SNRU JST
SNRU Journal of Science and Technology
Journal home page: snrujst.snru.ac.th

Synbiotic Microencapsulation From Corn Dust in Alginate-Chitosan Capsules Improves Survival in Simulated Gastro-Intestinal Conditions

Nuttaporn Chanchay*, Kornnikar Dittasuwannakul
Department of Agro-Industrial Biotechnology, Faculty of Maejo University Phrae Campus, Maejo University, Phrae, 54140 Thailand
*Corresponding Author: nuttapornchanchay@gmail.com

Received: 15 March 2021; **Revised:** 22 June 2021; **Accepted:** 15 July 2021; **Available online:** 1 September 2021

Abstract The research aims to study synbiotic microencapsulation from corn dust in alginate-chitosan capsules whether it improves survival in simulated gastro-intestinal conditions, by studying the survival of synbiotic made from a mixture of corn dust and two of strains probiotic microorganisms such as *Lactobacillus acidophilus* and *Lactobacillus lactis* which are not encapsulated and encapsulated in 2% of sodium alginate and coated with chitosan. The survival tested in simulated gastric juice (SGJ) shows that synbiotic with *L. acidophilus* encapsulated has the highest survival rate of 8.68%; moreover the synbiotic with *L. lactis* encapsulated has the highest survival rate of 5.43% resulting to *L. acidophilus* is more resistant to acidity than *L. lactis*. The survival test in simulated intestinal juice (SIJ) indicates that the survival rate of synbiotic with *L. lactis* encapsulated has the highest survival rate of 10.29%; in addition, synbiotic with *L. acidophilus* encapsulated has a survival rate of 5.86%. The difference of the synbiotic that are not encapsulated ($p \leq 0.05$) when *L. lactis* will be resistant as well in bile salt. As a result, the survival rate is higher than the *L. acidophilus* when replication is in simulated intestinal juice allowing synbiotics can be used as animal feed.
Keywords: encapsulation; simulated gastro-intestinal conditions; synbiotic; *Lactobacillus acidophilus*; *Lactobacillus lactis*

1. Introduction

Currently, agricultural products have been processed into various products and corn is one of the most popular agricultural products. In the process, after the corn kernels have been removed, a large amount of dust is released and causing global warming pollution. Corn dust contains high cellulose content. Therefore, it can be used to produce animal feed. According to previous study, corn waste, husk, stalk and corn dust, was studied to produce prebiotic with enzyme and the results showed that corn dust gave the best results for prebiotic [1].

Farm animals and aquaculture have found chemical residual that resulting to environment and animals. Moreover, animals that use of antibiotic maybe causing antibiotic resistance. For that reason, researchers are looking for solutions and study the use of probiotics in the animal feeding production. Modulation of the gut microbiota with zoo-technical feed additives such as prebiotics and probiotics for host protection to support animal husbandry, including livestock, poultry, and fish farming, is the key to maximize productivity and maintain animal health and welfare [2]. In addition, probiotics are increasingly used in commercial animal production operations to advantageously alter gastrointestinal flora, thereby improving animal health and productivity. The major outcomes from using probiotics include improvement in growth, reduction in mortality, and improvement in feed conversion

efficiency [3]. Although probiotics are effective and useful in the field of animal growth and treatment but probiotic will be digested in the gastrointestinal tract, and most of them will be almost digested in the stomach of aquatic animals. In addition, prebiotic are chosen to stimulate some intestinal flora (*Bifidobacterium* spp. and Lactobacilli) but may reduce the population of some beneficial bacteria (*Aeromonas* spp. and *Carnobacterium* spp.) in the colon [4].

Researchers have used the term synbiotic to describe the use of prebiotic and probiotic mixtures that may benefit animal or human gastrointestinal (GI) systems [5] but synbiotic refers to a state of coexistence of two or more elements. It's another alternative of aquaculture in the way of organic aquaculture [6].

Microencapsulation is a means of packaging, separating, and storing materials in microscopic capsules for later release under controlled conditions. The versatile technologies that have resulted from the evolution of this science have been widely used in the food industry, both to improve product performance and provide better controlled delivery of ingredients [7]. Several researches reviewed that the bacterial microencapsulation technique was enhance the survival rate in population of some beneficial bacteria [8 – 13].

Synbiotic encapsulated in alginate-chitosan capsules make to enhance the survival of probiotic bacteria and preservation of prebiotic during exposure to adverse conditions in the gastrointestinal tract, including gastric juice in the gastrointestinal acidity and may destroy microbes probiotic before arriving in the stomach. [14 – 16].

This study was conducted by synbiotic encapsulated in alginate-chitosan capsules to study the survival rate in 2 conditions of simulated gastro-intestinal (simulated gastric juice (SGJ) and simulated intestinal juice (SIJ)). The mixture of probiotic microbial (*L. acidophilus* and *L. lactis*) and corn dust was encapsulated in alginate-chitosan capsules. The survival rate were different in simulated gastric juice (SGJ) at time 5, 30, 60 and 120 min and simulated intestinal juice (SIJ) at 60, 90 and 120 min were evaluated [12, 14 – 15]. The number of colonies were counted by the Spread plate and incubated in anaerobic conditions at 37 °C for 2 days [7]. The results from this study provide important information on the use of synbiotic, which could be implemented in alternative choices in the future.

2. Materials and Methods

Preparation of raw materials

Corn dust samples were obtained from the production of corn forage and dried at 60°C for 8 – 10 hr. Digestion sample with an enzyme ACCELLERASE 1000 at 0.80 % (wv^{-1}) at 40 °C for 18 hr. as prebiotic [1]. *L. acidophilus* and *L. lactis* bacteria were fed into corn dust sample.

Microencapsulation and coating procedures

Synbiotic were incorporated into 10 ml (Mcfarland 0.5) of 20 gl^{-1} (2.00%) of sodium alginate.

Chitosan aqueous solution was prepared. In brief, chitosan was dissolved in 100 ml distilled water acidified with glacial acetic acid to achieve a final chitosan concentration of 0.4 % (wv^{-1}). $CaCl_2$ 0.10 M was added to the chitosan solution [15].

The extrusion technique of encapsulation was derived by using alginate as the supporting matrix. To form beads, the sodium alginate solution was extruded into a previous sterile chitosan solution and washed with sterile distilled water [16].

Survival assay and numeration of encapsulated synbiotic

Simulated gastric juice (SGJ) consisted of 9 gl^{-1} of NaCl with adjusted to pH 2.0 and hydrochloric acid. 0.20 g of encapsulated synbiotic or 0.20 ml of cell suspensions were mixed in 10 ml of SGJ and incubated for 5, 30, 60 and 120 min at 37 °C with constant agitation at 50 rpm [14]. Simulated intestinal juice (SIJ) was prepared by dissolving bile salted in intestinal solution (6.5 gl^{-1} NaCl, 0.835 gl^{-1} KCl, 0.22 gl^{-1} $CaCl_2$ and 1.39 gl^{-1} $NaHCO_3$) adjusted to pH 7.5 to final concentrations of 3.0 gl^{-1}. Triplicate samples were mixed, incubated at 37 °C and sampled 60, 90 and 120 min after addition of the beads with bacteria or cell suspensions [15]. From previous study, the pH values of SGJ and SIJ were different. The pH will affect the survival rate of the bacteria. Causing different incubation time required [14 – 15].

Surviving bacteria (*L. acidophilus* and *L. lactis*) were washed with 0.85% NaCl. (2 time). And washed with distilled water 1 time. Then, centrifuged and wrapped in alginate for made into a gel tablet. Finally, coated with chitosan and counted by spread plate counts in MRS agar anaerobically incubated at 37 °C for 2 days [12]

The survival rate (%) was calculated followed by previous study [14]:

$$\text{Survival rate (\%)} = \frac{\text{The number of bacteria remaining after acid infusion}}{\text{The number of bacteria remaining before acid infusion}} \times 100$$

Statistical analysis

Results are presented as means ± standard deviation (SD) of replicated determinations. Data were subjected to one-way analysis of variance (ANOVA) and multiple comparisons were performed by Duncan's test. Statistical significance was set at $p \leq 0.05$. All analyses were performed using SPSS version 22.0 for Windows.

3. Results and discussions

The survival of synbiotic made from a mixture of corn dust with two strains of encapsulated and non-encapsulated probiotic microorganism (*L. acidophilus* and *L. lactis*) was study by using synbiotic microencapsulation from corn dust in alginate-chitosan capsules in simulated gastrointestinal condition (simulated gastric juice and simulated intestinal juice).

In simulated gastric juice condition, the concentration of *L. acidophilus* and *L. lactis* bacteria were 2.39×10^9 CFU ml^{-1} and 2.38×10^9 CFU ml^{-1}, respectively and exposure time were 5, 30, 60 and 120 min. The result revealed that survival rate of bacteria in two strains was significantly higher in encapsulated group than non-encapsulation group, indicating that microencapsulation technique can preserve bacteria from gastro-intestinal conditions in simulated gastric juice (Table 1 and 2).

Table 1 Number of *L. acidophilus* and *L. lactis* bacteria to survive in a simulated gastric juice (SGJ) in different time

The number of initial infection (CFU ml^{-1}): *L. acidophilus* 2.39×10^9; *L. lactis* 2.38×10^9

Treatment (CFU ml^{-1})	Time (min)			
	5	**30**	**60**	**120**
L. acidophilus non-encapsulated	1.84×10^9	2.67×10^7	2×10^7	6.7×10^6
L. acidophilus encapsulated	2.11×10^9	1.82×10^9	1.13×10^9	2.07×10^8
L. lactis non-encapsulated	1.94×10^9	2×10^7	3.3×10^6	0
L. lactis encapsulated	2.10×10^9	1.73×10^9	8.67×10^8	1.3×10^8

Table 2 The survival rate of *L. acidophilus* and *L. lactis* bacteria in simulated gastric juice (SGJ) under different time condition. Different letters in the same column indicate significantly differences ($p \leq 0.05$)

Treatment (%)	Time (min)			
	5	30	60	120
L. acidophilus non-encapsulated	$77.13^{c} \pm 0.01$	$1.12^{c} \pm 0.01$	$0.84^{b} \pm 0.01$	$0.28^{b} \pm 0.01$
L. acidophilus encapsulated	$88.42^{a} \pm 0.01$	$76.29^{a} \pm 0.01$	$47.48^{a} \pm 0.10$	$8.68^{a} \pm 0.01$
L. lactis non-encapsulated	$81.79^{b} \pm 0.01$	$0.84^{c} \pm 0.01$	$0.14^{b} \pm 0.02$	$0^{b} \pm 0.00$
L. lactis encapsulated	$88.02^{a} \pm 0.01$	$72.69^{b} \pm 0.10$	$36.26^{a} \pm 0.10$	$5.43^{a} \pm 0.01$

The synbiotic with *L. acidophilus* encapsulated was highest survival rate followed by *L. lactis* encapsulated, indicating that *L. acidophilus* resistance to acidity than *L. lactis* bacteria. Under acidity conditions (pH 2.0) for 3 h, survival rate of *L. acidophilus* TISTR 1034 as 45.36% [8], while this study was done only 2 h with the survival rate was only 8.68±0.01%. In addition, *L acidophilus* encapsulated with calcium alginate survive under acidity conditions in gastric juice [12].

In simulated intestinal juice condition, the concentration of *L. acidophilus* and *L. lactis* bacteria were 2.39×10^{9} and 2.38×10^{9}, respectively and exposure time were 60, 90 and 120 min. The results showed that the survival rate of bacteria in encapsulated group was significantly higher than non-encapsulated group (Table 3 and 4) and the survival rate of synbiotic with *L. lactis* encapsulated was highest followed by synbiotic with *L. acidophilus* encapsulated, indicating that *L. lactis* able to withstand the acidity condition in bile salt better than *L. acidophilus*. In recent year, the researcher revealed that the resistance of *L. lactis* type B1-04 and *L. lactis* type Bi-07 to acid and heat in bile salt. The results showed that the encapsulated cells significantly increase the survival cells [12].

Table 3 Number of *L. acidophilus* and *L. lactis* bacteria to survive in a simulated intestinal juice (SIJ) in different time

The number of initial infection (CFU ml^{-1}) — *L. acidophilus* 2.39×10^{9}; *L. lactis* 2.38×10^{9}

Treatment (CFU ml^{-1})	Time (min)		
	60	90	120
L. acidophilus non-encapsulated	7×10^{7}	5×10^{7}	3.30×10^{6}
L. acidophilus encapsulated	1.79×10^{9}	4.30×10^{8}	1.40×10^{8}
L. lactis non-encapsulated	5.33×10^{7}	3.67×10^{7}	3.30×10^{6}
L. lactis encapsulated	1.76×10^{9}	1.01×10^{9}	2.45×10^{8}

Table 4 The survival rate of *L. acidophilus* and *L. lactis* bacteria in simulated intestinal juice (SIJ) under different time condition. Different letters in the same column indicate significantly differences ($p \leq 0.05$)

Treatment (%)	Time (min)		
	60	90	120
L. acidophilus non-encapsulated	$2.93^{b} \pm 0.01$	$2.09^{c} \pm 0.01$	$0.14^{c} \pm 0.02$
L. acidophilus encapsulated	$74.69^{a} \pm 0.01$	$17.99^{b} \pm 0.01$	$5.86^{b} \pm 0.01$
L. lactis non-encapsulated	$2.24^{b} \pm 0.01$	$1.54^{c} \pm 0.02$	$0.14^{c} \pm 0.01$
L. lactis encapsulated	$73.74^{a} \pm 0.01$	$42.44^{a} \pm 0.01$	$10.29^{a} \pm 0.01$

The encapsulation technique can enhance cell survival in simulated gastro-intestinal condition lead to the encapsulated synbiotic with *L. acidophilus* and *L. lactis* were increase with survival rate. On the other hand, the survival rates were low in the non-encapsulated group. The cells encapsulated

L. acidophilus TIRTR 1034 with 2% wv^{-1} sodium alginate resist to acidity condition (pH 1.5) for 3 h [10]. They also reported that encapsulated *L. acidophilus* TIRTR 1034 survive for a 5.37 log CFU ml^{-1} and the free cells survive for 3.38 log CFU ml^{-1}. Moreover, *L. acidophilus* encapsulated with Al and Al/Chi-microspheres showed higher survival rates after exposure to the gastrointestinal tract and better mucoadhesive abilities than the free cells [11]. In addition, encapsulation and double coating of probiotic bacteria culture (*L. acidophilus* and *L. rhamnosus*) can increase the viability of them in Doogh beverage and in simulated gastro-intestinal condition [9].

In this study, *L. acidophilus* bacteria are resistance to acidity condition than *L. lactis*. Therefore *L. acidophilus*, the rate of survival in conditions simulated gastric juice (SGJ), which has good acidity. Conversely, *L. lactis* is resistant to bile salt condition better than *L. acidophilus*. Thus, survival rate of *L. lactis* was higher than *L. acidophilus* in simulated intestinal juice condition.

4. Conclusion

In conclusion, synbiotic microencapsulation from corn dust in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions, can be used to encapsulated synbiotic also developed a synbiotic product synthesizer that can last up in the digestive tract of animals. To promote the survival rate and maintain a healthy balance in the digestive tract of the animal and corn dust is one of an alternative choice of the synbiotic production. The leadership of the utilized agricultural waste and reduce air pollution from the burning dust of corn farmers. This could increase the value of agricultural residues as well. Thus, it can be used corn dust for synbiotic as animal feed are cheap.

5. References

[1] N. Chanchay, K. Dittasuwannakul. Improvement of pre-treating corn dust with enzyme as prebiotics in animal feed, RMUTI J. Sci&Tech. 9 (2016) 214 – 223

[2] A. Anadon, I. Ares, M.R., Martínez-Larrañaga, M.A. Martinez, Prebiotics and probiotics in feed and animal health, in: R.C. Gupta, A. Srivastava, R. Lall (Eds.), Nutraceuticals in Veterinary Medicine, Springer, Cham, 2019, pp. 261 – 285.

[3] H. Yirga, The use of probiotics in animal nutrition, JPH. 3 (2015) 557 – 569.

[4] I.B. Okey, U.U. Gabriel, S.N. Deekae, The use of synbiotics (prebiotic and probiotic) in aquaculture development, J Biotechnol. 1 (2018) 51 – 60.

[5] S. Kolica, G.R. Gibson, Synbiotics in health and disease, in: D.J. McClements (Ed), Annual review of food science and technology, A Nonprofit Scientific Publisher, Palo Alto, 2011, pp. 373 – 393.

[6] N. Chanchay. Synbiotic production from soybean residue for aquaculture practices, J Fish tech. 5 (2011) 47 – 55.

[7] C. Chooprom, Cells encapsulated probiotics with prebiotic and studied survival in acidic conditions and bile salts in vitro, Master of Science, Songklanakarintara University, Songkla, 2016.

[8] H. Pourjafar, N. Noori, H. Gandomi, A.A. Basti, F. Ansari, Viability of microencapsulated and non-microencapsulated Lactobacilli in a commercial beverage, in: K. Bayer, M. Cao, B.W. Dijkstra, M. Faisal, Biotechnology reports, Lund University, Sweden, 2020, pp. 1 – 9.

[9] S.C. Siang, L.K. Wai, N.K. Lin, P.L. Phing. Effect of added prebiotic (Isomalto-oligosaccharide) and coating of beads on the survival of microencapsulated *Lactobacillus rhamnosus* GG, Food. Sci. Technol. 39 (2019) 601 – 609.

[10] S. Mandal, K. Singh, Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC-298, Int. Dairy J. 16(2006) 1190 – 1195.

[11] R. Sobel, R. Versic, A.G. Gaonkar. Introduction to microencapsulation and controlled delivery in foods, in: A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds.), A practical implementation guide, Elsevier Inc., London, 2014, pp. 3 – 12.

[12] V. Chandramoulia, K. Kailasapathya, P. Peirisb, M. Jones. An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions, J. Microbiol. Met. 56 (2004) 27 – 35.

[13] Y. Lee, Y.R. Ji, S. Lee, M.J. Choi, Y. Cho. Microencapsulation of probiotic *Lactobacillus acidophilus* KBL409 by extrusion technology to enhance survival under simulated intestinal and freeze-drying conditions, J. Microbiol. Biotechnol. 29 (2019) 721 –730.

[14] A.V. Rao, N. Shiwnavain, I. Maharaj, Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juiced, Can. Inst. Food Sci. Technol. J. 22 (1989) 45 – 346.

[15] C. Maria, M. Izaskun, A. Raquel, C.I. Francisco, M. Florencio, C.V. Maria del, Microencapsulation of a probitic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions, Int. J. Food Microbiol. 142 (2010) 185 – 189.

[16] Y. Zhou, E. Martins, A. Groboillot, C.P. Champagne, R.J. Neufeld. Spectrophotometric quantification of lactic bacteria in alginate and control of cell releasse with chitosan coating, J. Appl. Microbiol. 84 (1998) 342 – 348.