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The effect of encapsulating materials on the survival of probiotics during intestinal digestion: a review

Amal Bakr Shori

King Abdulaziz University, Faculty of Science, Department of Biological Sciences, Jeddah 21589, Saudi Arabia

Abstract

Many studies have demonstrated the sensitivity of probiotics to the bile salt solution. Encapsulation is a useful technique to protect probiotics from the bile salts and other constituent products that it encounters during gastrointestinal transit and improve the delivery of probiotics to large intestine in sufficient amounts for colonization and proliferation in order to exert a beneficial effect on the host. Encapsulation materials are recognized as safe ingredients and can be used in food applications. There is a widespread interest in the improvement of the physical and mechanical stability of the polymers use in probiotics encapsulation. Therefore, the objective of this study is to review the effect of various types of encapsulating materials on the protection and survival of probiotics during intestinal digestion.

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Keywords: probiotics; microencapsulation; viability; bile salts.

1. Introduction

Probiotics are live microorganisms that when present in sufficient amounts in the digestive tract may confer health benefits on the host [1]. Thus the management of intestinal microflora by probiotics has increased and the impacts of these probiotics are known including regulation of the gastrointestinal tract, enhancing immune system, reducing serum cholesterol levels and lactose intolerance and preventing cancer and cardiovascular disease [2,3].

Being capable to survive bile concentrations made in the human small intestines is one of the main problems of probiotics. Bile salts are one of the main components of intestinal fluid that manufactured from cholesterol in the liver and secreted into the upper duodenum through the bile duct. The average concentration of bile salt in the small intestine ranged between 0.2% and 2% (w/v). Bile salts function in the intestinal tract is to emulsify and dissolve the ingested fats. Furthermore, bile salts are found to enter the bacterial cells result in oxidative stress and damage to the DNA [4]. Therefore, the lethal action of bile salts on probiotics has commonly been observed [5-8].

Microencapsulation is a process in which the probiotic cells are entrap 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 [9]. Encapsulation is a useful tool to protect probiotics from the bile salts and other constituent products that it encounters during gastrointestinal transit and improve the delivery of probiotics to large intestine in adequate amounts for colonization and proliferation in order to exert a beneficial effect on the host [10]. Encapsulation materials generally recognized as safe ingredients can be used in food applications [11]. Food-grade polymers such as alginate, chitosan, carboxymethyl cellulose, xanthan gum, starch, carrageenan, gelatin and pectin are largely applied using different microencapsulation techniques [8,10,12-15]. There have been numerous efforts to the

* Corresponding author.

E-mail address: shori_7506@hotmail.com (Amal Bakr Shori).

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development of the physical and mechanical stability of the polymers use in the probiotics encapsulation. Therefore, the objective of this study is to review the effect of various types of encapsulating materials on the protection and survival of probiotics during intestinal digestion.

2. Survival of encapsulated probiotics during intestinal digestion

Bile salts are one of the major threats to probiotics survival in intestine. Bile acid and its derivatives from bile salts act as biological surfactants that pass inside the cell membrane and destructive the membrane integrity resulting in bacterial cell death [16]. With the objective of increasing survival of probiotic bacteria during intestinal digestion, the use of probiotic microencapsulation considered to be a successful process. Several factors can be effected the protection and survival of encapsulated probiotics such as bile resistance properties of probiotic strains, encapsulating materials and their concentrations, encapsulation methods, types of polymers incorporation in the matrix and various concentrations and sources of bile salts [12]. Numerous of studies have demonstrated the effect of microencapsulation on the protection and survival of probiotics during intestinal digestion (Table 1). Li et al. [10] have studied the effect of the bile salt on the viability of *Lactobacillus casei* ATCC 393 loaded in alginate–chitosan microcapsules (dry microspheres with size of 2200 μm; Table 1). Alginate–chitosan encapsulated *L. casei* showed higher survival rate (93% and 86%) than free cells (> 0.03%) after 3 hours exposure to bile salt at 0.5% and 1% respectively (Table 1). However, the addition of carboxymethyl chitosan (CMCS) to alginate–chitosan microcapsules enhanced ($p>0.05$) the tolerance of *L. casei* to bile salts solution with 95% and 91% survival rate after 3 hours exposure to bile salt at 0.5% and 1% respectively. Another study found that microencapsulation of *L. casei* 431 using pH-induced gelation of sodium caseinate and gellan gum (dry microspheres with size range from 40 to 1100 μm) have no significant differences in the survival rate (98%) when compared with free cells after 2 hours in bile salt solution [14]. Nevertheless, the survival rate of free cell had decreased to 24.3% after 6 hours of incubation while encapsulated *L. casei* remained almost constant over this period. Previous study demonstrated that incorporated of *L. gasseri* or *Bifidobacterium bifidum* into chitosan-coated alginate microspheres (dry microspheres) with encapsulation efficiency of 39.2% and 40.2% respectively increased the survival rate during simulated intestinal juice (SIJ) [13]. Both encapsulated *L. gasseri* and *B. bifidum* showed higher survival rate (100.6% and 97.7% respectively; $p<0.05$) compared to free probiotics (59% and 25% for *L. gasseri* and *B. bifidum* respectively) during 1 hour sequential incubation (37°C) in SIJ (pH 6.0). In addition, encapsulated probiotics were not significantly reduced ($p>0.05$) after extended the exposure to another 1 hour in SIJ (Table 1) whereas free probiotics reduced to more than 10 cfu/ml after 2 hours [13]. This observation suggested that the chitosan coating may possibly provide a good protection for encapsulated probiotics in bile salt solution caused by an ion exchange reaction between the beads and bile salt [17]. The interaction between positively charged chitosan and negatively charged alginate may led to form a semipermeable membrane that does not dissolve in the presence of Ca^{2+} chelators or antigelling agents and therefore limited the diffusion of bile salt into the beads and protected encapsulated probiotics from interacting with the bile salt [18]. Moreover, alginate-coated gelatin microspheres was effective in protecting *B. adolescentis* 15703T cells with higher levels of survival (91% and 75%) compared to free cells (68% and 62%) after 1 and 2 hours sequential incubation (37°C) in SIJ respectively [19]. On the other hand, encapsulated of *B. longum* BIOMA 5920 in 1.5% (w/v) alginate microspheres or 1.5% (w/v) alginate–2% (w/v) human-like collagen microspheres had no significant effect on probiotic bacteria survival rate compared to free cells during 2 hours in SIJ [20]. Likewise, Kanmani et al. [21] showed no released cells of *Enterococcus faecium* MC13 encapsulated into alginate–chitosan capsules in SIJ after 24 hours whereas free cells showed viable cell counts of 5 log cfu/ml.

Gelation of caseins in milk has been applied to encapsulate probiotic bacteria [22, 23]. Shi et al., [6] have used milk gelation as carrier for *L. bulgaricus* encapsulation. Milk is a natural vehicle for bioactive compounds and possesses high physico-chemical properties as delivery system. The yield of encapsulated *L. bulgaricus* alginate–milk microspheres (wet microspheres) was high (~100%) with size of 830 μm and 381 μm for nozzle 0.45 and 0.20 mm respectively (Table 1). *L. bulgaricus* is very sensitive to bile salt solution and the viability of free *L. bulgaricus* is dramatically lost to 0% after 1 hour

exposure to bile salt solution [6]. However, encapsulated *L. bulgaricus* in alginate–milk microspheres provides a good protection against the injury of the bile salt solution compared to free cells [6]. This is because encapsulated *L. bulgaricus* in alginate–milk microspheres showed survival rate of 93% and 85% after 1 hour incubation in 1 g/100 ml and 2 g/100 ml bile solution respectively (Table1). However, extended exposure to another 1 hour showed significant reduction ($p<0.05$) of encapsulated *L. bulgaricus* survival to 87% and 78% in 1 g/100 ml and 2 g/100 ml bile solution respectively. Another study conducted by Shi et al., [7] indicated that encapsulated *L. bulgaricus* in milk microspheres coated with a layer of a mixture of carrageenan-locust bean gums under wet condition have improved the stability and mechanical strength of milk microspheres with about 60% of encapsulation efficiency (Table 1). This technique was associated with higher survival rate of *L. bulgaricus* in bile salt solution. Based on the authors results, encapsulated *L. bulgaricus* showed about 90% and 85% survival after 2 hours incubation in 1 g/100 ml and 2 g/100 ml bile salt solution respectively (Table 1). The improvement of *L. bulgaricus* survival ability in bile salt solution may be related to the increase protection (lower porosity and thicker structure) via double layer encapsulation microspheres (milk as first layer and carrageenan-locust bean gums as second layer) that could prevent bile entrance to the microsphere and thus reducing the bacterial stress.

Recently, the effect of incorporating locust bean (LB) or xanthan (XT) gums into chitosan-coated alginate microcapsules containing high-density biofilm *L. rhamnosus* (HD capsules, size 400-600 μm) during SIJ is investigated by Cheow et al. [15]. The authors have reported that, there is a strong interaction between the chitosan and the alginate-LB or XT matrices causing limitation in the burst release of cells from the capsule surface during gastric acid exposure and improved the cell release characteristics in SIJ. The survival rate of *L. rhamnosus* in HD capsules was ~71% and ~61% for encapsulated *L. rhamnosus* with LB and XT respectively during SIJ (Table 1). Encapsulation of *S. boulardii* in chitosan/dextran sulfate multilayers (dry microspheres) via layer-by-layer technique (LbL) showed no significant differences in survival rate (61.2%) compared to free cells (50.9%) after 2 hours in SIJ [8]. However, the survival rate of encapsulated *S. boulardii* was significantly higher (59.3%; $p<0.05$) than free cells (34.4%) after 2 hours sequential incubation (37°C) in SIJ. This indicates that LbL technique may be selectively permeable to different molecular weights such as bile salt that prevented from entering the bacterial cells. Cai et al., [24] found that using ethylenediaminetetraacetate (EDTA) in encapsulated *L. acidophilus* CGMCC1.2686 in alginate-Ca-EDTA (wet microspheres) has low encapsulation yield (36.9%) and negative impact on *L. acidophilus* survival (0%) compared to free cells (6.90 ± 1.19*10-5%) after 30 minutes in SIJ. The increase chelating ability of EDTA with Ca ions at neutral pH may decrease the integrity of Ca-cross linked alginate network leading to a decreased mechanical strength of alginate capsules [24]. In addition, the antimicrobial effect of EDTA may cause some damage to *L. acidophilus*. EDTA decreases the stability of bacteria cell membrane by complexing divalent cations that acted as salt bridges between membrane macro molecules [25].

3. Conclusions and perspectives

Chitosan-coated alginate microspheres are the most effective in protecting probiotic bacteria from bile salt. Incorporation of milk with carrageenan-locust bean gums had significant effect to increase viability of probiotics during intestinal transit due to denser hydrogel network formed resulted in decrease the diffusion rate of bile salt into the microspheres. Since a number of studies regarding to the relationship between plant materials and probiotics have been successfully established to increase the viability of probiotics, locust bean and gellan gums could be a good choice as plant-based materials for encapsulation development to provide sufficient protection against bile salts. Functional foods including probiotics are increasingly subject. In this case, different food matrixes can be protective and lead to an increase in the survival of encapsulated probiotics during digestion. Further studies are needed to ensure the role of different food matrixes such as dairy products, meats products, beverages products, cereals products, vegetables and fruits products and bread products in increase the viability of encapsulated probiotics during intestinal transit.

Table 1. The effect of encapsulating materials on the protection and survival of probiotics during intestinal digestion.

Probiotic	Type of encapsulating material	Microsphere characterization			Bile salts concentration	Time of incubation	Survival rate of encapsulated probiotics (%)	Survival rate of free probiotics (%)	References
		condition	Yield (%)	Size (μm)					
Lactobacillus bulgaricus	alginate–milk microspheres	wet	~100	830 - 381	1 g/100 ml	1 hour	92.6	0	[6]
						2 hour	86.8		
					2 g/100 ml	1 hour	85	0	
						2 hour	78.4		
Lactobacillus bulgaricus	milk microspheres coated with carrageenan-locust bean gums	wet	60	n	1 g/100 ml	1 hour	95.2	0	[7]
						2 hour	90.4		
					2 g/100 ml	1 hour	90	0	
						2 hour	85		
Lactobacillus gasseri	chitosan-coated alginate microspheres	dry	39.2	362.0	3%	1 hour	100.6	59	[13]
						2 hour	98.8	> 13.2	
						1 hour	97.7	25	
Bifidobacterium bifidum			40.2	345.4		2 hour	96.7	> 12.4	
Lactobacillus casei ATCC 393	alginate–chitosan microspheres	dry	n	2200	0.5%		92.9	> 0.03	[10]
					1%	3 hour	85.8		
	Alginate–chitosan–carboxymethyl chitosan microcapsules				0.5%		95.3		
					1%		90.9		
Lactobacillus casei 431	sodium caseinate and gellan gum mixture gelled by gradually decreasing pH with glucono-δ-lactone	dry	89.5	40 - 1100	1%	2 hour	98.1	~ 97	[14]
Bifidobacterium adolescentis 15703T	Alginate-coated gelatin microspheres	-	41–43	49.0 - 53.1	n	1 hour	91.4	68	[19]
						2 hour	74.9	62	
Lactobacillus rhamnosus	chitosan and alginate- locust bean	dry	n	400 - 600	n	4 hour	~ 71	n	[15]
	chitosan and alginate- xanthan						~ 61		
Saccharomyces boulardii	chitosan/dextran sulfate multilayers	dry	n	n	3g/L	2 hour	61.2	50.9	[8]
Lactobacillus acidophilus CGMCC1.2686	alginate- calcium disodium ethylenediaminetetraacetate (EDTA)	wet	36.9	343	1%	30 min	0	$6.90*10^{-5}$	[24]

* n = not mentioned.

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