International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:9, 2014

Survival of Four Probiotic Strains in Acid, Bile Salt and After Spray Drying

Rawichar Chaipojjana, Suttipong Phosuksirikul, Arunsri Leejeerajumnean

Abstract**—**The objective of the study was to select the survival of probiotic strains when exposed to acidic and bile salts condition. Four probiotic strains (*Lactobacillus casei* subsp. *rhamnosus* TISTR 047, *Lactobacillus casei* TISTR 1500, *Lactobacillus acidophilus* TISTR 1338 and *Lactobacillus plantarum* TISTR 1465) were cultured in MRS broth and incubated at 35ºC for 15 hours before being inoculated into acidic condition (5 M HCl, pH 2) for 2 hours and bile salt (0.3%, pH 5.8) for 8 hour. The survived probiotics were counted in MRS agar. Among four stains, *Lactobacillus casei* subsp. *rhamnosus* TISTR 047 was the highest tolerance specie. *Lactobacillus casei* subsp. *rhamnosus* TISTR 047 reduced 6.74±0.07 log CFU/ml after growing in acid and 5.52±0.05 log CFU/ml after growing in bile salt. Then, double emulsion of microorganisms was chosen to encapsulate before spray drying. Spray drying was done with the inlet temperature 170ºC and outlet temperature 80ºC. The results showed that the survival of encapsulated *Lactobacillus casei* subsp. *rhamnosus* TISTR 047 after spray drying decreased from 9.63 ± 0.32 to 8.31 ± 0.11 log CFU/ml comparing with non-encapsulated, 9.63 ± 0.32 to 4.06 ± 0.08 log CFU/ml. Therefore, *Lactobacillus casei* subsp. *rhamnosus* TISTR 047 would be able to survive in gastrointestinal and spray drying condition.

Keywords**—**Probiotic, acid, bile salt, spray drying.

I. Introduction

IN current research many works related to the production of potential food for health, such as probiotic powder. The high potential probiotics particularly *Bifidobacterium* and *Lactobacillus* were chosen for commercial production. Many evidences were reported that the interaction of the intestinal microflora with the intestinal mucosa cells play a significant role in subsequent health, allergies, enchancement of the immune system, reduction of lactose intolerance, anti-cancer activity, hypocholesterolemic effect, decrease mutagenicity and gastrointestinal diseases [1]-[6]. However, many probiotic bacteria lacked the ability to adequately survive in gastrointestinal tract condition. So the survival improvement of probiotics can be done by using microencapsulation.

Probiotics defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" and nowadays probiotics become increasingly popular. It has been recommended that food containing probiotic bacteria should contain at least 10^6 live microorganisms per g or ml [7], [8].

Lactic acid bacteria (LAB) are widely used in the production of fermented food products due to their specific metabolic activities, which translate into technological, nutritional and health properties [9]. The growth activity of LAB is affected by fermentation conditions such as pH, temperature, medium composition and other factors. The LAB generally has limited biosynthetic ability, requiring multiple amino acids and vitamins for growth. Also, different approaches that increase the resistance of these sensitive microorganisms against adverse conditions have been proposed, including appropriate selection of acid and bile-resistant strains, use of oxygen-impermeable containers, 2 step fermentation and stress adaptation, incorporation of micronutrients such as peptides and amino acids and microencapsulation [10], [11].

Microencapsulation is a role technique for bacterial cell protection and several studies have been carried out research about this technique against adverse conditions to which probiotics can be exposed [12], [13]. The industrial production of food often requires the addition of functional ingredients. Adding bioactive ingredients to functional food presents many challenges, particularly with respect to the stability of the bioactive compounds during processing and storage [14].

Several microencapsulation methods have been developed and described. The most relevant are spray-coating, spray-drying, extrusion, emulsion and gel-particle technology. Each methodology has particular features and characteristics which allow the application to systems based on materials with peculiar mechanical and physicochemical properties [15].

The main aim of this study was to select the survival of probiotic strains when exposed to acid, bile salts and after spray drying. The encapsulation technique by double emulsion of microorganisms was chosen before spray drying.

II. Materials and Method

A. Materials

Lactobacillus casei subsp. *rhamnosus* TISTR 047, *Lactobacillus casei* TISTR 1500, *Lactobacillus acidophilus* TISTR 1338 and *Lactobacillus plantarum* TISTR 1465 (Thailand Institute of Scientific and Technological Research), bile salt (Fluka, New Zealand), Hydrocholic acid (Merck, Germany), Peptone (Merck, India), MRS Agar and MRS broth (Merck, Germany), spray dryer (APV, Denmark), centrifuge (Universal 16, Hettich zentrifugen), Incubator (MEMMERT BM 600, Germany), Spectrophotometer G 10 (Spectronic Unicam, UK), pH meter (Meter Lab, France), Autoclave (Tomy Seiko, Japan), Cuvette glass (Hellma, Germany), Vortex-genie (Mettler, Switzerland)

B. Preparation of Microorganisms' Cell

Four probiotic strains were cultured in MRS broth and incubated at 37ºC for 15 hours. The cells were harvested by

R. Chaipojjana, S. Phosuksirikul and A. Leejeerajumnean* are with the Department of Food Technology, Silpakorn University, Nakorn Pathom, Thailand (*corresponding author to e-mail: arunsrilee@yahoo.com).

centrifugation at 8,000 rpm for 10 min and were washed with sterile 0.1% peptone [16].

C. Selection of Probiotic Strains

1. Growth Curve

Four probiotic strains were cultured in MRS broth and incubated at 37ºC for 24 hours. Bacterial growth was observed by monitoring viable cell counts at 0, 2, 4, 6, 8, 10, 12, 15, 18, 21 and 24 h on MRS agar. The turbidity of cells was measured by spectrophotometer at 600 nm (OD_{600}).

2. Acid and Bile Salt Tolerance

Acid tolerance of probiotic strains were carried out according to the method of Ding and Shah [17]. Briefly, MRS broth was adjusted to pH 2 with 5M HCl. Approximately 10^{10} CFU/ml of each probiotic strain was inoculated into the acidified MRS broth and incubated at 37ºC for 2 hours and the samples were interval taken at 30 min. The cells in broth were sonicated for 5 s to disperse before performing serial dilutions. The survival of probiotics was counted in MRS agar.

Bile salt tolerance of probiotic strains were carried out according to the method of Ding and Shah [17]. Briefly, MRS broth containing 0.3% (w/v) bile salt was adjusted to pH 5.8 with 5M HCl. Approximately 10^{10} CFU/ml of each probiotic strain was inoculated into the MRS broth with bile salts and incubated at 37 ºC for 8 hours and samples were interval taken at 2 hrs. Then, the survival of probiotics was counted in MRS agar.

TABLE I
THE SURVIVAL OF PROBIOTIC STRAINS INTO ACID CONDITION (PH 2)

Strain	Viable of cells (log CFU/ml)				
	0 min	30 min	60 min	90 min	120 min
L. rhamnosus TISTR 047	10.92 ± 0.09^{aA}	9.03 ± 0.07^{bB}	7.70 ± 0.02^{aC}	5.23 ± 0.35^{aD}	4.19 ± 0.03^{aE}
L. casei TISTR 1500	10.99 ± 0.05^{aA}	8.98 ± 0.04^{bB}	7.71 ± 0.03^{aC}	5.10 ± 0.16^{aD}	4.18 ± 0.09^{aE}
L. acidophilus TISTR 1338	10.87 ± 0.11^{aA}	9.30 ± 0.07^{aB}	7.23 ± 0.06^{bC}	5.00 ± 0.03^{aD}	4.12 ± 0.08^{bE}
L. plantarum TISTR 1465	10.58 ± 0.14^{bA}	8.49 ± 0.02^{cB}	6.14 ± 0.03^{cC}	4.88 ± 0.18^{aD}	3.61 ± 0.01^{cE}

Data are mean ± SD

a, b, and c mean of different letter in the same column significant different ($P \leq 0.05$), n = 3

A, B, C, D, and E mean of different letter in the same row significant difference ($P \leq 0.05$), n = 3

D. Survival of Probiotic after Spray Drying

The double emulsion of microorganisms was chosen to encapsulate before spray drying. Spray drying was done with the inlet temperature 170ºC and outlet temperature 80ºC [18], [19]. The survival of probiotics was counted in MRS agar comparing between encapsulated and non-encapsulated cells.

III. RESULTS AND DISCUSSION

A. Probiotic Growth

The growth curves of all probiotic organisms were shown in Fig. 1. All the probiotic had lag phase for first four hours with the number of viable cells in the range of 3.4-4.0 log CFU/ml. Then, probiotic organisms reached into the log phase, which can be seen the duplicated number of viable cells. The stationary phase of all probiotic organisms initiated after incubation for 15 hours. The numbers of viable cells were constantly at 9.0 log CFU/ml.

The growth curve drew from the absorbance values which showed lag phase, log phase and stationary phase was the same as drawing from viable cell counts. Since the absorbance was conducted by increasing turbidity of bacteria suspension. Liew et al. [20] also reported that *L. rhamnosus* had the highest number of viable cells (stationary phase) after incubation for 12-16 hours. The stationary phase induced various physiological states within the cells due to exhaustion and no available food sources that triggering stress response to provide over phase survival of cells [21], [22]. Therefore, the proper time for probiotics incubation was 15 hours before testing the acid and bile salt tolerance or making powder by spray drying.

B. Acid and Bile Salts Tolerance

The effect of acid as gastronomical condition (pH 2) on probiotic survivability founded significantly decreased the number of viable cells when increasing times ($P \leq 0.05$). After 120 min, the result showed that *L. rhamnosus* TISTR 047 and *L. casei* TISTR 1500 were the most acid tolerance strains among 4 selected strains (Table I). Both of them were remained significantly higher than the others two strains.

Similarly to the condition with present of bile salts, *L. rhamnosus* TISTR 047 and *L. casei* TISTR 1500 were the most tolerance strains. Mimetic intestinal condition, presence of bile salts in pH 5.8 MRS broth was chosen to examine the tolerance of selected strains. We observed that viable cells of all selected strains were significantly decreased ($P \leq 0.05$) when increasing times (Table II). The survivability of viable probiotic organisms, after 8 hours incubation decreased more than a half of the initial numbers. The remained *L. rhamnosus* TISTR 047 and *L. casei* TISTR 1500 were significantly higher than the other strains. The different survivability of probiotic strains were due to intrinsic factors which related to the cell structure [23]-[24]. For further used, accepted probiotic bacteria must be sufficiently survived in the consumed product to facilitate colonization and pathogenic resistance capable [25]. Therefore, *L. rhamnosus* TISTR 047 and *L. casei* TISTR 1500 could be selected for production of spray dried probiotic powder.

TABLE II
THE SURVIVAL OF PROBIOTIC STRAINS INTO BILT SALTS CONDITION (PH 5.8)

Strain	Viable of cells (log CFU/ml)				
	0 h	2 h	4 h	6 h	8 h
L. rhamnosus TISTR 047	10.64 ± 0.05^{abA}	8.90 ± 0.61^{abB}	7.90 ± 0.08^{aC}	6.29 ± 0.03^{aD}	5.12 ± 0.03^{aE}
L. casei TISTR 1500	10.66 ± 0.01^{abA}	9.27 ± 0.05^{abB}	7.90 ± 0.09^{aC}	6.31 ± 0.03^{aD}	5.11 ± 0.06^{aE}
L. acidophilus TISTR 1338	10.69 ± 0.02^{aA}	9.42 ± 0.03^{aB}	7.51 ± 0.02^{bC}	6.34 ± 0.04^{aD}	4.92 ± 0.03^{bE}
L. plantarum TISTR 1465	10.57 ± 0.10^{bA}	8.67 ± 0.25^{bB}	6.67 ± 0.24^{cC}	5.79 ± 0.10^{bD}	4.62 ± 0.13^{cE}

Data are mean ± SD

a, b and c mean of different letter in the same column significant difference ($P \leq 0.05$), n = 3

A, B, C, D and E mean of different letter in the same row significant difference ($P \leq 0.05$), n = 3

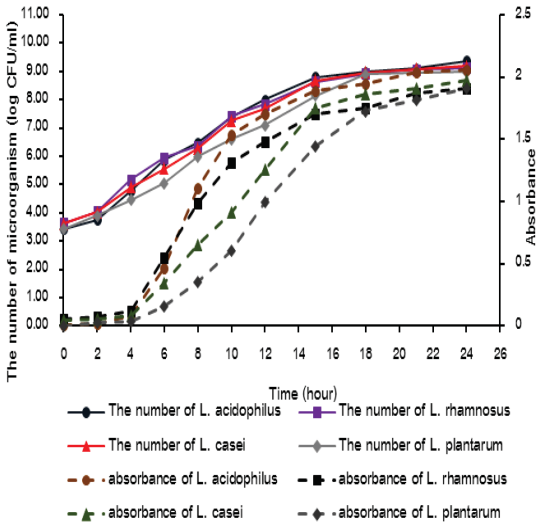

Fig. 1 Growth curve and absorbance (OD_{600}) of *Lactobacillus* counted in MRS agar and incubated at 37ºC for 24 hours

C. Survival of Probiotic after Spray Drying

The survival of probiotic organisms after spray drying were compared between encapsulated and non-encapsulation forms. The encapsulated probiotic organisms were obviously showed higher amount of survival organisms than non-encapsulated. The encapsulated was founded only one log cycle viable cells decreased after passed through spray drying condition. Whereas, the non-encapsulation decreased more than a half from the initial number (Table III). Pimentel-González et al. [26] indicated that encapsulation could protect the probiotic organisms from spray dried environmental effects. Moreover, encapsulation could be promoted survivability of probiotic organisms from acid and presence of bile salt in the host gastrointestinal [27].

TABLE III
THE SURVIVAL OF PROBIOTIC STRAINS AFTER SPRAY DRYING

Method	Viable of cells (log CFU/ml)			
	Initial	W/O	W/O/W	spray dry
non-encapsulate	9.63 ± 0.32^{aA}	7.96 ± 0.08^{aB}	6.05 ± 0.49^{aC}	4.06 ± 0.08^{bD}
encapsulate	9.63 ± 0.32^{aA}	8.94 ± 0.34^{aAB}	8.55 ± 0.21^{aB}	8.31 ± 0.11^{aB}

Data are mean ± SD
a and b mean of different letter in the same column significant difference ($P \leq 0.05$), n = 3
A, B, C and D mean of different letter in the same row significant difference ($P \leq 0.05$), n = 3

IV. CONCLUSIONS

Probiotic organisms decreased under acid and presence of bile salt condition when increasing time. Probiotic strains had different ability to survive under those conditions. *L. rhamnosus* TISTR 047 was the most tolerance strain. The encapsulation could prevent probiotics organisms from spray dried environment. Therefore, encapsulated *L. rhamnosus* TISTR 047 could be promoted survivability after consumed in gastrointestinal tract.

ACKNOWLEDGMENT

The author acknowledge the financial support given by The Thailand Research Fund (TRF) and Mighty interoperation (Thailand)

REFERENCES

[1] G. Veereman-Wauters, "Application of prebiotics in infant foods," The British Journal of Nutrition, vol. 93, pp. S57-S60, 2005.
[2] H. Parracho, A. McCartney, and G. R. Gibson, "Probiotics and prebiotics in infant nutrition," The Proceedings of the Nutrition Society, Vol. 66, pp. 405-411, 2007.
[3] J.M. Moreno Villares, "Prebiotics in infant formulars risks and benefits," Bioactive Food In Promoting Health: Probiotics and Prebiotics, Vol. 81, pp. 17-129, 2010.
[4] M. B. Roberfroid, "Prebiotics and probiotics: are they functional food," The American Journal of Clinical Nutrition, vol. 71, pp. 1682S-1687S, 2013.
[5] P. Capela, T. K. C. Hay, and N. P. Shah, "Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt," Food Research International, Vol. 39, pp. 203-211, 2006.
[6] V. Manojlovic, V. A. Nedovic, K. Kailasapathy, and N. J. Zuidam, "Encapsulation of probiotics for use in food products. In: N. J. a. V. Nedovic. (Ed.), Encapsulation Technologies for Active Food Ingredients and Food Processing. Springer, New York, pp. 269-302, 2010.
[7] R. R. Mokarram, S.A. Mortazavi, M.B.H. Naiafi and N.F. Shabidi, "The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice," Food Research International, Vol. 42, pp. 1040-1045, 2009.
[8] T. Heidebach, P. FÖrst, and U. Kulozik, "Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins," Food Hydrocolloids, vol. 23, no. 7, pp. 1670–1677. 2009.
[9] M. Papagianni, and S. Anastasiadou, "Encapsulation of *Pediococcus acidilactici* cells in corn and olive oil microcapsules emulsified by peptides and stabilized with xanthan in oil-in-water emulsion: Studies on cell viability under gastro-intestinal simulating conditions," Enzyme and Microbial Technology, Vol. 45, pp. 514-522, 2009.
[10] M. R. Gismondo, L. Drago, and A. Lombardi, "Review of probiotics available to modify gastrointestinal flora," International Journal of Antimicrobial Agents, Vol. 12, pp. 287-292, 1999.
[11] C. P. Tseng, and T. J. Montville, "Metabolic regulation of end product distribution in lactobacilli: Causes and consequences," Biotechnology Progress, Vol. 9, no. 2, pp. 113-121, 1993.
[12] T. Heidebach, P. FÖrst, and U. Kulozik, "Influence of casein-based microencapsulation on freeze-drying and stroge of probiotic cells," Journal of Food Engineering, Vol. 98, no. 3, pp. 309-316, 2010.
[13] G. B. Brinques, and M. A. Z. Ayub, "Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt," Journal of Food Engineering, vol. 103, pp. 123-128, 2011.
[14] C. P. Champagne and P. Fustier, "Microencapsulation for the improved delivery of bioactive compounds into foods," Current Opinion in Biotechnology, vol. 18, pp. 184 –190, 2007.
[15] M. Borgogna, B. Bellich, L. Zorzin, R. Lapasin, and A. Cesàro, "Food microencapsulation of bioactive compounds: Rheological and thermal characterization of non-conventional gelling system," Food Chemistry, Vol. 122, pp. 416-423, 2010.
[16] A. Sohail, M. S. Turner, A. Coombes, T. Bostrom, and B. Bhandari, "Survivability of probiotics encapsulated in alginate gel microbeads using a novel impinging aerosols method," International Journal of Food Microbiology, Vol. 145, pp. 162-168, 2011.
[17] W. K. Ding and N. P. Shah, "Effect of Various Encapsulating Materials on the stability of probiotic bacteria," Journal of Food Science, vol 74, pp. M100-M107, 2009.
[18] G. E. Gardiner, E. O'Sullivan, J. Kelly, M. A. Auty, G. F. Fitzgerald, and J. K. Collins, "Comparative survival rates of human derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray-drying," Applied and Environmental Microbiology, vol. 66, pp. 2605–2612, 2000.
[19] E. Ananta, M. Volkert, and D. Knorr, "Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG," International Dairy Journal, vol 15, pp. 399-409, 2005.

[20] S. L. Liew, A. B. Ariff, T. A. R. Raha, and Y. W. Hoa, "Optimization of composition for the production of a probiotic microorganism, Lactobacillus rhamnosus, using response surface methodology," Journal of Food Microbiology, Vol. 102, pp. 137-142, 2005.
[21] M. van de Guchte, P. Serror, C. Chervaux, T. Smokvina, S. D. Ehrlich, and E. Maguin, "Stress responses in lactic acid bacteria," Antonie Van Leeuwenhoek, vol 82, pp. 187–216, 2002.
[22] C. A. Morgan, N. Herman, P. A. White, and G. Vesey, "Preservation of microorganisms by drying: A review," Journal of Microbiological Methods, vol. 66, pp. 183–193, 2006.
[23] X. C. Meng, C. Stanton, G. F. Fitzgerald, C. Daly, and R. P. Ross, "Anhydrobiotics: The challenges of drying probiotic cultures," Food Chemistry, vol. 106. pp. 1406–1416, 2008.
[24] W. F. Tee, R. Nazaruddin, YN. Tan, and M.K. Ayob, "Effects of encapsulation on the viability of potential probiotic *Lactobacillus plantarum* exposed to high acidity condition and presence of bile salts," Food Science and Technology International, vol. 0, no. 0, pp. 1-6, 2014.
[25] X. Y. Li, X. G. Chen, Z. W. Sun, H. J. Park, and D. Cha, "Preparation of alginate/chitosan/carboxymethyl chitosan complex microcapsules and application in *Lactobacillus casei* ATCC 393," Carbohydrate Polymers, vol. 83, pp. 1479-1485, 2011.
[26] D. J. Pimentel-González, R. G. Campos-Montiel, C. Lobato-Calleros, R. Pedroza-Islas, and E. J. Vernon-Carter, "Encapsulationof *Lactobacillus rhamnosus* in double emulsions formulated withsweet whey as emulsifier and survival in simulated gastrointestinal conditions," Food Research International, vol. 42, pp. 292–297,2009.
[27] D. W. Olson and K. J. Aryana, "An excessively high Lactobacillus acidophilus inoculation level in yogurt lowers product quality during storage," Lebensmittel Wissenschaft und Technologie, vol. 41, pp. 911-918, 2008.