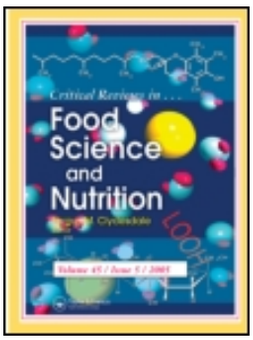

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Survival of Microencapsulated Probiotic Bacteria after Processing and during Storage: A Review

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The use of live probiotic bacteria as food supplement has become popular. Capability of probiotic bacteria to be kept at room temperature becomes necessary for customer's convenience and manufacturer's cost reduction. Hence, production of dried form of probiotic bacteria is important. Two common drying methods commonly used for microencapsulation are freeze drying and spray drying. In spite of their benefits, both methods have adverse effects on cell membrane integrity and protein structures resulting in decrease in bacterial viability. Microencapsulation of probiotic bacteria has been a promising technology to ensure bacterial stability during the drying process and to preserve their viability during storage without significantly losing their functional properties such acid tolerance, bile tolerance, surface hydrophobicity, and enzyme activities. Storage at room temperatures instead of freezing or low temperature storage is preferable for minimizing costs of handling, transportation, and storage. Concepts of water activity and glass transition become important in terms of determination of bacterial survival during the storage. The effectiveness of microencapsulation is also affected by microcapsule materials. Carbohydrate- and protein-based microencapsulants and their combination are discussed in terms of their protecting effect on probiotic bacteria during dehydration, during exposure to harsh gastrointestinal transit and small intestine transit and during storage.

Keywords Freeze drying, spray drying, gut, room temperature storage

INTRODUCTION

Probiotics have been considered as a functional food due to their abilities to provide health benefits (Lin, 2003; Sarkar, 2007) beyond nutrition. The use of probiotic bacteria is not limited to fermented milk, such as yogurt or yakult, but is extended to other forms of functional foods or beverages (Gibson, 2007; Prado et al., 2008). Consumption of probiotic bacteria in dried form is currently being developed with global market of worth $1.2 billion in 2007 with predictions to achieve $1.7 billion in 2013 (Anonymous, 2008). From many consumers, it is more practical to take dehydrated probiotic bacteria due to ease of convenience. In fact, most of probiotic bacteria supplement products has to be kept at refrigerator to keep the bacteria alive in high population (Amagase and Ide, 2007), which means high costs of transportation and storage. In addition, there is high expectation that probiotic bacteria should be still alive at certain population number during passage through gastrointestinal tract before adhering to lower intestinal tract and colon of the hosts. Microencapsulation is designed to cope with these adverse conditions. Studies have been conducted to produce microencapsulated probiotic bacteria which can easily be kept at room temperature and survive during exposure to harsh digestive systems (O'Riordan et al., 2001; Desmond et al., 2002; Sunny-Roberts and Knorr, 2009; Heidebach et al., 2010). The harsh conditions encountered by microencapsulated probiotic bacteria prior to exerting beneficial effects to the hosts are shown in Figure 1.

Microencapsulation methods of probiotic bacteria are based on hydrocolloid system or emulsion system followed by spray drying (O'Riordan et al., 2001; Crittenden et al., 2006), freeze drying (Bruno and Shah, 2003; Capela et al., 2006; Heidebach et al., 2010), vacuum desiccation (Efiuvwevwere et al., 1999; Xiaoyan and Xiguang, 2009), hybridization system (Ann et al., 2007) and extrusion followed by fluidized bed drying (Kim et al., 1988). Freeze drying method is the most common technique to dehydrate probiotic bacteria within coating materials or in dairy products (Meng et al., 2008). On the other hand, spray drying is

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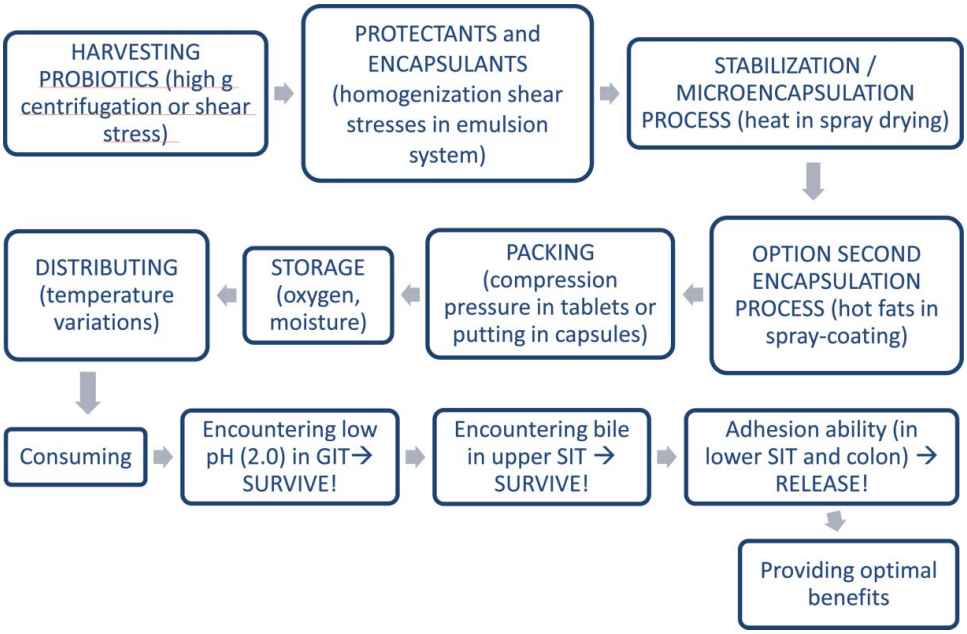

Figure 1 The 'long journey' of microencapsulated probiotic bacteria prior to exerting beneficial effects to the hosts.

popular in microencapsulation industries due to its economical and flexibility (Kailasapathy, 2002). A combination of encapsulating material(s) and suitable drying method with optimum setting conditions, for instance proportion of formulations, freezing temperature, time, temperature, and pressure of freeze drying, or inlet and outlet temperatures of spray drying, improves the stability of probiotic bacteria during storage and during exposure to harsh gastrointestinal and small intestinal tract before adhering to colon to exert beneficial effects. Reviews on probiotic microencapsulation technology including application of various substances as protectants have been carried out by Anal and Singh (2007), Mortazavian et al. (2007), Kailasapathy (2002), Poncelet (2006) and Rokka and Rantamäki (2010). Carvalho et al. (2004) reviewed more specifically about effects of freeze drying on probiotic bacteria, and Peighambardoust et al. (2011) emphasized on spray drying technique and its effect on lactic acid bacterial stability. Storage at ambient temperature has gained more attention due to its low storage cost as compared to that in refrigeration or frozen conditions. In addition, review on effectiveness of spray- and freeze-drying as part of microencapsulation in preserving probiotic bacteria protected by hydrocolloids, sugars, emulsion-based system, or their combinations during storage is lacking. Therefore, the present article is more focused on the effectiveness of microencapsulation of probiotic bacteria in improving survival including their acid and bile tolerance after freeze-drying and spray-drying and during subsequent storage with an emphasis on storage at room temperatures. Studies related to microencapsulation technology of probiotic bacteria are shown in Table 1.

STABILITY OF PROBIOTIC BACTERIA

Probiotic has been defined by FAO/WHO (2001) as microorganisms that when administered in adequate amount provide one or more health benefits to the hosts. The definition proposed by Tabbers and Benninga (2007) and Boirivant and Strober (2007) is that probiotics are 'single or mixed nonpathologic bacteria that have capability to alleviate inflammation when supplied into inflamed intestine.' They have abilities to release advantageous effects to the host such as maintaining the balance of bacteria thus improving strength of intestinal environment, enhancing the host's immune system resulting in reduction in intestinal infection, reducing the symptoms of lactose intolerance, reducing the risk of certain cancers, reducing inflammatory bowel disease and counteracting allergies, and providing antioxidants (Gilliland, 1990; Shah and Jelen, 1990; Gill and Guarner, 2004; Mottet and Michetti, 2005). In conjunction with those expectations, probiotic bacteria should be stable in gastric juice and bile salts of intestinal tracts, be able to adhere to human epithelial cells before releasing some benefits such as antimicrobial activity and prohibiting adhesion of pathogen to the epithelial cells (Dicks and Botes, 2010). Most probiotic bacteria belong to the species of *Lactobacillus* and *Bifidobacterium* (Lin, 2003), some *Lactococcus* strains have also been considered as probiotic based on their acid and bile tolerance (Kimoto et al., 1999; Kimoto et al., 2003).

Bifidobacterium

The first invention of *Bifidobacerium* was by Tissier of the Pasteur Institute in France in 1899 with an original name of

Table 1 Microencapsulation technology of some probiotic bacteria using some combination of encapsulants

Probiotic/ LAB bacteria	Basic microencapsulation technique	Microencapsulating material	Protective ingredients	Secondary coatings	Drying process	Functionality	References
L. acidophilus ATCC 43121	Hybridisation	A poly (vinylacetate) phthalate-based aqueous enteric coating system	Fructooligosaccharide, lactulose or raffinose	—	—	Acid, salt and heat tolerance	Ann et al. (2007)
Lactobacillus casei 81	Gel beads (Ca-alginate cross linking)	Sodium alginate blended with low-methoxyl pectin or modified starch	—	—	—	Viability in yoghurt	Calleros et al. (2007)
Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Bifidobacterium longum	Gel beads (Ca-alginate cross linking)	Sodium alginate	Fructooligosaccharides or	—	—	Storage, acid and bile tolerance	Chen et al. (2005)
L. rhamnosus type Lr-32, B. longum typeBl-05, L. salivarius type Ls-33, L. plantarum Lpc-37, L. acidophilus NCFM, L. paracasei Lp-115, B. lactis type Bl-04, and B. lactis type Bi-07	Emulsion combined with cross linking system	Alginate emulsion (with vegetable oil, Tween 80)		—	—	Acid, bile and heat tolerance	Ding and Shah (2007)
Lactobacillus rhamnosus, Bifidobacterium longum, L. salivarius, L. plantarum, L. acidophilus, L. paracasei, B. lactis type Bl-04, B. lactis type Bi-07, HOWARU L. rhamnosus, and HOWARU B. bifidum	Emulsion combined with cross linking system	Alginate, guar gum, xanthan gum, locust bean gum, or carrageenan gum (with vegetable oil, Tween 80)		—	—	acid and bile tolerance	Ding and Shah (2009a)
L. rhamnosus B. longum L. salivarius L. plantarum L. acidophilus L. paracasei B. lactis type Bl-O4 *B. lactis type* Bi-07	Emulsion combined with cross linking system		Alginate emulsion (with vegetable oil, Tween 80)	Palm oil and poly-L-lysine	—	acid and bile tolerance	Ding and Shah (2009b)

(Continued on next page)

Table 1 Microencapsulation technology of some probiotic bacteria using some combination of encapsulants *(Continued)*

Probiotic/ LAB bacteria	Basic microencapsulation technique	Microencapsulating material	Protective ingredients	Secondary coatings	Drying process	Functionality	References
Lactobacillus rhamnosus	Cross linking	Hydroxypropyloammonium starch (polycation)		Alginate (polyanion)	Freeze drying	Survival in acid and pH 7.0, 8.0	Goderska et al. (2003)
Lactobacillus acidophilus and Bifidobacterium lactis	Gel beads (Ca-alginate cross linking)	Alginate and Hi-Maize starch		—	—	Application to yoghurt, storage at 4°C	Kailasapathy (2006)
Lactobacillus rhamnosus	Gel beads (Ca-alginate cross linking)	Alginate	Pectin, carrageenan, whey protein isolate	—	Freeze drying	Survival in gastrointestinal tract model	Reid et al. (2005)
LAB cells	Gel beads (Ca-alginate cross linking)	Alginate	Gelatin and trehalose	—	—	Survival in acid-bile tract, storage at 4°C	Xiaoyan and Xiguang (2009)
Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus and Bifidobacterium spp.	Gel beads (Ca-alginate cross linking)	Alginate	Unipectine^{TM} RS 150, Hi-maize, FOS, or inulin	—	—	Application to yoghurt (fresh and freeze dried), survival during storage	Capela et al. (2006)
Lactobacillus paracasei ssp. *paracasei* F19, *Bifidobacterium lactis* Bb12	Cross linking (rennet) followed by emulsification (W/O system)	Casein		—	—	Survival, stability in low and neutral pH	Heidebach et al. (2009)
Lactobacillus F19 and Bifidobacterium Bb12	Cross linking	(Transglutaminase) followed by emulsification (W/O system)	Casein, Resistant starch	—	—	Freeze drying	Survival after drying and storage Heidebach et al. (2010)
Lactobacillus paracasei ssp. paracasei	Matrix		α-lactose monohydrate	—	Freeze drying	Storage stability at low a_w	Higl et al. (2007)
Lactobacillus rhamnosus	Spray drying (globular form)	Reconstituted skim milk only, reconstituted skim milk- polydextrose, polydextrose only	—	—	Spray drying	Storage stability	Corcoran et al. (2004)
Bifidobacterium infantis	O/W emulsion microcapsules	sodium caseinate, resistant starch	Glucose, fructooligosaccharides	—	Spray drying	Acid-bile tolerance, storage stability	Crittenden et al. (2006)
Lact. paracasei NFBC 338	Spray drying (globular form)	reconstituted skim milk, gum acasia	—	—	Spray drying	survival in gastric juice, storage stability	Desmond et al. (2002)
Bifidobacterium PL1	Spray drying (globular form)	modified waxy maize starch	—	—	Spray drying	Acid tolerance, storage, stability in food	O'Riordan et al. (2001)
Bifidobacterium bifidum	Spray drying (globular form)	Whey protein concentrate, gum arabic, mesquite gum, maltodextrin	local prebiotic	—	Spray drying	Storage stability	Rodríguez-Huezo et al. (2007)

Bacillus bifidus communis. It was classified as genus *Lactobacillus* based on its morphology and its characteristics, but was then declared as a sovereign genus namely *Bifidobacterium* in 1960s (Ishibashi et al., 1997). Currently, more than 30 *Bifidobacterium* species have been identified isolated from either human or animal intestines. *Bifidobacterium* isolated from human feces include *B. longum, B. breve, B. infantis, B. bifidum, B. adoltescentis*, and *B. pseudocatenulatum*, while those isolated from animal feces are *B. pseudolongum, B. thermophilus*, and *B. animalis* (Ishibashi et al., 1997). Among *Bifidobacterium, B. animalis* is more adaptive in acid environment; *B. animalis* that has encountered the genetic changes is known as *B. lactis* strains (Meile et al., 1997).

The bacteria of the genus *Bifidobacterium* are gram positive, immobile, and nonsporulated (Ballongue, 1998). They have rod and coccoid forms in the exponential and early stationary growth phases, and the cells are developed into branched and septated filaments, clubbed cell forms in the late stationary and death phases. The glucose fermentation end-products formed during their growth are acetic and lactic acids. They are not acid tolerant, however they are able to develop mechanism to adjust to the unfavorable environments including pH of 4.0–4.2 (Novik et al., 2001). Some strains of *B. animalis* and *B. thermacidophilum* are still capable of surviving at pH 3.5–4.0 (Dong et al., 2000). Bifidobacteria are also rigorously anaerobic, even though some strains such as *Bifidobacterium animalis* subsp. *lactis* and *Bifidobacterium thermophilum* are considered as microaerophilic (Von-Ah et al., 2007; Li et al., 2010).

Lactobacillus

Lactic acid bacteria are gram-positive, nonsporing, nonrespiring cocci or rods, and producing lactic acid as the major end-product during the fermentation of carbohydrates. *L. acidophilus* species is a microaerophilic having ability to ferment sugars (Axelsson, 1998) but some strains are capable of digesting sucrose more efficiently than lactose (Mital and Garg, 1992). Catabolic metabolism through Embden–Meyerhof–Parnas pathway occurs to digest glucose in order to produce the main product namely lactic acid; thus lactic acid bacteria are categorized as homofermentative bacteria (Axelsson, 1998).

Due to their health-promoting properties, some of them are classified as probiotic bacteria. Some of LAB species such as *L. acidophilus* has been recognized as probiotic bacteria due to their ability to adhere to animal or human intestines and to release health advantages for the hosts. In addition, the ability of LAB to bind mutagens has been hypothesized as a protecting mechanism against cancer (Ljungh and Wadström, 2006). *L. acidophilus* and *L. salivarius* could be good examples of LAB with their probiotic properties. They survive well in harsh environments such as very low pH and high bile concentration. They have ability to reduce the population of *Salmonella* by preventing their colonization on the epithelium (which is known as co-aggregation mechanism) (Del-Re et al., 2000), while they are able to adhere on it (Orłowski and Bielecka, 2006).

Lactococcus

Lactococcus strains, besides *Lactobacillus* strains, are also categorized as lactic acid bacteria. *Lactococcus* has an important role as a starter (or mixed cultures) in cheese and other fermented milk products owing to their high proteolysis and acidifying abilities (Monteagudo-Mera et al., 2011). These are gram-positive and nonspore forming; they forms pair or short chain on the media. They are categorized as homo-fermentative bacteria with lactic acid as a main product. They also produce nisin and cytokine which has a role in immune system (Nouaille et al., 2003; Elmarzugi et al., 2010). Two *Lactococcus* strains generally used in milk industry are *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris.* The difference between them is *L. lactis* ferments lactose, sucrose, glucose, maltose, galactose, and fructose; while *L. cremoris* metabolizes lactose, glucose, galactose, and fructose. However, none reacts with mannitol: a characteristic similar to that of *L. acidophilus* (Ahmed and Kanwal, 2004).

Current studies have shown that *L. lactis* has potential to be probiotic bacteria. Most of the studies showed that their functional properties such as survival in gastrointestinal tract as well as adherence ability to mucosal surface are highly strain-dependent (Drouault et al., 1999; Kimoto et al., 1999). Some lactococci strains can survive in intestinal tract of mice (Kimoto et al., 2003). A study on human feces showed that *L. lactis* was still able to survive in human gastrointestinal tract up to 3 days (Klijn et al., 1995). *L. lactis* has also ability to form proteins and antigen in order to improve mucosal vaccines (Nouaille et al., 2003). More detailed studies regarding the functional properties of *L. lactis* as probiotic bacteria have been established (Drouault et al., 1999; Kimoto et al., 1999; Kimoto et al., 2003; Sabir et al., 2010).

Stability of Probiotic Bacteria in the Gut

Viable probiotic bacteria are expected to improve microflora in the intestinal system and provide health benefits to the hosts. Therefore, probiotic bacteria are expected to survive during passage through gastrointestinal tract and adhere to mucosal layer of the hosts (Vinderola and Reinheimer, 2003). However, in particular cases, lysed probiotic bacteria might also be desirable for functionalities such as decreasing gut inflammation, and improvement in immunity and brain function (Ray et al., 2010). Stability of some probiotic bacteria in acid and bile environment is shown in Table 2. In general, they show different response to those environments depending upon their characteristics. Study on acid stability (pH 1.5–3.0, three-hour exposure) and on bile stability (bile salts 0–1.5%,

Table 2 Stability of free and microencapsulated probiotic bacteria in acid and bile environment

Probiotic	Products	Microencapsulant	Acid stability	Acid condition	Bile stability	Bile condition	References
L. acidophilus 2409	Freeze dried beads	2% alginate + Hi-maize resistant starch	56.2%	pH 2.0, 3 hours	80.2%	1%, 6 hours	Sultana et al. (2000)
L. acidophilus	Freeze dried beads	Sodium alginate 2% + xanthan gum 5% + glycerol 0.15% (w/v)	56.0%	pH 1.5, 3 hours, 37°C	86.4%	0.3% bile, after 24 hours, 37°C	Kim et al. (2008)
B. bifidum		Free	0%	3.0 g/L pepsin, pH 2.0, 120 minutes, 37°C	0%	Bile salt 3.0 g/L, pH 7.5, 120 minutes, 37°C	Chavarri et al. (2010)
		20 g/L of sodium alginate coated with chitosan 0.4% (w/v) solution	>90%		95.6%		
L. gasseri		Free	0%		0%		
		20 g/L of sodium alginate coated with chitosan 0.4% (w/v) solution	>85%		98.9%		
L. acidophilus		Free	36.1%	pH 1.55, 2 h	21.9%	0.6% bile salt, pH 8.25, 37°C, 2 hours	Krasaekoopt et al. (2004)
		Chitosan 1%	75.1%		55.5%		
		Alginate	60.4%		57.6%		
		poly-L-lysine 0.05% + alginate solution 0.17%	56.6%		52.2%		
B. bifidum		Free	All not detected		27.8%		
		Chitosan 1%			84.9%		
		alginate			49.5%		
		poly-L-lysine 0.05% + alginate solution 0.17%			39.1%		
L. acidophilus sp.	Free	—	3.4–5.6	pH 2.0, 3 hours, 37°C	45.7–79.9%	1%, 72 hours	Vinderola and Reinheimer (2003)
L. delbrueckii subsp. *bulgaricus*			6		0.0–5.1%		
L. lactis strains			6		0.9–61.3%		
B. bifidum			3.3–4.9		18.9–41.0%		
B. longum			>6.0		7.1–43.1%		
			(all in log CFU/mL decrease)				
L. acidophilus Z1L			79%	pH 2.0, 1 hours	81%	0.3%, 2 hours, 37°C	Sabir et al. (2010)
L. cremoris			71%		74%		
L. acidophilus	Free	3% alginate	35.3%	pH 2.0, 2 hours	40.2%	3%, 8 hours	Ding and Shah (2009)
	Wet beads		67.3%		63.0%		
L. bulgaricus KFRI 673	free		0%	pH 2, 1 hours	83.3%	Phosphate-buffered saline solution (pH 7.4) without pancreatin, 37°C, 120 min	Lee et al. (2004)
	Freeze dried beads	2% (w/v) sodium alginate, 5.5% (w/v) MRS broth, 5% (v/v) glycerol, 0.26% xanthan gum, 0.1% Tween 20, coated with chitosan	46.4%	pH 2.0, 3 hours, 37°C	77.9%		
L. plantarum 299	free		0%	pH 1.8, 90 minutes	—	0.9% sodium chloride + pancreatin + 1% trypsin + 0.3% of bile salts, pH 6.5, 180 min, 37°C	Gbassi et al. (2009)
	Freeze dried beads	alginate 0.2% coated with whey proteins 0.2% (all w/v)	67%	pH 1.8, 2 hours	34%		

L. acidophilus CSCC 2400	free		42.6%	pH 2.0; 3 hours	87.1%	1%, 6 h, 37°C	Chandramouli et al. (2004)
	wet beads	alginate 1.5%	71.1%	pH 2.0, 3 hours, 37°C	89.0%		
L. casei NCDC-298	free		44.7%	pH 1.5, 3 hours	77.1%	1% bile salt, 12 hours	Mandal et al. (2006)
	wet beads	2% alginate	63.8%		84.7%		
		4% alginate	80.1%		86.7%		
L. rhamnosus	Free		84.70%	pH 2.0, 3 hours	84.70%	MRS broth pH 7.0	Goderska et al. (2003)
	Freeze dried	Alginate and Hydroxypropyloammonium starch (No concentration given)	95.30%		100%		
L. casei	Dried under control air-flow, 4°C	Alginate bead (1.5%)	7.1	pH 2.0, 120 minutes	4.1	Bile 0.5% 6 hours	Li et al. (2011)
		Alginate bead–chitosan solution (1%)	7.4	7.3			
		Alginate bead–chitosan solution–carboxymethyl chitosan solution (0.5%)	7.9	7.9			
		(all in w/v)	(log CFU/g; no initial CFU given)		(log CFU/g; no initial CFU given)		
L. acidophilus (La-05)	Spray dried powder ($T_i/T_o = 130/75$)	Free	95.6%	pH 2.0, 120 minutes	103.1%	Bile 2%, 12 hours	Fávaro-Trindade and Grosso (2002)
		RSM 10% + cellulose acetate phthalate 10% + Glycerol 3.5% + Maltodextrin 2.0% + Raftilose 1.0% + Tween 80 0.1%	95.5%		103.1%		
B. lactis (Bb-12)	Spray dried powder ($T_i/T_o = 130/75$)	free	87.5%		94.4%		
		RSM 10% + cellulose acetate phthalate 10% + Glycerol 3.5% + Maltodextrin 2.0% + Raftilose 1.0% + Tween 80 0.1% (all in w/v)	100.0%		95.6%		
L. paracasei NFBC 338	Spray dried powder ($T_i/T_o = 170/95–100$)	RSM: Gum Acasia (10:10)%	49.1%	pH 3.0, 120 minutes, 37°C			Desmond et al. (2002)
		RSM 20%	18.2%				
B. PL1	Spray dried powder ($T_i/T_o = 100/45$)	Modified waxy maize starch 10% (w/v)	29.60%	pH 2.8; 0 hours; 37°C			O'Riordan et al. (2001)
			0%	pH 2.8; 3 hours; 37°C			
L. rhamnosus GG	Spray dried powder ($T_o = 65–70$)	Trehalose 20%	n.d.	pH 1.6, 37°C, 90 minutes	97.5%	5 mg/mL lysozyme + 1% bile	Sunny-Roberts and Knorr (2009)
		Tre20%+12.5 MSG (g/L)	0.60%		68.5%		
L. rhamnosus E-97800	Spray dried powder ($T_o = 65–70$)	Tre 20%	n.d.	pH 1.6, 37°C, 90 minutes	80.6%	5 mg/mL lysozyme + 1% bile	
		Tre20%+12.5 MSG (g/L)	0.01%		69.2%		

(Continued on next page)

Table 2 Stability of free and microencapsulated probiotic bacteria in acid and bile environment *(Continued)*

Probiotic	Products	Microencapsulant	Acid stability	Acid condition	Bile stability	Bile condition	References
L. casei	Free		45.8%	pH 2.0, without pepsin, 37°C, 100 rpm	75.7%	porcine bile 1%, 37°C	Nag (2011)
	Free		55.1%	pH 2.0, pepsin 0.32%, 37°C, 100 rpm			
	Wet microcapsule	0.25% (w/w) gellan gum+10%(w/w) sodium caseinate acidified by glucono-δ-lactone	72.0%	pH 2.0, without pepsin, 37°C, 100 rpm	100%	porcine bile 1%, 37°C	
			66.4%	pH 2.0, pepsin 0.32%, 37°C, 100 rpm			
B. lactis Bb12	Free		64.1%	pH 2.0, without pepsin, 37°C			Heidebach et al. (2009)
	Wet microcapsule	SM 35% (w/w) incubated with rennet	91.3%				
L.paracasei ssp. paracasei F19	free		51.5%				
	Wet microcapsule	SM 35% (w/w) incubated with rennet	59.2%				
B. breve R070	SD ($T_i/T_o = 185/85$)	Free	25.3%	pH 1.9; pepsin, 30′	63.3%	bile 15% + pancreatin, pH 7.5, 360′	Picot and Lacroix (2004)
		Harvested cells+denatured WPI 10% (w/v)	12.7%		91.1%		
B. longum R023		Free	0.0%		15.0%		
		Harvested cells+denatured WPI 10% (w/v)	0.0%		55.9%		
B. animalis ssp. *lactis* E-012010	Freeze dried powder	Free	65.2%	pH 2.5; no pepsin; 2 hours	70.4%	bile extract 1%; 3 hours	(Saarela et al. (2005)
		Sucrose (5% w/w)	69.6%		73.9%		
		RSM (5% w/w)	69.6%		—		
L. acidophilus (LAC 4)	free		70.5%	pH 1.0; 3 hours			Oliveira et al. (2007)
	Freeze dried powder	Pectin and casein (1 : 1, total solids content of 8%, w/v)	97.7%				
B. lactis (BI 01)	Free		58.8%				
	Freeze dried powder	Pectin and casein (1 : 1, total solids content of 8%, w/v)	89.3%				

three-hour exposure) of 6 *L. acidophilus* and 9 *Bifidobacterium* showed that the most robust strains surviving in both adverse conditions were *L. acidophilus* strains 2415, *Bifidobacterium pseudolongum* strain 20099, and *B. longum* strain 1941 (Lankaputhra and Shah, 1995). Acid stability of gram-positive bacteria was affected by ATPase activity which has the role in pumping proton out in order to maintain the pH of intracellular cells (Cotter and Hill, 2003; Corcoran et al., 2005). The presence of glucose as metabolizable sugar is necessary as ATPase substrate (Galazzo and Bailey, 1990; Corcoran et al., 2005).

Survival of probiotic and LAB in bile environment depends on concentration of bile, exposure time, and bacterial species and strains (Vinderola and Reinheimer, 2003). Bile tolerance of some probiotic is also shown in Table 2. In fact, bacteria get partially injured due to very low pH of gastric juice resulting in irregular responses to new harsh environment of bile salts (Dicks and Botes, 2010). All the bacteria showed ability to survive in 0.3% bile but some of them were not capable of surviving in 1% bile. Most *Lactobacillus* were sensitive to bovine and porcine bile (Ljungh and Wadström, 2006). All bifidobacteria survived well in the medium added with 0.5% conjugated bile salts; however, higher concentrations had a deteriorative effect (Noriega et al., 2006).

Adherence on epithelial surface might be a requirement for probiotic bacteria in conjunction with colonization in the lower intestinal tract or colon (Canzi et al., 2005). Its activity consists of 'receptor-specific binding and charge and hydrophobic interaction'; and was expressed as contact angle or adhesion to xylene (Ljungh and Wadström, 2006). Lugea et al. (2000) reported that due to the hydrophobic nature of the intestinal mucus layer, the hydrophobic bacterial surface is essential for nonspecific interface with mucin, the glycoprotein intestinal layer, the receptor on the intestinal epithelial cell with fatty acid binding sites (Ballongue, 1998).

Cell surface hydrophobicity (SHb) can be accurately measured by determining the adherence of bacteria to hydrocarbons (hexadecane, octane, and xylene) (Rosenberg et al., 1980; Pan et al., 2006; Rahman et al., 2008). Determination of microbial adhesion to hexadecane involved Van der Waals interactions and is affected by pH and 'vortexing' (Kiely and Olson, 2000). Several *Lactobacillus* species possess a surface layer protein comprising glyco-proteins (Vadillo-Rodríguez et al., 2004), the S-proteins with relative molecular weight between 40,000 and 200,000 (Sara and Sleytr, 2000), which help *Lactobacillus* to adhere to hexadecane via hydrophobic interactions (Greene and Klaenhammer, 1994; van der Mei et al., 2003). In addition, the presence of predominant apolar groups of bacterial membrane such as saturated fatty acids, monoenoic acids (Veerkamp, 1971), and lipoteichoic acids of bifidobacterial membrane (Op-den-Camp et al., 1985) may also support the adherence.

The adhesion ability varies with the type of bacteria and strain. Canzi et al. (2005) revealed that adhesion ability of *B. bifidum* strains with xylene or *n*-hexadecane was the highest (76–98%), *B. pseudocatenulatum* the lowest, *B. longum* and *B. adolescentis* low to moderate adhesion ability (2–48% and 4–58%, respectively); all were strain dependent. This observation was contradictory to that of Rahman et al. (2008) who found strains of *B. longum* with the highest SHb (surface hydrophobicity) (91.4 – 97.3%) except *B. longum* BB 536 (51.5%). On the other hand, SHb of *B. bifidum* was in the wide range of 51.9–92.8% depending on the strains. The highest SHb of *B. asteroides* and *B. pseudocatenulatum* was 37.2% and 32.1%, respectively; it was higher than SHb of *B. longum* (12.5%) and that of *B. animalis* (18.6%) (Pan et al., 2006). Wang et al. (2010) demonstrated that SHb of *B. animalis* Bb12, *L. acidophilus* NCFM, and *L. rhamnosus* GG were 50, 8, and 20%, respectively. SHb of *L. acidophilus* was 57–70% (strain-dependent); of L. *delbrueckii* subsp. *delbrueckii* and of *L. paracasei* was 90 and 90%, respectively; and of *L. plantarum* was 65 to 84% (strain-dependent) (Colloca et al., 2000). SHb of *L. acidophilus* M92 was high (71%); pH decrease resulted in decrease in SHb; and the reduced of pronase and pepsin removed SHb of *L. acidophilus* totally (Kos et al., 2003). However, any related factors such as different chemical composition of cell membranes, media (compositions, pH), and time of cultivation contributed large discrepancy of SHb between strains (Pan et al., 2006). In addition, adhesion might be reduced due to previous exposure to very low pH of gastric tract and bile salts environment of small intestinal tract (Dicks and Botes, 2010). Zavaglia et al. (2002) showed that surface hydrophobicity of *B. pseudolongum* and *B. bifidum* grown on MRS media (37°C, 15 hours) was in the range of 90.3–97%; whereas that of both bifidobacteria grown on MRS media supplied with 0.1% bile (37°C; 24 hours) decreased into 28–49%. Bile interaction with hydrophobic site of cell membrane of the strains might be the reason for decreased SHb of cells since bile acts as emulsifier (Ding and Shah, 2009b).

Stability of Probiotic Bacteria during Processing and Storage

Stability of probiotic in acid and bile environments has been described; however, extrinsic factors such as temperatures and oxygen also need to be considered. The optimal growth temperature of bifidobacteria is in the range of 36–38°C and 41–43°C for human and animal origin strains, respectively. However, *B. thermacidophilum* and *B. psycraerophilum* are still capable of growing at 49°C and at 4°C, respectively (Ruiz et al., 2011). Survival of *B. animalis* ssp. *lactis* JCM 7117, *B. animalis* ssp. *lactis* DSMZ 20105, and *B. animalis* ssp. *lactis* BB12 were 65.4, 1.3, and 1.2%, respectively, after heating at 60°C; while survival of those bifidobacteria after grown under aerobic condition (37°C, 24 hours) were 25.8; 24.9 and 25.6%, respectively. These strains appeared superior to other bifidobacterial strains such as *B. longum, B. thermophilum*, and *B. bifidum* which showed no growth at 60°C and very low survival in aerobic condition (0.90, 6.60 and 0.85%, respectively). These observations confirmed that bifidobacteria are more susceptible to heat instead

of aerobic environment (Simpson et al., 2005). Some heat shock proteins such as chaperones and proteins responsible to 'DNA and RNA synthesis and cell division' appeared to have developed at high temperatures (Schmidt and Zink, 2000). In fact, spray drying might have severe effects on bacteria not only due to heat stress but also osmotic stress related to dehydration and oxidative stress (Teixeira et al., 1997). High inlet temperature of spray drying can reduce viability depending upon exposure time. Decrease in viability could be due to heat damage of cell membrane substances such as fatty acids and S-layer proteins, or even intracellular proteins, ribosomes, and RNA (Teixeira et al., 1997).

Other process to extend the shelf life of probiotic bacteria is freeze drying. Some stages of freeze drying are considered as less harmful as compared to spray-drying due to the use of low temperature (Wang et al., 2004). However, disturbance on intact cells, ribosome functions, folding of proteins, and enzyme stability occurred due to storage at low temperatures (Mills et al., 2011). Besides, cell is damaged by formation of ice crystals and a high difference in osmolality due to solute concentration effects (Angelis and Gobbetti, 2004). Water movement from the cells to the environment induces loss of 'cell turgor pressure,' an increase in 'concentration of intracellular solutes' along with a decrease in 'cell volume' was also taken place during freeze dehydration (Angelis and Gobbetti, 2004). Bacteria are able to cope with temperature decrease by inducing a group of cold shock proteins such as CspA, CspB, CspG, RecA, dihydrolipoamide transferase, and pyruvate dehydrogenase (Phadtare, 2004); however, circumstances encountered by probiotic bacteria during freeze drying and storage are more severe and complex. Freezing of *L. delbrueckii* ssp. *bulgaricus* (also *L. delbrueckii bulgaricus*) L2 in water without any coating materials showed a very low survival i.e. 4%. After 14th day of freezing storage (−20°C) in water or ice milk, the number of the uncoated bacteria was 2% or 87% indicating a protective effect of milk proteins on the bacteria (Sheu et al., 1993). Stability of some probiotic bacteria after freeze drying and after storage at various conditions is shown in Table 3 and 4, respectively. It also appeared that cold storage of probiotic bacteria, particularly *Bifidobacterium* in fermented milk, was not a suitable storage method for bacterial viability (Fachin et al., 2008). The authors demonstrated that viability of *B. animalis* ssp. *lactis* Bb12 in MRS-LP media and *L. bulgaricus* in MRS media was 9.5 and 7.6 log CFU/mL, respectively, after 30 days of storage in the refrigerator; whereas that in yoghurt after the same storage conditions was undetectable. As comparison, population of *L. bulgaricus* in yoghurt after the above mentioned storage was 6.4 log CFU/mL (no initial number was given). These studies showed the importance of microencapsulation of probiotic bacteria to preserve them from drying process and to extend their shelf life without losing viability during storage.

Adverse effect of oxygen in bacterial toxicity takes place during fermentation, harvesting, processing, and upon storage. Formation of H_2O_2 due to an interaction of oxygen with moisture showed adverse effects on proteins, lipids, and DNA (Mills et al., 2011). A study on the effect of oxygen levels (0, 5, 10, 15, and 21%) on metabolic activities of *Lactobacillus acidophilus* and *Bifidobacterium* spp. has been carried out by Talwalkar and Kailasapathy (2003) who found that either lactic acid or lactate-to-acetate ratio decreased due to an increase in oxygen percentage. At level of 21% oxygen, activity of NADH oxidase, NADH peroxidase, and ability to decompose H_2O_2 of *Bifidobacterium* spp. increased significantly into 6.2–18.9 (units/per mg of total protein of the cell free extract), 6.1–16.9 (units/per mg of total protein of the cell free extract), and 3.7–13.3 (nmol H_2O_2), respectively; the results were strain dependent. However, those of *L. acidophilus* were high i.e. 27.3 (units/per mg of total protein of the cell free extract), 25.6 (units/per mg of total protein of the cell free extract) and 38.4 (nmol H_2O_2) at the same oxygen level introduced. Bifidobacterial strains are more susceptible to oxygen than *L. acidophilus*. In addition, the presence of oxygen resulted in a discrepancy of SHb, a decrease in level of carbon source as well as an increase in protein content of *L. acidophilus* LA5 and *B. lactis* Bb12 (Shakirova et al., 2010). The sensitiveness of bacteria to harsh external factors leads scientists to develop microencapsulation technology for probiotic bacteria.

MICROENCAPSULATION TECHNOLOGY

A bacterium in spore form is naturally resistant to temperature changes, radiation, toxic chemicals, and starvation (Sunde et al., 2009). The outermost part of spore known as 'coat' is consisted of several coatings of cross-linked proteins; the inside part is called 'cortex' consisted of cross-linked peptidoglycan matrix. Both layers are responsible for maintaining the dry-state of the 'core' and for protecting from oxygen, moisture, chemicals, and enzyme (Driks, 1999; Henriques and Moran, 2000). Based on this natural encapsulation phenomenon (Gibbs et al., 1999), the concept of microencapsulation of probiotic bacteria has been developed to improve stability during storage and passage through digestive systems.

Microencapsulation is an 'entrapment of a compound or a system inside a dispersed material for its immobilization, protection, controlled release, structuration, and functionalization' (Poncelet, 2006). According to this definition, microencapsulation can limit contact between protected substance and other parts in the system or in environment, can homogenize the small liquid core with high volume of microencapsulating materials and convert the mixture into powder, can release the active ingredient and display its functionality in targeted tract once microcapsule is ruptured. This technology allows entrapping probiotic bacteria and protecting them during oral delivery and during exposure to harsh digestive systems (Islam et al., 2010).

By applying microencapsulation technology, bacterial integrity can be maintained during passage through the harsh environment of digestion systems and be released when they

Table 3 Survival of free and microencapsulated probiotic bacteria after spray or freeze drying

Probiotic	Protectant	Drying method	Survival after drying	a_w/MC	
B. bifidum	20 g/L of sodium alginate coated with chitosan 0.4% (w/v) solution	FD	94.8%	—	Chavarri et al. (2010)
L. gasseri	As above	FD	96.1%		
L. lactis	Mannitol 10% - sucrose 10%	FD	16%	—	Berner and Viernstein (2006)
	mannitol 5% - sucrose 10%		26%		
	mannitol 10% - skim milk 10%		10%		
	mannitol 5% - MRS broth		62%		
L. acidophilus (La-05)	RSM 10% + cellulose acetate phthalate 10% + Glycerol 3.5% + Maltodextrin 2.0% + Raftilose 1.0% + Tween 80 0.1%	SD (Ti/To = 130/75)	98.3%	0.23/5.3%	Fávaro-Trindade and Grosso (2002)
B. lactis (Bb-12)	As above		78.7%		
Lactobacillus helveticus	Maltodextrin (19% w/v)	SD		—	Johnson and Etzel (1995)
		To = 82°C	15%		
		To = 120°C	0.08%		
		FD	48%		
		Freezing	54%		
L. reuteri-PS77	Non fat skim milk (20%w/v)	FD	66.7%	6.5–7.0%	Zamora et al. (2006)
		SD; $T_i/T_o = 170/85$	100%	5.8–6.7%	
L. lactis ssp. *cremoris*	230 g maltodextrin, 76g lactose, 3.5 g NaH2PO4zH2O, 7.1 g Na2HPO4 and 685 mL deionized water	FD	63%	—	To and Etzel (1997)
		SD ($T_o = 65°C$)	2.95%		
		SD ($T_o = 90°C$)	0.35%		
L. pseudoplantarum	As above	FD	71%	—	
		SD ($T_o = 65°C$)	14.7%		
		SD ($T_o = 70°C$)	13%		
B. lactis (Bb-12)	SPI-MD (1:1) of total 20% (w/v)	FD	79.0%	0.13 / 3.58	Chavez and Ledeboer (2007)
		SD; $T_i/T_o = 80/48$	44%	0.35 / 7.51	
		SD; $T_i/T_o = 80/48$ + vacuum (45°C)	23%	0.18 / 4.97	
B. pseudocatenulatum G4	SM (10% w/v)	SD; $T_i/T_o = 160/75$	0.26%	9.2%	Wong et al. (2010)
		SD; $T_i/T_o = 160/85$	0.05%	6.2%	
	Free	FD	87%	12.7%	
	SM (10%)	FD	82.1%	4.4%	
	SM (10%) + Lactose (5%)	FD	81.1%	5.1%	
B. lactis Bb12	Free	FD	43%	0.092	Heidebach et al. (2010)
	Casein 15% (w/w) + transglutaminase (10 U/g casein)	FD	42%	0.109	
	As above + Resistant Starch (1% w/w)	FD	45%	0.118 (MC 3–4%)	
L. paracasei ssp. *paracasei* F19	Free	FD	71%		
	Casein 15% (w/w) + transglutaminase (10 U/g casein)	FD	34%		
	As above + Resistant Starch (1% w/w)	FD	16%		
B. breve A71	Lactose 5%, gelatine 1.5% and glycerol 1% (all w/v)		83.3%	—	Trsic-Milanovic et al. (2001)
	Saccharose 8%, gelatine 1.5% and skim milk 10% (all w/v)		66.6%		
B. bifidum BbTD	Lactose 5%, gelatine 1.5% and glycerol 1% (all w/v)		73.9%		
	Saccharose 8%, gelatine 1.5% and skim milk 10% (all w/v)		68.2%		
L. salivarius I 24	Distilled water (control)		0.08%	—	Ming et al. (2009)

(Continued on next page)

Table 3 Survival of free and microencapsulated probiotic bacteria after spray or freeze drying *(Continued)*

Probiotic	Protectant	Drying method	Survival after drying	a_w/MC	
	Skim milk (20% w/v)		13.03%		
	Sucrose (20% w/v)		9.00%		
	Glycerol (5% w/v)		0.005%		
	Calcium carbonate (Ca^{2+}) (0.5% w/v)		0.00%		
L. lactis	Egg yolk	FD	44.6%	—	Nanasombat and Sriwong (2007)
	Glucose		46.3%		
	Lactose		64.2%		
	Skim milk		61.6%		
	Sorbitol		59.7%		
	Soy milk		60.4%		
	Sucrose		61.0%		
	Trehalose		55.7%		
	(each 9% w/v distilled water)				
L. paracasei subsp. *tolerance*	SM/Tre/asc(6/8/4)w/v	FD	82%	—	Jalali et al. (2011)
L. delbrueckii subsp. *bulgaricus*	SM/Tre/asc(6/8/4)w/v	FD	74%		
L. plantarum ATCC 8014	Control	FD	8%		De-Valdez et al. (1983)
	Adonitol (0.8 M)		72%	All <1%	
	Dulcitol (0.8 M)		8%		
	Glycerol (1 M)		33%		
	m-Inositol (0.8 M)		10%		
	Mannitol (0.8 M)		9.50%		
	Sorbitol (1 M)		11%		
	(all dissolved in 10% non fat skim milk; final concentration of each polyol was 0.32 M)				
L. rhamnosus GG	Free	FD	87.90%		Miao et al. (2008)
	Lactose		93.7	—	
	Trehalose		97.1		
	Maltose		96.4		
	Sucrose		88.4		
	Lactose-Trehalose (1:1)		97.5		
	Lactose-maltose (1:1)		98.7		
	(all 15%)				
L. salivarius subsp. *salivarius*	Free	FD	4%	—	Zayed and Roos (2004)
	Sucrose 4% (w/v)		13%		
	SM 18% (w/v)		22.4%		
	Trehalose 4% (w/v)		34%		
L. rhamnosus GG	RSM 20% (w/v)	SD;$T_o = 80$	65%	MC = 3.7%	Ananta et al. (2005)
	RSM/polydextrose (1:1; total 20% w/v)		56%	MC = 3.4%	
	RSM/raftilose P95 (1:1; total 20% w/v)		65%	MC = 3.7%	
L. rhamnosus GG	RSM (20%)	SD; $T_o = 85–90$	50%	—	Corcoran et al. (2004)
	RSM:raftilose (10:10)%		43%		
	inulin (20%)		0.25%		
L. paracasei NFBC 338	20% (wt/vol) RSM supplemented with 0.5% (wt/vol) yeast extract	SD; $T_o = 70–75$	97%	7.30%	Gardiner et al. (2000)
		SD; $T_o = 80–85$	65%	5%	

L. salivarius UCC 118	20% (wt/vol) RSM supplemented with 0.5% (wt/vol) yeast extract	SD; T_o = 70–75	11.30%	8.80%	
		SD; T_o = 80–85	1%	2.10%	
L. plantarum CIDCA 83114	SM 11%(wt/vol)	SD; Ti/T_o = 160/70	98.9%	—	Golowczyc et al. (2010)
L. paracasei NFBC 338	RSM + 1% glucose	SD; Ti/T_o = 170/80–85	11.80%		Kearney et al. (2009)
L. paracasei NFBC 338	RSM: Gum Acasia (10:10)%	SD; Ti/T_o = 170/95–100	1.40%	MC = 2.8%	Desmond et al. (2002)
		SD; Ti/T_o = 170/100–105	0.90%	MC = 2.5%	
	RSM 20%	SD; Ti/T_o = 170/95–100	1.70%	MC = 3.2%	
		SD; Ti/T_o = 170/100–105	0.01%	MC = 2.8%	
Bifidobacterium PL1	Free	SD; Ti/T_o = 100/45	29.60%	—	O'Riordan et al. (2001)
	Modified starch 10% (w/v) : cells (10:1)		30.20%		
	Modified starch 10% (w/v) : cells (5:1)		26.84%		
B. bifidum	WPC, whey protein concentrate; GA, gum arabic; MG, mesquite gum; MD, maltodextrin DE 10.			—	Rodrıguez-Huezo et al. (2007)
	WPC 17% + MG 17% + MD 66% + aguamiel 1.4%	SD; Ti/T_o = 155/70	25.10%		
	GA 17% +MG 66% +MD 17% + aguamiel 1.4%		19.90%		
	GA 50% + MG 50% + aguamiel 1.4%		10%		
	WPC 17% +MG 17% + MD 66%		19.90%		
	GA 17% + MG 66% + MD 17%		1.60%		
	GA 50% + MG 50%		1.30%		
	(all in w/w; aguamiel in w/v as cell re-suspension)				
L. rhamnosus GG	Tre 20%	SD; T_o = 65–70	68.8%	3.8–4.1%	Sunny-Roberts and Knorr (2009)
	Tre20%+12.5 MSG (g/L)		80.8%		
	SM 20%		75%		
L. rhamnosus E-97800	Tre 20%	SD; T_o = 65–70	23.4%		
	Tre20%+12.5 MSG (g/L)		89.3%		
	SM 20%		55%		
L. acidophilus DSM 20079	Mod. Starch Hylon VII 30% (w/v)	SD; Ti/T_o = 185/85	123.3%	—	Goderska and Czarnecki (2008)
B. bifidum DSM 20239	Mod. Starch N-Tack 30% (w/v)	SD; Ti/T_o = 185/85	121.6%		
L. rhamnosus R011	WPI gelled by $CaCl_2$, 20% (w/w) SM, 5%(w/w) sucrose, 1%(w/w)bacto casitone 0.35% (w/v) ascorbic acid	FD	9.5×10^8	—	Reid et al. (2007)
	SM (control)	1.5×10^{10}			
	un-gelled WPI + 25.6% (w/w) lactose + 13.9% (w/w) sucrose		2.3×10^{10}		
			(no initial population given)		
B. breve R070	Harvested cells+denatured WPI 10%	SD; Ti/T_o = 185/85	25.67%	0.16/1.95	Picot and Lacroix (2004)
	Harvested cells+milk fat+denatured WPI 10%		10.58%	0.16/1.40	
	Freeze dried cells+milk fat+denatured WPI 10%		0.71%	0.14/1.28	
B. longum R023	Harvested cells+denatured WPI 10%		1.44%	0.18/2.05	
	Harvested cells+milk fat+denatured WPI 10%		0.03%	0.16/2.07	
	Freeze dried cells+milk fat+denatured WPI 10%		0.03%	0.14/1.42	

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Table 3 Survival of free and microencapsulated probiotic bacteria after spray or freeze drying *(Continued)*

Probiotic	Protectant	Drying method	Survival after drying	a_w/MC	
B. animalis ssp. *lactis* E-012010	Sucrose (5% w/w)	FD	122.3%	2.70%	Saarela et al. (2005)
	RSM (5% w/w)		122.3%	2.80%	
L. casei	2% alginate + 2% rice starch + 1% lecithin	FD	95.3%	—	Donthidi et al. (2010)
L. casei	2% alginate + 2% hylon + 1% lecithin		92.5%		
	2% alginate + 2% maize + 1% lecithin		91.1%		
	2% alginate + 2% potato + 1% lecithin		83.3%		
	2% alginate + 2% wheat + 1% lecithin		96.1%		
B. lactis (BI 01)	Pectin and casein (1 : 1, total solids content of 8%, w/v)	SD; $Ti/T_o = 70/46$	93.0%	11%	Oliveira et al. (2007)
L. acidophilus (LAC 4)	Pectin and casein (1 : 1, total solids content of 8%, w/v)		95.3%	9.60%	
B. animalis ssp. *lactis* DSMZ 20105	RSM (20%, w/v)	SD; $T_i/T_o = 170/90$	87%	2.47%	Simpson et al. (2005)
B. animalis ssp. *lactis* BB12			79%	3.16%	
B. breve			23–38%	3.05–3.23%	
B. longum			20%	4.18%	
B. thermophilum			22–26%	3.38–3.78%)	

Table 4 Stability of microencapsulated probiotic bacteria during storage

Probiotic	Protectant	Drying method	Survival after storage	Storage conditions	aw/MC	References
B. lactis (Bb-12)	SPI-MD (1:1) of total 20% (w/v)	FD	4.9%	2 mo, 30°C	0.13	Chavez and Ledeboer (2007)
		SD; Ti/T_o = 80/48	n.d.		0.35	
		SD; Ti/T_o = 80/48 + vacuum (45°C)	5.1%		0.18	
L. reuteri-PS77	NFSM (20%)	FD	85.9%	5°C, 60 days	—	Zamora et al. (2006)
	NFSM (20%) + 0.5% yeast extract	SD; Ti/T_o = 170/85	33.1%			
L. reuteri-PS77	NFSM (20%)	FD	66.6%	20°C, 60 days		
	NFSM (20%) + 0.5% yeast extract	SD; Ti/T_o = 170/85	10.7%			
L. paracasei subsp. *tolerance*	SM/Tre/asc(6%/8%/4%)w/v	FD	76.0%	3 mo, 4°C	—	Jalali et al. (2011)
			37.0%	3 mo, 23°C		
L. delbrueckii subsp. *bulgaricus*	SM/Tre/asc(6%/8%/4%)w/v	FD	72.0%	3 mo, 4°C		
			35.0%	3 mo, 23°C		
B. longum 1941	Unipectin 2% (w/v)	FD	85.3%	−18°C, 20 mo	—	Bruno and Shah (2003)
			46%	4°C, 20 mo		
			n.d.	20°C, 5 mo		
L. bulgaricus KFRI 673	Free cells in 10% skim milk solution	FD	94.7%	4 weeks, 4°C	—	Lee et al. (2004)
	Alginate		89.0%			
	Alginate 2% + chitosan (MW 3.852 × 10^3)		91.3%			
	Alginate 2% + chitosan (MW 1.824 × 10^4)		94.0%			
	Alginate 2% + chitosan (MW 1.709 × $10^{5)}$		94.0%			
	Free cells in 10% skim milk solution		69.9%	4 weeks, 22°C		
	Alginate		72.6%			
	Alginate 2% + chitosan (MW 3.852 × 10^3)		79.4%			
	Alginate 2% + chitosan (MW 1.824 × 10^4)		83.6%			
	Alginate 2% + chitosan (MW 1.709 × $10^{5)}$		90.4%			
B. bifidum	free	FD	0%	28 days, 4°C	—	Chavarri et al. (2010)
	20 g/L of sodium alginate coated with chitosan 0.4% (w/v) solution		87.2%			
L. gasseri	Free		0%	28 days, 4°C		
	20 g/L of sodium alginate coated with chitosan 0.4% (w/v) solution		88.5%			

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Table 4 Stability of microencapsulated probiotic bacteria during storage *(Continued)*

Probiotic	Protectant	Drying method	Survival after storage	Storage conditions	aw/MC	References
Lactobacillus ssp. *paracasei* LMG9192T	Free	FD	20.0%	Vacuum-sealed aluminum foil, 150 days, 25°C	—	Coulibaly et al. (2010)
	Glycerol 3% (w/v) + Sorbitol 1.2% (w/v)		40.0%			
	Glycerol 3% (w/v) + MSG 1.2% (w/v)		25.0%			
L. plantarum CWBI-B1419	Free		30.0%			
	Glycerol 3% (w/v) + sorbitol 1.2% (w/v)		60.0%			
	Glycerol 3% (w/v) + MSG 1.2% (w/v)		43.0%			
Lactobacillus rhamnosus GG	Lactose + trehalose (1:1; total 15% w/v)	FD	42.7%	38 days, a_w = 0.0, 25°C	—	Miao et al. (2008)
	Lactose + maltose (1:1; total 15% w/v)		86.4%	38 days, a_w = 0.0, 25°C		
	Lactose + maltose (1:1; total 15% w/v)		100.0%	38 days, a_w = 0.11, 25°C		
	Lactose+trehalose (1:1; total 15% w/v)		18.0%	38 days, a_w = 0.11, 25°C		
	RSM 15% (w/v)		65.4%	38 days, a_w = 0.11, 25°C		
L. acidophilus CSCC 2409	Free	FD	44.0%	6 weeks, −20°C	—	(Kailasapathy and Sureeta (2004)
	Ca-alginate 2% (w/v)		71.3%			
	WP 10% (w/v)		66.6%			
B. infantis CSCC 1912	Free	FD	31.8%	6 weeks, −20°C		
	Ca-alginate 2% (w/v)		70.8%			
	WP 10% (w/v)		52.6%			
L. paracasei NFBC 338	20% (wt/vol) RSM supplemented ‘with 0.5% (wt/vol) yeast extract	SD; T_o = 80–85	92.0%	4°C, 2 months	—	Gardiner et al. (2000)
			11.0%	15°C, 2 months		
L. salivarius UCC 118	20% (wt/vol) RSM supplemented with 0.5% (wt/vol) yeast extract	SD; T_o = 80–85	13.0%	4°C, 2 months	—	Kearney et al. (2009)
			2.0%	15°C, 2 months		
			86.0%	15°C, 42 days		
			n.d.	37°C, 42 days		
L. paracasei NFBC 338	RSM: Gum Acasia (10:10)%	SD; Ti/T_o = 170/95–100	68.0%	4°C, 8 weeks	0.37	Desmond et al. (2002)
		SD; Ti/T_o = 170/100–105	19.0%		0.35	
	RSM 20%	SD; Ti/T_o = 170/95—100	3.5%		0.36	
		SD; Ti/T_o = 170/100–105	11.0%		0.36	
	RSM: Gum Acasia (10:10)%	SD; Ti/T_o = 170/95–100	0.0%	30°C, 8 weeks	0.44	
		SD; Ti/T_o = 170/100–105	0.0%		0.34	
	RSM 20%	SD; Ti/T_o = 170/95–100	0.0%		0.41	
		SD; Ti/T_o = 170/100–105	0.0%		0.34	

Bifidobacterium infantis CCRC 14633	SM 15%	SD; $Ti/T_o = 100/50$	54.0%	Glass, 25°C, deoxidant+ desiccant	—	Hsiao et al. (2004)
			44.6%	Polyester, 25°C, deoxidant+ desiccant		
			96.1%	Glass, 4°C, deoxidant+ desiccant		
			95.6%	Polyester, 4°C, deoxidant+ desiccant		
	Gum arabic 15%		0.0%	Glass, 25°C, deoxidant+ desiccant		
			0.0%	Polyester, 25°C, deoxidant+ desiccant		
			81.2%	Glass, 4°C, deoxidant+ desiccant		
			73.4%	Polyester, 4°C, deoxidant+ desiccant		
	Gelatin 15%		41.2%	Glass, 25°C, deoxidant+ desiccant		
			34.8%	Polyester, 25°C, deoxidant+ desiccant		
			72.4%	Glass, 4°C, deoxidant+ desiccant		
			69.9%	Polyester, 4°C, deoxidant+ desiccant		
	Soluble starch (proportion was not given)		30.9%	Glass, 25°C, deoxidant+ desiccant		
			19.8%	Polyester, 25°C, deoxidant+ desiccant		
			62.3%	Glass, 4°C, deoxidant+ desiccant		
			59.8%	Polyester, 4°C, deoxidant+ desiccant (all kept for 42 days)		

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Table 4 Stability of microencapsulated probiotic bacteria during storage *(Continued)*

Probiotic	Protectant	Drying method	Survival after storage	Storage conditions	aw/MC	References
Bifidobacterium PL1	Free	SD; $Ti/T_o = 100/45$	56.4%	20 days, (19–24°C RT)	—	O'Riordan et al. (2001)
	Modified waxy maize starch 10% (w/v); coating polymer : core = 10:1		44.9%			
B. animalis ssp. *lactis* DSMZ 20105	RSM (20%, w/v)	SD; $T_i/T_o = 170/90$	59%	90 days, 25°C	—	Simpson et al. (2005)
B. animalis ssp. *lactis* BB12			49%			
B. breve			47%			
B. longum			<25%			
B. thermophilum			32%			
B. bifidum	WPC, whey protein concentrate; GA, gum arabic; MG, mesquite gum; MD, maltodextrin DE 10.				—	Rodrıguez-Huezo et al. (2007)
	WPC 17% + MG 17% + MD 66% + aguamiel 1.4%	SD; $Ti/T_o = 155/70$	2.0%	5 weeks, 4°C, a_w 0.32		
	GA 17% +MG 66% +MD 17% + aguamiel 1.4%		1.0%			
	GA 50% + MG 50% + aguamiel 1.4%		0.6%			
	WPC 17% +MG 17% + MD 66%		<0.1%			
	GA 17% + MG 66% + MD 17%		0.0%			
	GA 50% + MG 50% (all in w/w; aguamiel in w/v as cell re-suspension)		<0.1%			
L. acidophilus DSM 20079	Mod. Starch Hylon VII	SD; $Ti/T_o = 185/85$	60.2%	4°C, 4 months	—	Goderska and Czarnecki (2008)
	Mod. Starch N-Tack		34.1%			
	Mod. Starch N-Lock		33.3%			
B. bifidum DSM 20239	Mod. Starch N-Tack	SD; Ti/To = 185/85	72.3%			
	Mod. Starch N-Lock (all 30% w/v)		36.1%			
L. acidophilus DSM 20079	SM	FD	100.0%			
	SM+5% sacch+0.35% ascorbic acid		92.5%			
	Saccharides (20%)		90.4%			

B. bifidum DSM 20239	SM		100.0%			
	SM+5% sacch+0.35% ascorbic acid		92.5%			
	saccharides (20%)		93.3%			
Bifidobacterium animalis ssp. *lactis* E-012010	Sucrose (5% w/w)	FD	91.3%	37°C, 2 mo	—	Saarela et al. (2005)
	RSM (5% w/w)		80.0%			
	sucrose (5% w/w)		99.1%			
	RSM (5% w/w)		98.3%			
	sucrose (5% w/w)		99.1%			
	RSM (5% w/w)		96.5%			
L. casei NCFB 161	Alginate (2% w/v) + gelatinized starches (2% w/v) + lecithin (1% w/v)	FD	73.5% / 24.9%	12 weeks / 24 weeks (23°C)	—	Donthidi et al. (2010)
L. plantarum DSM 12028			69.3% / 46.7%			
L. acidophilus NCFB 1748			88.4% / n.d.			
L. gasseri NCFB 2233			65.4% / n.d.			
L. bulgaricus NCFB 1489			72.2% / n.d.			
B. adolescentis NCIMB 702204			62.2% / n.d.			
Lactococcus lactis NCIMB 6681			93.8% / 68.8%			
B. lactis	Pectin and casein (1 : 1, total solids content of 8%, w/v)	SD; Ti/To = 70/46		37°C, 120 d	—	Oliveira et al. (2007)
				7°C, 120 d		
L. acidophilus	Pectin and casein (1 : 1, total solids content of 8%, w/v)			37°C, 120 d		
				7°C, 120 d		
L. rhamnosus GG	RSM 20%	SD; T_o = 85–90	88.9%	4°C, 8 weeks	—	Corcoran et al. (2004)
			74.4%	37°C, 8 weeks		
	RSM 10%+inulin 10%		43.4%	37°C, 8 weeks		
	Inulin 20%		42.7%	37°C, 1 week		

reach their target destination by the gradual breakdown of coating materials. Key objectives of microencapsulation are to increase the stability of the core, to control the release of the core into environmental destination and to facilitate ease of transportation and storage (Shahidi and Han, 1993). Application of microencapsulation technology for lactobacilli and bifidobacteria could improve their survival against harsh environments (Tannock, 1999).

A microcapsule comprises a semi permeable, round, thin, and strong membrane bordering a solid/liquid core, with a diameter in a range from a few microns to 1 mm (Anal and Singh, 2007); however, its characteristic depends upon some variables such as microencapsulating materials, techniques of microencapsulation, the presence of secondary coatings and drying process. The substance within the microcapsule is recognized as the core, internal phase, or fill, while the wall is named as shell, coating, wall material, or membrane. The walls can be single or even multiple, meanwhile the core can be a crystalline material, an emulsion, a suspension of solids, or a suspension of smaller microcapsules (Gharsallaoui et al., 2007). Based on the morphology, microcapsules can be divided into three elementary categories i.e. mono-cored in which single core is coated by protectants, poly-cored in which some cores are within protectants, and matrix types in which protectants form matrices with core entrapped therein (Yoshizawa, 2004). Sugars, polysaccharides, proteins, or their combinations are used as coating agents as shown in Table 1. Alginate-based materials are mostly applied for probiotic microencapsulation. Other polysaccharides such as modified starch, maltodextrin or prebiotics have been studied as a potential microencapsulant combined with different proteins (Table 1). The use of microencapsulating materials is to entrap or immobilize bacteria within microcapsule and to protect the bacteria, from damages due to drying process, which is the last stage of microencapsulation technique.

Stability of Microencapsulated Probiotic Bacteria during Freeze-drying or Spray-drying

Drying is used to form a structure of the micro-capsule technology and reduce the moisture content to ensure desirable shelf life of probiotic bacteria. The effect of drying on the viability varies with the characteristics of bacteria, type of drying, and the formulation used for microencapsulation. Both freeze- and spray-drying are used for this purpose. Freeze drying is preferred commercially due to its 'mild' characteristics avoiding thermal stress regardless its high production cost. Currently, spray drying has been developed as an alternative to freeze drying as it offers some advantages such as low cost and high production rate. Drying is usually carried out at as low a temperature as possible to maintain viability of cells. Since the reduction in viability is a function of temperature and the resident time used, the use of low inlet temperature resulting in low outlet temperature was successful in maintaining bacterial viability. From industrial point of view, the use of spray drying is more beneficial since its fixed cost and manufacturing cost were 12% and 20%, respectively, of that of freeze drying (Peighambardoust et al., 2011).

Due to sensitivity of probiotic bacteria toward extremely low- or high-drying temperatures, coating probiotic bacteria using cryoprotectants or thermoprotectants has been studied. Comparison between freeze-drying and spray-drying method as a final step of microencapsulation technology for probiotic bacteria has been carried out since more than 20 years ago initiated by Johnson and Etzel (1995); they found that freeze drying was more effective than spray drying to maintain viability (Table 3). Interestingly, spray drying (T_{outlet} of 82°C) retained high aminopeptidase and β-galactosidase of 85 and 17%, respectively, compared to those of frozen cells which were 15 and 2%, respectively. These enzymes were absent in freeze-dried or spray-dried cells with T_{outlet} 120°C. Similar results were obtained by Kim and Bhowmik (1990) and Wong et al. (2010). In contrast, Ying et al. (2010) and Zamora et al. (2006) did not find any difference in loss of viability after spray or freeze drying. Zamora et al. (2006) found 100% and 66.7% survival of *L. reuteri* after spray drying and freeze drying, respectively (Table 3). The contradictive results indicate effects of factors such as strains, growth conditions (medium, pH), growth phase, coating materials, different set-up of freeze or spray drying used (Johnson and Etzel, 1995; Ying et al., 2010) and extent of drying (moisture or a_w readings).

Loss in cell viability is mainly due to protein denaturation, changes in cell envelopes, and removal of water during evaporation. Those variables have an important role in stabilizing the structure of cells and in maintaining cell functional integrity (Brennan et al., 1986). Encapsulation of sensitive materials such as proteins, enzymes, and probiotic bacteria within polysaccharides and proteins based system using spray drying technique has been carried out to protect the core from thermal and dehydration inactivity (Broadhead et al., 1994; Adler and Lee, 1999; Desmond et al., 2002; Hsiao et al., 2004; Yadav et al., 2009).

A specific study on spray-coating method to microencapsulate *Lactobacillus rhamnosus* R0011 or *Bifidobacterium longum* ATCC 15708 with fat DP108 blend of fractionated palm kernel oil and palm oil has been carried out by Champagne et al. (2010); the microencapsulation method was according to Durand and Panes (2003). The cells were previously freeze dried using skim milk and sucrose as protectant. The precise viable count was not achieved due to incomplete rehydration of microencapsulated powder when they were exposed to water with gentle agitation. However, sample preparation using blender or 'generator probes high-shear homogenization' showed no difference on CFU determination. Viable count of microencapsulated bacteria was 10.2 and 10.6 log CFU/g depending upon sample preparation method. The powder mass recovered of spray-coated *Lactobacillus rhamnosus* R0011 with fats with particle size ranging from 53 to 250 μm was 95%; meanwhile that of commercial spray-coated

Lactobacillus rhamnosus R0011 (provided by Institut Rosell-Lallemand, Montréal, QC, Canada) within the same range of particle size was 64.4% (Champagne et al. 2010).

Microencapsulating Materials

The use of sugars, polysaccharides, protein-based system, or combinations to preserve probiotic bacteria during spray- or freeze-drying has been established. The following sections focus on the effectiveness of carbohydrate-based or protein-based systems and their combinations in protecting probiotic bacteria from microencapsulation processes and from harsh gastro- and intestinal environment.

Carbohydrate-based System

Among polysaccharides, alginate is common microencapsulation material due to it being nontoxic, relatively cheap and its easiness to create strong beads; thus its use is discussed more specifically. Calcium alginate in the form of gel beads has been widely used for the immobilization of probiotic bacteria (Sultana et al., 2000) due to its easy handling, nontoxic nature, low cost, gentle process condition, and easy to dissolve in intestine thus releasing entrapped cells (Reid et al., 2005; Mortazavian et al., 2007). Freeze drying has been commonly used as a final process of alginate microencapsulation. Frozen *Lactobacillus bulgaricus* L2 entrapped in 6% of alginate beads showed almost 100% survivals, while 1.5% and 3.0% of alginate beads showed 80% survival. However, high proportion of alginate was too dense to be applied in commercial scale. Combination of alginate with proteins, cryoprotectants, or antioxidants has been established to improve bacterial stability (Sultana et al., 2000; Cui et al., 2006; Gbassi et al., 2009). Alginate 3.6% combined with 6% of glycerol or mannitol as cryoprotectants improved the bacterial survival during freezing at −20°C for two weeks (95 and 90%, respectively) (Sheu et al., 1993). Data on acid and bile tolerance of freeze dried *Lactobacillus* strains coated with alginate base are shown in Table 2. Cui et al. (2006) demonstrated that addition of either yeast extract, cryoprotectants (glycerol or lactose), antioxidants ($NaHSO_3$ or ascorbic acid), or buffering agent ($Mg_3(PO_4)_2$) improved the survival of bifidobacteria-loaded alginate poly-l-lysine microparticles significantly during freeze drying as compared to control (cells entrapped in alginate poly-l-lysine without fortification).

Particle size of microcapsules also affected survival of probiotic bacteria. Ding and Shah (2009c) studied the effect of homogenization techniques (microfluidizer, the ultra-turrax homogenizer, and standard magnetic stirrer method) on size of alginate microcapsules and survival of the probiotic bacteria. Standard method using magnetic stirrer produced the largest size of microcapsules (120–132 μm) with the highest encapsulation efficiency (84.4–88.3%). On the other hand, ultra-turrax homogenizer set at 5 min at 4000 rpm resulted in higher size of microcapsules (90–97 μm) but lower encapsulation efficiency (59.8–69.6%) than microfluidizer set at 10 passes at 10,000 psi (size 72–80 μm; efficiency 76.2–80.6%). Viability of microencapsulated probiotic bacteria produced by standard method, microfluidizer, and ultra-turrax (the same settings) was 9.1–9.4 log CFU/mL, 8.6–9.0 CFU/mL, and 7.5–8.4 CFU/mL, respectively; all strain dependent. This experiment also showed that speed (rpm) and time of mixing controlled cell viability (Ding and Shah, 2009c). Similarly, Sheu et al. (1993) demonstrated that large- (102 μm) and medium-sized (30 μm) alginate beads were more effective in preserving bacterial viability than small beads (15 μm) ($p < 0.05$). In addition, increase in size of alginate beads (from 200 to 1000 μm) resulted in higher viability of *L. acidophilus* during three-hour exposure to pH 2.0 at 37°C (from ∼5.0 to ∼5.5 log CFU/mL) (Chandramouli et al., 2004).

In contrast, some studies revealed ineffectiveness of alginate matrix as cell coating material. Zohar-Perez et al. (2004) showed that bacterial distribution in alginate-beads was not homogenous; the cells tend to be on the surface of alginate beads instead of within the beads. Dianawati and Shah (2011b) demonstrated that alginate was efficient as a coating material, but was not effective in protecting *B. animalis* ssp. *lactis* Bb12 during freeze drying and during exposure to pH 2.0 for 2 h; a significant plummeting (10^4 log CFU/g) occurred. Alginate beads immersed in peptone solution was not effective in protecting *Streptococcus thermophilus* from freeze drying; a decrease of more than 99% of viable population occurred after freeze drying; similar results were shown for *Lactococcus lactis* in alginate beads coated with poly-L-lysine (Champagne et al., 1992; Champagne and Gardner, 2001). Meanwhile, Andrade et al. (2010) found no difference between alginate alone or alginate combined with nonmilk protein isolates in protecting *L. casei* from harsh gastric intestinal environment. Similarly, no *B. lactis* encapsulated with alginate was detected after 14 days of refrigerated storage of yoghurt; however, survival of *L. bulgaricus* encapsulated with alginate was 85.7% after storage at the same conditions (Grosso and Fávaro-Trindade, 2004). Krasaekoopt et al. (2004) demonstrated that chitosan-coated alginate beads provided a good protection only for *L. acidophilus* in acid (pH 1.5) and bile environment (0.6% bile salt) but not for *B. bifidum* (Table 2).

Polysaccharides such as cellulose acetate phthalate (used for medicinal market) (Fávaro-Trindade and C.R.Grosso, 2002), maltodextrin (Johnson and Etzel, 1995; To and Etzel, 1997), and modified waxy maize starch (O'Riordan et al., 2001) has also been applied as microencapsulants (Table 3). The results were highly varied; cellulose acetate phthalate preserved probiotic better than maltodextrin and modified starch. Two latter components appeared ineffective as protectants. It is because maltodextrin acts as inactive bulking compound which does not interact with cell envelopes of the bacteria (Oldenhof et al., 2005). However, those polysaccharides cannot be compared due to the use different probiotic species and

strain and microencapsulation technique applied in their studies.

The use of low molecular weight sugars (lactose, trehalose, maltose, sucrose) was effective in protecting *L. rhamnosus* (Miao et al., 2008); but sucrose and trehalose were not effective in protecting *L. salivarius* (Zayed and Roos, 2004) (Table 3). The use of sugar alcohols as protectant have been established by De Valdez et al. (1983) and Carvalho et al. (2003b). The effectiveness of mannitol and sorbitol in protecting bacteria was demonstrated by Mugnier and Jung (1985); Efiuvwevwere et al. (1999) and Santivarangkna et al. (2010). Study of Berner and Viernstein (2006) (Table 3) on microencapsulation of *L. lactis* indicated that the higher proportion of mannitol such as 10% (w/v) had an adverse effect on bacterial survival possibly due to the formation of crystalline mannitol, as suggested by Constantino et al. (1998). The mechanism of protection by sorbitol and mannitol has been explained by Santivarangkna et al. (2010) and Dianawati et al. (2012) which is in agreement with that of Leslie et al. (1995) and Oldenhof et al. (2005).

Protein-based System

Current studies have been developed by applying skim milk, caseins, and whey proteins as microencapsulating materials for probiotic bacteria using spray drying or freeze drying (Reid et al. 2005; Crittenden et al., 2006; Reid et al., 2007; Heidebach et al., 2009; Heidebach et al., 2010; Doherty et al., 2011); these studies used different encapsulation techniques such as emulsion, extrusion, or cross-linking. The use protein-based systems combined with relatively short carbon chains of sugars was effective in increasing survival of probiotic bacteria during spray drying, whereas incorporation of polysaccharides showed no or less effect (Gardiner et al., 2000; Corcoran et al., 2004; Ananta et al., 2005; Sunny-Roberts and Knorr, 2009). These are in contradiction with the results of Desmond et al. (2002) and Rodrıguez-Huezo et al. (2007)) who found that incorporation of polysaccharides into proteins improved bacterial stability significantly as shown in Table 3. Sodium caseinate provided excellent protection for bifidobacteria during spray drying and storage (Crittenden et al., 2006). Similarly, heat denatured 10% whey protein solution was effective to immobilize bifidobacteria using spray-drying technique (Picot and Lacroix, 2004). Maltodextrin blended with sodium caseinate, gelatin, or soy protein was also used to protect phospholipids during spray drying (Yu et al., 2007). The authors revealed that a high stability of emulsion comprising maltodextrin and sodium caseinate was attained when spray drying with an air inlet temperature (140°C), a solid concentration (20%), and feed temperature (30°C) were carried out; it retained 90% of phospholipids. This study indicates that this technique can have potential for microencapsulation of probiotic bacteria, as cell envelopes of bacteria are mainly consisted of phospholipid bilayers (Crowe et al., 1987).

Cell dehydration can have serious effects on membrane phospholipids such as fusion and transformation from crystal liquid of fatty acids into gel phase; which increases the membrane permeability (Crowe et al., 1987). The use of protective materials such as sugars, proteins, or their combinations is effective in preserving probiotics during freezing and freeze drying. This is due to interactions between sugars (sucrose, trehalose) or sugar alcohols (mannitol, sorbitol) and polar site of phospholipid bilayer of cell membranes occurs via hydrogen bond as demonstrated by Fourier Transform infrared (FTIR) spectroscopy (Leslie et al., 1995; Oldenhof et al., 2005; Santivarangkna et al., 2010; Dianawati et al., 2012). Some studies related to the use of sugars or their combination with various proteins to protect probiotic bacteria during freeze drying has been compiled in Table 3.

Stability of Microencapsulated Bacteria in Gastrointestinal Tract

Effectiveness of microencapsulation on protecting probiotic bacteria is dependent on some variables such as the type of microcapsulating substances, method of microencapsulation, and bacterial strains reflecting their different characteristics. Alginate is a common microencapsulant used for protecting probiotic bacteria from harsh acid environment (Table 2). Nevertheless, alginate was not successful in protecting *B. bifidum* from high acidity of gastric juice (pH = 1.55) (Krasaekoopt et al., 2004); this study was in agreement with Dianawati and Shah (2011b) who demonstrated that a significant plummeting ($>10^4$ log CFU/g) of freeze-dried *B. animalis* ssp. *lactis* Bb12 coated with alginate occurred during exposure to pH 2.0 for two hours. It is because alginate is hydrolyzed into D-mannuronic and L-guluronic acid during exposure to very acid environment (Heyraud and Leonard, 1990); hence the bacteria will be released before achieving the targets (lower intestinal tract or the colon).

Some studies have incorporated proteins to improve the protective effect of alginate on probiotic bacteria. Some strains of *L. plantarum* were successfully protected by Ca-alginate-based microcapsules layered by whey proteins (Gbassi et al., 2009). Similarly, the use of Ca-alginate coated with chitosan also improved survival during exposure to simulated gastrointestinal tract (Chavarri et al., 2010; Li et al., 2011b) (Table 2). Ding and Shah (2009b) found high viability of some species of *Lactobacillus* and *Bifidobacterium* microencapsulated with Ca-alginate coated with poly-L-lysine and palm oil. The application of Ca-alginate combined with other carbohydrates such as starch and glycerol (Sultana et al., 2000) or glycerol and xanthan gum (Kim et al., 2008) was also proven effective in increasing probiotic bacterial bile tolerance. However, some studies demonstrated that the use of polysaccharides such as alginate and starch as coating materials without any addition of protein was less effective in protecting *Lactobacillus* and *Bifidobacterium* from acid environment (Sultana et al., 2000; O'Riordan et al., 2001; Krasaekoopt et al., 2004). O'Riordan

et al. (2001) stated that starch was not able to protect spray dried *Bifidobacterium* PL1 at very low pH; no survivors was detected after 3 h exposure to pH 2.8 (37°C).

Modification method of microencapsulation using 'milk protein matrices' induced by rennet was successful in improving stability of *Lactobacillus paracasei* ssp. *paracasei* and *Bifidobacterium lactis* Bb12 during exposure to pH 2.5 for 1.5 hours (Heidebach et al., 2009). The use of whey proteins as microcapsule of spray-dried *Bifidobacterium breve* R070 and *Bifidobacterium longum* R023 also improved bacterial stability in simulated GIT; however, survival level was strain dependent (Picot and Lacroix, 2004). Similar result was reported by Doherty et al. (2011) using *Lactobacillus rhamnosus* GG as a model. Sudden drop of viability occurred when microencapsulated bifidobacteria strains were exposed to SGJ containing pepsin (pH 1.9) for 30 minutes (less than 1.0 log CFU/g), but they were able to grow when exposed to pancreatin pH 7.5 for six hours (achieving 7.5 and 4.0 log CFU/g for R070 and R023, respectively) (Rodríguez-Huezo et al., 2007).

Combination of proteins and carbohydrates were investigated to increase the effectiveness of microencapsulation. Casein provided the shielding effect on bifidobacteria from low pH of simulated gastric tract (Charteris et al., 1998; Crittenden et al., 2006). Survival in SGJ (pH 1.6, 60 min, 37°C) of spray-dried *L. rhamnosus* GG encapsulated with trehalose-MSG was 1.7×10^7 CFU/mL; but *L. rhamnosus* E800 was not able to survive (Sunny-Roberts and Knorr, 2009). The use of complex formulation comprising cellulose, maltodextrin, prebiotic and reconstituted milk as protectant also increased acid and bile tolerance of spray-dried *Lactobacillus acidophilus* and *Bifidobacterium lactis* (Bb-12) (Fávaro-Trindade and C.R. Grosso, 2002). Polysaccharides such as gum acacia (GA) combined with RSM improved survival of spray dried *L. paracasei* NFBC 338 as compared to RSM only (Desmond et al. 2002). Similarly, Fávaro-Trindade and Grosso (2002) demonstrated the effectiveness of skim milk (as carrier of the microorganisms) combined with cellulose acetate phthalate (as wall material), detail is shown in Table 2.

Enzyme Activities of Microencapsulated Bacteria

Determination of activity of enzymes is important since it relates to fermentation pathways; some enzymes are also related to probiotic functional properties. The cleavage of β-glycosidic linkage of glucosides, dissacharides, and oligosaccharides as well as the transformation of isoflavone glycoside (in soybean) into isoflavone aglycone takes place due to the activity of β-glucosidase (β-glu) (Izumi et al., 2000; Otieno et al., 2007; Yang et al., 2009). Lactose hydrolysis into glucose and galactose can take place due to β-galactosidase (β-gal) activity. This activity decreases the possibility of lactose intolerance (Vasiljevic and Jelen, 2003). β-Galactosidases are categorized as thermo-resistant enzymes; however, they have wide a range of thermal stability from 0 to 80°C depending on bacterial species (Asraf and Gunasekaran, 2010). Stability of β-gal of *B. longum* CCRC 15708 was optimum at 30°C for 40-minute exposure (98% remaining); an increase in exposure temperature to 40°C decreased enzyme activity to 80% (Hsu et al., 2006). The optimum temperature for activity of β-glu is 60°C (Xie et al., 2004); however, its stability can be preserved at 40°C for 150 minutes when it is protected by polysaccharide matrices (Rashid, 1997).

In fact, study on stability of some enzymes of microencapsulated probiotic bacteria is still very few; most studies observed microencapsulation or entrapment of enzymes instead of the bacteria. Sugars or sugar-protein combination have been applied as protecting ingredients to maintain high activities of some enzymes during dehydration (Carpenter and Crowe, 1988; Broadhead et al., 1994; Burin et al., 2002; Izutsu and Kojima, 2002; Okamoto et al., 2002; Vasiljevic and Jelen, 2003; Singh and Singh, 2003; Han et al., 2007; Santagapita and Buera, 2008; Yoshii et al., 2008; Izutsu and Kojima, 2002; Li et al., 2011a). Grosova et al. (2009) encapsulated β-gal using polyvinylalcohol hydrogel to produce D-galactose. The results indicated that the activity of entrapped β-gal was maintained and hence, shortened the production time. Different microencapsulation techniques namely precipitation, emulsion cross-linking and ionic gelation was used to preserve β-gal using chitosan as microencapsulating material (Biró et al., 2008). Ionotropic gelation using sodium sulphate as gelation agent resulted in the highest enzyme activity. Microencapsulated β-gal was preserved well after three weeks of storage in aqueous solution at 4°C and pH 7.0; decrease in activity was below 5%. Woodward et al. (1993) found that microencapsulated ß-glu using propylene glycol alginate/bone gelatin was stable during exposure to 40°C for couple months without losing its effectiveness. Li et al. (2011a) demonstrated that ATPase of *L. reuteri* protected by trehalose or RSM decreased significantly during freeze drying compared to pyruvate kinase and hexokinase.

Microencapsulation of bacteria and its effect on the activity of some enzymes were observed by Goel et al. (2006), Dianawati et al (2011a) and Dianawati et al (2013a). Encapsulation of *S. thermophilus* using of calcium alginate, carrageenan and gellan-xanthan enhanced the stability of β-gal of the bacteria at temperature of (>55°C) (Goel et al., 2006). Dianawati and Shah (2011a) observed some enzyme activities of microencapsulated probiotic bacteria using alginate-based system. Freeze drying decreased the activities of β-glu, β-gal, lactate dehydrogenase, pyruvate kinase, hexokinase, and ATPase of microencapsulated *B. animalis*; percent retention was enzyme dependent. Incorporation of mannitol into alginate system improved stability of the observed enzymes of microencapsulated bacteria after storage at a_w of 0.07 and 0.1 at 25°C. Alginate alone and alginate-mannitol microcapsules and a_w affected the retention of β-glu, β-gal, HK, and ATPase ($p < 0.05$), but not of LDH and PK. Emulsion system using caseins added by glucose and mannitol followed by spray drying also maintained high stability of β-gal of microencapsulated *L. acidophilus* and *L. cremoris* ssp. *lactis* after 10 weeks of storage

at 25°C at a_w of 0.07 and 0.1 (86.3 – 87.7% of enzyme retention) (Dianawati et al., 2013a).

STORAGE

We have discussed freeze drying and spray drying as a part of microencapsulation technology in subsection 3.1; in which spray drying with low outlet temperature could be more beneficial than freeze drying in terms of maintaining viability. However, it might result in a high residual moisture content of products due to inadequate drying. It might have a harmful effect on bacterial survival during storage, as water could be available as solvent required for physico-chemical reactivity. Removal of remaining free water of dehydrated microcapsules can be carried out through further treatments such as use of desiccants or vacuum drying in second stage. Freeze- or spray dried microcapsules with low a_w is desired for the purpose of long-term storage at room temperatures i.e. between 8 and 12 weeks (Corcoran et al., 2004; Donthidi et al., 2010) and storage up to 20 months at cold temperature (Bruno and Shah, 2003).

Materials and proportion of microencapsulant, temperature, a_w, and period of storage are factors that influence stability of microencapsulated bacteria during storage (Zamora et al., 2006; Chavez and Ledeboer, 2007; Higl et al., 2007; Miao et al., 2008; Kurtmann et al., 2009; Coulibaly et al., 2010; Savini et al., 2010; Ying et al., 2010). Storage of microencapsulated probiotic bacteria at low temperature (4–7°C) ensures high viability of the cells for long periods but results in increased cost of transportation and storage. As a result, transporting microencapsulated bacteria for a long distance becomes impracticable. The following part emphasizes on storage of freeze- or spray dried probiotic bacteria at room temperature, mainly at low water activity (a_w). Storage at frozen- and cold temperatures is also discussed briefly.

Storage at Cold and Frozen Temperatures

Storage at 4°C (Boza et al., 2004; Lee et al., 2004; Heidebach et al., 2010; Savini et al., 2010) or at −18°C (Bruno and Shah, 2003) has always been proven effective in lengthening shelf life of probiotic bacteria. Freeze dried *Bifidobacterium longum* 1941 protected with unipectin was only able to survive at freezing or cold temperatures but not at room temperature (Bruno and Shah, 2003). This was in contradiction with the results of Saarela et al. (2005); details of both studies are shown in Table 4. It demonstrated superiority of milk or low MW sugars as protectants as compared to polysaccharides. Similarly, SM + trehalose + ascorbic acid improved survival of freeze-dried *Lactobacillus* sp.; however, storage at 4°C was preferable (Jalali et al. 2011). On the contrary, survival of *L. acidophilus* and *B. infantis* encapsulated with whey proteins was lower than that encapsulated with Ca-alginate after six weeks of storage at −20°C (Kailasapathy and Sureeta, 2004); protein denaturation of whey proteins might occur during storage at freezing temperature (Bedu-Addo, 2004) reducing its effectiveness as microencapsulant. In spite of its benefit in maintaining high survival of microencapsulated bacteria, a high transportation and storage costs adds to the high price of products. Hence, storage of microencapsulated probiotic bacteria at room temperature is needed in order to decrease cost of handling and storage along with retaining high survival of the bacteria.

Storage at Room Temperature

Conventional storage of frozen probiotic bacteria in the freezer requires high handling and storage costs in addition to increased risk associated with thawing. Microencapsulation of probiotic bacteria using dehydration methods such as spray- or freeze-drying elongates storage periods and reduces the distribution and storage costs due to the convenience of storage of products at room temperature. Besides the type of coating materials, water activity (a_w) and glass transition affect probiotic survival during storage at room temperature (Peighambardoust et al., 2011). Water activity determines the accessibility of water for chemical reactions or the growth of microorganisms (Roos, 1995). Glass transition indicates 'a physical change in an amorphous material promoted by the addition of heat and/or the uptake of low molecular weight substances' (Bell and Hageman, 1994). Rahman (2010) suggested that both a_w and glass transition concepts are useful in determining food deterioration or food stability, and also in predicting shelf-life of food products during storage.

Water activity during storage needs to be adjusted at a lower value and kept at low water activity constantly for preserving dried probiotic bacteria. Storage at very low a_w such as 0.07 and 0.1 (Mugnier and Jung, 1985; Higl et al., 2007; Kurtmann et al., 2009) improved bacterial survival during storage at room temperature. Kearney et al. (2009) stated that a residual water content of 4% corresponding to a_w of 0.2 is required to extend the shelf life of probiotic bacteria in dried dairy products. In fact, a_w is more useful parameter than moisture content to determine quality of food products. The growth of microorganisms, chemical, enzymatic and physical reactions, and moisture migration in complex system of foods are well indicated by a_w instead of moisture content (Maltini et al., 2003). Stability of some microencapsulated probiotic bacteria during storage at room temperature is shown in Table 4.

Dianawati et al. (2013b) studied microencapsulation of *L. acidophilus* and *Lactococcus lactis* ssp. *cremoris* using casein-based emulsion system. The authors demonstrated that decrease in T_g occured along with an increase in residual moisture content. The type of desiccants such as NaOH, LiCl or silica gel influenced T_g of microcapsules after 10 weeks of storage. However, changes in cell envelopes and secondary

protein structures of microencapsulated bacteria still took place as confirmed by FTIR, even though no glass transition was observed at storage at 25°C. These might affect survival of microencapsulated *L. acidophilus* and *Lactococcus lactis* ssp. *cremoris* during storage (Dianawati et al. 2013a). Similar results were also confirmed by Chang et al. (1996), Ananta et al. (2005), Chavez and Ledeboer (2007) demonstrating that structural alteration of bacteria during freeze- drying or spray-drying contributed a further damage during following storage, even with storage temperature below T_g.

Some studies on stability of microencapsulated probiotic bacteria during storage at room temperature have been carried out by Ananta et al. (2005), Heidebach et al. (2010), and Crittenden et al. (2006). Even probiotic bacteria have been microencapsulated and kept at low a_w at room temperature for certain periods, but the bacterial strains, coating materials and microencapsulation techniques are different between studies. Therefore bacterial survival data could not be compared. Water activity plays an important role in controlling reaction rate during storage. Ying et al. (2010) compared the effectiveness of freeze drying (−18°C of freezing, 48 h of freeze drying) and spray drying (T_i/T_o = 160 and 65°C) on retaining the viability of *L. rhamnosus* GG microencapsulated with whey proteins and resistant starch during storage at 25°C at a_w of 0.32, 0.57, and 0.70. Spray-dried powder containing bacteria was more stable than freeze-dried powder during 37 days of storage. It was because spray-dried capsules had a stronger water-binding energy as measured by NMR spectroscopy.

In addition to proteins combined with sugars, alginate is common microencapsulating material for probiotic bacteria (Mortazavian et al., 2007) and was successfully applied in yoghurt (Sultana et al., 2000), but effectiveness of alginate in protecting probiotic bacteria during storage at room temperature was only carried out by Donthidi et al. (2010). The authors demonstrated that after 24 weeks of storage, some probiotic bacteria encapsulated with alginate+starch+lecithin kept at 23°C did not survive (Table 4). Incorporation of chitosan into alginate improved survival of *L. bulgaricus* during 4 weeks storage (4°C) (Lee et al., 2004) (Table 4). Mannitol incorporation into alginate improved freeze-dried bifidobacterial survival including their acid and bile tolerance during storage (25°C, 10 weeks) at a_w of 0.1, but at the end of the storage, the bacterial viability was only 5.2 log CFU/g (82.6% survival relative to that after freeze drying) (Dianawati and Shah, 2011b). This suggests that even though alginate has been widely applied as microencapsulant for probiotic bacteria, its use as single material is not effective in improving cell survival during storage at room temperature.

Reconstituted skim milk (RSM) was compared to disaccharides (lactose, trehalose, sucrose, maltose, lactose + maltose, and lactose + trehalose) to ascertain their effectiveness in protecting freeze-dried *L. rhamnosus* survival during storage at a_w of 0.0; 0.11; 0.22, 0.33, and 0.76 at room temperature (Miao et al., 2008). Results showed that trehalose and lactose + maltose were the most effective encapsulants protecting viability of bacteria during 38 days storage at 25°C at a_w of 0.00 and 0.11; the protective effect of lactose + maltose was higher than that of RSM (Table 4). This appears controversial with the result of Zayed and Roos (2004). They found that storage at a_w of 0.00 (using P_2O_5 as a desiccant) resulted in decrease in survival of freeze-dried *L. salivarius* ssp. *salivarius* protected with skim milk combined with sucrose or trehalose at 44% after one week of storage at room temperature. Further, decrease of viability by 72% was observed after 7 weeks of storage at a_w of 0.00; while no significant decline was detected when LiCl was used as a desiccant (a_w = 0.11). Higher a_w contributed crystallization of disaccharides; thus survival of encapsulated *Lactobacillus* decreased. The authors stated that T_g of disaccharides decreased at higher a_w, and vice versa; this result was in agreement with that of Higl et al. (2007) and Kurtmann et al. (2009). Water activity of 0.11 was capable of providing higher viability of freeze-dried *L. acidophilus* than a_w of 0.23 and of 0.43 during 10 weeks of storage at 20°C, and sucrose maintained higher viability than lactose (Kurtmann et al., 2009).

Besides skim milk, the use of sodium caseinate as coating material has also been proven effective in improving freeze-dried *Lactobacillus* F19 and *Bifidobacterium* Bb12 during storage at 25°C (Heidebach et al., 2010). The authors examined stability of freeze-dried *Bifidobacterium* Bb12 encapsulated with enzymatic cross-linked casein during storage at different temperature and a_w. An incorporation of resistant starch into microcapsule formulation made from enzymatic cross-linked casein showed an adverse effect on freeze-dried *Bifidobacterium* Bb12 and did not improve stability of *Lactobacillus*. Storage at 4°C of encapsulated freeze-dried *Bifidobacterium* Bb12 provided superior result as compared to that at 25°C; a_w of both storages was adjusted to 0.1. Survival of both encapsulated and free bacteria kept at 25°C decreased when a_w was increased into 0.3; whereas encapsulated bacteria kept at 4°C were not affected by the increase in a_w. Casein-based microcapsule showed significant protective effect of on freeze-dried *Bifidobacterium* BB12 regardless of a_w and temperature of storage. Spray-dried probiotic bacteria coated with casein-based system, however, showed less survival as compared to freeze-dried bacteria after drying process and after storage at 25°C at low a_w (Dianawati et al., 2013). This finding is in agreement with that of Wong et al. (2010), Zamora et al. (2006) and Johnson and Etzel (1995).

Savini et al. (2010) freeze-dried *Lactobacillus rhamnosus* IMC 501 and *Lactobacillus paracasei* IMC 502 using sugar alcohols (glycerine, mannitol, sorbitol), inulin, dextrin, and crystalean; semi-skimmed milk was used as a control. Glycerin, followed by mannitol, was the most effective sugars in protecting both strains during storage at room temperature for five months. Addition of sorbitol improved survival of freeze-dried *L. paracasei* ssp. *paracasei* LMG 9192 and *L. plantarum* CWBI-B1419 after 150 day storage at 25°C in the vacuum sealed bags; increase in unsaturated fatty acids indicated an adaptation mechanism of the bacteria to survive (Coulibaly

et al., 2010) (Table 4). Similarly, Mugnier and Jung (1985) found that mannitol, an isomer of sorbitol, was more effective in protecting dehydrated gram-positive bacteria than glycerol, reducing sugars or higher MW sugars during storage at room temperature at a_w of 0.07. This result was in agreement with that of Carvalho et al. (2003a) who reported that an incorporation of sucrose, a disaccharides, as cryoprotectant was ineffective in increasing the survival of freeze-dried *L. bulgaricus* during storage at 20°C.

Reconstituted skim milk (RSM) combined with prebiotic (raftilose or polydextrose) and RSM alone as control (20% total solids) has been compared to establish their effectiveness in improving survival of *Lactobacillus rhamnosus* GG (ATCC 53103) during spray drying and during storage at room temperature (Ananta et al., 2005). Incorporation of prebiotics did not influence bacterial survival during spray drying (T_{outlet} = 80°C) with survival of 55–67%; partial replacement of prebiotics to RSM had an adverse impact on bacterial survival during 6 weeks of storage at 25 or 37°C compared to RSM only (% survival was not shown). These results were similar to those of Corcoran et al. (2004). Similarly, the use of waxy maize starch as microencapsulant appeared effective only in protecting *Bifidobacterium* strain during spray drying (T_{inlet} = 100°C; T_{outlet} = 45°C), but was ineffective in protecting the bacteria from acid environment and during 20 days of storage at room temperature (O'Riordan et al., 2001). The use of RSM and gum acacia as growth media instead of microencapsulant for *L. paracasei* NFBC 338 was carried out by Desmond et al. (2002). Both bacteria and milk-based media were then spray dried (air inlet temperature of 170°C; outlet temperature was 95–100°C and 100–105°C). The result showed that stability of spray-dried bacteria was good only during storage at 4°C, but more than 99.9% decrease occurred when spray dried bacteria in RSM-gum acacia were kept at 30°C for eight weeks.

The role of sugars with high molecular weight raises the question whether they can function as encapsulating materials, just as a carrier or space filler without any interaction with the core (Oldenhof et al., 2005) or in the contrary, they had an adverse effect on bacterial survival as proposed by Hincha et al. (2002) and Ananta et al. (2005). The presence of prebiotic (10% w/v) in skim milk (10% w/v) decreased bacterial viability during storage at 37°C as compared to skim milk alone (20% w/v) (Ananta et al., 2005). The presence of large size of polymers (such as prebiotic) might cause the 'steric hindrance' preventing them to interact with dehydrated proteins and membrane lipids (Hincha et al., 2002). Ananta et al. (2005) proposed that skim milk alone was capable of interacting with polar headgroups of membrane phospholipids and protecting cell membranes during spray drying and storage. However, this mechanism might be difficult due to high molecular weight of milk proteins. On the other hand, it was proven that the presence of sorbitol or mannitol as sugar alcohol was able to interact with polar site of phospoholipids of probiotic bacteria via hydrogen-bond (Santivarangkna et al., 2010; Dianawati et al., 2012).

Other studies also demonstrated that mostly combination between proteins, low MW sugars and high MW sugars were effective in protecting bifidobacteria, but the results vary depending upon strains. Chavez et al. (2007) found that soy isolate proteins combined with maltodextrin contributed to the highest viability of *B. lactis* BB12 during 3 months of storage at 30°C, while skim milk + trehalose and skim milk + arabic gum provided lower stability. Crittenden et al. (2006) found that high survival of spray-dried *Bifidobacterium infantis* Bb-02 was achieved when they were encapsulated using formulation containing sodium caseinate, fructooligosaccharides and resistant starch. However, survival of bifidobacteria after spray drying and after storage at 25°C was strain-dependent (Table 4) (Simpson et al., 2005). Sunny-Roberts and Knorr (2009) observed the protection effect of trehalose and trehalose + MSG on *Lactobacillus rhamnosus* strains after spray drying (T_{outlet} = 60–75°C) and during storage at 25 or 37°C. The results showed that T_{outlet} optimum was 65–70°C providing highest viability of bacteria after spray drying (1.8×10^9 CFU/mL; moisture content (MC) = 3.8% w/w); MC ranged between 3.57 to 4.43%, depending on strains. Storage at 25°C for six weeks at a_w equals 0.11 with the presence of trehalose and MSG provided highest stability of spray-dried *L. rhamnosus* strains (10^8 CFU/mL); while encapsulation using trehalose without MSG or storage at 37°C decreased bacterial stability significantly. The protection of MSG was likely due to its antioxidation potential (Sunny-Roberts and Knorr, 2009). Overall, some studies did not determine moisture content or a_w of their products (Table 3 and 4); this could compromise the accuracy of the survival data.

Survival of spray-dried bifidobacteria ($T_{inlet/outlet}$ = 100/50°C) in skim milk, gum arabic, gelatin, or soluble starch with or without oxygen absorber and desiccant was observed (Hsiao et al., 2004). The presence of absorber and desiccant increased the survival of microencapsulated cells when kept at 25°C (42 days). Population reduction was the lowest when skim milk was used, and was the highest when gum arabic was used; this difference was more apparent during storage at 25°C (Table 4). The diffusion of oxygen through microcapsule might still occur resulting in adverse effects such as changes in cell membrane structure (Hsiao et al., 2004). Among probiotic organisms, bifidobacteria were the most susceptible to oxygen due to their anaerobic characteristic (Talwalkar and Kailasapathy, 2003). In agreement with the result of Ananta et al. (2005), a combination of casein with resistant starch decreased the stability of *Bifidobacterium* during storage (Heidebach et al. 2010). The authors hypothesized that protein matrix consistency might be disturbed due to the existence of resistant starch; hence, its function to protect the bacteria from oxygen diffusion decreased. For *Lactobacillus* F19 which is more oxygen tolerant, the presence of RS in casein-matrix had no effect. The difference in bacterial strains or species, a_w of storage, sugars or proteins as encapsulant materials and drying method have an influence on bacterial retention during storage at room temperature.

CONCLUSION

Microencapsulation of probiotic bacteria is carried out to improve the bacterial stability during transportation and storage and during passage through harsh environment of gastrointestinal tract before adhering onto colon of the host in order to provide several health benefits. Various encapsulating materials prepared from carbohydrates (sugar alcohols, reducing sugars, polysaccharides, hydrocolloids), or proteins (milk- or non-milk based) or their combination have been studied to ascertain their effectiveness in protecting probiotic bacteria during process of microencapsulation, storage, and passage through simulated gastric or bile juice. Skim milk and casein and/or sugars such as mannitol, sorbitol, trehalose, and sucrose were proven effective as encapsulating materials for *Lactobacillus* and *Bifidobacterium*. On the other hand, the use of polysaccharides showed varied results depending on bacterial strains and method of microencapsulation including the freeze- or spay-drying stage. Each of these must be optimized to increase the shelf life of probiotic bacteria without any significant changes in functional characteristics.

Storage at cold and frozen temperatures is ideal in maintaining bacterial viability. However, it requires high cost of transportation and storage, thus increasing the product price. Storage at room temperature (usually between 20 and 30°C) can be a cheap alternative required to be developed. Water activity and glass transition are critical factors that will trigger adverse physico-chemical reactions causing bacterial inactivation. Viability is favored in an amorphous state which is metastable and can only be achieved at low a_w storage. Storage at ambient temperature at low a_w of 0.07 and 0.1, and under vacuum are effective in improving bacterial survival for long period assuring health benefits associated with consumption.

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