HAYATI Journal of Biosciences 24 (2017) 1–5

HOSTED BY

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Review article

Microencapsulation Improved Probiotics Survival During Gastric Transit

Amal Bakr Shori

King Abdulaziz University, Faculty of Science, Department of Biological Sciences, Jeddah, Saudi Arabia.

ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form
9 December 2016
Accepted 9 December 2016
Available online 6 January 2017

KEYWORDS:
microencapsulation,
probiotics,
simulated gastric condition,
viability

ABSTRACT

Several studies have demonstrated differences among strains of probiotic bacteria with regard to their survival in acid environment. Probiotics must survive in gastric acids to reach the small intestine and colonize the host for appropriate prevention and management of several gastrointestinal diseases. To improve the survival rates of probiotic microorganisms during gastric transit, microencapsulation is considered to be a promising process. A variety of polymers are commonly used for microencapsulation. Thus, there is a widespread interest in the improvement of the physical and mechanical stability of the polymers use in probiotics encapsulation. In addition, there is a developing trend toward the use of milk proteins as encapsulation device. To fulfill many demands of a successful probiotics encapsulation, different techniques have been applied to increase the resistance of these sensitive microorganisms against gastric conditions. Therefore, the objective of this study is to review the effect of microencapsulation on survival of probiotics in an *in vitro* model simulating gastric transit.

1. Introduction

Probiotics are live microorganisms that when present in sufficient amounts in the digestive tract may confer health benefits on the host (Lourens-Hattingh and Viljoen 2001). To promote their beneficial effects in the host, probiotics must survive transit through the harsh acidic conditions of gastric environment and being capable of reaching the large intestine in adequate amounts to enable colonization and proliferation (Li *et al.* 2011). It has been recommended that food containing probiotic bacteria should be in the range of 10^8–10^9 colony forming unit (cfu)/g right before ingestion to ensure that sufficient therapeutic minimum of 10^6–10^7 cfu/g could reach the colon (Nazzaro *et al.* 2009). Unfortunately, most of the probiotics lack the ability to survive in high quantity because of low pH (pH = 2) in gastric juice and/or exposure to oxygen that limited their effectiveness in most functional foods. Microencapsulation is a process in which the probiotic cells are incorporated into an encapsulating matrix or membrane that can protect the cells from degradation by the damaging factors in the environment and release at controlled rates under particular conditions (Desai and Park 2005). The purpose of microencapsulation of probiotics is to protect them from the low pH, bile salts, and other constituent products that it encounters during gastrointestinal transit (Muthukumarasamy *et al.* 2006). A microcapsule comprises a semipermeable or nonpermeable, spherical, thin, and strong membrane surrounding a solid and liquid core with very small diameter varying between a few microns and 1 mm. Encapsulation materials are generally recognized as safe ingredients that can be used in food applications (Ei-Salam and Ei-shibiny 2012). Food-grade polymers, such as alginate, chitosan, carboxymethyl cellulose, xanthan gum, starch, carrageenan, gelatin, and pectin, are largely applied using different microencapsulation techniques (Sultana *et al.* 2000; Muthukumarasamy *et al.* 2006; Anal and Singh 2007; Ding and Shah 2009; Mokarram *et al.* 2009; Chavarri *et al.* 2010; Cook *et al.* 2011, 2013). In addition, there is a developing trend toward the use of encapsulation in milk proteins such as casein (Oliveira *et al.* 2007; Heidebach *et al.* 2009a,b) and whey protein (Doherty *et al.* 2012). Alginate is a natural polymer that is applied successfully as a pH-sensitive material for the microencapsulation of probiotic bacteria (Allan-Wojtas *et al.* 2008). However, to fulfill many demands of a successful probiotics encapsulation, different techniques have been applied to increase the resistance of these sensitive microorganisms against gastric conditions including incorporation of some food-grade polymers into the matrix of alginate (Sultana *et al.* 2000; Muthukumarasamy *et al.* 2006; Anal and Singh 2007; Ding

E-mail address: shori_7506@hotmail.com.
Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2016.12.008

and Shah 2009; Mokarram *et al.* 2009; Chavarri *et al.* 2010; Cook *et al.* 2011, 2013). Therefore, a vast number of publications were used to review the effect of microencapsulation on survival of probiotics in an *in vitro* model simulating gastric transit.

2. Milk Proteins-Based Microencapsulation

Coatings and mixtures of suitable biopolymers such as alginate and k-carrageenan are of nondairy origin. Therefore, they are undesirable to use in dairy products in several countries (Picot and Lacroix 2004). In the case of calcium alginate (Ca-alginate), there is no pronounced barrier effect given at very low pH because of the low density of the forming gel network (Crittenden *et al.* 2006; Mortazavian *et al.* 2008). Alginate microspheres with porous structure permit the diffusion of acid in and out of microspheres. Besides, the large size of the produced beads (diameters between 1 and 3 mm) may induce contrary effects on the sensorial quality of the enriched product. Carrageenan is used as an encapsulation carrier because of its gelation property. It is a natural polysaccharide including a crosslinking structure by D-galactose-4-sulfate and 3,6-dehydrated-D-galactose. However, probiotic bacteria, such as *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Bifidobacterium infantis*, were encapsulated in k-carrageenan microcapsules or alginate microcapsules with 2% resistant starch for supplementation of cheddar cheese (Godward and Kailasapathy 2003). The authors found that the viable cell numbers of all probiotic strains were higher in free cells cheddar cheese than encapsulated cells during storage. This indicated that the physiological conditions in a hydrocolloid matrix such as k-carrageenan or alginate are less appropriate for the probiotic cells. Caseins, whey proteins, and milk fat globule membrane proteins present in milk are recognized as safe materials with high nutritional and good sensory properties. The milk proteins also have properties such as binding small molecules, self-assembly, excellent gelation, pH-responsive gel swelling behavior, and ability to interact with other polymers for the formation of complexes (Livney 2010). Thus, milk proteins, especially whey proteins, show a potential in the encapsulation of lactic acid bacteria. For example, encapsulated yield of *Lactobacillus bulgaricus* in skim milk-alginate microspheres was about 100% (Pan *et al.* 2013). Similarly, Hébrard *et al.* (2009) obtained 95% of encapsulation efficiency of probiotic yeast (*Saccharomyces boulardii*)-loaded microspheres with whey protein and alginate in a ratio of 62:38. This can be attributed to the excellent interaction between milk proteins and alginate-based microspheres in Ca^{2+} solution for the formation of complexes. In addition, the protective effects of the rennet cheese dense protein matrix toward probiotic bacteria at low pH 2–2.5 have been reported (Boylston *et al.* 2004). Heidebach *et al.* (2009a) investigated the probiotic cells microencapsulated in milk protein matrices by means of an enzymatic-induced gelation with rennet. The authors reported that the survival rate of *B. lactis* and *Lactobacillus paracasei* encapsulated in milk protein significantly improved compared with free cells at pH 2.5. The viable cell counts of encapsulated *B. lactis* and *L. paracasei* were higher by 2.8 and 0.8 log units cfu/g, respectively, than free cells after 90 minutes of exposure to low pH (2.5) at 37°C (Table 1). It has been shown that *L. paracasei* is more sensitive to acid than *B. lactis*, which means the survival rate of probiotics in low pH is strain dependent. According to Muthukumarasamy *et al.* (2006), each probiotic strain has different response mechanisms to tolerate low pH, which cannot be fully recovered by microencapsulation. However, both strains achieved higher survival rate of cells because of the entrapment in a protective environment of the microcapsules (Heidebach *et al.* 2009a). Another study used sodium caseinate gelled with transglutaminase (TGase) enzyme for *L. paracasei* and *B. lactis* encapsulation (Heidebach *et al.* 2009b). This study found that the free cells of *L. paracasei* and *B. lactis* were reduced by more than 5 and 3.3 log cfu/g, respectively, after 90 minutes in simulated gastric fluid (SGF) at pH 2.5. However, when the cells encapsulated in sodium caseinate gelled with TGase, the reductions were about 3.0 and 2.7 log cfu/g for *L. paracasei* and *B. lactis*, respectively (Table 1). The improved survival of encapsulated cells could be explained by the reaction of TGase to induce crosslinks built on a covalent network resulting in water-insoluble and physically stable gels resistant against disbanding at low pH. *L. casei* cells were effectively entrapped into sodium caseinate and gellan gum mixture gelled by gradually decreasing pH using glucono-δ-lactone (Nag *et al.* 2011). The viability of encapsulated cells was reduced by only 3.1 log cfu/g, whereas free cells showed 6.1 log cfu/g reductions after 120 minutes in SGF. Several factors may act together to cause high viability of encapsulated *L. casei* cells such as the synergistic effect between gellan gum and sodium caseinate to provide additional gel strength and preadaptability of bacterial cells in low pH caseinate gels (Nag *et al.* 2011).

L. bulgaricus is very sensitive to low pH, and the viability of free *L. bulgaricus* is dramatically lost on exposure to acidic condition. Encapsulation of *L. bulgaricus* in alginate-milk microspheres showed significant ($p < 0.05$) improvement in the survival of *L. bulgaricus* in SGF (Shi *et al.* 2013a). This encapsulation maintained full viability of *L. bulgaricus* after 120 and 30 minutes of incubation in SGF with pH 2.5 and 2.0, respectively (Table 1). The buffering capacity of milk in microspheres may be responsible for the excellent pH tolerance of encapsulated *L. bulgaricus*. Moreover, Shi *et al.* (2013a) demonstrated that high milk concentration may lead to form denser hydrogel network that could decrease the diffusion rate of acid into the microspheres. The viability of encapsulated *L. bulgaricus* reduced to around 1 log cfu/g in alginate:milk ratio of 1:4, whereas the reduction was more than 8 log cfu/g in alginate:milk ratio of 1:1 after 120 minutes in SGF with pH 2.0.

Encapsulation of *Lactobacillus rhamnosus* GG in microparticles consisting of micellar casein and denatured whey proteins showed 99% of bacterial survival and 97% of encapsulation rate (Burgain

Table 1. The effect of milk proteins-based microencapsulation on the protection and survival of probiotics during simulated gastric fluid (pH 2.5) at 37°C

Probiotic	Type of encapsulating material	Probiotic viability (log cfu/g)	Time of incubation (min)	References
B. lactis *L. paracasei*	Milk protein matrices with rennet	2.8 log cfu/g↑ 0.8 log cfu/g↑	90	Heidebach *et al.* (2009a)
L. paracasei *B. lactis*	Sodium caseinate gelled with transglutaminase	2 log cfu/g↑ 0.6 log cfu/g↑	90	Heidebach *et al.* (2009b)
L. casei	Sodium caseinate and gellan gum mixture gelled by glucono-δ-lactone	3 log cfu/g↑	120	Nag *et al.* (2011)
L. bulgaricus	Alginate-milk	10^{10} log cfu/g*	120	Shi *et al.* (2013a)
L. rhamnosus GG	Micellar casein and denatured whey proteins	~10^{11} cfu/g*	120	Burgain *et al.* (2013)

B. lactis = *Bifidobacterium lactis*; cfu = colony forming unit; *L. bulgaricus* = *Lactobacillus bulgaricus*; *L. paracasei* = *Lactobacillus paracasei*; *L. casei* = *Lactobacillus casei*; *L. rhamnosus* = *Lactobacillus rhamnosus*; ↑ = higher than free cells.

* No change from initial counts..

Table 2. The effect of alginate-based microencapsulation on the protection and survival of probiotics during simulated gastric fluid (pH 2.0) at 37°C

Probiotic	Type of encapsulating material	Survival rate (%)	Time of incubation (min)	References
L. acidophilus CGMCC1.2686	Alginate-$CaCO_3$	22	120	Cai *et al.* (2014)
	Alginate-Ca-ethylenediaminetetraacetate	7.1		
L. rhamnosus GG ATCC 53103	Ca-alginate	43	180	Guimarães *et al.* (2013)
B. animalis DN-173 010		72		
B. longum BIOMA 5920	Alginate and human-like collagen	51	120	Su *et al.* (2011)
B. adolescentis 15703T	Gelatin microspheres with alginate	~30	120	Annan *et al.* (2008)

B. adolescentis = *Bifidobacterium adolescentis*; *B. animalis* = *Bifidobacterium animalis*; *B. longum* = *Bifidobacterium longum*; *L. acidophilus* = *Lactobacillus acidophilus*; *L. rhamnosus* = *Lactobacillus rhamnosus*.

et al. 2013). The improvement of encapsulation efficiency of *L. rhamnosus* GG referred to micellar casein and the interaction between probiotic strain and whey proteins.

3. Alginate-Based Microencapsulation

Alginate is a natural polymer that is applied successfully as a pH-sensitive material for the microencapsulation of probiotic bacteria (Allan-Wojtas *et al.* 2008). Alginate is a polysaccharide extracted from algae composed of various amounts and sequential distribution of β-D-mannuronic (M) and α-L-guluronic acids (G) (block copolymer containing both MM, GG, and irregular sequences of M and G units) that can affect functional properties of alginate as a supporting material (Burgain *et al.* 2011). When sodium alginate solution containing cell suspension is poured into a calcium solution, the bound ions interact with other GG blocks to form a complex that leads to gel formation and the probable release of entrapped cells in the intestinal tract (Prakash and Jones 2005). Alginate microencapsulation strategies have been established for the ability to protect probiotic viability in the gastrointestinal digestion (Hansen *et al.* 2002). Cai *et al.* (2014) demonstrated that the protection efficiency of alginate-$CaCO_3$ microcapsule for *L. acidophilus* CGMCC1.2686 was stronger than alginate-Ca-ethylenediaminetetraacetate (EDTA). The encapsulated *L. acidophilus* in alginate-$CaCO_3$ showed survival rate of 22% compared with 7.1% in alginate-Ca-EDTA after SGF for 2 hours (Table 2). High performance and productivity of alginate-$CaCO_3$ microcapsule for *L. acidophilus* against acidic injury might be explained by the improvement in mechanical properties and denser structure of the alginate-$CaCO_3$ microcapsules. One possible reason for higher mechanical strength for alginate-$CaCO_3$ microcapsules than alginate-Ca-EDTA could be the neutral pH of alginate capsules that was adjusted after gelation and solidification by using phosphate buffer. However, the increase in chelating ability of EDTA with Ca ions at neutral pH may decrease the integrity of Ca-crosslinked alginate network leading to a decreased mechanical strength of alginate capsules (Cai *et al.* 2014). Moreover, the antimicrobial effect of EDTA may cause some damage to *L. acidophilus* by decreasing the stability of bacterial cell membrane through complexing divalent cations that acted as salt bridges between membrane macromolecules (Chang *et al.* 2012). Another study developed new probiotic beads similar to fish eggs to use in oriental cuisine (Guimarães *et al.* 2013). *L. rhamnosus* GG ATCC 53103 and *Bifidobacterium animalis* DN-173 010 were encapsulated in beads produced by the extrusion encapsulation technique with Ca-alginate. Both free probiotic strains failed to survive at low pH 2.0 and 2.5 after 180 minutes in SGF, whereas encapsulated cells of *L. rhamnosus* and *B. animalis* showed viability of 3.4 and 5.7 log cfu/g, respectively, at pH 2.0 (Guimarães *et al.* 2013; Table 2). Su *et al.* (2011) developed microspheres based on alginate and human-like collagen for improving the survival of *Bifidobacterium longum* BIOMA 5920 in SGF. They found that none of free *B. longum* survived after exposure to SGF for 2 hours. However, encapsulated *B. longum* in 1.5% (w/v) alginate and 2% (w/v) human-like collagen survived about 4.81 log cfu/mL of 9.47 cfu/mL (four log cycles reduction) after 2 hours in SGF. This level of survival was better than using alginate alone that showed about five log cycles reduction (from 9.02 to 3.77 log cfu/mL) during 2 hours of exposure to SGF. Previous study found that coating of gelatin microspheres with alginate provided significant protection for *Bifidobacterium adolescentis* 15703T from the harsh acidic conditions of SGF (Annan *et al.* 2008). Encapsulated *B. adolescentis* showed two log units higher in survival rates compared with free cells after incubation in SGF at pH 2.0 for 2 hours (Table 2).

4. Chitosan-Based Microencapsulation

Cationic polymers such as chitosan can form gels with polyphosphate or sodium alginate (nontoxic multivalent anionic counterions) by ionic crosslinking (Lucinda-Silva *et al.* 2010). The coating of alginate beads and its efficiency in protecting probiotics has been widely studied over a period of years. Previous study has found that coating alginate microcapsules with chitosan had significant effect on the stability of the alginate beads and thus increased the survival rate of the encapsulated probiotics (Krasaekoopt *et al.* 2003). According to Chavarri *et al.* (2010), coating alginate beads with chitosan develops chitosan with alginate complex. This complex decreases the porosity of alginate beads, reduces the leak of the encapsulated probiotic, and shows stability at low pH. Kanmani *et al.* (2011) showed no released cells of *Enterococcus faecium* MC13 encapsulated into alginate-chitosan capsules in SGF for 144 hours (Table 3). Similarly, Chavarri *et al.* (2010) reported that the alginate-chitosan capsules enhanced

Table 3. The effect of chitosan-based microencapsulation on the protection and survival of probiotics during simulated gastric fluid (pH 2.0) at 37°C

Probiotic	Type of encapsulating material	Probiotic viability	Time of incubation	References
Enterococcus faecium MC13	Alginate-chitosan	Nil	144 hr	Kanmani *et al.* (2011)
L. gasseri	Alginate-chitosan	7 log cfu/mL	120 min	Chavarri *et al.* (2010)
B. bifidum				
L. casei	Alginate-chitosan	7.38 log cfu/g	120 min	Li *et al.* (2011)
	Alginate-chitosan-carboxymethyl chitosan	7.91 log cfu/g		
B. breve	Alginate-chitosan and fluid-bed drying	6.6 log cfu/mL	60 min	Cook *et al.* (2011)
B. breve	Poly D,L-lactic-co-glycolic acid containing prebiotic galactooligosaccharides incorporated into an alginate-chitosan matrix	>4 log cfu/mL	60 min	Cook *et al.* (2014)
Saccharomyces boulardii	Chitosan/dextran sulphate	7.19 log cfu/100 mg	120 min	Thomas *et al.* (2014)

B. bifidum = *Bifidobacterium bifidum*; *B. breve* = *Bifidobacterium breve*; *L. casei* = *Lactobacillus casei*; *L. gasseri* = *Lactobacillus gasseri*.

Table 4. The effect of plant materials-based microencapsulation on the protection and survival of probiotics during simulated gastric fluid at 37°C

Probiotic	Type of encapsulating material	pH of gastric fluid	Survival rate (%)	Time of incubation (min)	References
B. adolescentis	Pea protein isolate-alginate with fructo-oligosaccharides	pH 2.0	88.9	120	Klemmer *et al.* (2011)
L. bulgaricus	Carrageenan-LB gum-coated milk	pH 2.5	100	120	Shi *et al.* (2013b)
		pH 2.0	80		
L. rhamnosus	Chitosan-coated alginate capsules with LB	pH 3.0	92	60	Cheow *et al.* (2014)

B. adolescentis = *Bifidobacterium adolescentis*; *L. bulgaricus* = *Lactobacillus bulgaricus*; LB = locust bean; *L. rhamnosus* = *Lactobacillus rhamnosus*.

($p < 0.05$) the survival rate of *Lactobacillus gasseri* and *B. bifidum* (10^7 cfu/mL) when compared with free cells (10 cfu/mL) in simulated gastric conditions after 2 hours. Alginate-chitosan and alginate-chitosan-carboxymethyl chitosan microcapsules of *L. casei* were found to maintain high viable cell counts (7.38 and 7.91 log cfu/g, respectively) after 2 hours in SGF (Li *et al.* 2011; Table 3). This may indicate that chitosan-coated alginate is very effective in protecting probiotics in highly acidic environment. In contrast, encapsulation into Ca-alginate without chitosan failed to protect probiotic cells such as *L. plantarum*, *L. acidophilus*, *L. rhamnosus*, and bifidobacteria in SGF (Sultana *et al.* 2000; Truelstrup Hansen *et al.* 2002; Gbassi *et al.* 2009; Mokarram *et al.* 2009). The impeding of Ca-alginate matrix without chitosan coating happens in phosphate buffer solution by chelating action of phosphate ions in pH exceeding 5.5 (Dainty *et al.* 1986). The encapsulation techniques used are very important to guarantee long-term delivery of stable cultures in terms of viability (Carvalho *et al.* 2003). It has been found that free *Bifidobacterium breve* showed zero survival after 1 hour exposure to SGF (Cook *et al.* 2011). However, the survival rate of encapsulated *B. breve* in alginate-chitosan and fluid-bed drying reduced by only 1 log cfu/mL and thus guaranteed higher viable cell counts of about 6.6 ± 0.5 log cfu/mL after 1 hour in SGF (Cook *et al.* 2011). A recent study was conducted to investigate the effect of incorporated *B. breve* into a multiparticulate consisting of poly D,L-lactic-co-glycolic acid microcapsules containing prebiotic galactooligosaccharides incorporated into an alginate-chitosan matrix (Cook *et al.* 2014). Results showed significant reduction (5 log cfu/mL) in the viable cell counts of the chitosan-coated multiparticulate system after exposure to gastric conditions at pH 2 for 1 hour. This technique gives low viability of *B. breve* in gastric condition (>4 log cfu/mL) compared with alginate-chitosan and fluid-bed drying technique mentioned earlier (Cook *et al.* 2011; Table 3). However, chitosan-coated multiparticulate system showed latter significant increase in the viability of cells in intestine section to about 8.0 ± 0.3 log cfu/mL, which could be associated with the release of prebiotics (galactooligosaccharides) that stimulated the growth of cells.

Recently, *Saccharomyces boulardii* was encapsulated by layer-by-layer technique with oppositely charged polyelectrolytes such as chitosan and dextran sulfate to protect it from low pH in SGF (Thomas *et al.* 2014). The cell counts of encapsulated *S. boulardii* were reduced by only 0.5 log cfu/100 mg to reach 7.19 ± 2.0 log cfu/100 mg, whereas unencapsulated cells showed about 1.3 log cfu/100 mg reductions after 2 hours in SGF of pH 2 (Table 3). The possible reason for high survival of encapsulated *S. boulardii* is the strong electrostatic interaction between the chitosan and dextran sulfate polymer layers that led to dense structure for protecting the yeast cells.

5. Plant Materials-Based Microencapsulation

Encapsulation of probiotics using plant-based materials may play a role in food applications. Klemmer *et al.* (2011) reported that viability of *B. adolescentis* in pea protein isolate-alginate microspheres with fructo-oligosaccharides was highly improved (10^8 cfu/mL) compared with free cells (10 cfu/mL) after 2 hours of incubation in SGF with pH 2.0 (Table 4). Shi *et al.* (2013b) have used pure milk as an inner layer for encapsulated *L. bulgaricus* in addition to carrageenan-locust bean (LB) gum that was used as an outer layer for coating the milk microspheres. Carrageenan-LB gum-coated milk microspheres with double layer structure were produced to enhance the number of viable probiotic bacterial cells. However, the authors discovered relatively low encapsulation yield (~60%), which is caused by the poor film formation ability of milk protein. Furthermore, high Ca^{2+} concentration (16 g/100 mL) in the solution used for formulating microbeads may cause damage to probiotics viability (Shi *et al.* 2013b). However, encapsulated *L. bulgaricus* in carrageenan-LB gum-coated milk improved pH stability with full and 8 log cfu/g viability of *L. bulgaricus* preserved after 2 hours of incubation in pH 2.5 and 2.0 SGF, respectively (Table 4). The improvement in survival numbers of encapsulated probiotics can probably be explained by the buffering capacity of the proteins-based capsule-matrix, dense structure, and low porosity on the surface of the microspheres.

Recently, the effect of adding LB gum to chitosan-coated alginate capsules on *L. rhamnosus* release profiles in SGF was investigated (Cheow *et al.* 2014). Chitosan-coated alginate-LB capsules of *L. rhamnosus* provided higher cells protection (92%) compared with chitosan-coated alginate-xanthan and chitosan-coated alginate (47% and 9%, respectively) after 1 hour in SGF with pH 3. In addition, chitosan-coated alginate-LB capsules released the smallest amount of cells (4.0 log cfu/mg) compared with chitosan-coated alginate capsules and chitosan-coated alginate-xanthan capsules (4.4 and 4.9 log cfu/mg) after 1 hour incubation in the SGF (Cheow *et al.* 2014). The authors suggested that a strong interaction between chitosan and alginate-LB leads to more extensive chitosan coating on the alginate-LB capsules, and high swelling capacity caused dense structure that can withstand the low pH environments and delay the onset of bulk capsule dissolution.

6. Conclusions and Perspectives

Several factors can effect the protection and survival of encapsulated probiotics during gastric transit such as acid resistance properties of probiotic strains, encapsulating materials and their concentrations, encapsulation methods, and types of polymers incorporation in the matrix. Encapsulation of probiotics in biocompatible multilayers using layer-by-layer technique is very effective to improve the protection and viability of the probiotics in gastric tract. Furthermore, chitosan-coated alginate microencapsulation significantly enhanced the stability of alginate in low acidic environments. Incorporation of milk, whey protein, and micellar casein with alginate had significant effect to increase viability of probiotics during gastric transit because of their pronounced buffering capacity. In addition, the use of enzymes such as rennet and TGase as gelling agents during probiotics encapsulation in dairy-based matrices provides many promising opportunities for enhancing probiotics protection in harsh acidic conditions of the stomach. Since then, a number of studies regarding the relationship between plant materials and probiotics have been successfully established to increase the viability of probiotics. LB gum could be a good choice as plant-based material for encapsulation

development to provide sufficient protection against gastric acid. However, few data are available to describe the effects of phenolic compounds as a natural antimicrobial and adequate growth medium into encapsulated probiotics. Food additives with prebiotic properties have enhanced and stimulated the growth of cells in encapsulated probiotics during gastric digestion. The mechanical stability of capsules with probiotics could increase when applied into a food matrix, considering that the presence of micronutrients (glucose) may enhance the survival of probiotics during the gastric digestion. In addition, this mechanism could be applied to improve the shelf life of probiotics dairy products with low pH such as yogurt.

Conflict of interest

The authors declare no conflict of interest.

References

Allan-Wojtas P, Hansen LT, Paulson AT. 2008. Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. *LWT Food Sci Technol* 41:101–8.

Anal AK, Singh H. 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends Food Sci Technol* 18:240–51.

Annan NT, Borza AD, Hansen LT. 2008. Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions. *Food Res Int* 41:184–93.

Boylston TD, Vinderola CG, Ghoddusi HB, Reinheimer JA. 2004. Incorporation of bifidobacteria into cheeses: challenges and rewards. *Int Dairy J* 14:375–87.

Burgain J, Gaiani C, Francius G, Revol-Junelles AM, Cailliez-Grimal C, Lebeer S, Tytgat HL, Vanderleyden J, Scher J. 2013. In vitro interactions between probiotic bacteria and milk proteins probed by atomic force microscopy. *Coll Surf B Biointer* 104:153–62.

Burgain J, Gaiani C, Linder M, Scher J. 2011. Encapsulation of probiotic living cells: from laboratory scale to industrial applications. *J Food Eng* 104:467–83.

Cai S, Zhao M, Fang Y, Nishinari K, Phillips GO, Jiang F. 2014. Microencapsulation of *Lactobacillus acidophilus* CGMCC1.2686 via emulsification/internal gelation of alginate using Ca-EDTA and $CaCO_3$ as calcium sources. *Food Hydrocoll* 39:295–300.

Carvalho AS, Silva J, Ho P, Teixeira P, Malcata X, Gibbs P. 2003. Effect of various growth media upon survival during storage of freeze-dried *Enterococcus faecalis* and *Enterococcus durans*. *J Appl Microbiol* 94:947–52.

Chang Y, Gu W, McLandsborough L. 2012. Low concentration of ethylenediaminetetraacetic acid (EDTA) affects biofilm formation of *Listeria monocytogenes* by inhibiting its initial adherence. *Food Microbiol* 29:10–7.

Chavarri M, Maranon I, Ares R, Ibanez FC, Marzo F, Villaran Mdel C. 2010. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *Int J Food Microbiol* 142:185–9.

Cheow WS, Kiew TY, Hadinoto K. 2014. Controlled release of *Lactobacillus rhamnosus* biofilm probiotics from alginate-locust bean gum microcapsules. *Carbohydr Polym* 103:587–95.

Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. 2011. Production and evaluation of dry alginate-chitosan microcapsules as an enteric delivery vehicle for probiotic bacteria. *Biomacromolecules* 12:2834–40.

Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. 2014. Microencapsulation of a synbiotic into PLGA/alginate multiparticulate gels. *Int J Pharmaceut* 466:400–8.

Cook MT, Tzortzis G, Khutoryanskiy VV, Charalampopoulos D. 2013. Layer-by-layer coating of alginate matrices with chitosan-alginate for the improved survival and targeted delivery of probiotic bacteria after oral administration. *J Mat Chem B* 1:52–60.

Crittenden R, Weerakkody R, Sanguansri L, Augustin M. 2006. Synbiotic microcapsules that enhance microbial viability during nonrefrigerated storage and gastrointestinal transit. *Appl Environ Microbiol* 72:2280–2.

Dainty AL, Goulding KH, Robinson PK, Simpkins I, Trevan MD. 1986. Stability of alginate-immobilized algal cells. *Biotechnol Bioeng* 28:210–6.

Desai KGH, Park HJ. 2005. Recent developments in microencapsulation of food ingredients. *Drying Technol* 23:1361–94.

Ding WK, Shah NP. 2009. Effect of various encapsulating materials on the stability of probiotic bacteria. *J Food Sci* 74:M100–7.

Doherty SB, Auty MA, Stanton C, Ross RP, Fitzgerald GF, Brodkorb A. 2012. Survival of entrapped *Lactobacillus rhamnosus* GG in whey protein micro-beads during simulated ex vivo gastro-intestinal transit. *Int Dairy J* 22:31–43.

Ei-salam MH, Ei-shibiny S. 2012. Formation and potential uses of milk proteins as nano delivery vehicles for nutraceuticals: a review. *Int J Dairy Technol* 65:13–21.

Gbassi GK, Vandamme T, Ennahar S, Marchioni E. 2009. Microencapsulation of *Lactobacillus plantarum* spp in an alginate matrix coated with whey proteins. *Int J Food Microbiol* 129:103–5.

Godward G, Kailasapathy K. 2003. Viability and survival of free and encapsulated probiotic bacteria in cheddar cheese. *Milchwissen-Milk Sci Int* 58:624–7.

Guimarães RR, Vendramini ALDA, Santos ACD, Leite SGF, Miguel MAL. 2013. Development of probiotic beads similar to fish eggs. *J Funct Foods* 5:968–73.

Hansen LT, Ian-Wojtas PM, Jin YL, Paulson AT. 2002. Survival of Caalginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiol* 19:35–45.

Hébrard G, Hoffart V, Beyssac E, Cardot JM, Alric M, Subirade M. 2009. Coated whey protein/alginate microparticles as oral controlled delivery systems for probiotic yeast. *J Microencapsul* 27:292–302.

Heidebach T, Först P, Kulozik U. 2009a. Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. *Food Hydrocoll* 23:1670–7.

Heidebach T, Först P, Kulozik U. 2009b. Transglutaminase-induced caseinate gelation for the microencapsulation of probiotic cells. *Int Dairy J* 19:77–84.

Kanmani P, Kumar RS, Yuvaraj N, Paari KA, Pattukumar V, Arul V. 2011. Effect of cryopreservation and microencapsulation of lactic acid bacterium *Enterococcus faecium* MC13 for long-term storage. *Biochem Eng J* 58-59:140–7.

Klemmer KJ, Korber DR, Low NH, Nickerson MT. 2011. Pea protein-based capsules for probiotic and prebiotic delivery. *Int J Food Sci Technol* 46:2248–56.

Krasaekoopt W, Bhandari B, Deeth H. 2003. Evaluation of encapsulation techniques of probiotics for yoghurt: a review. *Int Dairy J* 13:3–13.

Li XY, Chen XG, Sun ZW, Park HJ, Cha D-S. 2011. Preparation of alginate/chitosan/carboxymethyl chitosan complex microcapsules and application in *Lactobacillus casei* ATCC 393. *Carbohydr Polym* 83:1479–85.

Livney YD. 2010. Milk proteins as vehicles for bioactives. *Curr Opin Coll Interf Sci* 15:73–83.

Lourens-Hattingh A, Viljoen BC. 2001. Yogurt as probiotic carrier food. *Int Dairy J* 11:1–17.

Lucinda-Silva RM, Salgado HRN, Evangelista RC. 2010. Alginate-chitosan systems: in vitro controlled release of triamcinolone and in vivo gastrointestinal transit. *Carbohydr Polym* 81:260–8.

Mokarram RR, Mortazavi SA, Najafi MBH, Shahidi F. 2009. The influence of multistage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. *Food Res Int* 42:1040–5.

Mortazavian AM, Azizi A, Ehsani MR, Razavi SH, Mousavi SM, Sohrabvandi S, Reinheimer JA. 2008. Survival of encapsulated probiotic bacteria in Iranian yogurt drink (Doogh) after the product exposure to simulated gastrointestinal conditions. *Milchwissen* 63:427–9.

Muthukumarasamy P, Allan-Wojtas P, Holley RA. 2006. Stability of *Lactobacillus reuteri* in different types of microcapsules. *J Food Sci* 71:20–4.

Nag A, Han KS, Singh H. 2011. Microencapsulation of probiotic bacteria using pH-induced gelation of sodium caseinate and gellan gum. *Int Dairy J* 21:247–53.

Nazzaro F, Fratianni F, Coppola R, Sada A, Orlando P. 2009. Fermentative ability of alginate-prebiotic encapsulated *Lactobacillus acidophilus* and survival under simulated gastrointestinal conditions. *J Funct Foods* 1:319–23.

Oliveira AC, Moretti TS, Boschini C, Baliero JCC, Freitas O, Favaro-Trindade CS. 2007. Stability of microencapsulated *B. lactis* (BI 01) and *L. acidophilus* (LAC 4) by complex coacervation followed by spray drying. *J Microencapsul* 24:673–81.

Pan LX, Fang XJ, Yu Z, Xin Y, Liu XY, Shi LE, Tang ZX. 2013. Encapsulation in alginate-skim milk microspheres improves viability of *Lactobacillus bulgaricus* in stimulated gastrointestinal conditions. *Int J Food Sci Nutr* 64:380–4.

Picot A, Lacroix C. 2004. Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *Int Dairy J* 14:505–15.

Prakash S, Jones ML. 2005. Artificial cell therapy: new strategies for the therapeutic delivery of live bacteria. *J Biomed Biotechnol* 1:44–56.

Shi LE, Li ZH, Li DT, Xu M, Chen HY, Zhang ZL, Tang ZX. 2013a. Encapsulation of probiotics *Lactobacillus bulgaricus* in alginate-milk microspheres and evaluation of survival in simulated gastrointestinal conditions. *J Food Eng* 117:99–104.

Shi LE, Li ZH, Zhang ZL, Zhang TT, Yu WM, Zhou ML, Tang ZX. 2013b. Encapsulation of *Lactobacillus bulgaricus* in carrageenan-locust bean gum coated milk microspheres with double layer structure. *LWT Food Sci Technol* 54:147–51.

Su R, Zhu X, Fan D, Mi Y, Yang C, Jia X. 2011. Encapsulation of probiotic *Bifidobacterium logum* BIOMA 5920 with alginate-human-like collagen and evaluation of survival in simulated gastrointestinal conditions. *Int J Biol Macromol* 49:979–84.

Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K. 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int J Food Microbiol* 62:47–55.

Thomas MB, Vaidyanathan M, Radhakrishnan K, Raichur AM. 2014. Enhanced viability of probiotic *Saccharomyces boulardii* encapsulated by layer-by-layer approach in pH responsive chitosan-dextran sulfate polyelectrolytes. *J Food Eng* 136:1–8.

Truelstrup Hansen L, Allan-Wojtas PM, Jin YL, Paulson AT. 2002. Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiol* 19:35–45.