of them is the gastric–small intestinal system TIM-1 (Minekus et al., 1995), which consists of four serial compartments simulating the stomach and the three segments of the small intestine: the duodenum, jejunum, and ileum. Another one, the TIM-2 model, is a more sophisticated *in vitro* model of fermentation in the proximal large intestine. It consists of a series of linked glass vessels containing flexible walls which allow simulation of peristalsis (De Preter et al., 2011). The simulator of the human intestinal microbial ecosystem (SHIME) was developed to simulate the entire human gastrointestinal system (Molly et al., 1993). SHIME consists of a series of five temperature- and pH-controlled vessels that simulate the stomach; small intestine; and ascending, transverse and descending colon, respectively. The SHIME harbors a microbial community resembling that from the human colon both in fermentation activity and in composition (De Preter et al., 2011). Yet another model of the digestive system has been developed by such as TNO to mimic human physiological conditions in the stomach and small intestine (Blanquet et al., 2001). The major limitations of those systems is that digestion products are not removed during the incubation, and they may have a potential inhibitory effect on enzyme activities and on probiotic survival (Pitino et al., 2010). Furthermore, such systems ignore key GIT physical processes, including the temporal nature of gastric and duodenal processing, structure of food, pattern of mixing, particle size reduction and shear, which all affect the digestion rate (Shah 2000; Sumeri et al., 2008).

Effects of probiotics are strain specific. Strain identity is important in order to link a strain with a specific health effect, as well as to enable accurate surveillance and epidemiological studies (Ganguly et al., 2011). It is very important to be able to identify specifically and unambiguously the particular probiotic LAB strains from clinical fecal and intestinal biopsy specimens and from food samples (Tilsala-Timisjärvi & Tapanialtossava, 1998). Identification of bacterial species and strains from commercialized probiotics has been conducted mostly using molecular methods (Holzapfel et al., 2001; Schillinger et al., 2003; Huys et al., 2006; Sheu et al., 2009).

Verdenelli et al. (2009) investigated the probiotic potential of 11 *Lactobacillus* strains isolated from the faeces of elderly Italians. For this purpose, the researchers identified the *Lactobacillus* strains and examined them for resistance to gastric acidity and bile toxicity, adhesion to HT-29 cells, antimicrobial activities, antibiotic susceptibility and plasmid profile. They also examined the survival of the strains as they moved through the human intestine in a 3-month human feeding trial. According to the results, *L. rhamnosus* IMC 501 and *L. paracasei* IMC 502 present favourable strain-specific properties for their utilisation as probiotics in functional foods. Both *in vitro* and *in vivo* studies confirm the high adhesion ability of *L. rhamnosus* IMC 501 and *L. paracasei* IMC 502, used in combination, indicating that the two bacterial strains could be used as health-promoting bacteria.

Başyiğit Kılıç & Karahan (2010) isolated one hundred seven strains of human originated LAB identified by 16S rRNA analysis and examined them for resistance to acidic pH, bile salts and antibiotic susceptibility. They found that *L. plantarum* (AA1–2, AA17–73, AC18–88, AK4–11, and AK7–28), *L. fermentum* (AB5–18, BB16–75, and AK4–180), *Enterococcus faecium* (AB20–98 and BK11–50) and *E. durans* (AK4–14 and BK9–40) are potentially good probiotic candidates for use as health-promoting bacteria. In another study, the *L. plantarum* strains were examined for resistance to gastric acidity in simulated gastric juice at pH 2.0, 2.5, 3.0 and 3.5; 0.4% phenol; production of H_2O_2; adhesion to Caco-2 cell line; and antimicrobial activities. The researchers determined that the artificial gastric juice, even at pH 2.0, did not significantly change the viability of the cultures, and all *L. plantarum* strains showed good resistance to 0.4% phenol. They also reported antimicrobial activity and good adhesion of *L. plantarum* strains to Caco-2 cells. The researchers concluded that all of the strains showed probiotic properties, but *L. plantarum* AB6-25, AB7-35, AA13-59, AB16-65, BC18-81 and AK4-11 were the best potential probiotic strains for human use, given their ability to survive in gastric conditions, strong resistance to phenol, and the ability to adhere to the Caco-2 cell line (Başyiğit Kılıç et al., 2011a).

Lo Curto et al. (2011) investigated the survival of three commercial probiotic strains (*L. casei* subsp. *shirota*, *L. casei* subsp. *immunitas*, *L. acidophilus* subsp. *johnsonii*) in the human upper GIT. They used a dynamic gastric model (DGM) of digestion followed by incubation under duodenal conditions. The DGM is a computer-controlled gastric model which incorporates the chemical, biochemical, physical environment and processes of the human stomach; the model is based on kinetic data derived from the Echo planar-MRI and data on the rates of GI digestion obtained from human studies (Marciani et al., 2001; 2003; 2005; 2006). The researchers used water and milk as food matrices, and survival was evaluated in both

logarithmic and stationary phases. The researchers found that the % of recovery in the logarithmic phase ranged from 1.0% to 43.8% in water for all tested strains, and from 80.5% to 197% in milk. They observed higher survival rates in the stationary phase for all strains. *L. acidophilus* subsp. *johnsonii* showed the highest survival rate in both water (93.9%) and milk (202.4%).

The safety of probiotic bacteria must be carefully assessed, with particular attention to transferable antibiotic resistance (Mathur & Singh, 2005). In the last decade, increasing concern has arisen about the safe use of LAB cultures for food and feed applications, in light of the latest knowledge about their possible role as an antibiotic-resistant gene reservoir. Particular concern is due to evidence of widespread occurrence in this bacterial group of conjugative plasmids and transposons (Clementi & Aquilanti, 2011). It is known that lactobacilli have a high natural resistance to bacitracin, cefoxitin, ciprofloxacin, fusidic acid, kanamycin, gentamicin, metronidazole, nitrofurantoin, norfloxacin, streptomycin, sulphadiazine, teicoplanin, trimethoprim/sulphamethoxazole, and vancomycin (Danielsen & Wind, 2003).

One of the primary benefits associated with probiotic bacterial cultures is that they can exclude pathogenic bacteria from the small and large intestine (Kos et al., 2008). Another benefit is that in food products, antimicrobial activity of probiotic bacteria may contribute to an improvement in the quality of fermented foods. This may result from control of spoilage and pathogenic bacteria, extension of shelf life, and improvement of sensory quality (Wei et al., 2006; Siripatrawan & Harte, 2007). Kos et al. (2008) used overnight cultures and cell-free supernatants of the three probiotic strains *L. acidophilus* M92, *L. plantarum* L4, and *E. faecium* L3 for determining the antagonistic effect against *Listeria monocytogenes*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Acinetobacter calcoaceticus*. The researchers determined that probiotic strains *L. acidophilus* M92, *L. plantarum* L4, and *E. faecium* L3 demonstrated anti-*Salmonella* activity. *L. acidophilus* M92 was also shown to have antilisterial activity, as demonstrated by *in vitro* competition test.

Production of antimicrobial compounds, which may take part in the inhibition of intestinal pathogens, is another criterion for classifying a potentially probiotic bacteria (Hutt et al., 2006). The inhibition of pathogenic microorganisms by selected probiotic strains may occur via a) production of antibiotic-like substances, b) bacteriocins and bacteriocin-like inhibitory substances such as acidophilin and reuterin, c) lowering of pH by producing organic acids such as acetic, lactic and phenyllactic acid, d) production of hydrogen peroxide and short chain fatty acids, e) decreasing the redox potential, and f) consumption of available nutrients (Holzapfel et al., 1995; Ouwehand, 1998; Tharmaraj & Shah, 2009).

The ability of LAB to adhere to epithelial cells and mucosal surfaces is thought to be an important property of many bacterial strains used as probiotics (FAO/WHO, 2001). Cell adhesion is a complex process involving contact between the bacterial cell membrane and interacting surfaces. Difficulties experienced in studying bacterial adhesion *in vivo*, especially in humans, have stimulated interest in the development of *in vitro* models for preliminary screening of potentially adherent strains (Duary et al., 2011). Attachment and

colonization of the gut epithelium prolongs the time for microorganisms to influence the immune system and microbiota of the host (Forestier et al., 2001). HT-29 and Caco 2 cells, the two colonic adenocarcinomas, are derived from human intestinal epithelium. Because they have structural and functional features of normal human enterocytes, they have been extensively used as *in vitro* models in the study of human enterocytic function (Moussavi & Adams, 2009).

The ability of probiotic bacteria to adhere to Caco-2 cells can be determined by plate counting or real time PCR (Matijasic et al., 2003; Candela et al., 2005). Nawaz et al. (2011) used both of these methods and did not find a statistically significant difference. Gaudana et al. (2010) investigated the ability of four different isolates (*L. plantarum* CS23, *L. rhamnosus* CS25, *L. delbrueckii* M and *L. fermentum* ASt1) and two standard strains (*L. plantarum* ATCC 8014 and *L. rhamnosus* GG) to stimulate three types of cells (Caco-2 cells, human peripheral blood mononuclear cells [PBMC] and THP-1 cells). The researchers reported that child faecal isolate CS23 showed high binding ability, high tolerance to acidic pH and bile salts, and significant immunomodulation; therefore they concluded that CS23 can be a good potential probiotic candidate. Duary et al. (2011) determined the colonization potentials of five human faecal *L. plantarum* isolates to the Caco-2 cells. Based on direct adhesion to epithelial cells, *L. plantarum* Lp91 was the most adhesive strain to the Caco-2 cell lines, with adhesion values of approximately 10.2%. They also mentioned that the percentage of adhesion to Caco-2 and HT-29 cell lines was higher among the strains isolated from the human faecal samples and buffalo milk than that which had been isolated from cheese.

3. Technological usage of probiotics

The use of starter cultures in the production of fermented food is necessary for guaranteeing safety and standardizing properties. LAB functions primarily to drop the pH of the batter; lower pH a) promotes product safety by inactivating pathogens, b) creates the biochemical conditions to attain the final sensory properties through modification of the raw materials, and c) improves the product stability and shelf life by inhibiting undesirable changes brought about by spoilage microorganisms or abiotic reactions (Ammor & Mayo, 2007).

Functional starter cultures are defined as microbes that possess at least one inherently functional property aimed at improving the quality of the end product (De Vuyst, 2000). The use of probiotics in food has reinforced the acclaimed healthy properties and given rise to an increased consumption of these products in Europe and the USA (Kristo et al., 2003). Probiotics have been evaluated as functional starter cultures in various types of fermented food products such as yoghurt, cheese, dry sausage, salami, and sourdough. They have also been studied in therapeutic preparations to assess their positive effects on physico-chemical properties of foods and their impact on the nutritional quality and functional performance of the raw material (Knorr, 1998; Rodgers, 2008).

Fermented dairy products are widely-accepted, healthy food products and valued components of diets. The incorporation of probiotic bacteria as adjuncts in various fermented milk products is currently an important topic with industrial and commercial

consequences. A number of dairy products containing probiotic bacteria are currently on the market. Fermented milk and cheeses have been described as the most suitable carriers, because they enhance the transit tolerance of bacteria (Saarela et al., 2000; Lourens-Hattingh & Viljoen, 2001). Some strains of *Lactobacillus* and *Bifidobacterium* have been shown to tolerate acidic stress when ingested with milk products (Mater et al., 2005). Lactobacilli (e.g. *L. acidophilus*, *L. casei* subsp. *casei*, *L. gasseri*, *L. paracasei*, *L. reuteri* and *L. rhamnosus*) and bifidobacteria (e.g. *Bifidobacterium adolescentis*, *B. bifidum*, *B. breve*, *B. infantis* and *B. longum*) constitute a significant proportion of probiotic lactic acid bacterium cultures used in the dairy industry (Wood & Holzapfel, 1995; Klein et al., 1998). It is also important to determine the technological features of the strains because they could greatly affect food quality. Further, probiotic starter cultures need to be tested for large-scale production feasibility in regard to acidification, proteolysis, and aroma formation. They must accomplish this without losing viability and functionality or creating unpleasant flavor or texture (De Vuyst, 2000; Lacroix & Yildirim, 2007).

Although the number of cells required to produce therapeutic benefits is not known and might vary as a function of the strain and the health effect desired, in general a minimum level of more than 10^6 viable probiotic bacteria per mililitre or gram of food product is accepted (Ouwehand & Salminen, 1998). The study of new probiotic strains for their technological relevance and use in food products is important for trade and industry. The search for strains which show resistance to biological barriers of the human GIT, and which possess physiological characteristics compatible with probiotic properties among LAB isolated from food, may eventually lead to the discovery of new probiotic strains for functional food products (Bude-Ugarte et al., 2006).

Studies of fermented food products as a source of new isolates are rapidly accumulating. For example, a mixture of human-derived probiotic strains was tested in the manufacture of ice cream; some of the ice cream was sweetened with sucrose and some was sweetened with aspartame (Başyiğit et al. 2006). The results showed that neither frozen conditions during the storage period nor the type of sweeteners used had any undesired effect on the survival of the probiotic cultures. Georgieva et al. (2009) studied technologically relevant properties of eight candidate probiotic *L. plantarum* strains isolated from cheeses. Researchers tested their capacity to survive over extended shelf-times at refrigerated temperatures and their growth viability in the presence of preservatives widely used in food processing. The researchers determined that the cultures' acidifying and coagulating abilities and enzyme activity make them appropriate for diverse food applications, but especially for dairy products. In another study, the survival of the probiotic strains *L. fermentum* (AB5-18 and AK4-120) and *L. plantarum* (AB16-65 and AC18-82), all derived from human faeces, was investigated in Turkish Beyaz cheese production (Başyiğit Kılıç et al., 2009). The researchers determined the viability of probiotic bacteria in Turkish Beyaz cheese during 4 months of ripening and the bacteria's effect on chemical properties of the cheese. The results of the study revealed that the test probiotic culture mix was successful for cheese production and did not adversely affect cheese quality during ripening.

Essid et al. (2009) characterized 17 strains of *L. plantarum* isolated from traditional Tunisian salted meat products to select the most suitable for use as starters for fermenting meat.

Critical characteristics included acidification and enzymatic activities responsible for final sensory properties; also important were safety characteristics, including antagonistic activity against spoilage strains and antibiotic resistance. The researchers determined that all strains of *L. plantarum* had good acidifying activity; however they showed some differences in antimicrobial, proteolytic and enzymatic activities. Başyiğit Kılıç et al. (2011b) investigated the technological properties of twenty *L. plantarum* strains to evaluate their potential usage as starter cultures in the dairy industry. During two months in cold storage, there were no significant changes in the number of bacteria or the pH of the skim milk inoculated with *L. plantarum* strains. The authors suggested that *L. plantarum* AC3-10 and AB6-25 can be used in industrial yogurt manufacture, based on their technological properties such as proteolitic activity, acidifying ability, and production of flavour compounds.

Floros et al. (2012) tested 19 facultatively heterofermentative lactobacilli from Feta, Kasseri, and Graviera cheeses for potential probiotic strains. Data from this study revealed that isolates B1, G16, G22, E22, E35, and H30 from Feta; PB2.2 from Kasseri; and 631 from Graviera have promising probiotic properties *in vitro*. β-galactosidase, low proteolytic and coagulation activities, and antibacterial activities make them promising candidates as adjunct cultures for the food industry. In another study, yoghurt was produced using a mixture of potential probiotic *L. plantarum* AB6-25, AC18-82, AK4-11 and a commercial starter culture. The yoghurt was divided into four experimental batches to which were added 0.25%, 0.5%, 1%, and 1.5% β-glucan. The survivability of these potential probiotic strains and the physico-chemical properties of the yoghurts were analyzed during a 21-day storage period. The highest *L. plantarum* count was found in the yoghurt containing 0.25% β-glucan. The study found the best physico-chemical properties to be in the 0.25% and 0.5% β-glucan containing yoghurts. Therefore, the researchers suggested using 0.25% and 0.5% β-glucan in yoghurts produced using these potential probiotic bacteria and commercial starter culture (Başyiğit Kılıç, 2012).

Wang et al. (2010) identified and established the functional and technological characteristics of potential probiotic *Lactobacillus* strains isolated from two sources: the faeces of breast-fed infants and traditional Taiwanese pickled cabbages. The authors selected the strains *L. reuteri* F03, *L. paracasei* F08 and *L. plantarum* C06 for producing probiotic fermented milk, due to their acid and bile tolerance and ability to adhere to Caco-2 cells. The milks were fermented with these 3 strains separately, and rats were fed a daily dose of 10^8 CFU/day for 14 days. After the consumption of the *Lactobacillus*-fermented milk, the rats showed increased faecal lactobacilli counts, while the counts of coliform and *C. perfringens* were significantly decreased. On the other hand, Başyiğit Kılıç et al. (2010) investigated the effects of a probiotic culture mix (*L. fermentum*, *L. plantarum* and *E. faecium*) and alfa-tocopherol administration on the microbial flora in rat GIT and faeces during a 14-day feeding period. The results indicated that the probiotic culture and alfa-tocopherol administration had no significant effects on the microbial flora of the rat intestinal tract during the 14 days of intake. Minelli et al. (2004) reported that in rats administered milk fermented with L. casei, the faecal *E. coli* counts remained stable, but *Clostridia* counts decreased significantly. Yang et al. (2005) also reported decreased faecal coliform counts as one of advantages of *Lactobacillus* and *Bifidobacterium* proliferation in the rat

gut. Such potentially probiotic bacteria colonizing the intestinal mucosa provide a barrier effect against pathogens by using a variety of mechanisms, such as occupation of niches, competition for nutrients, and production of antimicrobials (Ouwehand et al., 2001).

3.1. Methods to increase survival and viability of probiotics

Researchers have long been encouraged to find new, efficient methods of improving the viability of probiotics in food products (especially fermented types), since viability can be affected by the acidic-bile conditions of the gastrointestinal tract (Mortazavian et al., 2007). The latest developments focus on fermentation technologies for producing probiotic bacteria; new approaches for enhancing the performance of these fastidious organisms during fermentation, downstream processing, and utilization in commercial products; and improving functionality in the gut. Processes to optimize survival and functionality in the gut include sublethal stress applications during cell production and new fermentation technologies, such as immobilized cell biofilm-type fermentations, are promising in this respect (Lacroix & Yildirim, 2007).

3.1.1. Immobilized cell biofilm

Cell immobilization in fermentations is an attractive and rapidly expanding research area because of its technical and economic advantages, compared to a free cell system (Stewart & Russell, 1986). The immobilization method is cheap, simple and easy (Kourkoutas et al., 2006). The technology of cell immobilization allows an increase in cell stability and a decrease of the lethal effect on the microbial cells, providing protection from the conditions of the environment (Champagne et al., 1994; Grosso & Fávaro-Trindade, 2004). Thus immobilization techniques could provide protection to acid-sensitive LAB and increase their survival rate during the shelf life of the yoghurt and during their passage through the gastrointestinal tract (Cui et al., 2000; Fávaro-Trindade & Grosso, 2002). Kushal et al. (2006) determined that the process of co-immobilization of probiotic strains of *L. acidophilus* NCDC 13 and *B. bifidum* NCDC 255 resulted in better protection of the viability of the cultures during transit through the gastrointestinal tract. In another study conducted by Kourkoutas et al. (2006), *L. casei* cells were immobilized on apple pieces and the immobilized biocatalysts were used separately as adjuncts in producing probiotic fermented milk. The results showed that the immobilized biocatalyst was able to ferment after storage for 15, 98 and 129 days at 4 °C, while no infection was reported during storage periods. Denkova et al. (2007) determined that the immobilization of the cells of *L. acidophilus* A., *L. helveticus* H., *L. casei* subsp. *casei* C. and *L. plantarum* 226-15 in chitosan resulted in preparations with high concentration of viable cells. The immobilized LAB in the chitosan gel beads was resistant to the model conditions of digestion: low and neutral values of pH, enzyme presence, and high concentrations of bile salts.

3.1.2. Encapsulation

Encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall as a core of encapsulated material (Kailasapathy,

2002). Encapsulation occurs naturally when bacterial cells grow and produce exo-polysaccharides. The microbial cells are entrapped within their own secretions that act as a protective structure or a capsule, reducing the permeability of material through the capsule, and making it less exposed to adverse environmental factors. Many LAB synthesise exo-polysaccharides, but they produce insufficient amounts to encapsulate themselves fully (Shah, 2002). Encapsulating probiotics in hydrocolloid beads has been investigated as a means of improving their viability and survival in food products and in the intestinal tract (Picot & Lacroix, 2004). Other benefits of encapsulation include reduction of cell injury, protection of probiotics from bacteriophages (Steenson et al., 1987), increased survival during freeze-drying and freezing (Kim & Yoon, 1995), and greater stability during storage (Kebary et al., 1998). Several methods of encapsulation have been used on probiotics in fermented milk products and biomass production: emulsion or two phase systems, the extrusion or droplet method, and spray drying and spray coating (Mortazavian et al., 2007). The common materials used for microencapsulation of probiotics are alginate and its derivatives, starch, mixtures of xanthan-gelan, carrageenan and its mixtures, gelatin, cellulose acetate phethalate, chitosan, and miscellaneous compounds such as whey proteins, soybean oil, gums, wax, and calcium chloride (Rao et al., 1989, Picot & Lacroix, 2004, Chandramouli et al., 2004).

Hou et al. (2003) demonstrated that encapsulation of *L. delbrueckii* spp. *bulgaricus* increased their bile tolerance, and viability was elevated by approximately four log units after encapsulation within artificial sesame oil emulsions. Encapsulation in spray dried whey protein microcapsules improved survival of *B. breve* R070 but not that of *B. longum* R023 during refrigerated storage in yoghurt (Picot & Lacroix, 2004). Ding & Shah (2007) stated that encapsulation improved the survival of probiotic bacteria including *L. rhamnosus*, *B. longum*, *L. salivarius*, *L. plantarum*, *L. acidophilus*, *L. paracasei*, *B. lactis* type Bl-O4, and *B. lactis* type Bi-07 when exposed to acidic conditions, bile salts, and mild heat treatment. Capela et al. (2006) found improved viability of probiotic organisms encapsulated in 3% v/w sodium alginate in freeze-dried yogurt after 6 months of storage at 4 and 21°C. Ozer et al. (2009) studied the viability of encapsulated bacteria in white-brined cheese; the researchers used *B. bifidum* BB-12 and *L. acidophilus* LA-5 that had been encapsulated in Na-alginate by either an extrusion or an emulsion technique. Both encapsulation techniques were found to be effective in keeping the numbers of probiotic bacteria higher than the level of the therapeutic minimum. While the counts of non-encapsulated probiotic bacteria decreased approximately by 3 logs, the decrease was more limited in the cheeses containing microencapsulated cells (approximately 1 log). Khater et al. (2010) tested the ability of twelve non-encapsulated and encapsulated lactic acid and bifidobacteria strains to assimilate cholesterol and to survive at a low pH and fairly high bile concentrations. The results obtained declared that encapsulation effectively protected the microorganisms from the hostile environment in the GIT, thus potentially preventing cell loss. The assimilative reductions of cholesterol by non-encapsulated and encapsulated strains were clearly different, varying from 32.6% to 89.3% and 27.9% to 85.1% respectively. Kim et al. (2008) stated that encapsulation reduces the ability of LAB to assimilate cholesterol.

4. Effects of probiotics on human health

Probiotics have the potential for contributing greatly to human and animal health via a wide range of applications. Historically, probiotics have been used in food for humans and animals without any side effects, while providing for the balance of intestinal flora (Holzapfel & Wood, 1998). The health-promoting effects of probiotics have been widely explored and include stabilization of the indigenous microbial population, boosting of the immune system, inhibition of the growth of pathogenic organisms, prevention of diarrhea from various causes, alleviation of lactose intolerance, increased nutritional value of foods, reduction of serum cholesterol levels, antimutagenicity and anticarcinogenicity, reduction of the risk of inflammatory bowel conditions, improvement of digestion of proteins and fats, synthesis of vitamins, and detoxification and protection from toxins (Klaenhammer, 1998; Perdigon et al., 2002; Gaudana et al., 2010).

Anderson & Gilliland (1999) conducted two controlled clinical studies to test effects of yoghurt on heart-related health. They reported an average reduction of serum cholesterol by 2.9% with regular consumption of yoghurt containing *L. acidophilus* and a 6-10% decrease in cardiac complications due to hypercholesterolemia. A study by Ouwehand et al. (2002) found that a multi-strain probiotic mixture composed of *L. reuteri, L. rhamnosus* and *Propionibacterium freudenreichii* proved effective in both increasing the number of bowel movements and decreasing mucin secretion in elderly subjects. The probiotic mixture was more effective than *L. reuteri* alone, although unfortunately it is difficult to draw conclusions about mixtures versus individual probiotics, since only one component of the mixture was tested and its dose was over 10 times lower than the total bacterial dose in the mixture. Agarwal & Bhasin (2002) have reported that the strain *L. casei* DN-114001 reduced diarrhoeal morbidity by 40% in children.

Isolauri *et al.* (1999) found significant improvement when a supplement of either *L. rhamnosus* or *B. lactis was given* to children from 4 to 6 years of age who had atopic eczema. Another study involving pregnant women and newborns suggested that consumption of probiotic *L. rhamnosus* GG reduced the rate of newborns having atopic dermatitis (Kalliomaki et al., 2001). In an Australian study, 178 newborns of women with allergies who received either *L. acidophilus* LAVRI-A1 or placebo daily for the first 6 months of life showed no difference in atopic dermatitiS. However, at 12 months, the rate of sensitization was significantly higher in the probiotic group. These results suggested that the probiotic treatment had increased the risk of subsequent cow's milk sensitization (Taylor et al., 2007).

Can (2003) used an experimental animal model to study the effects of a probiotic mixture and *L.* GG on immune responses in allergy. The OVA specific IgE levels of the study groups which were administred probiotics and reference strain were found lower than the skim milk fed groups. A double-blind, randomized, placebo controlled trial study was conducted by Abrahamsson et al. (2007) on 188 subjects with allergic disease, in which the mothers received *L. reuteri* ATCC 55730 daily from gestational week 36 until delivery, and their babies continued with the probiotic until 12 months. Probiotic supplemented babies showed less IgE-associated eczema during the second year. Several probiotic effects are mediated

through immuneregulation, particularly through establishing and maintaining a balance between pro-and anti-inflammatory cytokines (Isolauri et al., 2001). TNF-a and IL-6 are pro-inflammatory cytokines, which are produced by the host in response to bacterial colonisation or invasion and hence are central to the host defense mechanism against pathogens (Solis-Pereyra et al., 1997). Though lipopolysaccharide of Gram-negative bacteria is known to stimulate their production, Miettinen et al. (1996) have reported an increase in IL-6 and TNF-a production in human PBMC exposed to lactobacilli and thereby suggested the use of probiotics as vaccine vectors and for the purpose of stimulating non-specific immunity. Kailasapathy & Chin (2000) proved that the synthesis of cytokines is increased as the probiotics adhere to the intestinal epithelium.

Ziarno et al. (2007) studied cholesterol assimilation by commercial starter cultures, reporting *L. acidophilus* monocultures to assimilate cholesterol by 49-55%. In another study involving hypercholesterolemic mice, the probiotic potential of *L. plantarum* PHO4 was established by Nguygen et al. (2007). The mice were fed with 10^7 CFU per day over two weeks. These mice had 7 to 10% lesser serum cholesterol and triglycerides than the control mice deprived of the probiotic feed.

Many probiotic species have been identified to be effective in children suffering from rotaviral diarrhea (Saavedra, 2000). Longdet et al. (2011) investigated the probiotic efficacy of *L. casei* isolated from human breast milk in the prevention of shigellosis in albino rats infected with clinical strains of *Shigella dysenteriae*. The results showed that the experimental rats infected with *S. dysenteriae* but not treated suffered from shigellosis, while the test groups infected and treated with the *L. casei* showed no sign of the disease as well as no clinical effect on the liver.

Senol et al. (2011a) investigated the protective effect of a probiotic mixture of 13 different bacteria and a-tocopherol on 98% ethanol-induced gastric mucosal injury. Levels of gastric mucosal pro-and anti-inflammatory cytokines, malondialdehyde, and secretory immunglobulin A were measured. Results showed that probiotic pretreatment significantly suppressed the ethanol-induced increase of gastric mucosal interleukin-4 levels. Pretreatment with either probiotic or a–tocopherol inhibited the ethanol-induced increase of mucosal malondialdehyde concentration. Probiotic pretreatment enhanced the gastric mucosal secretory immunoglobulin A concentration. The researchers indicated that the probitic mixture and a-tocopherol reduced ethanol-induced gastric mucosal lipid peroxidation, suggesting that these probiotics may be beneficial for helping heal gastric lesions induced by lower ethanol concentration. In another study, the role of a probiotic mixture, including 13 different bacteria, in the prevention of aspirin-induced gastric mucosal injury was investigated. Pretreatment with the probiotic mixture reduced aspirin-induced gastric damage and exerted a tendency toward downregulation of proinflammatory cytokines elicited by aspirin. Researchers also found that the probiotic mixture increased sIgA production approximately 7.5-fold in the stomach, and significantly reduced the malondialdehyde increase in the gastric mucosa elicited by aspirin. Additionally, pretreatment with the probiotic mixture alleviated aspirin-induced reduction

of mast cell count in the gastric mucosa. Probiotic mixture pretreatment attenuates the aspirin-induced gastric lesions by reducing the lipid peroxidation, enhancing mucosal sIgA production, and stabilizing mucosal mast cell degranulation into the gastric mucosa (Senol et al., 2011b).

5. Final remarks

Significant data have been accumulated on probiotics and their beneficial health effects. Furthermore, more insights and key findings on the impact of processing and storage on probiotic viability and stability have been gained. A variety of microorganisms, typically food grade LAB, have been evaluated for their probiotic potential and are applied as adjunct cultures in various types of food products or in therapeutic preparations. In addition, further studies are needed to determine if preventive probiotic strategies are safe with regard to development of probiotic infections. Cooperation amongst food technologists, medical and nutrition scientists, and anticipation of future consumer demands are crucial for future success in probiotics.

Author details

Gülden Başyiğit Kiliç
Mehmet Akif Ersoy University, Department of Food Engineering, Faculty of Engineering-Architecture, Burdur, Turkey

6. References

Abrahamsson, T.R., Jakobsson, T., Bottcher, M.F., Fredrikson, M., Jenmalm, M.C. & Bjorksten, B. (2007). Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial, *Journal of Allergy and Clinical Immunology* 119: 1174 1180.

Agarwal, K.N. & Bhasin, S.K. (2002). Feasibility studies to control acute diarrhoea in children by feeding fermented milk preparations, Actimel and Indian Dahi, *European Journal of Clinical Nutrition* 56: 56-59.

Ammor, M.S. & Mayo, B. (2007). Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update, *Meat Science* 76: 138–146.

Anderson, J.W. & Gilliland, S.E. (1999). Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolemic humans, *Journal of the American College of Nutrition* 18(1): 43-50.

Başyiğit, G., Kuleaşan, H. & Karahan, A.G. (2006). Viability of human derived probiotic lactobacilli in ice-cream produced with sucrose and aspartame, *Journal of Industrial Microbiology and Biotechnology* 33: 796-800.

Başyiğit Kılıç, G., Kuleaşan, H., Eralp, I. & Karahan, A.G. (2009). Manufacture of Turkish Beyaz cheese added with probiotic strains, *LWT - Food Science and Technology* 42: 1003-1008.

Başyiğit Kılıç, G., Kılıç, B., Kuleaşan, H. & Karahan, A.G. (2010). Effect of Probiotics and α-tocopherol applications on microbial flora of rat gastrointestinal tract, *Journal of Animal and Veterinary Advances* 9(14): 1972-1977.

Başyiğit Kılıç, G. & Karahan, A.G. (2010). Identification of lactic acid bacteria isolated from the fecal samples of healthy humans and patients with dyspepsia and determination of their pH, bile and antibiotic tolerance properties, *Journal of Molecular Microbiology and Biotechnology* 18: 220-229.

Başyiğit Kılıç, G., Kuleaşan, H. & Çakmak, V.F. (2011a). Determination of probiotic properties of *L. plantarum* strains isolated from the human fecal samples, *Proceedings of Novel Approches in Food Industry, International Food Congress*, 26-29 May 2011, Çeşme-İzmir, Turkey, pp. 56.

Başyiğit Kılıç, G., Kuleaşan, H., Akpınar, D., Çakmak, V.F. (2011b). Characterization of technological properties of human originated probiotic *L. plantarum* strains, *Proceedings of International Scientific Conference on Probiotics and Prebiotics-IPC*, Slovakia, pp. 14.

Başyiğit Kılıç, G. (2012) (unpublished data). Determination of probiotic and technological properties of some *Lactobacillus plantarum* strains, TUBITAK 109 O 623, Ongoing Project.

Blanquet, S., Marol-Bonnin, S., Beyssac, E., Pompon, D., Renaud, M. & Alric, M. (2001). The "biodrug" concept: an innovative approach to therapy, *Trends in Biotechnology* 19(10): 393–400.

Bude-Ugarte, M., Guglielmotti, D., Giraffa, G., Reinheimer, J.A. & Hynes, E. (2006). Non starter lactobacilli isolated from soft and semi hard Argentinean cheeses: genetic characterization and resistance to biological barriers, *Journal of Food Protection* 69: 2983-2991.

Can, R. (2003). The effects of probiotics on allergy, *PhD Thesis*, Süleyman Demirel University, Medicinal Faculty, Departmant of Microbiology, Isparta, Turkey, p. 75 (unpublished).

Candela, M., Seibold, G., Vitali, B., Lachenmaier, S., Eikmanns, B.J. & Brigidi, P. (2005) Real-time PCR quantification of bacterial adhesion to Caco-2 cells: competition between bifidobacteria and enteropathogens, *Research in Microbiology* 156(8): 887-895.

Capela, P., Hay, T.K.C. & Shah, N.P. (2006). Effect of cryoproyectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt, *Food Reseach International* 39: 203-211.

Carr, F.J., Chill, D. & Maida, N. (2002). The lactic acid bacteria: a literature survey, *Critical Reviews on Microbiology* 28: 281-370.

Champagne, C.P., Lacroix C. & Sodini-Gallot, I. (1994). Immobilized cell technology for the dairy industry, *Critical Reviews in Biotechnology* 14: 109-134.

Chandramouli, V., Kalasapathy, K., Peiris, P. & Jones, M. (2004). An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions, *Journal of Microbiological Methods* 56: 27-35.

Charteris, W.P., Kelly, P.M., Morelli, L. & Collins, J.K. (1998). Development and application of an *in vitro* methodology to determinate the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract, *Journal of Applied Microbiology* 84: 759-768.

Clementi, F. & Aquilanti, L. (2011). Recent investigations and updated criteria for the assessment of antibiotic resistance in food lactic acid bacteria, *Anaerobe* 17: 394-398.

Corzo, G. & Gilliland, S.E. (1999). Bile salt hydrolase activity of three strains of *Lactobacillus acidophilus*, *Journal of Dairy Science* 82: 472-480.

Cui, J.H., Goh, J.S., Kim, P.H., Choi, S.H. & Lee B.J. (2000). Survival and stability of bifidobacteria loaded in alginate poly-l-lysine microparticles, *International Journal of Pharmacy Research* 210: 51-59

Danielsen, M. & Wind, A. (2003). Susceptibility of *Lactobacillus* spp. to antimicrobial agents, *International Journal of Food Microbiology* 82: 1–11.

Denkova, Z., Slavchev, A., Blazheva, D. & Krastanov, A. (2007). The effect of the immobilization of probiotic lactobacilli in chitosan on their tolerance to a laboratory model of human gut, *Biotechnology & Biotechnology Equipment* 21 (4),442-450.

De Preter, V., Hamer, H.M., Windey, K. & Verbeke, C. (2011). The impact of pre-and/or probiotics on human colonic metabolism: Does it affect human health? *Molecular Nutrition & Food Research* 55: 46–57.

De Vries, M.C., Vaughan, E.E., Kleerebezem, M. & De Vos, W.M. (2006). *Lactobacillus plantarum* – survival, functional and potential probiotic properties in the human intestinal tract, *International Dairy Journal* 16: 1018–1028.

De Vuyst, L. (2000). Technology aspects related to the application of functional starter cultures, *Food Technology and Biotechnology* 38(2): 105–112.

Duary, R.K., Rajput, Y.S., Batish, V.K. & Grover, S. (2011). Assessing the adhesion of putative indigenous probiotic lactobacilli to human colonic epithelial cells, *The Indian Journal of Medical Research* 134: 664-671.

Essid, I., Medini, M. & Hassouna, M. (2009). Technological and safety properties of *Lactobacillus plantarum* strains isolated from a Tunisian traditional salted meat, *Meat Science* 81: 203–208.

Fávaro-Trindade, C.S. & Grosso, C.R.F. (2002). Microencapsulation of *L. acidophilus* (La-05) and *B. lactis* (Bb-12) and evaluation of their survival at the pH values of the stomach and in bile, *Journal of Microencapsulation* 19: 485-494.

FAO/WHO (2001). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Rome, Italy: Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report.

FAO/WHO (2002). Guidelines for the evaluation of probiotics in food, *Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food*, London Ontario, Canada.

Fávaro-Trindade, C.S. & Grosso, C.R.F. (2002). Microencapsulation of *L. acidophilus* (La-05) and *B. lactis* (Bb-12) and evaluation of their survival at the pH values of the stomach and in bile, *Journal of Microencapsulation* 19: 485-494.

FB 1046, 2009. Probiotic Market- Advanced Technologies and Global Market (2009 - 2014), By: markets and markets.com. Publishing Date: September 2009. Report Code: FB 1046. [accessed on March 26, 2012]. URL: www.marketsandmarkets.com/Market-Reports/probiotic-market-advanced-technologies-and-global-market-69.html.

Floros, G., Hatzikamari, M., Litopoulou-Tzanetaki, E. & Tzanetakis, N. (2012). Probiotic and technological properties of facultatively heterofermentative lactobacilli from Greek traditional cheeses, *Food Biotechnology* 26: 85–105.

Forestier, C., De Champs, C., Vatoux, C. & Joly, B. (2001). Probiotic activities of *Lactobacillus casei rhamnosus*: in vitro adherence to intestinal cells and antimicrobial properties, *Research in Microbiology* 152(2): 167-173.

Ganguly, N.K., Bhattacharya, S.K., Sesikeran, B., Nair, G.B., Ramakrishna, B.S., Sachdev, H.P.S., Batish, V.K., Kanagasabapathy, A.S., Muthuswamy, V., Kathuria, S.C., Katoch, V.M. Satyanarayana, K., Toteja, G.S., Rahi, M., Rao, S., Bhan, M.K., Kapur, R. & Hemalatha, R. (2011). CMR-DBT guidelines for evaluation of probiotics in food, *The Indian Journal of Medical Research* 134(1): 22–25.

Gaudana, S.B., Dhanani, A.S., Bargchi, T. (2010). Probiotic attributes of *Lactobacillus* strains isolated from food and of human origin, *British Journal of Nutrition* 103: 1620–1628.

Georgieva, R., Iliev, I., Haertlé T., Chobert, J.M., Ivanova, I. & Danova, S. (2009). Technological properties of candidate probiotic *Lactobacillus plantarum* strains, *International Dairy Journal* 19: 696–702.

Grosso, C.R.F. & Fávaro-Trindade, C.S. (2004). Stability of free and immobilized *Lactobacillus acidophilus a*nd *Bifidobacterium Lactis i*n acidified milk and of immobilized *B. lactıs* in yoghurt, *Brazilian Journal of Microbiology* 35: 151-156.

Hobbs, C. (2000). Pro-life therapy with probiotics. Health World Online 2000. URL: healthy.net/asp/templates/article.asp?id=953.

Holzapfel, W. H., Geisen, R. & Schillinger, G. (1995). Biological-preservation of foods with reference to protective cultures, bacteriocins, and food grade enzymes, *International Journal of Food Microbiology* 24: 343-362.

Holzapfel, W.H. & Wood, B.J.B. (1998). *The genera of lactic acid bacteria*, Blackie Academic and Professional, London.

Holzapfel, W.H., Haberer, P., Geisen, R., Bjorkroth, J. & Schillinger, U. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition, *American Journal of Clinical Nutrition* 73: 365-373.

Hou, R.C., Lin M.Y., Wang, M.M. & Tzen, J.T. (2003). Increase of viability of entrapped cells of *Lactobacillus delbrueckii* spp. *bulgaricus* in artificial sesame oil emulsions, *Journal of Dairy Science* 86: 424-428.

Hutt, P., Shchepetova, J., loivukene, K., Kullisaar, T. & Mikelsaar, M. (2006). Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens, *Journal of Applied Microbiology* 100:1324-1332.

Huys, G., Vancanneyt, M., D'Haene, K., Vankerckhoven, V., Goossens, H. & Swings, J. (2006). Accuracy of species identity of commercial bacterial cultures intended for probiotic or nutritional use, *Research Microbiology* 157: 803–810.

Isolauri, E., Arvola, T., Sutas, Y. & Salminen S. (2001). Probiotics: effects on immunity, *American Journal of Clinical Nutrition* 73(2): 444-450.

Kailasapathy, K. & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp., *Immunology and Cell Biology* 78: 80–88.

Kailasapathy, K. (2002). Microencapsulation of probiotic bacteria: technology and potential applications, *Current Issues on Intestinal Microbiology* 3: 39-48.

Kalliomaki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P. & Isolauri, E. (2001). Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial, *Lancet* 357: 1076-1079.

Kebary, K.M.K., Hussein, S.A. & Badawi, R.M. (1998). Improving viability of *Bifidobacteria* and their effect on frozen ice milk, *Egyption Journal of Dairy Science* 26: 319-337.

Khater, K.A.A., Ali, M.A. & Ahmed, E.A.M. (2010). Effect of encapsulation on some probiotic criteria, *Journal of American Science* 6(10): 836-845.

Kim, K.I. & Yoon, Y.H. (1995). A study on the preparation of direct vat lactic acid bacterial starter, *Korean Journal of Dairy Science* 17: 129-134.

Kim, S.J., Cho, S.Y., Kim, S.H., Song, O.J., Shin, S., Chu, D.S. & Park, H.J. (2008). Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121, *LWT Food Science and Technology* 41: 493-500.

Kitazawa, H., Toba, T., Itoh, T., Kumano, N., Adachi, S. & Yamaguchi, T. (1991). Antitumoral activity of slime-forming encapsulated *Lactococcus lactis* subsp. *cremoris* isolated from Scandinavian ropy sour milk, "viili", *Animal Feed Science andTechnology* 62: 277-283.

Klaenhammer, T.R. (1998). Functional activities of *Lactobacillus* probiotics: genetic mandate, *International Dairy Journal* 8: 497–505.

Klein, G., Pack, A., Bonaparte, C. & Reuter, G. (1998). Taxonomy and physiology of probiotic lactic acid bacteria*International Journal of Food Microbiology*41: 103–125.

Kos, B., Šušković, J., Beganović, J., Gjuračić, K., Frece, J., Iannaccone, C. & Canganella, F. (2008). Characterization of the three selected probiotic strains for the application in food industry, *World Journal of Microbiology and Biotechnology* 24(5): 699–707.

Kourkoutas, Y., Kanellaki, M. & Koutinas, A.A. (2006). Apple pieces as immobilization support of various microorganisms, *LWT Food Science and Technology* 39: 980–986.

Kristo, E., Biliaderis, C.G. & Tzanetakis, N. (2003). Modelling of rheological, microbiological and acidification properties of a fermented milk product containing a probiotic strain of *Lactobacillus paracasei, International Dairy Journal*, 13: 517–528.

Kushal, R., Anand S.K. & Chander H. (2006). *In vivo* demonstration of enhanced probiotic effect of co-immobilized *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, *International Journal of Dairy Technology* 59: 265-271.

Lacroix, C. & Yildirim, S. (2007). Fermentation technologies for the production of probiotics with high viability and functionality, *Current Opinion in Biotechnology* 18: 176–183.

Leroy, F. & deVuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry, *Trends in Food Science and Technology* 15: 67-78.

Lim, S.M. & Im, D.S. (2009). Screening and characterization of probiotic lactic acid bacteria isolated from Korean fermented foods, *Journal of Microbiology and Biotechnology* 19(2): 178-186.

Lo Curto, A., Pitino, I., Mandalari G., Dainty J.R., Faulks, R.M. & Wickham John, M.S. (2011). Survival of probiotic lactobacilli in the upper gastrointestinal tract using an *in vitro* gastric model of digestion, *Food Microbiology* 7: 1359-1366.

Longdet I.Y., Kutdhik, R.J. & Nwoyeocha, I.G. (2011). The probiotic efficacy of *Lactobacillus casei* from human breast milk against Shigellosis in Albino rats, *Advances in Biotechnology & Chemical Processes* 1: 12-16.

Lourens-Hattingh, A. & Viljoen, B.C. (2001). Review: yoghurt as probiotic carrier in food, *International Dairy Journal* 11: 1–17.

Marciani, L., Ramanathan, C., Tyler, D.J., Young, P., Manoj, P., Wickham, M.S.J., Fillery Travis, A., Spiller, R.C. & Gowland, P.A. (2001). Fat emulsification measured using NMR transverse relaxation, *Journal of Magnetic Resonance Imaging* 153: 1-6.

Marciani,L., Wickham, M.S.J., Wright, J., Bush, D., Faulks, R.M., Fillery Travis, A., Gowland, P.A. & Spiller, R.C. (2003). Magnetic resonance imaging (MRI) insights into how fat emulsion stability alters gastric emptying, *Gastroenterology* 124: A581-1.

Marciani, L., Bush, D., Wright, P., Wickham, M.S.J., Pick, B., Wright, J., Faulks, R.M., Fillery Travis, A.J., Spiller, R.C. & Gowland, P.A. (2005). Monitoring of gallbladder and gastric coordination by EPI. *Journal of Magnetic Resonance Imaging* 21: 82-85.

Marciani, L., Wickham, M.S.J., Bush, D., Faulks, R.M., Wright,J., Fillery-Travis, A., Spiller, R. & Gowland, P. (2006). Magnetic resonance imaging of the behaviour of oil-in water emulsions in the gastric lumen of man, *British Journal of Nutrition* 95: 331-339.

Mater, D.D.G., Bretigny, L., Firmesse, O., Flores, M.J., Mogenet, A., Bresson, J.L. & Corthier, G. (2005). *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* survive gastrointestinal transit of healthy volunteers consuming yogurt, *FEMS Microbiology Letters* 250: 185-187.

Mathur, S. & Singh, R. (2005). Antibiotic resistance in food lactic acid bacteria--a review, *International Journal of Food Microbiology* 105(3): 281-295.

Matijasic, B.B., Narat, M. & Zoric, M. (2003) Adhesion of two *Lactobacillus gasseri* probiotic strains on Caco-2 cells, *Food Technology and Biotechnology* 41(1): 83-88. Miettinen, M., Vuopio-Varkila, J. & Varkila, K. (1996). Production of human tumor necrosis factor a, interleukin-6, and interleukin-10 is induced by lactic acid bacteria, Infection and Immunity 64: 5403–5405.

Minekus, M., Marteau, P., Havenaar, R. & Huisin't Veld, J.H.J. (1995). A multi compartmental dynamic computer-controlled model simulating the stomach and smal lintestine, *Atla* 23: 197–209.

Minelli, E.B., Benini, A., Marzotto, M., Sbarbati, A., Ruzzenente, O. & Ferrario, R., (2004). Assessment of novel probiotic Lactobacillus casei strains for the production of functional dairy foods, *International Dairy Journal* 14: 723-736.

Molly, K.,Vande Woestyne, M. & Verstraete, W. (1993). Development of a5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem, *Applied Microbiology and Biotechnology* 39: 254–258.

Mortazavian, A., Razavi, S.H., Ehsani, m.R. & Sohrabvandi, S. (2007). Principles and methods of microencapsulation of probiotic microorganisms, *Iranian Journal of Biotechnology* 5:1.

Moussavi, M. & Adams, M.C. (2009). An *in vitro* study on bacterial growth interactions and intestinal epithelial cell adhesion characteristics of probiotic combinations, *Current Microbiology* 60: 327-35.

Nawaz, M., Wang, J., Zhou, A., Ma, C., Wu, X. & Xu, J. (2011) Screening and characterization of new potentially probiotic lactobacilli from breast-fed healthy babies in Pakistan, *African Journal of Microbiological Researches* 5(12): 1428-1436.

Nguygen, T.D.T., Kang, J.H. & Lee, M.S. (2007). Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol lowering effects, *International Journal of Food Microbiology* 113(3): 358-361.

Ouwehand, A. C. (1998). Anti-microbial components from lactic acid bacteria, *in* Salminen, S. & Von wright, A. (ed). *Lactic Acid Bacteria: Microbiology and Functional Aspects*, 2nd Edition, Marcel Dekker Inc., NewYork, pp. 139-160.

Ouwehand, A.C., Salminen, S.J. (1998). The health effects of cultured milk products with viable and non-viable bacteria, *International Dairy Journal* 8: 749-758.

Ouwehand, A.C., Tuomola, E.M., Tölkkö, S. & Salminen, S. (2001) Assessment of adhesion properties of novel probiotics strains to human intestinal mucus, *International Journal of Food Microbiology* 64: 119-126.

Ouwehand, A.C., Lagstrom, H., Suomalainen, T. & Salminen, S. (2002). Effect of probiotics on constipation, fecal azoreductase activity and fecal mucin content in the elderly, *Annals of Nutrition and Metabolism* 46(3–4): 159–162.

Ozer, B., Kirmaci, H. A., Senel, E., Atamer, M. & Hayaloglu. A. (2009). Improving the viability of Bifidobacterium bifidum BB-12 and Lactobacillus acidophilus LA-5 in white-brined cheese by microencapsulation, *International Dairy Journal* 19: 22-29.

Park, Y.S., Lee, J.Y., Kim, Y.S. & Shin, D.H. (2002). Isolation and characterization of lactic acid bacteria from feces of newborn baby and from dongchimi, *Journal of Agricultural and Food Chemistry* 50: 2531–2536.

Perdigon, G., Maldonado Galdeano, C. & Valdez, J.C. & Medici, M. (2002). Interaction of lactic acid bacteria with the gut immune system, *European Journal of Clinical Nutrition* 56(4): 21–26.

Picot, A., & Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt, *International Dairy Journal* 14 (6): 505-515.

Pitino, I., Randazzo, C.L., Mandalari, G., Lo Curto, A., Faulks, R.M., Le Marc, Y., Bisignano, C., Caggia, C. & Wickham, M.S.J. (2010). Survival of *Lactobacillus rhamnosus* strains in the upper gastrointestinal tract, *Food Microbiology* 27: 1121-1127.

Prasad, J., Gill, H., Smart, J. & Gopal, P.K. (1998). Selection and characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotic, *International Dairy Journal* 8: 993–1002.

Rao, A.V., Shiwnarin, N. & Maharij, I. (1989). Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices, *Canadian Institution of Food Science and Technology Journal* 22: 345-349.

Reid, G., Jass, J., Sebulsky, M.T. & McCormick, J.K. (2003). Potential uses of probiotics in clinical practice, *Clinical Microbiological Reviews* 16(4): 658–72.

Rodgers, S. (2008). Novel applications of live bacteria in food services: probiotics and protective cultures, *Trends in Food Science and Technology* 19: 188-197.

Rönkä E., Malinen E., Saarela M., Rinta-Koski M., Aarnikunnas J., Palva A. (2003). Probitic and milk technological properties of *Lactobacillus brevis*, *International Journal Food Microbiology* 83: 63–74.

Saarela, M., Mogensen, G., Fonden, R., Matto, J. & Mattila-Sandholm, T. (2000). Probiotic bacteria: safety, functional and technological properties, *Journal of Biotechnology* 84: 197–215.

Saavedra, J. (2000). Probiotics and infectious diarrhea, *American Journal of Gastroenterology* 95: 16-18.

Salminen, S., von Wright, A., Morelli L., Marteau P., Brassart D., de Vos, W.M., Fondén, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S.E. & Mattila-Sandholm T. (1998). Demonstration of safety of probiotics – a review, *International Journal Food Microbiology* 44: 93–106.

Sandeep, B., Gaudana, Akhilesh, S., Dhanani & Tamishraha, B. (2010). Probiotic attributes of *Lactobacillus* strains isolated from food and of human origin, *British Journal of Nutrition* 103: 1620–1628.

Sanders, M.E. & Huis in't Veld, J.H.J. (1999). Bringing a probiotic containing functional food to the market: microbiological, product regulatory and labeling issues, *in* Konings, W.N., Kuipers, O.P. & Huis in't Veld, J.H.J. (ed.), *Proceedings of the 6th symposium on lactic acid bacteria: genetics, metabolism and applications*, Kluwer Academic Publishers, Antonie van Leeuwenhoek, Veldhoven, The Netherlands, pp. 293–316.

Sanders, ME. (2000). Considerations for use of probiotic bacteria to modulate human health, *Journal of Nutrition* 130: 384-390.

Sanders, M.E. (2008). Probiotics: Definition, sources, selection, and uses, *Clinical Infectious Diseases* 46: 58–61.

Schillinger, U., Yousif, N.M., Sesar, L. & Franz, C.M. (2003). Use of group-specific and RAPD-PCR analyses for rapid differentiation of Lactobacillus strains from probiotic yogurts, *Current Microbiology* 47: 453–456.

Shah, N.P. (2000) Probiotic bacteria: selective enumeration and survival in dairy foods, *Journal of Dairy Science* 83: 894-907.

Shah, N. (2002). The exopolysaccharides production by starter cultures and their influence on textural characteristics of fermented milks, *Proceeding of Symposium on New Developments in Technology of Fermented Milks*, International Dairy Federation, Comwell Scanticon, Kolding, Denmark, p. 5.

Sheu, S.J., Hwang, W.Z., Chen, H.C., Chiang, Y.C. & Tsen, H.Y. (2009.) Development and use of tuf gene-based primers for the multiplex PCR detection of *Lactobacillus acidophilus, Lactobacillus casei* group, *Lactobacillus delbrueckii*, and *Bifidobacterium longum* in commercial dairy products, *Journal of Food Protection* 72: 93–100.

Senol, A., Isler, M., Karahan, A.G., Kilic, G.B., Kuleasan, H., Kaya, S., Keskin, M., Goren, İ., Saritas, U., Aridogan, C.B. & Delibas, N. (2011a). Preventive effect of probiotics and alpha-tocopherol on the ethanol-induced gastric mucosal injury in rats, *Journal of Medicinal Food* 14(1-2): 173-179.

Senol, A., Isler, M., Karahan, A.G., Basyigit Kilic, G., Kuleasan, H., Goren, İ., Saritas, U., Kaya, S., Ciris, M., Akturk, O., Aridogan, C.B., Demirin, H. & Cakmakci, M.L. (2011b). Effect of the probiotics on aspirin-induced gastric mucosal lesions, *Journal of Turkish Gastroenterology* 22 (1):18-26.

Stewart, G.G. & Russell, I. (1986). One hundred years of yeast research and development in the brewing industry, *Journal of the Institute of Brewing* 92: 537–558.

Siripatrawan, U. & Harte, B.R. (2007). Solid phase microextraction/gas chromatography/mass spectrometry integrated with chemometrics for detection of *Salmonella typhimurium* contamination in a packaged fresh vegetable. *Analytica Chimica Acta* 581: 63–70.

Solis-Pereyra, B., Aattouri, N. & Lemonnier, D. (1997). Role of food in the stimulation of cytokine production, *American Journal of Clinical Nutrition* 66: 521–525.

Steenson, L.R., Klaenhammer, T.R. & Swaisgood, H.E. (1987). Calcium alginate-immobilized cultures of lactic streptococci are protected from attack by lytic bacteriophage, *Journal of Dairy Science* 70: 1121-1127.

Sumeri, I., Arike, L., Adamberg, K. & Paalme, T. (2008). Single bioreactor gastrointestinal tract simulator for study of survival of probiotic bacteria, *Applied Microbiology and Biotechnology* 80: 317-324.

Šušković, J., Kos, B., Goreta, J. & Matošić, S. (2001). Role of lactic acid bacteria and bifidobacteria in symbiotic effect, *Food Technology and Biotechnology* (39): 227–235.

Taylor, A.L., Dunstan, J.A. & Prescott, S.L. (2007). Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial, *Journal of Allergy and Clinical Immunology* 119: 184-191.

Tharmaraj, N. & Shah, N.P. (2009). Antimicrobial effects of probiotics against selected pathogenic and spoilage bacteria in cheese-based dips, *International Food Research Journal* 16: 261-276.

Tilsala-Timisjärvi, A. & Tapanialtossava, T. (1998). Strain-specific identification of probiotic *Lactobacillus rhamnosus* with Randomly Amplified Polymorphic DNA-Derived PCR Primers, *Applied and Environmental Microbiolog* 64(12): 4816–4819.

Vaillancourt, J. (2006). Regulating pre- and probiotics: a U.S. FDA perspective, *Proceedings of ending the war metaphor: the changing agenda for unraveling the host-microbe relationship*. *in* Institute of Medicine of the National Academies. Washington, DC: National Academies Press, pp. 229–237.

Verdenelli, M.C., Ghelfi, F., Silvi, S., Orpianesi, C., Cecchini, C. & Cresci, A. (2009). Probiotic properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faeces, *European Journal of Nutrition* 48: 355–363.

Wang, C., Lin, P., Ng, C. & Shyu, Y. (2010). Probiotic properties of *Lactobacillus* strains isolated from the feces of breast-fed infants and Taiwanese pickled cabbage, *Anaerobe* 16: 578-585.

Wei, H., Wolf, G. & Hammes, W.P. (2006). Indigenous microorganisms from iceberg lettuce with adherence and antagonistic potential for use as protective culture, *Innovative Food Science Emerging Technologies* 7: 294–301.

Wood, B.J.B. & Holzapfel W.H. (1995). *The Lactic Acid Bacteria*, *Vol. 2, The Genera of Lactic Acid Bacteria*, Blackie Academic & Professional, London, pp 55-124.

Yang, S.C., Chen, J.Y., Shang, H.F., Cheng, T.Y., Tsou, S.C. & Chen, J.R. (2005). Effects of symbiotics on intestinal microflora and digestive enzyme activies in rats, *World Journal of Gastroenterology* 11:7413-7417.

Zago, M., Fornasari, E., Carminati, D., Burns, P., Suàrez, V., Vinderola, G., Reinheimer, J. & Giraffa, G. (2011). Characterization and probiotic potential of *Lactobacillus plantarum* strains isolated from cheeses, *Food Microbiology* 28: 1033-1040.

Ziarno, M., Sekul, E. & LafrayaAguado, A. (2007). Cholesterol assimilation by commercial yoghurt starter cultures, *ACTA Scientiarum Polonorum-Food Science and Human Nutrition* 6(1): 83-94.