## **Research Article**

## Methods of Increasing Probiotic Survival in Food and Gastrointestinal Conditions

Maleki D<sup>1</sup>, Azizi A<sup>2</sup>, Vaghef E<sup>3</sup>, Balkani S<sup>4</sup> and Homayouni A<sup>5\*</sup>

## Abstract

Several studies have shown that the therapeutic value of live probiotic bacteria is more than unviable cells. Although the researches on the therapeutic value of dead probiotic cells and their metabolites are not sufficient, most researchers believe that the survival of probiotic bacteria is very important for their therapeutic effects. Thus, attempts have been made to increase the survival of probiotics. Some studies have been done to evaluate the tolerance of probiotics against low pH and high bile, conditions that probiotic bacteria must face in gastrointestinal tract. More investigations are required to study the survival of probiotics during processing and storage. Dairy products are largely used as vehicles for probiotic bacteria. Type of dairy food, its temperature and content of air can affect the viability of probiotics. International Dairy Federation (IDF) suggests that a probiotic product should contain a minimum of 107 live probiotic bacteria per gram of the product, at the time of consumption. Factors affecting probiotic survival have been widely studied in yoghurt but further studies are warranted in other dairy products such as ice cream. The method of increasing probiotic survival depends on the nature of the food product which is to be used as probiotic carrier. Selection of resistant probiotic strains to production, storage and gastrointestinal tract conditions is one of the most important methods. Adjusting the condition of production and storage for more survival rates is another priority to be considered. The physical protection of probiotics by microencapsulation is a novel technique to increase the survival of probiotics.

#### Keywords

Probiotic; Survival; Ice cream; Microencapsulation; Genetic modification

### Introduction

### **Probiotics**

The word probiotic is derived from the Greek word "probios", meaning "for life" that is the opposite of antibiotic [1]. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" by the FAO/WHO. This restricts probiotic foods to products that contain live microorganisms and improve health and well-being of humans or animals, so that the probiotics must be viable and available at a high concentration (minimum 107cfu/g) to convey health benefits [2,3].

Most commercially available probiotics are non-spore forming lactic acid bacteria, namely Lactobacilli, Bifidobacteria, and Enterococci. A variety of strains have been demonstrated to possess beneficial (probiotic) properties. Bacilli, including Bacillus licheniformis, Bacillus subtilis, and Bacillus toyoi, have been evaluated to a more limited extent, and are less widely available. One of the most attractive properties of Bacilli is their ability to form resistant spores that should be better able to withstand the rigors of processing and storage. Nonpathogenic strains of E. coli have been evaluated in some species as a means of competitively excluding pathogenic strains of E. coli. Yeasts also have been evaluated for probiotic properties; however, most yeast supplements act as nutritional supplements, not probiotics. Saccharomyces boulardii is nonpathogenic yeast shown effective for the prevention of antibiotic associated diarrhea and treatment of recurrent Clostridium difficile diarrhea in people. This effect is thought to be due to direct effects on C. difficile, in addition to secretion of a protease that affects toxins [4].

Some important species of probiotic bacteria in dairy foods are related to genus of *Lactobacillus* (*L. acidophilus* and *L. johnsonii* or *paracasei*) and *Bifidobacterium* (*B. longum* and *B. bifidum*) [5-7].

### Prebiotics

Prebiotics are defined as selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal micro biota that confers benefits upon host well-being and health [8]. A number of food ingredients can act as prebiotics; however, the most commonly evaluated prebiotics are those which stimulate the lactic acid bacteria (*Lactobacilli, Bifidobacteria*, and *Enterococci*) component of the micro flora. After passing through the small intestine essentially unchanged, they are a nutritional substrate for certain types of colonic and fecal bacteria [4].

The most important prebiotic compounds are Fructo-Oligo-Saccharids (FOS), Galacto-Oligo-Saccharides (GOS in cow milk), Xylo-Oligo-Saccharides (XOS) and Trans Galactosylated Oligosaccharides [2].

### Synbiotic

As mentioned before, probiotic foods contain live microorganisms. A food product containing both of probiotics and prebiotics is called a synbiotic food. Synbiotic describes a combination of a probiotic and a prebiotic. Addition of an appropriate prebiotic may improve survival and establishment of a probiotic organism by providing a readily available nutritional source that might not be used by competing organisms [4].

There is a synergy effect between probiotics and prebiotics in synbiotic products. Prebiotic compounds are consumed by probiotics as a carbon or energy source. This results in increase of probiotic count and reduction of pathogen microorganisms in the gut [9,10].

### Health benefits of probiotics

Many of the suggested benefits of probiotic dairy foods are based on the involvement of the gastrointestinal micro flora in resistance to disease. The benefits of probiotics include inflammatory



# La Prensa MedicaArgentina

<sup>\*</sup>Corresponding author: Aziz Homayouni, Associate professor, Department of Food Science and Technology, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran, Tel: +98 411 3357581; E-mail: homayounia@tbzmed.ac.ir

Received: May 19, 2014 Accepted: December 05, 2014 Published: December 10, 2015

Citation: Maleki D, Azizi A, Vaghef E, Balkani S, Homayouni A (2015) Methods of Increasing Probiotic Survival in Food and Gastrointestinal Conditions. Prensa Med Argent 101:4.

disease control, treatment and prevention of allergies, cancer prevention, immune stimulation, respiratory disease reduction, nutritional enhancement with synthesis of Vit K and Vit B group and improvement of mineral absorption (Ca, Zn, Fe, Mn, Cu and P). Probiotics are used for treatment of lactose maldigestion, diarrhea disease, high blood cholesterol, constipation, hepatic encephalopathy and endotoxemia [11-13].

Lactose intolerance or maldigestion causing abdominal discomfort affects approximately 70% of the world's population, to varying degrees. A probiotic bacterium, L. acidophilus, has been clinically shown to alleviate the symptoms of lactose intolerance. Rotavirus is one of the leading causes of gastroenteritis worldwide. Gastroenteritis characterized by acute diarrhea and vomiting is a leading cause of death and illness among children, affecting approximately 16.5 million children annually. Ample evidence has shown that the probiotic strain L. GG reduces the duration and severity of rotavirus infection. Oral administration of B. bifidum has been shown to be potentially beneficial in reducing the incidence of diarrhea in infants hospitalized for rotavirus infection. Other researchers suggest that the probiotic bacteria B. longum and Sacharomyces boulardii prevent antibioticassociated diarrhea. The role of lactic acid bacteria in reducing the incidence of DNA damage and other carcinogenic changes has also been investigated. Probiotics might suppress the growth of bacteria that convert pro-carcinogens to carcinogens. An important index of carcinogenic activity is the activity of enzymes that convert procarcinogens to carcinogens. Several studies have shown an inhibitory effect on these enzyme activities following the consumption of probiotics. Additional research on the prevention or delay of tumor development by probiotic bacteria suggests that they might bind to mutagenic compounds in the intestinal tract. A reduction of mutagens in urinary excretions was found when meals were supplemented with fermented milk containing L. acidophilus. This suggests that lactobacilli were binding to the mutagenic compounds, thereby reducing their absorption in the intestine [2,14-20].

### Probiotic dairy products

Probiotic bacteria may be incorporated into foods or available in dietary supplement forms. The majority of foods containing probiotic bacteria are dairy products due to the historical association between lactic acid bacteria and fermented milk. Probiotics have been incorporated into fermented milks, yoghurts, soft semi-hard and hard cheeses, ice-cream, frozen fermented dairy desserts, quark and cottage cheese as well as salami and bread [5,21-27].

## Probiotic ice cream

Probiotic bacteria have been incorporated into ice-cream and fermented ice-cream which are ideal vehicles of delivery for these organisms in human diet [5,21-22,28]. Incorporation of *B. bifidum* and *L. acidophilus* into ice-cream mixes that are fermented to the desired pH, then frozen and stored for 17 weeks resulted in high survival level in probiotics counts. Post-freezing counts were above  $1\times108$  cfu/g, decreasing to  $4\times106$  for *L. acidophilus* and  $1\times107$  for *B. bifidum* at the trial completing [5]. Another trial studying these two microorganisms added the cultures to ice-cream mix via fermented milk in levels up to 50% without further fermentation. The freezing process caused a decrease of up to one log unit in viable count of microorganisms, with a further reduction after 16 weeks. Ultimate counts after this period were approximately 0.5-1.0×107 cfu/g [29]. A low-fat formulation provides better survival rates than the full-fat formulation in ice-cream [30].

Volume 101 • Issue 4 • 1000154

The low storage temperature of frozen dairy foods is ideal for the long term preservation of bacteria [5,21]. The frozen yoghurt type dairy desserts must adhere to the standards of yoghurt and have a pH less than or equal to 4.5 which affects the viability of *L. acidophilus* and Bifidobacterium spp. [28]. Incorporation of probiotic bacteria into ice-cream, does not pose the same problem as the pH of 6.5-6.6 is more ideal for these organisms [21]. However, the incorporation of air affects the viability of anaerobic organisms [21,28].

Ice-creams and frozen yoghurts have been developed containing both probiotic bacteria and prebiotic carbohydrates [5,22,28-30]. Resistant starch has been used as prebiotic ingredient in ice-cream [30]. Once frozen; the studies showed that the viability of the probiotics in ice-cream changed little over a one-year period. As for cheese, ice-cream provided a stable matrix for the consumption of probiotic strains.

### **Probiotic cheese**

The incorporation of probiotic bacteria into cheese has been described recently [23]. *Bifidobacterium spp.* survives well in Cheddar and Gouda cheeses, which may be the ideal delivery vehicle for probiotic bacteria in the human diet. Cheese has advantages over yoghurt being lower in acidity and higher in fat content and having more solid consistency offering more protection to the organisms during storage and travel through the gastrointestinal tract [26].

Cheese starter cultures including lactic acid bacteria are required to begin the fermentation of lactose to lactic acid, which reduces the pH of the product. However, during ripening of the cheese, conditions for survival of starter cultures are not favorable. High salt in moisture, low pH, lack of fermentable carbohydrates and low storage temperature lead to a decline in numbers of the starter culture in cheese. As the starter culture population declines, the non-starterlactic acid bacteria (NSLAB) proliferate as the cheese ripens. The adjunct bacteria incorporated for probiotic effect must then survive the relatively long ripening period of 6-24 months [26].

Since cheese is an effective delivery vehicle for probiotic bacteria, it is important for commercial application that the technology for probiotic cheese manufacture remains simple [26]. Commercial lyophilized *B. bifidum* can be added to Cheddar cheese curd at the milling stage. This was done to overcome some of the obstacles of the cheese-making process, cooking and cheddaring, at the presence of starter culture organisms [23]. Bifidobacterium spp. does not affect the normal aerobic micro flora of the cheddar cheese and do not change the normal proteolytic activity during the ripening period [26]. However, the *Bifidobacterium spp*. is able to remain viable throughout the ripening period at 6-7°C even though the optimal temperature for growth of Bifidobacterium spp. is 37°C.

The final numbers of *L. acidophilus* in probiotic Turkish white cheeses were greater than the minimum (107 cfu/g), required to produce health benefits claimed for probiotic cheese. Furthermore, *L. acidophilus* can be used to give good flavor, texture, and a high level of proteolysis. In addition, probiotic cheese which was vacuum packed following salting was more acceptable than the corresponding cheese which was stored in brine following salting [31].

The resistance of probiotic strains to passage through the gastrointestinal tract, when delivered in a whey cheese matrix, is strain-dependent. The best viability profiles throughout the period of exposure to the combination of artificial gastric juice and bile salts were obtained for *L. brevis*, *B. animalis Bo* and *B. animalis Bb-12. L.* 

*acidophilus* LAC-1 and *L. acidophilus* Ki resisted exposure to artificial gastric juice, but showed different behavior in the presence of bile salts. *L. paracasei ssp.* paracasei LCS-1 and *B. animalis* BLC-1 were the least resistant to artificial gastric juice plus bile salts [32].

Several parameters may determine the extent of probiotic strains' survival through the upper gastrointestinal tract, such as the degree of stomach acidity and the time of exposure, as well as the concentration and the time of exposure to bile salts. However, in vivo studies are still necessary to fully validate all previous in vitro studies, such as microbiological analyses of faecal samples after feeding of the inoculated products are required, as other factors (e.g. pancreatic juice) also play a role [32].

## **Probiotic yoghurt**

Probiotic yoghurts may be produced using *L. acidophilus, B. bifidum, B. longum* or *L. casei* in any combination with or without the normal starter organisms for traditional yoghurt. Therapeutic yoghurt production is more difficult without the presence of traditional starter organisms. Because acid production by therapeutic starters is low and more care must be taken not to allow contamination. Therapeutic properties may be enhanced when both sets of starter cultures are used in combination [24].

Large range of probiotic yoghurts are produced worldwide. But there are not very high levels of probiotic organisms to be found in these products and their activity may be less than ideal [33-41]. There are international standards which require that any cultured products sold with health claims must contain a minimum of 107 cfu/g viable probiotic bacteria at the consumption time [42]. Many cultured dairy products currently on the market fail to meet these standards because the strains of probiotic bacteria used in production cannot survive the acidity of the product [35,37,39]. Even if there are enough viable cells at production time, the acidity of the product can increase when it contains lactic acid producing bacteria, eventually inhibiting their own viability [37]. Also resistance to acetate may be important for survival in dairy products since acetate is a major byproduct of Bifidobacterium spp. fermentation [43]. L. acidophilus and Bifidobacterium spp. are affected by the acidity of yoghurt and a decline in the population is inevitable as growth of L. acidophilus ceases at pH=4.0, while that of Bifidobacterium spp. at pH=5.0; thus, probiotic bacteria are unstable in yoghurt [35,44,45]. Postacidification of yoghurt during the storage period is a large contributor to the death of probiotic cells in the product. There are other factors contributing to probiotic cell death including hydrogen peroxide production by the yoghurt fermenting cultures which reduces the viability of probiotic bacteria [35,40,45].

## Probiotic survival in dairy foods

The importance of probiotic survival: All definitions of probiotics emphasize the importance of viability of the bacteria to their efficacy of action [40,41,46-47]. The probiotic bacteria must not only survive in the food during the shelf life of the product, but must then survive the transit through the gastrointestinal tract and the acidity of the stomach as well as enzymes and bile salts in the intestine before reaching the site of action [41]. Therefore there is a standard set that any food sold with health claims due to the inclusion of probiotic bacteria must contain at least 107 cfu/g viable probiotic bacteria at the used-by date [26,48]. If viability is crucial, then the high numbers of viable cells will decline in the food as well

as during transition through the gastrointestinal tract [49]. There is some evidence that non-viable probiotics and bacterial components also have some health benefits; however, no comparisons have been made between the efficiency of viable cells against non-viable cells [42,48]. The best documented health effects are available for viable cells although some benefits of using non-viable cells include fewer safety concerns, no risk of infection, excellent shelf life and no risk of transferring antibiotic resistance [48].

Factors that affect probiotic survival: There are several factors which have been reported to affect the viability of probiotic cultures in fermented milk products including titratable acidity, pH, hydrogen peroxide, dissolved oxygen content, storage temperature, species and strains of associative fermented dairy product organisms, concentration of lactic and acetic acids and buffers such as whey protein concentrates which have been identified to have an effect during manufacture and storage of dairy products [28,33-34,45,50-60]. The organisms' survival in dairy products is not the only issue at stake. In fact, food provides a buffer to protect the microorganisms in the gut [61]. The bacterial strains selected as probiotics must also tolerate the gastric acidity (pH=1-4), bile salts, enzymes such as lysozyme present in the intestine, toxic metabolites including phenols produced during digestion, bacteriophage, antibiotics and anaerobic conditions. Furthermore, the human gastrointestinal tract and stomach have the highest acidity; the probiotic bacteria must not only survive these conditions but are required to colonize the gut. Therefore probiotic strain selection is very important as many strains of L. acidophilus and Bifidobacterium spp. cannot survive these conditions [53,62-65]. L. casei and B. lactis have been shown to be more resistant to the gut pH and low temperatures, when compared to L. acidophilus and B. longum [66].

Monitoring survival level of probiotic: Several media are available for the selective enumeration of L. acidophilus and Bifidobacterium spp. For L. acidophilus the available media includes bile medium, Rogosa agar, cellobiose-esculin agar, agar based on X-Glu., modified Lactobacillus selective agar (mLBS), Lactobacillus selective medium (mLSM) and deMan Rogosa Sharpe (MRS) medium with salicin, maltose, raffinose or melibiose instead of glucose or dextrose [57,67,68]. For Bifidobacterium spp. the available media includes modified Bifidobacterium spp. iodoacetate medium (mBIM), MRS with cystine, bile and dicloxacillin (MRS + BCD), Reinforced Clostridial Prussian Blue agar (RCPB), Reinforced Clostridial Prussian Blue agar adjusted to pH=5 (RCPBpH5), modified Columbia agar (mCol), modified Rogosa's agar (RMS), Arroyo, Martin and Cotton agar (AMC), Lithium chloride-sodium propionate agar (LP), blood-glucose-liver agar with Oxgall and gentamycin (BL-OG), modified VF-Bouillon agar plus Lithium chloride, sodium lauryl sulfate, sodium propionate and neomycin sulfate, and nalidixic acid-neomycin sulfate-Lithium chlorideparomomycin sulfate agar (NNLP, some strains do not grow on this medium) [22,45,57,67-70].

Various media are suitable for growing L. acidophilus and Bifidobacterium spp.; however, they may not be suitable for selective enumeration of one species in the presence of the other or in the presence of starter culture organisms. Another concern is that different strains within the same species display different sugar fermentation characteristics as well as tolerance to selective ingredients such as bile or antibiotics making a true determination of the viable count in dairy products more difficult [45,57].

Citation: Maleki D, Azizi A, Vaghef E, Balkani S, Homayouni A (2015) Methods of Increasing Probiotic Survival in Food and Gastrointestinal Conditions. Prensa Med Argent 101:4.

MRS-Maltose has been used for the selective enumeration of L. acidophilus from yoghurt products without Bifidobacterium spp. incorporated. Different media may be more suitable for dairy products with both L. acidophilus and Bifidobacterium spp. incorporated depending on the sugar utilization of the specific strain used in the product [45,57]. Incorporation of S. thermophilus, L. delbrueckii subsp. bulgaricus, L. acidophilus and different Bifidobacterium species to salicin, cellobiose, fructose, manitol, sorbitol and glucose showed that all strains of bacteria were able to use either glucose or fructose; however, S. thermophilus, L. delbrueckii subsp. bulgaricus were not able to use salicin, cellobiose, manitol or sorbitol. L. acidophilus strains were all able to use all these sugars; however, very few species of Bifidobacteria were able to utilize cellobiose, manitol or sorbitol and none could use salicin. Thus nutrient agar with salicin as the carbon source at 0.5% concentration was found suitable for selective enumeration of L. acidophilus from samples containing yoghurtfermenting cultures and Bifidobacterium spp. [45,67]. When dairy products contain both L. acidophilus and L. casei as probiotic adjuncts, MRS-salicin or MRS-sorbitol agar may be used for enumerating both organisms together. At the same time LC-agar used to determine the viability of L. casei alone and the subtraction method may then be used to determine the count of L. acidophilus alone. Likewise a subtraction method has been used for the determination of Bifidobacterium spp. viability in a mixed culture [57]. Recently, spectrophotometricaly measurement of optical density at 580 nm has been shown to be the simple, rapid and inexpensive method for evaluation of probiotic growth as well [71].

Methods of increasing probiotic survival: Substances such as simple sugars, minerals and vitamins have been shown to stimulate the growth of L. acidophilus and Bifidobacterium spp. [37]. The growth of L. acidophilus may be stimulated by the addition of simple sugars such as glucose and fructose and minerals such as magnesium and manganese, which could be in the form of tomato juice or papaya pulp and result in higher viable counts, shorter generation times and improved sugar utilization. A similar result can be seen with the addition of acetate or by supplementing milk with casitone, casein hydrolyzates and fructose [41]. The growth of Bifidobacteria is stimulated by vitamins, dextrin and maltose. Bifidobacteria grow poorly in milk but the survival of B. longum is improved with the inclusion of 0.01% bakers' yeast. The use of oligosaccharides as prebiotic allows favored growth of probiotic bacteria in the colon. Raffinose, stachyose, fructo-, isomalto- and galactooligosaccharides are good substances for Bifidobacteria to be metabolized to acetic and lactic acids [37,41,72]. Increasing protein content has been shown to enhance probiotic survivability as well [73]

In dairy products it would also be possible to increase the survival of probiotic bacteria by manipulation of production and storage conditions. This could be achieved by completing the fermentation process at a higher pH than normal (>5) allowing better survival of *Bifidobacterium spp*. [24]. Storing the product at a lower temperature (below 3-4°C) increases the survival of AB culture [74]. Addition of whey protein concentrate and acid casein hydrolyzate to yoghurt mix increases its buffering capacity, thus the probiotic cultures survive better [40,57,59]. Application of hydrostatic pressure (200-300 MPa for 10 minutes at room temperature) can prevent after-acidification and maintain the number of viable LAB [75]. Heat treatment (5 minutes at 58°C) to prevent excess acid production and to maintain a constant acidity during storage would mean that the pH cannot reduce further and cause a decline in probiotic viability [76]. Lower

incubation temperatures [37°C rather than 40-42°C) would increase incubation time and growth of Bifido bacterium spp. Use of yoghurt starter cultures without L. delbrueckii subsp. Bulgaricus can also reduce post-acidification of the product and overcome severe losses in viability of probiotic bacteria. Storage of dairy products in glass containers rather than plastic containers would decrease the dissolved oxygen content and allow more anaerobic organisms to survive [57]. Two-step fermentation of yoghurt, where the probiotic cultures are added first and allowed to establish their populations in the first stage of fermentation, followed by a second stage of fermentation with the yoghurt starter cultures resulted in higher viability of probiotic organisms [38,57]. Probiotic bacteria viability may also be achieved by stress adaptation of the culture to the harsh conditions of the fermented dairy products, particularly the low pH of yoghurt [57]. Fortification of products with ascorbic acid (vitamin C) or L-cysteine hydrochloride to act as an oxygen scavenger and to lower the redox potential increases the viability of L. acidophilus [36,56]. Rupturing of yoghurt starter cultures to release the intracellular  $\beta$ -galactosidase and to reduce their viable counts increases the viability of the probiotic cultures L. acidophilus and Bifidobacterium spp. [57,77]. Microencapsulation is a technique which has been used to increase survival of probiotic bacteria in human gastric and intestinal juices [37,41,57,78,79].

**Genetic modification:** Lactic Acid Bacteria (LAB) are a genetically diverse group of bacteria, including species of the genera *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Onecoccus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella*. The genus Lactobacillus remains heterogeneous, with 60 species having guanosine and cytosine (GC) contents of between 34% and 53%, about a third of which are strictly hetero fermentative [80].

Since there are a number of characteristics that are ideal for a probiotic culture and it is unlikely that any particular strain would have all those traits, it would be possible to derive a new strain by genetic manipulation. The ideal probiotic culture would be a strain that could survive in the human gastrointestinal tract as well as colonize the gut. The perfect strain should inhibit pathogens in the gut as well as contributing to the health of the host by synthesizing essential nutrients not found in the diet or by auto-digesting dietary substances that the host cannot process himself. The ideal probiotic would be suitable for cultivation on a large scale in industrial conditions as well as be suitable for preservation for storage before retail sale or incorporation into food. Crucially, the ideal probiotic strain should also be a virulent and not have any metabolic traits that could compromise the host's health. Finally, all of the mentioned ideal probiotic characteristics need to be stable properties of the cell, which if occurs naturally would be too costly to screen for. It would be possible to derive the perfect strain by gene technology. The initial microbe chosen as the target of the probiotic can be selected from human originating strains. Human gastrointestinal tract is unique in terms of the large proportion of it being free from permanent colonization as well as having large populations in the terminal ileum and large bowel.

Colonization of probiotics in the gastrointestinal tract is ideal; however, the perfect strain would need to be able to overcome gastric acidity, mucus secreting mucosa lining the entire gastrointestinal tract as well as rapid peristaltic propulsion of digesta through the small bowel. The nature of the probiotic would also be determined by whether the strain is to provide long-term colonization in the gut to be able to continually exert its influences on the bowel or if frequent delivery is desired to specific locations within the gastrointestinal tract in prescribed doses, which would give more choice in the original microbe. The most important consideration with genetic manipulation of gastrointestinal micro flora would be the stability of the recombinant DNA, ensuring that the plasmids cannot be transferred to other organisms in the gut [81].

Some possible probiotic developments by gene technology would be strains that can immunize, particularly from mother to infant while feeding, delivering strains of other characteristics such as specific molecules to particular regions of the intestinal tract. Another development could be of probiotics for nutrition, such as those that enable the host to utilize more energy from the diet. The three crucial factors to assess genetically modified gut organisms would be the access, expression and damage. Access means the probability of the organism escaping and entering the human body, surviving and entering susceptible tissues. Expression relates to how efficiently the new gene will be expressed and damage is an estimation of the probability that the product of the foreign gene will cause physiological damage to the host [81]. Regulation and consumer acceptance, again become the prime issues thus more inventive ways of increasing survival of probiotics must be investigated.

Adjustment of production and storage condition: Production condition may play an important role in probiotic survival. Adjustment of production and storage condition is a main method for increasing probiotic survival. For instance; single-step fermentation, two-step fermentation and fermentation beginning with a neutralized yoghurt mix were used for this aim. Two-step fermentation means introducing the probiotic bacteria into the product mix 2 hours earlier than the yoghurt culture in order to stabilize probiotic culture. The neutralized mix procedure involved adjusting the pH of the product mix from 6.6 to 6.9 before fermentation such that it should take longer to achieve pH=4.5, thereby giving more time for the probiotic cultures to grow. The viable count of L. acidophilus 2409 and B. longum 1941 were higher initially and were maintained higher in yoghurts that were produced by Two-step fermentation as well as neutralized mix. Fermentation time for the products to reach pH=4.5 was the longest for the two-step fermentation and shortest for the commercially used single-step fermentation. The neutralized mix took longer to reach pH=4.5 by 20 minutes for each 0.1 pH unit adjustment [38].

## Physical protection by encapsulation:

**Definition:** Encapsulation is defined as "the process of forming a continuous, thin coating around encapsulants (solid particles, droplets of lipids or gas cells) which are wholly contained within the capsule wall as a core of capsulated material". The distinction is made from entrapment, which refers to "the trapping of encapsulants within or throughout a matrix [e.g. a gel or crystal etc)", different from encapsulation as some of the entrapped ingredients may be exposed at the surface of the particle [82]. The material to be coated is called the active, internal phase, fill or core material while the coating material is referred to as the shell, coating, wall material, carrier or encapsulants and may vary in thickness and number of layers [83-84].

Microencapsulation as a strict term would apply to particles of size 0.2-5000 $\mu$ m while those larger than 5000  $\mu$ m are classified as macroand those smaller than 0.2 $\mu$ m are classified as nano-microcapsules. If the core material is very large, then the process is referred to as "coating" [83]. Ideally the encapsulated particle is spherical; however, this is influenced by the structure of the core material [84].

Encapsulated food ingredients include enzymes, flavors, flavor enhancers, sweeteners, antioxidants, food preservatives, acidulates, amino acids, colorants, and edible oils, fats, leavening agents, vitamins, minerals and microorganisms. Microencapsulation is not only used in the food industry; the process also has applications in the biotechnology, pharmaceutical and chemical industries [83,85].

Microencapsulation of various bacterial cultures including probiotics has been a common practice for extending their storage life and converting them into a powder form for ease of their use. There are several techniques such as spray drying, freeze drying, fluidized bed drying for encapsulating the cultures and converting them into a concentrated powdered form. However, the bacteria encapsulated by these techniques are completely released in the product. In this case, the cultures are not protected from the product environment or during the passage through the stomach or intestinal tract. Encapsulation in hydrocolloid beads entraps or immobilizes the cells within the bead matrix, which in turn provides protection in such an environment [78].

Encapsulation of probiotic bacteria: Microencapsulation of bacterial cells is currently gaining attention to increase viability of probiotic bacteria in acidic products such as yoghurt [86-87]. Microencapsulation segregates the cells from adverse environment, thus potentially reducing cell injury. There is a need for encapsulation or enteric coatings for probiotic bacteria to survive human gastric juice in the stomach, where the pH can be as low as 2. It has been reported that microencapsulation using calcium-induced alginatestarch polymers [79,87], in potassium induced kappa-carrageenan polymers [88] and in whey protein polymers [89] have increased the survival and viability of probiotic bacteria in yoghurt during storage. Also, probiotic bacteria when encapsulated in calcium alginate beads had 30% increased survivability in ice cream [90]. The encapsulate materials used in these studies such as alginate, starch, carrageenan and whey protein are commonly used food stabilizers in the manufacture of stirred yoghurts to prevent syneresis. For example, alginate is a natural polysaccharide extracted from brown seaweeds and it enhances viscosity and binds water hence reduces syneresis in stirred yoghurts.

Microbial exopolysaccharides (EPS) are also employed as additives to a wide variety of food products, where they serve as thickening, stabilizing, emulsifying or gelling agents. EPS produced by lactic acid bacteria, which carry the GRAS status, are used in situ to improve body and texture of fermented products. It has been reported that most lactic acid bacteria produce a small amount of EPS [91]. By incorporating polymer encapsulated bacteria it may be possible to not only increase viability but also improve viscosity/gel properties of yoghurt. Microencapsulation enhanced the survival of probiotic cultures compared to free cells in yogurts stored over 7 weeks [92].

Microencapsulation is a process in which the cells are retained within an encapsulating membrane to reduce cell injury or cell loss. The encapsulation techniques applied to probiotics for the use in fermented milk products or biomass production can be classified into 2 groups, depending on the method used to form the beads: extrusion (droplet method) and emulsion or two phase system. Both extrusion and emulsion techniques increase the survival of probiotic bacteria by up to 80–95% [93-96]. **Extrusion technique:** Extrusion is the oldest and most common approach to making capsules with hydrocolloids [83]. It simply involves preparing a hydrocolloid solution, adding microorganisms to it, and extruding the cell suspension through a syringe needle in the form of droplets to free-fall into a hardening solution or setting bath



Figure 1: Flow diagram of encapsulation of bacteria by the extrusion and emulsion techniques.

(Figure 1). The size and shape of the beads depend on the diameter of the needle and the distance of free-fall, respectively. The size of the beads can vary between 2-5 mm. This method is the most popular due to its ease, simplicity, low cost, and gentle formulation conditions ensuring high retention of cell viability.

**Emulsion technique:** In this technique, a small volume of the cellpolymer suspension is added to a large volume of a vegetable oil such as soybean oil, sunflower oil, canola oil or corn oil. The mixture is homogenized to form a water-in-oil emulsion. Once the water-in-oil emulsion is formed, the water-soluble polymer must be insolubilized (cross-linked) to form tiny gel particles within the oil phase (Figure 1). The smaller the internal phase particle size of the emulsion, the smaller the final micro particles will be. The insolubilization method chosen depends on the type of supporting material used. The beads are harvested later by filtration. The size of the beads is controlled by the speed of agitation, and can vary between  $25\mu$ m and 2 mm. This technique has been used successfully to encapsulate lactic acid bacteria for batch and continuous fermentation [97,98].

The traditional method of preparing calcium alginates was by adding calcium chloride drop wise to sodium alginate solution; however, other methods have been used for specific application. Ca-EDTA can be used in place of calcium chloride to provide the calcium ions for gelation [99]. Also water/oil emulsion of alginate (3%) and culture mixture can be added drop wise to vegetable oil containing 0.2% Tween 80 [100]. The capsules are then hardened with calcium in the form of calcium chloride (Table 1). The beads can be collected by centrifugation and washed with sterile water. This method has been used for the preservation of probiotic bacteria in dairy products [94-96]. This method can be used in the reverse order, first mixing the cells with sterile calcium chloride solution and dropping this cell suspension into 0.6% sodium alginate solution emulsified with 0.1% Tween 20. The excess sodium alginate can be removed by washing with sterile saline and the capsules left to harden in a 1% calcium chloride solution [101,102]. In Figure 2 scanning electron

 Table 1: Strains of major probiotic bacteria with published clinical data.

Strain	Clinical application(s)		
Lactobacillus rhamnosus GG (Valio)	Lactose intolerance, immune response modulation, Helicobacter pylori, bacterial vaginitis, atopic eczema and food allergy, rotaviral diarrhea, antibiotic-associated diarrhea, traveller's diarrhea, other acute bacterial diarrhea		
Lactobacillus paracasei Shirota (Yakult)	Lactose intolerance, bladder cancer, chronic constipation, immune response modulation, antibiotic-associated diarrhea, other acute bacterial diarrhea		
Bb12 Bifidobacterium lactis (Chr. Hansen)	Lactose intolerance, immune response modulation, Clostridium difficile pseudo-membranous colitis, chronic constipation, immune response modulation, chronic constipation, antibiotic-associated diarrhea, traveler's diarrhea, rotaviral diarrhea, other acute bacterial diarrhea		
Saccharomyces cereviseae boulardii (Biocodex)	Chronic constipation, bacterial vaginitis, Clostridium difficile pseudo-membranous colitis, antibiotic-associated diarrhea, traveler's diarrhea, other acute bacterial diarrhea		
Lactobacillus acidophilus La5 (Chr. Hansen)	Lactose intolerance, chronic constipation, antibiotic-associated diarrhea, traveler's diarrhea, other acute bacterial diarrhea		
Lactobacillus johnsonii La1 (Nestle)	Lactose intolerance, chronic constipation, Helicobacter pylori, immune response modulation		
Enterococcus faecium SF68 (Cernelle)	Cholesterol, irritable bowel syndrome, antibiotic-associated diarrhea, other acute bacterial diarrhea		
Lactobacillus reuteri (Biogaia)	Rotaviral diarrhea, other acute bacterial diarrhea		
Bifidobacterium longum BB536 (Morinaga)	Antibiotic-associated diarrhea, chronic constipation		
Bifidobacterium breve (Yakult)	Other acute bacterial diarrhea		
Lactobacillus acidophilus NFCM (Rhodia, US)	Lactose intolerance, other acute bacterial diarrhea		
Lactobacillus plantarum 299v (ProViva, Sweden)	Irritable bowel syndrome, other acute bacterial diarrhea		
Lactobacillus acidophilus LB (Lacteol)	Rotaviral diarrhea, antibiotic-associated diarrhea		
Lactobacillus rhamnosus GR-1 (Urex Biotech.)	Uro-genital infections		
Lactobacillus fermentum RC-14 (Urex Biotech.)	Uro-genital infections		



 Table 2: Encapsulation of probiotic bacteria by emulsion technique for use in Ice

 Cream and Frozen Desserts.

Bacteria	Bead size (diameter)	Application	Reference
Lactobacillus delbrueckii spp. bulgaricus	-	Ice Cream	[94]
Lactobacillus delbrueckii spp. bulgaricus	30µm	Frozen Dessert	[94]
Lactobacillus delbrueckii spp. bulgaricus	25-35µm	Frozen Ice Milk	[95]
Bifidobacterium bifidum Bifidobacterium infantis	-	Ice Milk	[97]
Lactobacillus acidophilus Bifidobacterium lactis	0.5-1mm	Ice Cream	[103]

photomicrograph of microcapsules is shown [79]. Table 2 represents encapsulation of probiotic bacteria by emulsion technique for use in ice cream and frozen dairy desserts.

#### References

- MR Gismondo, Drago, A Lombardi (1999) Review of probiotics available to modify gastrointestinal flora. International Journal of Antimicrobial agents 12: 287-292.
- YH Hui (2004) Handbook of Food and Beverage Fermentation Technology. Marcel Decker Inc New York USA.
- CP Champagne, RP Ross, M Saarela, KF Hansen, D Charalampopoulos (2011) Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. International journal of food microbiology 149: 185-193.
- WJ Scott (2002) Probiotics, prebiotics, and synbiotics. Journal of Equine Veterinary Science 22: 357-360.
- Hekmat, DJ McMahon (1992) Survival of Lactobacillus and Bifidobacterium bifidum in ice cream for use as a probiotic food. Journal of Dairy Science 75: 1415-1422.
- RH Davidson, SE Duncan, CR Hackney, WN Eigel, JW Boling (2000) Probiotic culture Survival and implications in fermented frozen yogurt characteristics. Journal of Dairy Science 83: 666-673.
- C Alamprese, R Foschino, M Rossi, C Pompei, L Savani (2002) Survival of Lactobacillus johnsonii La1 and influence of its addition in retail-manufactured ice cream produced with different sugar and fat concentrations. International Dairy Journal 12: 201-208.
- 8. GR Gibson, HM Probert, JV Loo, RA Rastall, MB Roberfroid (2004) Dietary

modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research reviews 17: 259-275.

- GR Gibson, MB Roberfroid (1998) Dietary modulation of the human colonic microbiota: Introduction to the concept of probiotics. Journal of Nutrition 125: 1401-1412.
- JK Collins, G Thornton, GO Sullivan (1998) Selection of probiotic strains for human applications. International Dairy Journal 8: 487-490.
- 11. HS Ejtahed, J Mohtadi-Nia, A Homayouni-Rad, M Niafar, M Asghari-Jafarabadi, et al. Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. J Dairy Sci 94: 3288-3294.
- Homayouni Rad A, Vaghef Mehrabany E, Alipoor B, Vaghef Mehrabany L, Javadi M (2012) Do probiotics act more efficiently in foods than in supplements? Nutrition 28: 733-736.
- HS Ejtahed, J Mohtadi-Nia, A Homayouni-Rad, M Niafar, M Asghari-Jafarabadi, et al. (2002) Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 28: 539-543.
- NP Shah (2007) Functional cultures and health benefits. International dairy journal 17: 1262-1277.
- Mai V, Draganov PV (2009) Recent advances and remailning gaps in our knowledge of associations between gut microbiota and human health. World J Gastroenterol 15: 81-85.
- HS Lye, CY Kuan, JA Ewe, WY Fung, MT Liong (2009) The improvement of hypertension by probiotics: effects on chelesterol, diabetes, renin and phytoesterogens. International Journal of Dairy Sciences 10: 3755-3775.
- SJ Allen, EG Martinez, GV Gregorio, LF Dans (2010) Probiotics for treating acute infectious diarrhea. Chocrane Collaboration 11: 1-124.
- HS Ejtahed, A Homayouni (2010) Effects of Probiotics on the Prevention and Treatment of Gastrointestinal Disorders. Microbial biotechnological journal of Islamic Azad University 2: 53-60.
- S Lebeer, J Vanderleyden, SCJ Keersmaecker (2010) Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. Nature Reviews Microbiology 8: 171-184.
- NM Roos, MB Katan (2000) Effects of probiotic bacteria on diarrhea, lipid metabolism and carcinogenesis: a review of papers published between 1988 and 1998. American journal of clinical nutrition 71: 405-411.
- HW Modler, RC Mckellar, HD Goff, DA Mackie (1990) Using ice cream as a mechanism to incorporate Bifidobacteria and Fructooligosaccharides into the human diet. Cultured Dairy Products Journal 25: 4-9.
- S Laroia, JH Martin (1991) Effect of pH on survival of Bifidobacterium bifidum and Lactobacillus acidophilus in frozen fermented dairy desserts. Cultured Dairy Products Journal 26: 13-21.
- P Dinakr, VV Mistry (1994) Growth and viability of Bifidobacterium bifidum in Cheddar cheese. Journal of Dairy Science 77: 2854-2864.
- 24. AH Varnam (1994) JP Sutherland: Milk and milk products: Technology. Chemistry and Microbiology Chapman and Hall London UK.
- 25. AMP Gomes, FX Malcata (1999) Bifidobacterium spp and Lactobacillus acidophilus: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trend in Food Science and Technology 10: 139-157.
- 26. C Stanton, G Gardiner, PB Lynch, JK Collins, G Fitzgerald, et al. (1998) Probiotic cheese. International Dairy Journal 8: 491-496.
- 27. RDCS Randheera, SK Baines, MC Adams (2010) Importance of food in probiotic efficacy. Food Research International 43: 1-7.
- RR Ravula, NP Shah (1998) Effect of acid casein hydrolyzates and cysteine on the viability of yogurt and probiotic bacteria in fermented frozen dairy desserts. Australian Journal of Dairy Technology 53: 174-179.
- PS. Christiansen, D Edelsten, JR Kristiansen, EW Nielsen (1996)\_Some properties of ice cream containing Bifidobacterium bifidum and Lactobacillus acidophilus. Milschwissenschaft 51: 502-504.

- IN Haynes, MJ Playne (2002) Survival of probiotic cultures in low-fat ice cream. The Australian Journal of Dairy Technology 57: 10-14.
- A Kasimoglu, M Goncuoglu, S Akgun (2004) Probiotic white cheese with Lactobacillus acidophilus. International Dairy Journal 14: 1067-1073.
- 32. AR Madureiraa, CI Pereiraa, K Truszkowskab, AM Gomesa, ME Pintadoa, et al. (2005) Survival of probiotic bacteria in a whey cheese vector submitted to environmental conditions prevailing in the gastrointestinal tract. International Dairy Journal 15: 921-927.
- A Samona, RK Robinson (1994) Effect of yoghurt cultures on the survival of Bifidobacteria in fermented milks. Journal of the Society of Dairy Technology 47: 58-60.
- S Rybka, K Kailasapathy (1995) The survival of culture bacteria in fresh and freeze-dried AB yoghurts. Australian Journal of Dairy Technology 50: 51-57.
- 35. NP Shah, WVE Lankaputhra, ML Britz, WSA Kyle (1995) Survival of Lactobacillus acidophilus and Bifidobacterium bifidum in commercial yoghurt during refrigerated storage. International Dairy Journal 5: 515-521.
- RI Dave, NP Shah (1997) Effect of level of starter culture on viability of yoghurt and probiotic bacteria in yoghurts. Food Australia 49: 164-168.
- K. Kailasapathy, S. Rybka, Lactobacillus acidophilus and Bifidobacterium spp.-their therapeutic potential and survival in yoghurt. Australian Journal of Dairy Technology 52 (1997) 28-35.
- WEV Lankaputhra, NP Shah (1997) Improving viability of Lactobacillus acidophilus and Bifidobacteria in yoghurt using two step fermentation and neutralized mix. Food Australia 49: 363-366.
- S Rybka, K Kailasapathy (1998) Effect of freeze drying and storage on the microbiological and physical properties of AB-yoghurt. Milschwissenschaft 52: 390-395.
- 40. RI Dave, NP Shah (1998) Ingredient supplementation effects on viability of probiotic bacteria in yogurt. Journal of Dairy Science 81: 2804-2816.
- 41. K Kailasapathy, J Chin (2000) Survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp. Immunology and Cell Biology 78: 80-88.
- AC Ouwehand, PV Kirjavainen, C Shortt, SJ Salminen (1999) Probiotics: mechanisms and established effects. International Dairy Journal 9: 43-52.
- HS Chung, YB Kim, SL Chun, GE Ji (1999) Screening and selection of acid and bile resistant Bifidobacteria. International Journal of Food Microbiology 47: 25-32.
- 44. DB Hughes, DG Hoover (1995) Viability and enzymatic activity of Bifidobacteria in milk. Journal of Dairy Science 78: 268-276.
- WEV Lankaputhra, N.P. Shah, M.L. Britz, Evaluation of media for selective enumeration of Lactobacillus acidophilus and Bifidobacterium species, Food Australia, 48(3) (1996) 113-118.
- 46. S Salminen, C Bouley, MC Boutron-Ruault, JH Cummings, A Franck, et al., (1998) Functional food science and gastrointestinal physiology and function. British Journal of Nutrition 80: 147-171.
- AS Naidu, WR Bidlack, RA Clemens (1999) Probiotic spectra of lactic acid bacteria (LAB). Critical Reviews in Food Science and Nutrition 39: 13-126.
- AC Ouwehand, SJ Salminen (1998) The health effect of cultured milk products with viable and non-viable bacteria. International Dairy Journal 8: 749-758.
- 49. JH Martin (1996) Technical considerations for incorporating Bifidobacteria and bifidogenic factors into dairy products. Bulletin of the IDF 313: 49-51.
- 50. S Shimamura, F Abe, N Ishibashi, H Miyakawa, T Yaeshima, et al., (1990) Endogenous oxygen uptake and polysaccharide accumulation in Bifidobacterium. Agricultural and Biological Chemistry 54: 2896-2874.
- 51. S Shimamura, F Abe, N Ishibashi, H Miyakawa, T Yaeshima, et al., (1992) Relationship between oxygen sensitivity and oxygen metabolism of Bifidobacterium species. Journal of Dairy Science 75: 3296-3306.
- 52. PA Clark, LN Cotton, JH Martin (1993) Selection of Bifidobacteria for use as dietary adjuncts in cultured dairy foods: II-Tolerance to stimulated pH of Human stomachs. Cultured Dairy Products Journal 28: 11-14.

- WEV Lankaputhra, NP Shah (1995) Survival of Lactobacillus acidophilus and Bifidobacterium spp. in the presence of acid and bile salts. Cultured Dairy Products Journal 30: 2-7.
- 54. K Kailasapathy, D Supriadi (1996) Effect of whey protein concentrate on the survival of Lactobacillus acidophilus in lactose hydrolyzed yoghurt during refrigerated storage. Milschwissenschaft 51: 565-568.
- 55. K Kailasapathy, D Supriadi, JA Hourigan (1996) Effect of partially replacing skim milk powder with whey protein concentrate on buffering capacity of yoghurt. Australian Journal of Dairy Technology 51: 89-93.
- 56. RI Dave, NP Shah (1997) Effect of Cysteine on the viability of yoghurt and probiotic bacteria in yogurts made with commercial starter cultures. International Dairy Journal 7: 537-545.
- NP Shah (2000) Probiotic Bacteria: Selective enumeration and survival in dairy foods. Journal of Dairy Science 83: 894-907.
- RD Sleator, C Hill (2008) New frontiers in probiotic research. Letters in applied microbiology 46: 143-147.
- M Bazrafshan, A Homayouni (2010) Effect of environmental stress factors on the survival of Lactobacillus acidophilus: A review. Microbial Biotechnology Journal of Islamic Azad University 2: 47-56.
- 60. A Homayouni, MR Ehsani, A Azizi, SH Razavi, MS Yarmand (2008) Growth and survival of some probiotic strains in simulated ice cream conditions. Asian Network for Scientific Information 8: 379-382.
- 61. D Supriadi, K Kailasapathy, JA Hourigan (1994) Effect of partial replacement of skim milk powder with whey protein concentrate on buffering capacity of yogurt. Book of abstracts of the 24th International Dairy Congress Melbourne Australia 418.
- 62. A Gopal, NP Shah, H Roginski (1996) Bile tolerance, taurocholate deconjugation and cholesterol removal by Lactobacillus acidophilus and Bifidobacterium spp. Milschwissenschaft 51: 619-623.
- BR Goldin (1998) Health benefits of probiotics. British Journal of Nutrition 80: 203-207.
- 64. MV Tejada-Simon, JH Lee, Z Ustunol, JJ Pestka (1999) Ingestion of yoghurt containing Lactobacillus acidophilus and Bifidobacterium to potentiate immunoglobulin A responses to cholera toxin in mice. Journal of Dairy Science 82: 649-660.
- 65. X Wang, IL Brown, AJ Evanc, PL Conway (1999) The protective effects of high amylase maize (amylomaize) starch granules on the survival of Bifidobacterium spp. in the mouse intestinal tract. Journal of Applied Microbiology 87: 631-639.
- A Homayouni (2011) Selection of an appropriate probiotic strain for producing functional ice cream. Microbial Biotechnology Journal of Islamic Azad University 3: 49-56.
- RI Dave, NP Shah (1996) Evaluation of media for selective enumeration of S. thermophilus, L. delbrueckii ssp. Bulgaricus, L. acidophilus and Bifidobacteria. Journal of Dairy Science 79: 1529-1536.
- 68. Ingham SC (1999) Use of modified Lactobacillus selective medium and Bifidobacterium iodoacetate medium for different enumeration of Lactobacillus acidophilus and Bifidobacterium spp. in powdered nutritional products. Journal of Food Protection 62: 77-80.
- 69. Rybka, Kailasapathy K (1996) Media for the enumeration of yoghurt bacteria. International Dairy Journal 6: 839-850.
- Payne JF, Morris AEJ, Beers P (1999) Evaluation of selective media for the enumeration of Bifidobacterium spp. in milk. Journal of Applied microbiology 86: 353-358.
- Homayouni MR, Ehsani A Azizi, Razavi SH, Yarmand MS (2008) Spectrophotometricaly Evaluation of Probiotic Growth in Liquid Media. Asian Journal of Chemistry 20: 2414-2420.
- Mitsuka T (1992) Role of intestinal flora in health with special reference to dietary control of intestinal flora. In: Microbiology Applications in Food Biotechnology, B.H. Nga, Y.K. Lee (Eds.), Elsevier Applied Science Michigan USA 135-140.
- A Alizadeh, A Homayouni, MR Ehsani (2008) Probiotic Survival in Yogurt Made from Ultrafiltered Skim Milk During Refrigeration Storage. Research Journal of Biological Sciences 3: 1163-1165.

Citation: Maleki D, Azizi A, Vaghef E, Balkani S, Homayouni A (2015) Methods of Increasing Probiotic Survival in Food and Gastrointestinal Conditions. Prensa Med Argent 101:4.

- K Sakai, C Mishinama, T Tachiki, H Kumagai, T Toichikura (1987) Mortality of bifidobacteria in boiled yoghurt. Journal of Fermentation Technology 65: 215-220.
- T Tanaka, K Hatanaka (1992) Application of hydrostatic pressure to yogurt to prevent its after acidification. Journal of the Japanese Society of Food Science and Technology 9: 73-77.
- VM Marshall (1992) Inoculated ecosystems in a milk environment. Journal of Applied Bacteriology Symposium Supplement 73: 127-135.
- NP Shah, WEV Lankaputhra (1997) Improving viability of L. acidophilus and Bifidobacterium spp. in yoghurt. International Dairy Journal 7: 349-356.
- AG Zavaglia, G Kociubinski, AG Perez (1998) Isolation and characterization of Bifidobacterium Strains for probiotic formulation. Journal of Food Protection 61: 865-873.
- K Sultana, G Godward, N Reynolds, R Arumugaswamy, P Peiris, et al. (2000) Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. International Journal of Food Microbiology 62: 47-55.
- ME Stiles, WH Holzapfel (1997) Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol 36: 1-29.
- GW Tannock (1992) Genetic manipulation of gut microorganisms. In: Probiotics: The scientific basis. R. Fuller Chapman and Hall London UK.
- 82. AH King (1995) Encapsulation of food ingredients: a review of available technology, focusing on hydrocolloids. In: Encapsulation and controlled release of food ingredients. SJ Risch, GA Reineccius (Eds.) American Chemical Society Washington USA 26-39.
- 83. BR Bhandari, BR D'Arcy (1996) Microencapsulation of flavor compounds. Food Australia 48: 549-551.
- 84. J Dziezak (1988) Microencapsulation and encapsulated ingredients. Food Technology 42: 136-151.
- HHY Kim, IC Baianu (1991) Novel liposome microencapsulation techniques for food applications. Trends for Food Science and Technology 2: 55-61.
- W Krasaekoopt, B Bhandari, H Deeth (2002) Review: Evaluation of encapsulation techniques of probiotics for yogurt. International Dairy Journal 13: 3-13.
- G Godward, K Kailasapathy (2003) Viability and survival of free, encapsulated and co-encapsulated probiotic bacteria in yoghurt. Milk Science International (Milschwissenschaft) 58: 396-399.
- K Adhikari, A Mustapha, I., Grun, L. Fernando, Viability of microencapsulated bifidobacteria in set yogurt during refrigerated storage. Journal of Dairy Science 83 (2000) 1946-1951.

- 89. A Picot, C Lacroix (2004) Encapsulation of bifidobacteria in whey protein based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. International Dairy Journal 14: 505-515.
- A Homayouni, A Azizi, MR Ehsani, MS Yarmand, SH Razavi (2008) Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. Food Chemistry 111: 50-55.
- L De Vuyst, B Degeest (1999) Heteroploysaccharides from lactic acid bacteria. FEMS Microbiology Reviews 23: 153-177.
- 92. K Kailasapathy (2006) Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. Food Science and Technology 39: 1221-1227.
- P Audet, C Paquin, C Lacroix (1988) Immobilized growing lactic acid bacteria with k-carrageenan-locust bean gum gel. Applied Microbiology and Biotechnology 29: 11–18.
- 94. TY Sheu, RT Marshall (1991) Improving culture viability in frozen dairy desserts by microencapsulation. Journal of Dairy Science 74: 102-107.
- 95. TY Sheu, RT Marshall (1993) Microentrapment of Lactobacilli in calcium alginate gels. Journal of Food Science 54: 557-561.
- 96. TY Sheu, RT Marshall, H Heymann (1993) Improving survival of culture bacteria in frozen desserts by microentrapment. Journal of Dairy Science 76: 1902-1907.
- KMK Kebary, SA Hussein, RM Badawi (1998) Improving viability of Bifidobacteria and their effect on frozen ice milk. Egyptian Journal of Dairy Science 26: 319-337.
- 98. P Audet, C Lacroix, C Paquin (1992) Continuous fermentation of a supplemented whey permeate medium with immobilized Streptococcus salivarius ssp. Thermophilus. International Dairy Journal 2: 1-15.
- U N Muramatsu, T Kondo (1998) An approach to prepare microparticles of uniform size. Journal of Microencapsulation 12: 129-136.
- 100.ME Sanders (1999) Probiotics. Food Technology 53: 67-75.
- 101. IK Yoo, GH Seong, HN Chang, JK Park (1996) Encapsulation of Lactobacillus casei cells in liquid-core alginate capsules for lactic acid production. Enzyme and Microbial Technology 19: 428-433.
- 102.A Homayouni, MR Ehsani, A Azizi, MS Yarmand, SH Razavi (2007) Effect of Lecithin and Calcium Chloride Solution on the Microencapsulation Process Yield of Calcium Alginate Beads. Iranian Polymer Journal 16: 597-606.
- 103. K Kailasapathy, K Sultana (2003) Survival and β-D-galactosidase activity of encapsulated and free Lactobacillus acidophilus and Bifidobacterium lactis in ice cream. The Australian Journal of Dairy Technology 58: 223-227.

## Author Affiliations

<sup>1</sup>Hematology and Oncology ward, Urmia University of Medical Sciences, Urmia, Iran

<sup>2</sup>Department of Food Engineering, Ministry of Jihad Agriculture, Karaj, Iran <sup>3</sup>Department of Nutrition, Faculty of Nutrition, Tabriz University of Medical Sciences, Iran

<sup>4</sup>Faculty of chemical Engineering, Kish Pardis, Tehran University, Kish, Iran <sup>5</sup>Associate professor, Department of Food Science and Technology, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran

Тор