Microencapsulation of Probiotic Bacteria

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Abstract The global popularity of functional foods containing probiotics has generated increased interest in developing protective materials for the bacteria during food processing, storage and consumption. To confer beneficial effects to the host, a probiotic product should contain at least 10^6 CFU/g or ml of the product. Probiotic microorganisms are sensitive to food processing environments and to conditions in the gastrointestinal tract. Microencapsulation technology can be used to protect the probiotic bacteria against adverse conditions. This chapter discusses the potential of using various microencapsulation techniques to protect probiotic bacteria.

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1 Introduction

The interest in using live probiotics for health maintenance and disease prevention through their incorporation into foods has been continuously increasing in the food industry (Ouwehand et al. 2002; Picot and Lacroix 2003). *Bifidobacterium* and *Lactobacillus* species have been the focus of probiotic interest since the presence of a large population of these bacteria in the gastrointestinal (GI) tract is indicative of a healthy microbiota (Crittenden et al. 2001; Picot and Lacroix 2003). However, some studies have shown poor viability of probiotic bacteria in functional foods when exposed to intrinsic and extrinsic factors such as moisture, oxygen, light, relative humidity, product composition and heat during food processing and storage (Kailasapathy 2002; Ding and Shah 2009; Peighambardoust et al. 2011). A significant proportion of probiotics is destroyed by high stomach and bile acids during their passage through the GI tract (Sultana et al. 2000; Krasaekoopt et al. 2003). Since probiotics are required at specific target sites in the host, it is therefore essential that probiotics are able to withstand the host's natural barriers against the ingested bacteria and remain viable (Kailasapathy and Chin 2000; Kailasapathy 2002; Picot and Lacroix 2003, 2004). In order for probiotics to confer beneficial effects on human health by improving the balance of intestinal microflora, high levels of viable cells are recommended in probiotic foods for efficacy (Gardiner et al. 2002). According to the FAO/WHO (2002), the level of probiotic bacteria required in a food product at consumption should be at least $>10^6$ cells/g or ml.

Microencapsulation is a potential technology that protects sensitive lactic cultures against harsh conditions during their transit through the GI tract of the host (Kailasapathy 2002; Mortazavian et al. 2007; Solanki et al. 2013). Encapsulating live cells within a shell material can minimize cell injury and enhance cell viability. Different encapsulation methods such as spray-drying, spray freezing, extrusion, emulsion and fluidized bed coating have been successfully used to encapsulate probiotics using various materials to protect them and extend their shelf life.

2 Probiotic Bacteria

Probiotic bacteria can be defined as live microorganisms that are beneficial to human health by positively influencing the intestinal microbial balance (Crittenden et al. 2001; Ananta et al. 2005). Previous studies have demonstrated numerous beneficial effects of probiotics in human health, including the prevention of diarrhoea caused by certain pathogenic bacteria and viruses (Andersson et al. 2001; Isolauri 2001). The term "probiotic" includes a large range of microorganisms mainly bacteria, but yeasts are also included (Ouwehand et al. 2002; Mortazavian et al. 2007). With the ability to remain alive until they reach the colon and provide beneficial effects to the host, selected lactic acid bacteria, non-lactic acid bacteria and yeasts can all be considered as probiotics.

2.1 Types of Probiotic Microorganisms

Probiotics can be classified into three types, namely, lactic acid bacteria (LAB), non-lactic acid bacteria and yeasts. LABs are mainly gram-positive and usually live in anaerobic environments, but they can also grow under aerobic conditions. Bifidobacteria can grow at a pH range of 4.5–8.5, but the most important characteristic of this group of LAB is that they are strictly anaerobic. Other LABs such as *Lactococcus lactis*, non-lactic acid bacteria (e.g. *Escherichia coli* Nissle 1917 and *Enterococcus faecium*) and some yeasts (e.g. *Saccharomyces cerevisiae*, *Saccharomyces boulardii*) are considered as probiotics (Burgain et al. 2011). However, only strains classified as LAB are considered important in food and nutrition (Mortazavian et al. 2007). The characteristics and optimum growth conditions of probiotic cultures vary with different microorganisms.

2.2 Health Beneficial Roles of Probiotics

There is strong evidence that probiotics have the potential to exert beneficial effects to human health (Isolauri 2001; Isolauri et al. 2001; Kalliomäki et al. 2001; Ouwehand et al. 2002). The effects of probiotics are strain specific, and therefore, it is important to specify the genus and species of probiotic bacteria when claiming health benefits (Saarela et al. 2000). The probiotic health benefits may be due to various factors, such as the production of bacteriocins, competition for nutrients with pathogens and enhanced immune system (Isolauri 2001; Peighambardoust et al. 2011). However, the exert mechanisms of how probiotics confer health benefits to human health are still not well studied.

Probiotics play significant therapeutic roles in human nutrition (O'Riordan et al. 2001). The main therapeutic and health benefits of most probiotics include enhancing the immune system against intestinal infections; prevention of diarrheal diseases, colon cancer, hypercholesterolaemia and upper gastrointestinal tract diseases; and stabilization of gut mucosal barrier (Kailasapathy and Chin 2000; Isolauri 2001; Isolauri et al. 2001; Kalliomäki et al. 2001; Desmond et al. 2002; Ouwehand et al. 2002; Krasaekoopt et al. 2003). Probiotic bacteria not only compete with the growth of pathogens and suppress "unhealthy fermentations" in the human intestine, but also confer several beneficial effects on the host by improving its intestinal microbial balance (Kailasapathy and Chin 2000).

3 Microencapsulation Techniques

Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Anal and Stevens 2005). Encapsulation can be used for many applications in the food industry, including stabilization of materials, control of oxidative reaction and degradation, sustained and/or controlled release, masking flavours, colours or odours, extension of shelf life and protection of components against nutritional loss. A variety of different techniques are available that can be used for encapsulation including spray-drying, spray-congealing, fluidized bed coating, extrusion and coacervation (Anal and Singh 2007).

Microencapsulation techniques can be classified into encapsulation process and drying process (Solanki et al. 2013). In the encapsulation process, probiotics are commonly encapsulated by a method involving cell immobilization through their physical entrapment in polymeric networks, such as extrusion and emulsion. Immobilization of LAB can offer many advantages for biomass and metabolite production compared with free-cell systems, such as high cell concentration, reuse of biocatalysts, improved resistance to contamination and bacteriophage attack, enhancement of plasmid stability and resistance to washing out during continuous cultures as well as physical and chemical protection of cells (Champagne et al. 1994; Doleyres and Lacroix 2005). For food applications, cell entrapment in a food-grade porous gel matrix has been widely used.

In the dairy industry, immobilized cells can be utilized for continuous yoghurt fermentation and optimized continuous biomass production through re-harvest but are not directly incorporated in foods (Heidebach et al. 2009a). This could be because these microcapsules are not optimized towards the requirements for capsules intended to pass through the human GI tract. In the drying process for microencapsulation of microorganisms, spray-drying has been widely investigated, particularly for the preservation of LAB (Santivarangkna et al. 2007). The ideal cell culture for distribution is in a dry form, sufficiently active to produce rapid growth when added to foods and yet dormant that it can be stored for extended periods without losing its activity. This section describes the preparation of microcapsules entrapping probiotics by using cell immobilization techniques, such as emulsion and extrusion, and spray-drying.

3.1 Cell Immobilization for Microencapsulation

3.1.1 Emulsion Technique

The emulsion technique allows the encapsulation of probiotic living cells by using hydrocolloids (e.g. alginate, carrageenan and pectin) as encapsulating materials. The principle of this technique is based on the addition of a cell-polymer suspension

(e.g. alginate) into a vegetable oil (e.g. soybean oil, corn oil) to form a water-in-oil emulsion (Burgain et al. 2011). The emulsion containing small droplets is formed by agitating the mixture usually with a magnetic stirrer to form tiny gel particles within the oil phase. The solidified beads are then formed by adding a cross-linking agent (e.g. calcium chloride) to the solution while stirring. The calcium alginate gel beads formed can be further introduced into a second polymer solution to create a coating layer that provides added protection to the encapsulated cells (Burgain et al. 2011). An advantage of the emulsion technique for microencapsulation of probiotics is that the process is relatively easy to scale up and gives high survival rates of microbial cells (Burgain et al. 2011). However, the main disadvantage of this method is that it provides relatively large sizes of beads (Burgain et al. 2011) ranging from 200 to 1000 μm in diameter that can influence the texture and mouthfeel when added into food products (Capela et al. 2007).

The size of capsules or beads entrapping cells varies and is affected by various factors, such as the type, concentration and viscosity of wall material polymers used, and the shear stress and pressure applied. In most instances, hydrocolloids, such as alginate, κ-carrageenan, gellan gum and xanthan gum, have been used as matrix materials for the immobilization of probiotics using the emulsion technique. The high viscosities of the aqueous solutions of these polymers even at low concentrations hinder the formation of small droplets in the continuous oil phase, resulting in the formation of large gel beads. Some methods that could be employed in reducing the bead size is to use homogenization using mechanical devices such as high shear mixer and high pressure valve homogenizer that generate intensive disruptive forces. In a study by Capela et al. (2007), the effect of homogenization on the size of calcium alginate beads was demonstrated using different types of homogenizers (e.g. ultra-turrax, Silverson mixer and Avestin piston homogenizer). It was shown that homogenization induced the formation of small beads from 3 % (w/v) alginate solution with average diameters between 30 and 200 μm. However, the disadvantage of these processes involving homogenization can be its negative impact on the encapsulation efficiency and viability of probiotics as the study showed a significant loss in the survival of probiotics (35.6–94.5 %) due to the shearing stress. This was also affected by the type of homogenizers used. Individual species of probiotic microorganisms may vary in their sensitivity to external stresses, such as those encountered with high shear force and pressure during homogenization. It is therefore essential to ensure that the method and conditions selected for the microencapsulation process of probiotic microorganisms is gentle to sensitive probiotic microorganisms.

Novel encapsulation techniques based on emulsification and enzymatic cross-linking or cold gelation were also introduced for the delivery of heat-sensitive bioactives including probiotics using proteins as encapsulating matrix agents. Milk protein, such as sodium caseinate, was used for encapsulation of probiotic micro-organisms using similar principle and technique as described above, but gelation of caseins was induced by using enzymes (transglutaminase or rennet) during the emulsifying process (Heidebach et al. 2009a, b). The studies demonstrated small capsules lower than 200 μm in diameter being formed by gentle agitation using a

magnetic stirrer without the addition of extra emulsifiers or application of high shearing during the emulsifying process. This could be attributed to good emulsifying properties of milk proteins and the low viscosity of the milk concentrate regardless of having a high solid concentration of 35 % (Heidebach et al. 2009a). Cold gelation provides an alternative gel matrix development method based on adding cations to a preheated protein suspension (Maltais et al. 2005). With this method, a heating step is required where whey proteins are denatured and polymerized into soluble aggregates. This is followed by a cooling step and subsequent salt addition which results in the formation of a network via Ca^{2+}-mediated interactions of soluble aggregates (Roff and Foegeding 1996). The formation of cold-set gels opens interesting opportunities for whey proteins as carriers of heat-sensitive bioactive compounds (Chen and Subirade 2007).

3.1.2 Extrusion Technique

Extrusion can be used to encapsulate probiotic living cells and uses hydrocolloids (alginate and carrageenan) as encapsulating materials because of their ability to form gels under mild conditions. Extrusion is a simple and cheap method that uses a gentle operation which does not damage probiotic cells and gives high probiotic viability. With this method, the solution containing cells is projected through a nozzle at high pressure into a hardening solution (e.g. calcium chloride), and beads with a few millimetres in diameter are produced (Burgain et al. 2011). Extrusion leads to a relatively narrow particle size distribution which is determined by the geometry of the nozzle (Heidebach et al. 2009a). The inner diameter of the nozzle or openings influences the size of capsules formed (De Vos et al. 2010).

Large-scale droplet production can be achieved by multiple-nozzle systems, spinning disc atomizer or jet-cutter techniques (De Vos et al. 2010; Kailasapathy 2002). The advantage of extrusion technology is that it is in most cases a true encapsulation procedure and not just an immobilization technology (De Vos et al. 2010). Extrusion technology has many advantages for encapsulation of microbes. This technology does not involve the use of deleterious solvents or oils and can be conducted under both aerobic and anaerobic conditions. This is especially advantageous with anaerobic microorganisms by placing an extrusion device in a sterile cabinet where oxygen is substituted for nitrogen (De Vos et al. 2010).

3.1.3 Encapsulating Materials for Cell Immobilization

Entrapment of LAB in calcium alginate beads has been commonly used for cell immobilization (Rowley et al. 1999). Alginate is a naturally derived polysaccharide extracted from brown sea weeds which is composed of α-L-guluronic and β-D-mannuronic acids (Koo et al. 2001). The simple, mild aqueous-based gel formation of alginate is completed in the presence of divalent cations such as Ca^{2+} (Koo et al. 2001). Calcium alginate beads entrapping cells are made by extruding a

solution of alginate mixed with cells into a calcium chloride solution. Calcium chloride solution containing cells can also be dropped as tiny droplets into alginate solution while being stirred and then instantly wrapped with calcium alginate gel membrane. The characteristics of hydrogel membranes such as thickness, pore size, surface charge and mechanical strength can be fabricated by varying some processing conditions and ingredient formulations. These include reaction time for gelation, concentration of alginate and calcium ions and different types of gel-forming polymers (Nigam et al. 1988).

Encapsulation of bacteria in alginate gel can improve survival rates of bacteria by one log compared to free cells when stored in skim milk for 24 h (Rokka and Rantamaki 2010). Alginate gel cross-linked with calcium ions is preferred for encapsulating probiotics due to its simplicity, non-toxicity, biocompatibility and low cost (Krasaekoopt et al. 2003). However, some disadvantages may be attributed to its sensitivity to strong acidic environment which is not resistant to decomposition in the stomach conditions (Mortazavian et al. 2007). Calcium alginate capsules are also chemically unstable upon contact with cation chelating agents, such as phosphate, citrate and lactate, which can cause disruption or dissolution of alginate gel matrix (Castroa et al. 2009). In addition, the microparticles formed with alginate are porous which is a disadvantage for good protection of cells. Since alginate is anionic, cationic polymer coatings, such as polylysine, polyvinylamine and chitosan, have been used to increase the stability of alginate capsules or to minimize the loss of encapsulated material. However, these can be compensated by mixing alginate with starch that has been commonly used to improve the effectiveness of probiotic encapsulation (Sultana et al. 2000; Hansen et al. 2002; Krasaekoopt et al. 2003).

Gellan gum is a microbial polysaccharide derived from *Pseudomonas elodea*. It is composed of a repeating unit of tetrasaccharide consisting of glucose, glucuronic acid, glucose and rhamnose. Xanthan gum is also a microbial synthesized polysaccharide. A mixture of xanthan gum and gellan gum has been used to encapsulate probiotic cells, and contrary to alginate, the mixture presents high resistance towards acid conditions (Sultana et al. 2000).

κ-carrageenan is a natural polysaccharide extracted from marine macroalgae (e.g. red seaweeds). Elevated temperatures (60–80 °C) are required to dissolve the polymer at concentrations ranging from 2 % to 5 %. Cooling the mixture to room temperature can result in gelation, and the addition of potassium ions stabilizes the microparticles (Krasaekoopt et al. 2003). However, the produced gels tend to be brittle and are not able to withstand potential stresses. Locust bean gum, at a ratio of carrageenan to locust bean gum of 2:1, increases the strength of gels through specific interaction of galactomannan chains with carrageenan (Krasaekoopt et al. 2003).

Chitosan is a positively charged linear polysaccharide formed by the deacetylation of chitin extracted from crustacean shell. It is water soluble below pH 6 and forms a gel by ionotropic gelation. Chitosan, a polycation with amine groups, can be cross-linked by anions or polyanions, such as polyphosphate and polyaldehydrocarbonic acid. Chitosan has not shown good efficiency for increasing

cell viability but exhibited inhibitory effects on different types of LAB (Groboillot et al. 1993). To overcome viability problems with chitosan, the polymer is used as a coat for alginate beads to deliver the viable non-LAB cells to the colon.

Gelatin is a protein that is useful for making a thermo-reversible gel for probiotic encapsulation. Due to its amphoteric nature, it cooperates well with anionic polysaccharides like gellan gum. These hydrocolloids are miscible at pH > 6 since they both carry net negative charges and repel each other. However, when the pH is adjusted below the isoelectric point of gelatin, the net charge on the protein becomes positive, and this causes a strong interaction with the negatively charged gellan gum (Anal and Singh 2007; Krasaekoopt et al. 2003).

Milk proteins are natural vehicles for probiotic cells owing to their structural and physicochemical properties. Milk proteins are also widely available, inexpensive, natural and generally regarded as safe (GRAS) raw materials with high nutritional value and good sensory properties. They have many different structural properties and functionalities which make them highly suitable as vehicles for delivering various bioactives. Milk proteins such as caseins have excellent gelation properties (Heidebach et al. 2009a, b) that can be caused by micelle aggregation, based on their isoelectric precipitation and the proteolytic cleavage of κ-casein's hydrophilic "hairy layer" from casein micelles. This process is effective for the encapsulation of probiotic bacteria. Milk proteins also have excellent buffering capacity which provides good shielding for probiotic microorganisms against the harsh environment in the stomach.

3.2 Spray-Drying of Probiotics

Drying of bacterial cultures without losing their activity has been investigated to convert them into a dry state and facilitate storage and transportation (Anal and Singh 2007). Large-scale production of freeze-dried cultures is an expensive process with low yields. However, spray-drying represents a good low-cost alternative yielding higher production rates (Meng et al. 2008). Spray-drying of freshly prepared probiotic cell concentrates in various protein solutions with and without carbohydrates (maltodextrin, oligosaccharides, hydrocolloids) is widely used for the entrapment and drying of probiotic microorganisms in a single step (Corcoran et al. 2004; Desmond et al. 2001; Gardiner et al. 2000),whereas water-based dispersions are usually applied to spray-drying probiotics in water-soluble polymer matrices (De Vos et al. 2010).

Spray-drying is defined as the removal of water by vaporization from a solution of a non-volatile solid (Santivarangkna et al. 2007). The spray-drying process involves the injection of spray-drying medium at high velocities into the direction of the flow of hot air (typically, 150–200 °C). The atomized droplets have a very large surface area in the form of millions of micrometre-sized droplets (10–200 μm), which result in a very short drying time when exposed to hot air in the drying chamber (Santivarangkna et al. 2007). Water-based dispersions are usually

applied in spray-drying. Therefore, the matrix should have very high solubility in water. The microencapsulation can be achieved with biopolymers of various sources; however, typical wall materials for microencapsulation by spray-drying are low molecular weight carbohydrates, milk or soy proteins, gelatin and hydrocolloids like gum arabic.

Ananta et al. (2005) reported the application of spray-drying in the production of skim milk-based preparations (20 % w/v) containing *L. rhamnosus* GG (ATCC 53103). Using a range of outlet temperatures between 70 and 100 °C, bacteria survival rate of >60 % was achieved at an outlet temperature of 80 °C (Ananta et al. 2005). Similarly, another study by Corcoran et al. (2004) showed good survival rates of 25–41 % with ~10^9 CFU/g of *L. rhamnosus* E800 when subjected to spray-drying conditions at inlet temperature of 170 °C and outlet temperature of 85–90 °C. Similarly, Gardiner et al. (2000) evaluated the potential of 20 % skim milk in producing powders with *L. paracasei* NFBC 338. After subjecting the slurry to spray-drying conditions at outlet temperatures between 80 and 85 °C, 65 % of the cells retained viability. The probiotic viability was further assessed for over a period of two months where the maximum survival rates for *L. paracasei* NFBC 338 of 92 % of the initial cells remained viable. Carbohydrates are also used as encapsulating wall materials including gum arabic and starches because they tend to form spherical microparticles during the drying process (De Vos et al. 2010).

Spray-drying is rapid and relatively low cost. This technique is also highly reproducible and also suitable for industrial applications. However, the major hurdle of this technique is that it is an immobilization technology than an encapsulation technology which implies that some of the encapsulating core materials may be exposed on the surface of spray-dried microcapsules. This is especially problematic when considering the encapsulation of probiotics, where the bacteria may leak into the product when some hydration occurs.

Bifidobacteria are sensitive to high inlet temperatures (O'Riordan et al. 2001). Thus, it is necessary to investigate the sensitivity of probiotics before spray-drying. Protectants, such as trehalose, can be used to improve the survival of probiotics and reduce the deleterious effect of bile salts present in the acidic environment of stomach (Burgain et al. 2011).

The performance of a variety of probiotics during spray-drying and, in general, the survival rate of probiotic cultures depend on several factors, such as type of probiotic strains, inlet and outlet temperatures and drying medium. The tolerance of different bacterial species varies with spray-drying conditions. However, improved viability could be achieved by maintaining spray-dried powders at preferred moisture of ~3.5 % (Teixeira et al. 1995a).

3.2.1 Effect of Spray-Drying Conditions on Survival of Bacteria

The survival of probiotic cells during spray-drying can be affected by various factors relating to process parameters (inlet and outlet temperatures, drying time), product parameters (type of carrier medium and concentrations) and pretreatments

of cells (bacterial strain) (Desmond et al. 2001; O'Riordan et al. 2001; Lian et al. 2002).

Inlet and Outlet Temperatures

In order to enhance the efficiency of microencapsulation process with a suitable encapsulating wall material, optimal spray-drying conditions must be used. The main factors in spray-drying that need to be optimized are feed temperature, air inlet temperature and air outlet temperature. Feed temperature modifies the viscosity and fluidity of feed solutions, thus resulting in alteration in their capacity to be homogeneously sprayed (Fang and Bhandari 2012). When the feed temperature is increased, the size of droplets being sprayed in the dryer decreases due to a decrease in the feed viscosity, but high temperatures can cause degradation of some heat-sensitive ingredients to be encapsulated (Gharsallaoui et al. 2007; Medina-Torres et al. 2013). Air inlet temperature is directly related to the drying rate of droplets and the final water content of particles. High inlet temperatures increase the rate of droplet drying, thus facilitating rapid drying inside the drying chamber and leading to shorter residence times for the particles being dried (Bhandari et al. 2008). The inlet and outlet temperatures used during spry-drying vary, depending on the type and purpose of products being spray-dried. In food applications, high inlet (160–300 °C) and low outlet air temperatures (60–100 °C) are used to achieve the high thermal efficiency of the drier (Bhandari et al. 2008).

An increase in air inlet temperature normally decreases cell viability, but the bacterial cell survival is highly correlated to the outlet temperature (Peighambardoust et al. 2011). This means that the higher inlet temperature does not have a direct correlation to the inactivation of bacterial cells and has only a slight effect. This is due to the fact that the extent of cell inactivation is largely dependent on the drying temperature–time combinations. During spray-drying, the temperature of spray-dried particles increases but does not reach the inlet air temperature because of an evaporative cooling effect that occurs owing to the instant removal of moisture, and the subsequent exposure time of dried particle to the high temperature is very short (Fichtali and Namal Senanayake 2010). Therefore, it is important to maintain an optimum drying time (residence time) required for the removal of moisture without causing an increase in temperature of the dried particles for the survival of encapsulated bacterial cells during spray-drying (Santivarangkna et al. 2007).

In addition to the inlet air temperature, the outlet air temperature is another major drying parameter affecting the viability of spray-dried starter cultures (Gardiner et al. 2000, 2002). However, it is largely influenced by the inlet air temperature, air-flow rate, product-feed rate and the atomized droplet sizes. These factors highlight the importance of optimization of process parameters, in particular, inlet and outlet temperatures. Due to the difficulty in setting these variables and in turn the stabilization of outlet air temperature, there is often a great variation in the viability of the dried cultures (Ananta et al. 2005; Desmond et al. 2002).

Gardiner et al. (2000) reported that the survival rates for *L. paracasei* NFBC 338 were affected by outlet temperatures. It was 97 % at 70–75 °C, while the survival rate was close to 0 % when the outlet temperature was increased to 120 °C. The survival rates were better than for *L. salivarius* UCC 118 that had only 11 % even at the lowest outlet temperatures of 60–65 °C. These findings might be attributed to the greater thermal tolerance of strain NFBC 338. The survival rate of NFBC 338 during spray-drying was also considerably higher than the survival rate previously obtained for *L. acidophilus* or *L. curvatus* spray-dried under similar conditions. In many situations, the lower outlet air temperature correlates with higher cell viability (Santivarangkna et al. 2007). A low outlet air temperature is desirable to maintain high stability during storage. However, if the outlet air temperatures are too low, it may cause high residual moisture contents that exceed the required level for prolonged powder storage life and stability (4 %) (Gardiner et al. 2000).

Carrier Medium and Concentration

The use of different carriers has an impact on the viability of spray-dried cultures. In a study by Lian et al. (2002), the effect of different carriers (10 %, w/w) on the survival of various *Bifidobacterium* strains was demonstrated. The survival rate of *B. infantis* CCRC 14633 after spray-drying at inlet and outlet air temperatures of 100 and 50 °C, respectively, was 15.99 % for skim milk, 2.15 % for gum arabic, 1.30 % for gelatin and 0.92 % for soluble starch, while the survival of *B. longum* B6 was 83 % for reconstituted skim milk powder, 41 % for gum arabic, 64 % for gelatin and 29 % for soluble starch. Skim milk has potential for effective spray-drying of probiotic cultures (Corcoran et al. 2004; Desmond et al. 2002; Ananta et al. 2005) as skim milk proteins can prevent cellular injury by stabilizing cell membrane constituents (Ananta et al. 2005).

Soluble solid concentrations of the liquid feed can vary from 10 to 50 %, depending on the properties of feed (e.g. viscosity and heat sensitivity), the type of atomizer and the final product requirements. Higher feed concentrations improve the commercial viability of the process through thermal efficiency, but it also affects the survival of bacteria after spray-drying. Lian et al. (2002) also reported the effect of different concentrations of carriers on the survival rate of bifidobacteria. When the concentration of gum arabic, gelatin or soluble starch was increased from 10 to 20 % (w/w) or more, the survival rate was significantly lowered. For *B. infantis* CCRC 14633, the survival was 0.65, 0.52 and 0.09 %, respectively, after spray-drying with gum arabic, gelatin and soluble starch. For *B. longum* B6, it was 6.51, 2.07 and 1.56 %, respectively. However, the effect of skim milk concentration was not reported in their study.

A 10 % carrier concentration is generally considered ideal for increasing viability in spray-dried cultures (Morgan et al. 2006). Increasing feed concentration of carriers from 10 to 20 % or more can cause a reduction in the viability of spray-dried cultures. Lower viabilities at high feed concentrations may be caused by higher solid content that can result in larger particles that require longer drying

times, thus subjecting the entrapped microorganisms to more heat damage (Santivarangkna et al. 2007). However, in the case of reconstituted skim milk, the total solid content of 20 % has been frequently used and considered optimum for retaining high residual viability of different strains of lactic acid bacteria (Desmond et al. 2001; Gardiner et al. 2000). In fact, the storage stability of dried powder was reduced as the amount of skim milk solids in the carrier was decreased (Ananta et al. 2005).

Combinations of different carriers can be used to improve the survival of spray-dried probiotics. For instance, a combination of skim milk and gum arabic was shown to result in good survival rates of *B. lactis* BB12 after spray-drying and also during storage in vacuum at 30 °C, compared to the control sample prepared from skim milk without adding gum arabic (Chavez and Ledeboer 2007). Desmond et al. (2002) also used gum arabic to protect probiotic cultures of *L. paracasei* NFBC 338 during spray-drying, storage and gastric transit. It was demonstrated that a mixture of reconstituted skim milk (RSM, 10 % w/v) and gum arabic (10 % w/v) rendered tenfold greater survival than the control prepared with RSM (20 % w/v). Gum arabic has emulsifying properties and exhibits high solubility and low viscosity in aqueous solution compared to other hydrocolloid gums, thus facilitating the spray-drying process.

Stress Response Factors

To improve the viability of probiotics, several approaches have been attempted, including stress adaptation technique and selection of more resistant strains from various sources (Krasaekoopt et al. 2003). Adaptive cellular response could be induced, prior to dehydration, with the exposure of microorganisms to sublethal or gradually increasing doses of stress. This enhances the resistance of bacterial cells to stressful conditions and enables them to survive during dehydration. The bacteria respond to changes in their surroundings by a metabolic programming which leads to a cellular state of enhanced resistance (Meng et al. 2008).

Most bacteria exhibit stress sensing systems as defensive mechanisms against various stresses allowing them to survive under severe conditions. The induction of these defence systems influences the tolerance against harsh conditions such as heat or osmotic stress during drying. Adaptation to heat could be induced by heat-shock treatments by placing cells in sublethal high temperatures (50 °C for 30 min) (Teixeira et al. 1995a) before spray-drying. However, heat shock has little significance to stationary cells compared to cells from the exponential phase (Teixeira et al. 1995a). Meanwhile, the exposure of cells to other nonhomologous sublethal agents such as salt can render tolerance to heat and spray-drying even at high outlet temperatures of 100–105 °C (Santivarangkna et al. 2007; Desmond et al. 2001). Starved cells show multi-resistances against stresses particularly to heat and oxidative tolerances with increasing duration of starvation (Santivarangkna et al. 2007).

Protective Substances

The inclusion of protective agents to starter cultures is a common means to protect cells during drying and storage. The excipients added can be in the form of compounds used as suspending media or carriers, such as skim milk, whey, gum acacia and gelatin (Santivarangkna et al. 2007). The effectiveness of a given protectant varies largely with each type of culture. The most extensively investigated compound is trehalose. This may be due to the phenomenon called anhydrobiosis, where organisms in nature can survive for a long and extreme dehydration period by accumulating a large amount of disaccharides, especially trehalose. The presence of trehalose has the ability to raise the glass transition temperature (T_g) of the dry matrix (Santivarangkna et al. 2007).

Prebiotics are non-digestible carbohydrates (e.g. lactulose, inulin and some oligosaccharides) that benefit the host by selectively stimulating the growth and activity of beneficial bacteria in the colon (Burgain et al. 2011). It was shown that the partial substitution of solid content of skim milk powder with Raftilose (P95) and/or polydextrose enhanced the survival of *L. rhamnosus* GG (ATCC 53103) during spray-drying, but the storage stability of the bacteria was decreased during long-term storage (Ananta et al. 2005). This might be because some oligosaccharides present in these prebiotic substances are inadequate in replacing water molecules in the dehydrated skim milk. Thus, the maintenance of structural and functional integrity of bacterial cell membrane is not as effective as in the presence of skim milk alone (Ananta et al. 2005).

4 Post-drying Conditions

Several intrinsic and extrinsic factors affect the stability of probiotics during storage. Storage conditions, such as temperature (storage), moisture content of powders, water activity, relative humidity, powder composition, oxygen content, exposure to light and storage materials, have significant impact on the survival of probiotics in dried powders. Temperature is, however, one single most important factor affecting the stability of probiotics during storage. Stability of spray-dried samples decreases during storage, and higher microbial survival rates are maintained at low storage temperatures (Corcoran et al. 2004; Desmond et al. 2002). It is therefore essential to use correct storage conditions to maintain viable populations of spray-dried probiotic bacteria (Meng et al. 2008).

Moisture content and water activity are directly affected by the efficiency of the drying process and the quality of packaging materials used. The glass transition temperature (T_g) is another factor that impacts on the survival of probiotics in dried powders as it is a thermodynamic property of materials which is altered by the presence of water. Mass transfer rates are slower in a glassy state matrix. Storage of dried cultures at temperatures lower than their T_g increases their stability as it (T_g) retards the mobility of molecules and reaction rates (Chavez and Ledeboer 2007).

Relative humidity of the storage environment has a significant effect on the survival of dried probiotic cultures (Ying et al. 2010). High relative humidity can cause the caking phenomenon in dried powders. This phenomenon which is associated with the transition of powders from a glassy state to a rubbery state is one of the most undesirable conditions for the survival of probiotics. Therefore, it is essential that the relative humidity is kept to the critical equilibrium value that corresponds to the glass/rubber transition.

Lipid oxidation of cell membrane fatty acids is a possible cause for cell death during storage (Ananta et al. 2005). The onset of membrane lipid oxidation during storage has a detrimental effect to cells. Addition of antioxidant materials such as ascorbic acid and monosodium glutamate can protect cells during storage at 4 °C (Peighambardoust et al. 2011). However, the addition of such antioxidant materials is not recommended at storage temperature of about 20 °C as this can lead to high death rate of the culture due to pro-oxidant activity of ascorbic acids at higher temperature (Santivarangkna et al. 2007).

Proper packaging for storage of the cultures is important. Packaging under vacuum or nitrogen replacement is suitable for storing anaerobic probiotics such as bifidobacteria (Peighambardoust et al. 2011). Storage of cultures under vacuum is better than storage under nitrogen or air. Since vacuum packaging also removes air humidity, packaging of dried probiotics under vacuum is recommended (Chavez and Ledeboer 2007). The package should prevent the transmission of oxygen, moisture and light which are detrimental to the dehydrated cultures. Spray-dried *S. thermophilus* and *B. longum* can survive better in laminated pouches, followed by glass bottles and polyethylene terephthalate (PET) bottles (Wang et al. 2004). Skimmed milk powders containing cells can also be stored in polythene bags and kept in aluminium-coated paper bags (Simpson et al. 2005; Chavez and Ledeboer 2007).

5 Applications of Encapsulated Probiotics in Food Products

Microencapsulation is important for the survival of probiotics during storage and also its passage through the digestive tract depending on the type of microencapsulation system used. Addition of microcapsules to food matrices should not affect the sensory properties of food products when the size of the capsules is kept below 100 μm (Heidebach et al. 2009b). Encapsulated probiotic bacteria can be used in fermented dairy products, such as liquid fermented milks (yoghurt, cultured cream), Cheddar cheese and frozen dairy desserts, and for biomass production. The use of encapsulated probiotic cells, particularly in cheese, is common. Cheddar cheese has particular advantage of being a good carrier of encapsulated probiotics because of its high pH (5.5), good buffering capacity and relatively high fat content which can protect probiotic bacteria (Burgain et al. 2011). Spray-dried powder of *L. paracasei*

NFBC 338 was successfully used in probiotic Cheddar cheese manufacture, retaining good viability levels (up to 10^8 CFU/g) for 6–8 months during ripening without affecting quality (Gardiner et al. 2002).

Due to the low pH of yoghurt (~ pH 4.5), the viability of unprotected probiotics is often affected, although the use of acid-tolerant strains may be possible. Using encapsulated probiotic bacteria would be better for their survival in liquid yoghurt without making major modifications of the traditional fermentation process. Gellan–xanthan gum can be used to increase probiotic tolerance in acidic environments. The incorporation of encapsulated bifidobacteria into stirred yoghurt can lead to the defect of grainy texture, which affects the sensory quality and consumer acceptance of the product (Adhikari et al. 2003).

Microencapsulation technology has created opportunities to introduce probiotic microorganisms into products such as frozen desserts with high acidity and high osmotic pressure and containing incorporated air introduced during the freezing step. In ice cream, high viable cells of probiotics can be further protected by adding resistant starch (Homayouni et al. 2008).

The incorporation of probiotic cells encapsulated by spray-coating technology has been successfully used in chocolate. Probiotic viability in the small intestine was three times higher when incorporated in chocolate than in dairy products. Encapsulation of cells into chocolate acts as an excellent protectant against environmental stress conditions. The lipid fraction of cocoa butter protects bifidobacteria (Lahtinen et al. 2007). Encapsulated probiotics can be also protected against bacteriophages and harsh environments such as freezing and gastric solutions (Krasaekoopt et al. 2003).

6 Conclusions and Future Trends

While there has been significant progress in the development of encapsulation technology of probiotics, there is still a lot scope for more research. There are very few encapsulation materials that fulfil all the requirements to protect probiotics and deliver the microorganisms to the site of action. Ideal properties of microencapsulation materials include protecting live cells from sublethal damages, being easily digestible in the target site of GI tract, and providing protection to cells during handling and processing. Further, the materials should not affect the sensory profile of products. Therefore, the development of new encapsulation materials and techniques is desirable. Although there is an abundance of information on in vitro studies of probiotics, there is very little published data on in vivo investigations. Information on the optimum cell density in the microcapsules is important to avoid quorum sensing (cell-to-cell interactions) which express different genes. Consequences of morphological changes of encapsulated probiotics have not been reported. There is also renewed interest in induced stress resistance of probiotics caused by adverse conditions such as high temperature and acids. Whether the induced stress resistance is transient or permanent is unknown.

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