Indian Journal of Animal Sciences **92** (1): 136–138, January 2022/Short communication
https://doi.org/10.56093/ijans.v92i1.120941

Microencapsulation of *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 in alginate blended with starch by extrusion technique

CHOL-SU KWOAK[1], SONG-JUN KIM[1]✉ and CHOL-SOK KIM[1]

Institute of Microbiology, the State Academy of Sciences, Unjong, Pyongyang City, DPR Korea

Received: 4 August 2021 ; Accepted: 20 October 2021

Keywords: Alginate, *B. bifidum*, Encapsulation, Extrusion, *L. plantarum*

Probiotics are defined as live microorganisms that exert beneficial health effect on the host by improving the intestinal microbial balance (Hill *et al*. 2014). The most often used microorganisms as probiotics are of the genera *Bifidobacterium* or *Lactobacillus* (Taranu *et al*. 2018). To exert the beneficial effect of probiotics, they must arrive in intestines alive and in sufficient numbers which is suggested at 10^6–10^7 cfu/g products (Nazzaro *et al*. 2009, Chávarri *et al*. 2010). However, the survival of probiotics is very low, as most of all probiotics die during passage through the low *p*H of the stomach and high bile salt conditions of the intestine (Krasaekoopt *et al*. 2014, García-Ceja *et al*. 2015). In order to increase the viability of probiotics, microencapsulation techniques have been developed, which is a process that surrounds probiotics in a polymeric membrane, protecting them from harmful factors and, allowing them to release under specific conditions (Gerez *et al*. 2012). The methods for encapsulation of probiotics include extrusion, emulsion, spray drying and freeze drying (Solanki *et al*. 2013). As materials for microencapsulation, alginate, k-carrageenan, cellulose acetate phthalate, xanthan, milk fat and/or whey proteins, lipids and starch are used (Kailasapathy K *et al*. 1997). Alginate is the linear polysaccharides formed by β-1, 4-glycoside bonds of β-D-mannuronic acid and α-L-guluronic acid residues (Draget 2009). The starch has been reported as effective copolymers of alginate for the encapsulation of probiotics. By blending with starch, the survival of encapsulated probiotics in alginate was improved (And C I *et al*. 2005, Fuentes-Zaragoza *et al*. 2011). In this study, we microencapsulated *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 in alginate-blended starch and evaluated the encapsulation efficiency of probiotics in alginate-blended starch, the survival of encapsulated cells in SGF and SIF, the release of cells from capsules, and the storage stability during storage at 4°C and 20°C.

Microencapsulation: Probiotic bacteria were encapsulated by using a modified method described by Chen *et al.* (2007). For microencapsulation, firstly 2 g of sodium alginate and 2 g of starch were dissolved in 80 mL of distilled water under constant mechanical stirring and heating at 80°C The solution was autoclaved at 121°C for 15 min and cooled to 40°C. After cooling, 20 mL of the probiotics suspension was added and homogenized. The homogenous suspensions were extruded dropwise through 300 µm nozzle into 100 mL sterile 0.1 M $CaCl_2$ solution. The resultant capsules were allowed to harden in the $CaCl_2$ solution for 30 min and then washed three times with distilled water.

Survival of free and microencapsulated probiotics in SGF and SIF: The survival of free and microencapsulated probiotics in SGF and SIF was determined by using the method described by Gbassi G K (2009).

Release of cells from capsules: The release of cells from Capsules was determined by using the method by Mandal S (2006).

Evaluation of the storage stability: To determine the storage stability of the encapsulated bacteria, the number of viable cells was counted after every 10 day interval for a period of 2 months at 4 and 20°C.

Statistical analysis: The statistical analysis of data was performed by using STATISTICA data analysis software system. The results obtained in this study are presented in Table 1 and Figs 1 to 3.

Microencapsulation efficiency: The highest efficiency were observed to be 87.5%, 68.3% in *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 respectively where the concentration of sodium alginate was kept as 2% and alginate : starch ratio was kept as 1:1 (Table 1). Interestingly, results showed that the addition of starch into alginate solution significantly increased the EE. Thus 2% of alginate concentrations and 1:1 of alginate: starch ratio were used for further study.

Survival of free and microencapsulated probiotics in SGF and SIF: The survival of *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 decreased to 64.6%, 49.2% in SGF after 2 h of incubation respectively. Meanwhile, the survival rates for *Lactobacillus*

Present address: [1]Institute of Microbiology, the State Academy of Sciences, Unjong, Pyongyang City, DPR Korea.
✉Corresponding author email: ksj1971@star-co.net.kp

Table 1. Encapsulation efficiency (%) of *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 encapsulated in different concentrations of alginate and starch by extrusion method (P<0.05)

Microorganism	Alginate concentration (%)	Alginate : starch ratio	Number of probiotics (log cfu/g microcapsule)		Microencapsulation efficiency (%)
			Before microencapsulation	After microencapsulation	
	1	1:0	9.63±0.11	5.88±0.18	61.1±1.26
		1:0.5	9.34±0.12	7.35±0.07	78.8±2.17
		1:1	9.59±0.09	7.61±0.04	79.4±1.79
	2	1:0	9.25±0.08	6.26±0.08	67.7±0.91
		1:0.5	9.13±0.11	7.95±0.13	87.1±1.57
		1:1	9.05±0.08	7.91±0.93	87.5±1.88
	3	1:0	9.41±0.18	6.26±0.52	66.6±1.43
		1:0.5	9.68±0.16	8.05±0.17	83.2±1.15
		1:1	9.47±0.12	8.58±0.43	85.8±1.33
Bifidobacterium bifidum ATCC 1903	1	1:0	8.71±0.15	3.51±0.83	40.4±1.08
		1:0.5	8.54±0.05	4.99±0.59	58.5±3.87
		1:1	8.33±0.10	5.11±0.56	61.3±1.07
	2	1:0	8.76±0.15	4.18±0.18	47.8±1.11
		1:0.5	8.98±0.09	5.82±0.24	64.9±0.82
		1:1	8.66±0.18	5.91±0.65	68.3±0.65
	3	1:0	8.17±0.12	4.07±0.33	49.9±0.86
		1:0.5	8.59±0.02	5.52±0.71	64.3±1.16
		1:1	8.05±0.07	5.28±0.98	65.7±0.98

Means are not significantly different (P<0.05).

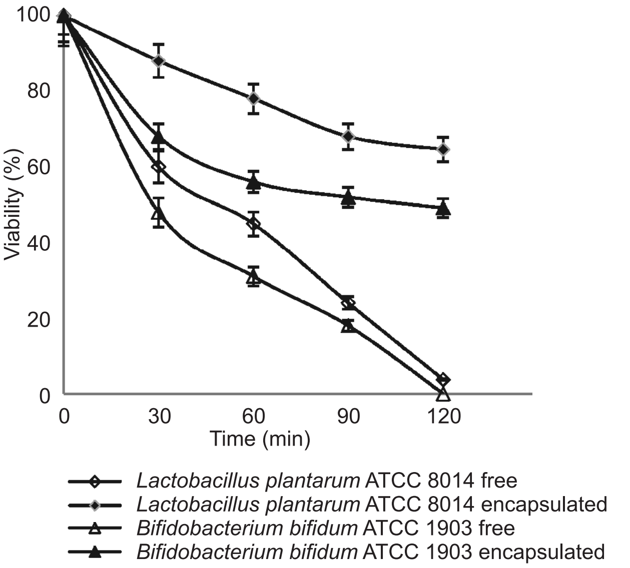

Fig. 1. Survival of free and encapsulated cells of *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 in simulated gastric fluid (SGF) (P<0.05).

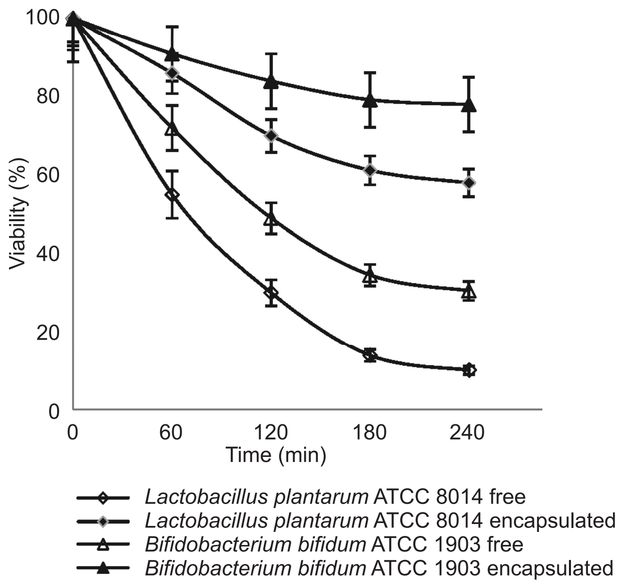

Fig. 2. Survival of free and encapsulated cells of *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 in simulated intestinal fluid (SIF) (P<0.05).

plantarum ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 in SIF after 4 h were 61.2%, 79.2% respectively (Figs 1 and 2). The survival of free probiotic cells in SGF and SIF were very lower than that of microencapsulated cells.

Release of cells from capsules: The number of released probiotic bacteria increased to 90% within 180 min in contact with buffer. No significant difference (P< 0.05) was observed from after 180 min.

Evaluation of the storage stability: The viability of encapsulated probiotics for 10 days interval for a period of 2 months at 4°C and 20°C was shown in Fig. 3. As shown in Fig. 3, after 20 days of storage at 20°C, the survival of encapsulated *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 decreased 0%. Meanwhile, after 60 days of storage at 4°C, the viability of encapsulated probiotics maintained the 80% level of survival.

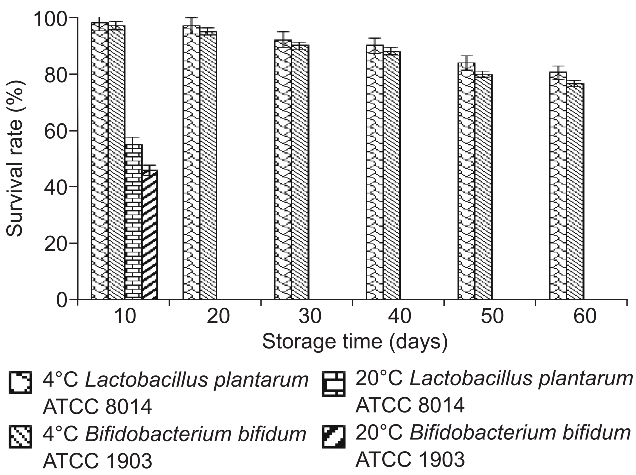

Fig. 3. Storage stability of encapsulated *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 during 60 days storage at 4°C and 20°C. Means(n=3)±SD. Means are significantly different (P<0.05).

SUMMARY

Lactobacillus plantarum ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 were encapsulated in alginate blended with starch by extrusion. The reinforcement effect of starch on the EE was significant. When the concentration of sodium alginate and alginate:starch ratio were kept as 2% and 1:1 respectively, the highest EE were observed. Encapsulation improves the survival of encapsulated cells of *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 in SGF and SIF significantly. The encapsulation efficiency of *L. plantarum* ATCC 8014 and *B. bifidum* ATCC 1903 were found to be 87.5%, 68.3% respectively. The complete release of the cells from the capsules was achieved in at least 3 h and microencapsulated probiotics were stable at 4°C for 60 d. In conclusion, the encapsulation of *Lactobacillus plantarum* ATCC 8014 and *Bifidobacterium bifidum* ATCC 1903 in alginate blended with starch by extrusion would be useful in delivery of probiotics to the intestine.

REFERENCES

And C I and Kailasapathy K. 2005. Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria under *in vitro* acidic and bile salt conditions and in yogurt. *Journal of Food Science* **70**: M18–M23.

Chávarri M, Marañón I, Ares R, Ibáñez F C, Marzo F, and Villarán, M D C. 2010. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *International Journal of Food Microbiology* **142**(1): 185–89.

Chen M, Chen K and Kuo Y. 2007. Optimal thermotolerance of *Bifidobacterium bifidum* in gellan–Alginate microparticles. *Biotechnology and Bioengineering* **98**(2): 411–19.

Draget K I. 2009. Alginates, pp. 807–828. (Eds) Phillips G O and Williams P A. *Handbook of Hydrocolloids* (2nd ed). Cambridge, UK.

Fuentes-Zaragoza E, Sánchez-Zapata E, Sendra E, Sayas E, Navarro C, Fernández-López J and Pérez-Alvarez J A. 2011. Resistant starch as prebiotic: A review. *Starch-Stärke* **63**: 406–15.

García-Ceja A, Mani-López E, Palou E and López-Malo A. 2015. Viability during refrigerated storage in selected food products and during simulated gastrointestinal conditions of individual and combined lactobacilli encapsulated in alginate or alginate-chitosan. *LWT Food Science and Technology* **63**(1): 482–89.

Gbassi G K, Vandamme T, Ennahar S and Marchioni E. 2009. Microencapsulation of *Lactobacillus plantarum* spp. in an alginate matrix coated with whey proteins. *International Journal of Food Microbiology* **129**: 103–05.

Gerez C L, Font de Valdez G, Gigante M L and Grosso C R F. 2012. Whey protein coating bead improves the survival of the probiotic *Lactobacillus rhamnosus* CRL 1505 to low *p*H. *Letters in Applied Microbiology* **54**: 552–56.

Hill C, Guarner F, Reid G, Gibson G R, Merenstein D J, Pot B, Morelli L, Canani R B, Flint H J and Salminen S. 2014. Expert consensus document. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology* **11**: 506–14.

Kailasapathy K and Rybka S. 1997. *Lactobacillus acidophilus* and *Bifidobacterium* spp.: Their therapeutic potential and survival in yoghurt. *Australian Journal of Dairy Technology* **52**: 28–35.

Krasaekoopt W and Watcharapoka S. 2014. Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT–Food Science and Technology* **57**(2): 761–66.

Mandal S, Puniya A K and Singh K. 2006. Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC-298. *International Dairy Journal* **16**: 1190–95.

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

Solanki H K, Pawar D D, Shah D A, Prajapati V D, Jani G K, Mulla A M and Thakar P M. 2013. Development of microencapsulation delivery system for long-term preservation of probiotics as biotherapeutics agent. *BioMed Research International*. doi: 10.1155/2013/620719.

Taranu I, Marin D E, Braicu C, Pistol G C, Sorescu I, Pruteanu L L, Berindan Neagoe I and Vodnar D C. 2018. *In vitro* transcriptome response to a mixture of *lactobacilli* strains in intestinal porcine epithelial cell line. *International Journal of Molecular Sciences* **19**: 1923.