Viability of Probiotic Lactic Acid Microencapsulated with Maltodextrin in the Simulated Gastric Juice and Bile Salt

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Abstract. Probiotic lactic acid bacteria (LAB) consisted of Lactobacillus murinus Ar3, Streptococcus thermophilus Kp2, and Pediococcus acidilacti Kd6 were encapsulated with skimmed milk-maltodextrin. In vitro studies were used to determine the effects of simulated gastric juice (SGJ) and bile salt on the viability of encapsulated probiotics. The results showed that the viability of microencapsulated LAB decreased 0.45 log units during 3-h exposure to SGJ with pepsin at pH 2.5. After 4-h exposure in bile salt solutions at pH 6.5, the number of microencapsulated LAB increased by 1.10 log units. Microencapsulated LAB was completely released from the coated after exposure to bile salt during 4-h. This study confirmed that maltodextrin in microencapsulation might be an effective method that could allow viable probiotic bacteria to reach the large intestine.

1. Introduction
Probiotics are defined as living microorganisms which when administered in adequate amount confer a health benefit on the host [1]. To achieve the health benefits, however probiotic bacteria must be stable as well as survive in large numbers through the digestive tract, to the appropriate location and have beneficial affects for the host [2]. Without protection, phage might not survive gastric passage and thus not be infective in the intestine [3]. Microencapsulation is the technique of providing physical protective layer of barrier to the microorganism to improve its viability [4]. Microencapsulation has been applied to enhance the viability of probiotic bacteria during processing and also for targeted delivery to the gastrointestinal tract [5,6]. Spray drying is one of microencapsulation techniques. Spray drying could make small size particles and easy application on an scale with low cost. In the previous studies skimmed milk-maltodextrin showed effectiveness in improving the viability of probiotic bacteria [7,8]. The objective of the study were to determine the effects of simulated gastric juice (SGJ) and bile salt on the viability of encapsulated probiotics.

2. Material and methods

2.1. Bacteria strain and culture conditions
Probiotic lactic acid bacteria (LAB) consisted of Lactobacillus murinus Ar3, Streptococcus thermophilus Kp2, and Pediococcus acidilacti Kd6. According to Muttaqin (with modifications) [9], the isolates were inoculated in sterile peptone glucose yeast (PGY) broth, and incubating at 37°C. Mix
probiotic LAB were harvested from 1800 ml of a 18 h culture (log phase) by centrifugation at 3000 rpm. Then, the cells were used in the microencapsulation process in the next step.

2.2. Microencapsulation culture of probiotic LAB
Microencapsulated probiotic LAB were prepared according to Mutukumira (with modified) [10]. Skim solution containing 20% of skim in distilled water (w/v) was prepared and sterilized (115°C for 10 min). Maltodextrin solution containing 20% of maltodextrin in distilled water (w/v) was prepared and sterilized (121°C for 15 min). The cells was mixed with skimmed milk-maltodextrin solution. The solution was homogenized with a magnetic stirrer during spray drying process. Spray dryer operating conditions as follows: inlet/outlet temperature 160/80°C. Colony of microencapsulated probiotic LAB obtained from spray dryer was count by the total plate count (TPC) method. The survival rate of microencapsulated probiotic LAB after spray drying was evaluated by the following equation:

$$\text{Survival (\%)} = \left( \frac{\sum \log \text{CFU after spray drying}}{\sum \log \text{CFU before spray drying}} \right) \times 100\%$$

3. Analysis methods

3.1. Survival of microencapsulated probiotic LAB in SGJ and bile salt
Resistance to simulated gastric fluid was determined by adding 1 g of the microencapsulated bacteria into flasks containing 10 mL of the SGJ, which consisted of 0.3% pepsin (Sigma) and 0.5% sodium chloride (Nakalai, Kyoto, Japan) adjusted to pH 2.5 with 1 N HCl. Resistance to bile salts was determined by adding microcapsules to the bile-salt solution, which consisted of 2% ox gall powder (Sigma). Both resistance treatments took place in agitated flasks (100 rpm) at 37 °C for 4 h.

3.2. Determination of probiotic viability
To determine the probiotic viability count, the entrapped probiotics were released from the microcapsules according to the method of Sheu and Marshall [11]. One gram of the microcapsules was resuspended in 9 mL of phosphate buffer (0.1 M, pH 7.0) followed by homogenization in a stomacher Seward Stomacker 400C, Brinkmann, Westbury, N.Y., U.S.A.) for 15 min. The suitability of the media was tested by plating decimal dilutions of the probiotic cultures. Thus, a 1-g sample was decimally diluted into sterile peptone water (0.1%), and then 0.1-mL aliquot dilutions were plated onto the different media, in triplicate. Plates of PGY agar were incubated aerobically for 72 h at 37 °C. The population, in colony-forming units (CFU), and the characteristics of the colonies were recorded. The survival rate of microencapsulated probiotic LAB was evaluated by the following equation:

$$\text{Survival (\%)} = \left( \frac{\sum \log \text{CFU after incubation}}{\sum \log \text{CFU before incubation}} \right) \times 100\%$$

4. Results and discussion

4.1. The effect of spray drying process on the viability of microencapsulated probiotic LAB
The viability of microencapsulated probiotic LAB before and after spray drying is shown in Table 1.

| Process                  | Log CFU/g |
|--------------------------|-----------|
| Before spray drying      | 1.1 x 10⁶ |
| After spray drying       | 6.2 x 10⁶ |
| Result spray drying (g)  | 86        |

In the present study showed that skimmed milk-maltodextrin can protect microencapsulated probiotic LAB during spray drying. Mutukumira et al. [10] reported that skimmed milk-maltodextrin can protect the viability of Lactobacillus casei-01. According to Kartheek et al. [12] maltodextrin
showed protective effect which can reduce the caking and stickiness to the spray dryer’s walls. Adja et al. [7] indicated that maltodextrin, milk protein and fat use as microencapsulating agents may have protected the microorganisms during the spray drying process.

4.2. The viability of microencapsulated probiotic LAB in GSJ and bile salt
The viability of microencapsulated probiotic LAB in GSJ and Bile salt is shown in Table 2.

Table 2. Viability of microencapsulated probiotic LAB in SGJ and bile salt during 3 hours

| Solution     | Duration (h) (log CFU/g) |
|--------------|--------------------------|
|              | 1           | 2           | 3           |
| SGJ          | 7.82±0,18  | 7.48±0,69  | 7.37±0,58  |
| Bile salt    | 8.81±0,04  | 9.13±0,02  | 9.68±0,02  |

SGJ: simulated gastric juice

Lactic acid bacteria isolated from the digestive tract of animals and humans are functional bacteria as a probiotic with the ability to survive in various limiting conditions along the digestive tract from the mouth to the intestine, such as low pH in the stomach and the presence of bile salts in the duodenum [12,13].

Table 2 showed that microencapsulated probiotic LAB had good viability on pH 2.5 and 6.5 in SGJ and bile salt. Lin et al. [14] stated that the survival of LAB ≥ 50% at pH 2, this was assumed to be due to gastric fluid, while LAB survival in bile salt stress was considered good if the viability was ≥ 60%. In this study showed that maltodextrin’s role as prebiotic potential and high buffer properties of whey protein may improve the viability of microencapsulated probiotic LAB in SGJ and bile salt conditions. Arslan et al. [15] stated that the survivability of microencapsulated S. boulardii using maltodextrin against simulated gastric solution was 63.62% at pH 2. The ability to tolerate gastric juice was one of an indicator, because good probiotics must be able to pass stresses in the gastrointestinal tract such as pH stress and gastric juice [16].

5. Conclusion
In conclusion, the present study indicated that viability of microencapsulated probiotic LAB was affected significantly by microencapsulated agents during spray drying and in SGJ and bile salt. Microencapsulated LAB was completely released from the coated after exposure to bile salt during 4-h. Maltodextrin’s role not only as a wall material in microencapsulation but also as a prebiotic potential. This study confirmed that maltodextrin in microencapsulation might be an effective method that could allow viable probiotic bacteria to reach the large intestine.

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