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Full Length Research Paper

Effect of heat, pH and coating process with stearic acid using a fluidized bed granulator on viability of probiotic *Lactobacillus reuteri* C 10

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This study was conducted to investigate the use of a fluidized bed granulator to coat a probiotic *Lactobacillus reuteri* C 10 with stearic acid with a view to enhance its survival rate during storage. *L reuteri* C 10 cells of two treatments, namely, freeze-dried cells incorporated with trehalose and sucrose as cryoprotectants prior to freeze-drying, and freeze-dried cells without any incorporation of cryoprotectants were evaluated. Since the pH of stearic acid is 5.5 and the melting point is 57.23°C, and the inlet temperature of the fluidized bed granulator could be as high as 70°C, a preliminary study was initiated to determine the tolerance of *L. reuteri* C 10 cells to heat exposure from 58 to 70°C and acidic conditions of pH 4 to 6 for 60 min, during which the cell viabilities were determined every 15 min. In the coating process, 2:3 ratio of freeze-dried *L. reuteri* C 10 cells and stearic acid, fluidization air of 20 to 50 rpm, coating rate of 40 to 80 g/min and inlet and outlet temperatures of between 50 to 70°C were assessed for optimization of the fluidized bed granulator. Results of the preliminary study showed that freeze-dried *L. reuteri* C 10 cells incorporated with cryoprotectants exhibited significantly ($P < 0.05$) less cell loss than cells without cryoprotectants when exposed to 62°C for 15 to 60 min, 64 °C for 15 to 30 min, 66°C for 30 min and 68°C for 15 to 30 min. Freeze-dried *L. reuteri* C 10 cells with cryoprotectants were also able to survive for 15 min at 70°C, but not freeze-dried *L. reuteri* C 10 cells without cryoprotectants. Freeze-dried *L. reuteri* C 10 cells with or without cryoprotectants could tolerate acidic conditions and there was growth and increase in cell viability at pH 4, 5 and 6. However, cells with cryoprotectants had significantly ($P < 0.05$) more growth when exposed to pH 5 for 30 to 60 min, and pH 6 for 15 to 60 min than cells without cryoprotectants. The application of a fluidized bed granulator to coat *L. reuteri* C 10 cells with or without cryoprotectants with melted stearic was not successful in this study because the fluidized bed granulator could not maintain the temperature of stearic acid above its melting point which led to clogging of the tube and spray nozzle of the fluidized bed granulator or resulted in the formation of a big lump of stearic acid and *L. reuteri* C 10 cells instead of uniform coated cell granules. Installation of a temperature jacket on the fluidized bed granulator may be necessary to control the temperature of stearic acid in the tube and spray nozzle above melting point.

Key words: Coating, fluidized bed granulator, *Lactobacillus reuteri* C10, stearic acid.

INTRODUCTION

In the last five decades, the vast availability of antibiotics has resulted in their extensive use as therapeutic antibiotics and growth promoters in the livestock industry. However, the widespread use of antibiotics as growth promoters increased the risk for emergence of new, more virulent or more resistant bacteria. The prolonged use of antibiotics at low dosage will not kill the bacteria instead it will promote their resistance towards antibiotics. With

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increased concern on the emergence of antibiotic resistant bacteria, many countries have banned the use of growth promoting antibiotics in livestock production. The use of probiotics is one of several approaches that have potential to be an alternative to antibiotic growth promoters for improving livestock production. Probiotic has been defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Fuller, 1989). It has been reported to exert many beneficial effects such as stimulation of the immune system, reduction in allergy, serum cholesterol concentration and blood pressure, improvement of livestock growth rate, improvement of lactose tolerance, regression of tumors and as treatments for human intestinal barrier dysfunctions (Sawada et al., 1990; Isolaurie et al., 1993; Kailasapathy and Chin, 2000; Patterson and Burkholder, 2003; Anal and Singh, 2007).

The ability of probiotic microorganisms to remain viable and stable during processing, storage and digestion is very crucial for their positive contribution to the host. In order for the host to attain the beneficial effects of probiotics, the probiotic bacteria must be viable at high concentration of at least 10^6 to 10^7 cfu/g (Shah et al., 2000). Viernstein et al. (2005) have reported that even under excellent storage conditions with sophisticated formulations, a probiotic product may lose 90% of its active microorganisms within a year. Probiotic survival in a product is affected by a range of factors, including pH, moisture content, acid production during refrigerated storage (post-acidification), oxygen permeation through packaging, storage temperatures and sensitivity to antimicrobial substances produced by the bacteria (Dave and Shah, 1995). Even if the probiotic bacteria manage to remain viable after processing and during storage, some of them do not survive when passing through the upper digestive tract. For probiotics that are incorporated into animal feed as one of the specific additives, the major problems are high temperatures and pressure during processing. The processing temperature may rise up to 85°C which would result in cell death as most of the probiotic bacterial strains are intolerant to high temperatures since they are mesophilic microorganisms (Kosin and Rakshit, 2006).

One of the most common methods to preserve probiotic microorganisms is by using drying techniques such as spray drying (Mauriello et al., 1999; Desmond et al., 2002) or freeze-drying (Todor and Tsonka, 2002; Algeria et al., 2004). These techniques are basically designed to evaporate water from the microorganisms. However, the hygroscopic nature of a dried probiotic is a major limitation of using drying techniques as there is a drastic loss of cell viability during storage especially at room temperature. According to Champagne et al. (1996), as storage temperature increases, mortality of cultures also increases. Lim (2006) observed a total loss of viability of freeze-dried *L. salivarius* I 24 after 3 months of storage at 30ºC. Tsvetkov and Brankova (1983) also reported a rapid decrease of *Micrococcus varians* and *L. plantarum* viabilities during storage at room temperature of 20ºC, with a total loss of viability at 3 months.

Microencapsulation technology, as one of the advanced and newest methods, offers an ideal solution to the destabilization problem of sensitive probiotic micro-organisms. Microencapsulation is defined as the process of entrapment of microorganisms by means of coating them in order to segregate the cells from the surrounding environment (Sultana et al., 2000; Krasaekoopt et al., 2003; Picot and Locroix, 2003). Fluidized bed coating, as one of the newest microencapsulation techniques, is one of the few technologies that is capable of coating particles with basically any kind of coating materials including polysaccharides, proteins, emulsifiers, fats, complex formulations, enteric coating and powder coating (Gouin, 2004). The use of melted fats, such as stearic acid, for coating is a promising and interesting concept. A hydrophobic wall surrounding the microorganisms may act as a barrier to prevent easy diffusion of moisture in and out of the fatty matrix. This technology is suitable for microorganisms that are used as feed additives for livestock as they are generally stored for a long time at room temperature.

In this study, we investigated the use of a fluidized bed granulator to coat a probiotic *Lactobacillus reuteri* C 10 with stearic acid, with a view to enhance its stability and survival during storage. *L. reuteri* C 10 is part of a multi-strain probiotic for chickens, which has been used as an alternative to antibiotic growth promoters to improve the growth performance, feed efficiency and immune response as well as reduce fat and cholesterol contents of chickens (Jin et al., 1996a, 1998a, b, 2000; Zulkifli et al., 2000; Kalavathy et al., 2003, 2005, 2006, 2008, 2009). A preliminary study was also carried out to evaluate the tolerance of *L. reuteri* C 10 to high temperatures of 58 to 70ºC and acidic conditions of pH 4 to 6, since the melting point of stearic acid is 57.23ºC and pH is approximately 5.5, and input temperatures of the fluidized bed granulator could be up to 70ºC.

MATERIALS AND METHODS

Bacterial strains and maintenance

A pure culture of *L. reuteri* C 10 was kindly donated by Stellar Gen Ltd (Kuala Lumpur, Malaysia). *L. reuteri* C10 was originally isolated from the gastrointestinal tract of a local chicken by Jin et al. (1996 b), and was identified as *L. brevis* C 10. However, recently, it was reclassified as *L. reuteri* C 10 by sequencing the 16S rRNA gene and 16S-23s rRNA gene intergenic spacer region (Lee et al., 2008). The strain was maintained by routine culture in de Man-Rogosa-Sharpe (MRS) broth (Oxoid, Hampshire, England) and incubated under anaerobic conditions using anaerobic jar (Oxoid Ltd) and gas generating kit (Oxoid Ltd) at 37°C. The culture was stored in MRS with 20% glycerol (v/v) and kept at -80°C. The strain was sub-cultured three times in MRS broth prior to experimental use.

Preparation of *L. reuteri* C 10 cells

For inoculum preparation, *L. reuteri* C 10 was sub-cultured onto MRS agar plates, and incubated for 48 h at 37°C. Single colonies were picked from the plates and transferred to an inoculum medium containing (/l): 20 g glucose, 0.2 g magnesium sulfate hepta-hydrate, 0.05 g manganese sulfate monohydrate, 2 g dipotasium hydrogen phosphate anhydrous, 5 g sodium acetate trihydrate (all above chemicals from Merck, Darmstadt, Germany), 22 g yeast extract (Oxoid, Hampshire, England) and 1 g Tween 80 (Merck, Hohenbrunn, Germany), and incubated for 16 to 18 h at 37°C. A 50-l fermenter (Biostat D 50 Stirred Tank Fermenter, B Braun Biotech International, Germany) was used as a fermenter vessel. The basal fermentation medium used consisted of (/l): 56.7 g glucose, 41.24 g yeast extract, 0.21 g magnesium sulfate heptahydrate, 0.05 g manganese sulfate monohydrate and 1.03 g Tween 80. Sterilization of the medium was carried out *in-situ* at 121°C for 15 min, except for glucose which was autoclaved separately. The inoculum (2.5%) was transferred aseptically into the 50-l fermenter vessel. An agitation speed of 150 rpm, anaerobic conditions, pH 5.5 and temperature of 37°C were kept constant throughout the fermentation period. After 9 h of fermentation (early stationary growth phase), the *Lactobacillus* cell suspension was centrifuged at 17000 x *g* at 4°C using a continuous centrifuge (Alfa Laval, Sweden). The cell pellets were mixed with or without a solution of cryoprotectants [2% (w/v) trehalose and 1% (w/v) sucrose] in a ratio of 2:1 (cell pellets : cryoprotectants) (Lim, 2006). The cell mixture was freeze-dried for 48 to 72 h using a freeze-dryer (Labconco, USA), and then ground using a 1 mm sieve.

Heat and acid tolerance

In this preliminary study, two cell treatments were used: (i) freeze-dried *L. reuteri* C 10 cells incorporated with cryoprotectants and (ii) freeze-dried cells without any incorporation of cryoprotectants. In the heat tolerance experiment, 1 g of freeze-dried cells with or without cryoprotectants was inoculated separately into tubes containing 9 ml of MRS broth. The tubes were placed in separate water bath with test temperatures of 37 (normal temperature for growth), 58, 60, 62, 64, 66, 68 or 70°C, respectively. In the acid tolerance experiment, the initial pH of MRS broth was adjusted to pH 4, 5 and 6 with either 1 N HCl or NaOH before sterilizing at 121°C for 15 min. One g of freeze-dried cells with or without cryoprotectants was inoculated separately into tubes containing 9 ml of MRS broth with different pH values, and incubated in a water bath at a constant temperature of 37°C. The incubation period for both heat and acid tolerance experiments was 60 min with enumeration of viable cells at 15-min intervals using the spread plate technique. Briefly, the cultures were vortexed for 15 s and serially diluted (10^{-1} to 10^{-8}) using peptone (1% w/v) and distilled water. From each dilution, 0.1 ml was spread on MRS agar and incubated anaerobically at 37°C for 48 h, after which colonies were counted. The number of viable cells in the samples was calculated from the total number of colonies counted on a plate multiply by the dilution factor involved. Viable cells were expressed as $\log_{10}$ cfu/g. The samples were analysed in duplicate. The experiment was carried out twice.

Fluidized-bed granulator coating process

Stearic acid (Pacific Oleochemicals Ltd, Malaysia) was used as the coating material. The coating process was carried out using a fluidized bed dryer spray granulator (N.R Industry FBS1, Thailand). The modified methods of Rutherford et al. (1992) and Durand and Panes (2003) were adopted for the optimization parameters of the coating process. In each run, 40 g of freeze-dried *L. reuteri* C 10 cells, with or without cryoprotectants, and 60 g of stearic acid were used. Freeze-dried cells (with or without cryoprotectants) were filled separately into the bed and blown by the fluidization air from the bottom distribution plate at a flow rate of between 20 to 50 rpm. Stearic acid was melted on a hot plate at 70°C and the melted stearic acid was pumped through a tube (5 mm diameter) [Figure 1(a)] into an air-atomizing spray nozzle located at the top of the bed [Figure 1(b)]. The melted stearic acid was sprayed [Figure 1(c)] at a rate of 40 g/min. The inlet and outlet temperatures were maintained at 50 to 70°C. The coating continued until all the coating solution (stearic acid) was dispatched and proceeded for another 5 min for drying. The formation of granules inside the bed chamber was observed and recorded. Viabilities of cells were determined before and after the coating process. Enumeration of *L. reuteri* C 10 in coated samples was carried out according to the modified methods of Brachkova et al. (2008) and Sabikhi et al. (2008). Briefly, 1 g of coated bacterial sample was rehydrated in 9 ml of sterilized depolymerization solution (0.05 sodium phosphate buffer, pH 7.4). The solution was incubated at 37°C for 10 min followed by vortexing at high speed for 5 min to disperse the bacteria in the buffer. The suspension was serially diluted (10^{-3} to 10^{-8}), and 0.1 ml was spread on MRS agar and incubated anaerobically at 37°C for 48 h. Colonies on the plates were counted and cell viability was expressed as $\log_{10}$ cfu/g. All analyses were performed in triplicate, and the experiment was carried out twice.

Statistical analysis

Experimental data of the heat and pH tolerance experiments were analyzed using the SPSS statistical package program, version 17.0 (SPSS Inc., Chicago, IL, USA). Significant differences between values of each incubation period at each temperature or pH were tested using T – test. Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Viability of freeze-dried *L. reuteri* C 10 cells at high temperatures

The results on the viabilities of freeze-dried *L. reuteri* C 10 cells with and without cryoprotectants incubated at temperatures of 37 to 70°C for 0 to 60 min are shown in Table 1. At 37°C, there was an increase in growth of *L. reuteri* C 10 cells with or without cryoprotectants when incubated for 15 to 60 min, and, generally, growth was significantly ($P < 0.05$) more in cells without cryo-protectants (increase of 0.23 log cfu/g with cryo-protectants and 0.46 log cfu/g without cryoprotectants at 60 min of incubation). When the temperature was raised to 58 and 60°C, *L. reuteri* C 10 cells with or without cryoprotectants exhibited losses in cell viability at the different incubation periods, with significantly ($P < 0.05$) more loss in cells with cryoprotectants. However, *L. reuteri* C 10 cells with cryoprotectants exhibited signifi-cantly ($P < 0.05$) less loss in cell viability than cells without cryoprotectants when incubated at 62°C for 15 to 60 min, 64°C for 15 to 30 min, 66°C for 30 min and 68°C

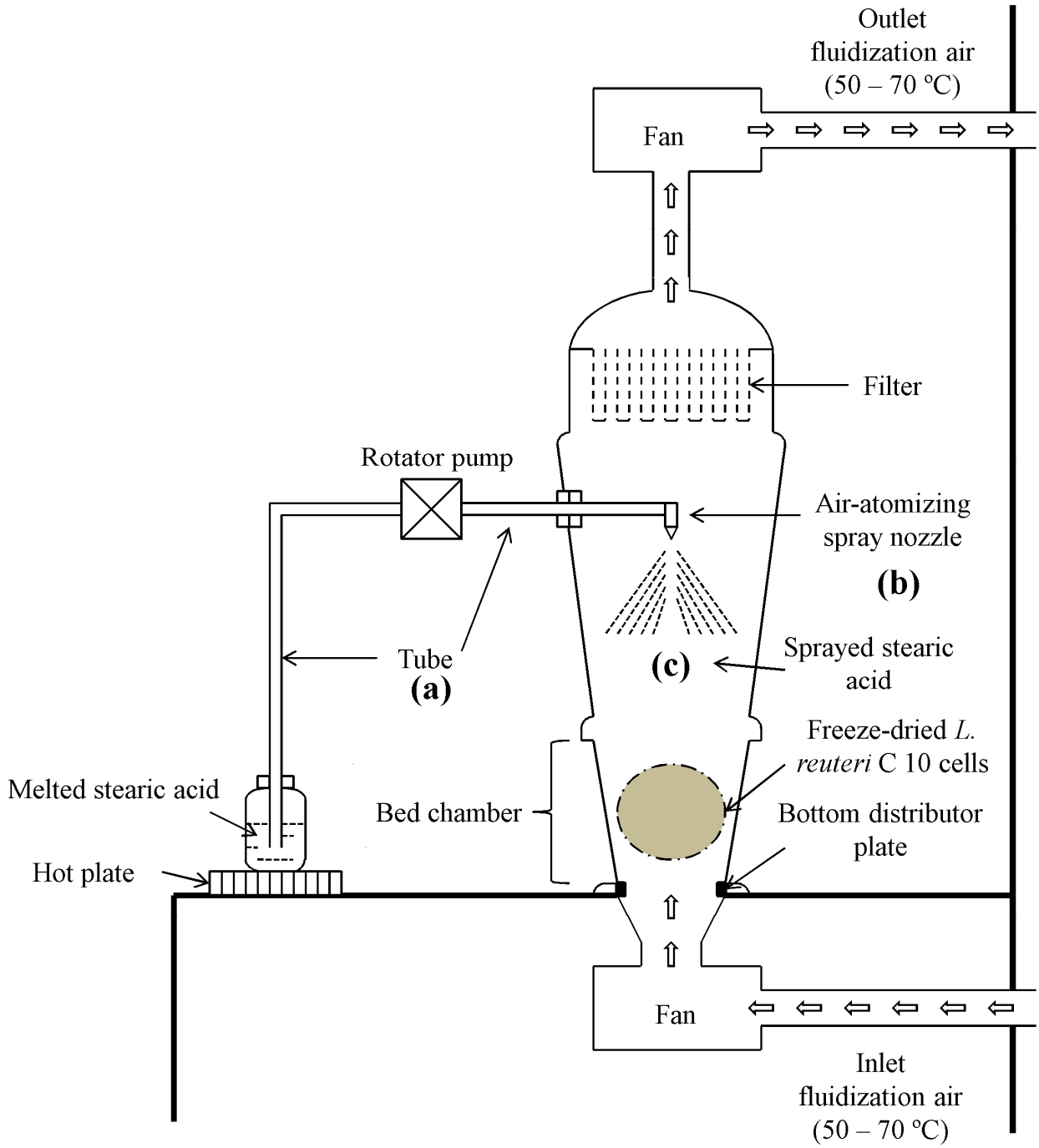

Figure 1. Schematic diagram of a fluidized bed granulator equipped with a hot plate for melting of stearic acid.

for 15 to 30 mins. At 70°C, there was a loss of 3.57 log cfu/g of freeze-dried *L. reuteri* C 10 cells with cryoprotectants after 15 min of incubation and no viable cells were detected at 30 min of incubation. However, for freeze-dried cells of *L. reuteri* C 10 without cryoprotectants, the cells were completely destroyed after 15 min of incubation at 70°C.

The results show that overall, freeze-dried *L. reuteri* C 10 cells incorporated with cryoprotectants exhibited better survival rates when exposed to high temperatures than freeze-dried cells without any incorporation of cyroprotectants. This indicated that freeze-dried cells of *L. reuteri* C 10 with cryoprotectants should be used for the coating process in a fluidized bed granulator, in which

Table 1. Reductions or increases (+) in cell viability (log cfu/g) of freeze-dried probiotic *L. reuteri* C 10 with or without cryoprotectants exposed to temperature of 37 °C(normal temperature for growth) to 70 °C at different incubation periods.

Incubation period (min)	Treatment	Temperature (°C) [1]							
		37	58	60	62	64	66	68	70
15	C	+ 0.01 [b]	0.97 [a]	1.35 [a]	2.13 [b]	2.52 [b]	2.77 [a]	2.84 [b]	3.57
	N	+ 0.23 [a]	0.72 [b]	1.04 [b]	2.30 [a]	2.96 [a]	2.89 [a]	3.25 [a]	-
30	C	+ 0.07 [b]	1.43 [a]	2.19 [a]	3.86 [b]	4.47 [b]	4.56 [b]	4.40 [b]	-
	N	+ 0.31 [a]	0.98 [b]	1.92 [b]	4.03 [a]	5.12 [a]	5.22 [a]	4.80 [a]	-
45	C	+ 0.21 [a]	1.84 [a]	2.25 [b]	3.89 [b]	-	-	-	-
	N	+ 0.31 [a]	1.49 [b]	2.81 [a]	5.07 [a]	-	-	-	-
60	C	+ 0.23 [b]	2.95 [a]	3.89 [a]	5.32	-	-	-	-
	N	+ 0.46 [a]	2.18 [b]	3.70 [b]	-	-	-	-	-

[1] Results are mean values from duplicate replications. [a-b] Means within a column of each temperature at each incubation period with no common superscript differ significantly ($P < 0.05$). - Complete loss of cell viability that is no viable cells detected. C, freeze-dried *L. reuteri* C 10 with cryoprotectants; N, freeze-dried *L. reuteri* C 10 without cryoprotectants

Table 2. Increases in cell viability (log cfu/g) of freeze-dried probiotic *L. reuteri* C 10 with or without cryoprotectants exposed to pH of 4 to 6 at different incubation periods.

Incubation period (min)	Treatment	pH [1]		
		4	5	6
15	C	0.02 [a]	0.11 [a]	0.24 [a]
	N	0.00 [a]	0.01 [a]	0.03 [b]
30	C	0.04 [a]	0.20 [a]	0.34 [a]
	N	0.02 [a]	0.05 [b]	0.05 [b]
45	C	0.08 [a]	0.25 [a]	0.37 [a]
	N	0.05 [a]	0.05 [b]	0.07 [b]
60	C	0.09 [a]	0.35 [a]	0.51 [a]
	N	0.05 [a]	0.12 [b]	0.13 [b]

[1] Results are mean values from duplicate replications. [a-b] Means within a column of each pH at each incubation period with no common superscript differ significantly ($P < 0.05$). C, Freeze-dried *L. reuteri* C 10 with cryoprotectants. N, freeze-dried *L. reuteri* C 10 without cryoprotectants

temperatures during the process could be up to 70°C with the maximum allocation time of 15 min for mixing, spraying and drying. Leslie et al. (1995) have reported that trehalose and sucrose are the best protection agents for drying and rehydration purposes. However, even though trehalose and sucrose do provide some protection for the cells at high temperatures, the protection was insufficient, especially when exposure to high temperatures exceeded 15 min. Similar findings were reported by Sunny-Roberts and Knorr (2009), in which there was a drastic reduction of *L. rhamnosus* GG and *L. rhamnosus* E97800 cells suspended in trehalose when exposed to 65°C for 30 s.

Viability of freeze-dried *L. reuteri* C 10 cells at low pH

The viabilities of freeze-dried *L. reuteri* C 10 cells with and without incorporation of cyroprotectants incubated in acidic conditions are shown in Table 2. Freeze-dried cells with or without cryoprotectants showed tolerance to acidic conditions and there was growth and increase in cell viability at pH 4, 5 and 6. At pH 4, the increases in viabilities of freeze-dried *L. reuteri* C 10 cells with cryoprotectants in the different incubation periods were numerically more than those of cells without cryoprotectants. At pH 5 (incubated for 30 to 60 min) and 6 (incubated for 15 to 30 min), cells with cryoprotectants exhibited significantly ($P < 0.05$) better growth than cells without cryoprotectants. These results show that cells of *L. reuteri* C 10 with or without cyroprotectants were able to survive and grow in acidic environment, but the cryoprotectants seemed to have provided some protection for the cells against acidic conditions as these cells exhibited better growth rate than those without cryoprotectants. The ability of *L. reuteri* C 10 cells to tolerate acidic conditions is not surprising as the strain was isolated from the gastrointestinal tract (pH 2 to 6) of

a local chicken and has been used as part of a multi-strain probiotic for chickens. Similar result was reported by Taheri et al. (2009), who observed that *L.* johnsonii Lt 17, previously isolated from a chicken, showed total resistance to pH 3 when incubated for 30 min. Gaudana et al. (2010) also reported that *L. rhamnosus* CS25 and *L. plantarum* CS23 showed good tolerance to low acidic environments of pH 2 and 2.5 for 2 h. Generally, *Lactobacillus* strains used as probiotics have to withstand highly acidic environment when passing through the gastrointestinal tract of the host.

The results of the preliminary study showed that cells of *L. reuteri* C 10 with cryoprotectants were able to tolerate pH values of 4 to 6 without any adverse effects on cell viability, and could also survive high temperatures of up to 70°C for 15 min and, thus, could be coated with melted stearic acid in a fluidized bed granulator.

Coating process of *L. reuteri* C 10 with stearic acid using a fluidized bed granulator

For a successful coating process, stearic acid which was melted at 70°C on a hot plate had to be maintained in a liquid form throughout the spraying and coating process. However, when the coating process was carried out, the heat of the melted stearic acid dissipated very quickly to the environment during the flowing process in the tube [Figure 1 (a)] and when it reached the spray nozzle, the temperature of the melted stearic acid was below the melting point, resulting in solidification and clogging of the spray nozzle [Figure 1 (b)]. Furthermore, when the spraying process was carried out, the compressed atomized air that was used to spray the melted stearic acid into the bed chamber increased the heat loss and accelerated solidification of the melted stearic acid.

Apart from the heat lost to the environment, another problem during the coating process was in achieving a coating rate of 40 g/min. Durand and Panes (2003) suggested that 40 g/min was the optimum rate to achieve a uniform coating of cells. However, in order to achieve the optimum coating rate of 40 g/min, the pump rate for the flow of melted stearic acid into the spray nozzle had to be reduced by 50% (from 30 rpm to 15 rpm). The slower flow rate inside the tube resulted in higher heat loss which further contributed to solidification of stearic acid and clogging of the tube [Figure 1 (a)] even before the melted stearic acid reached the spray nozzle [Figure 1 (b)]. According to Gao et al. (2002), coating rate of the coating solution is one of the most important factors affecting granule size distribution. Hence, in order to achieve a uniform size of coated cells, the amount of coating solution being sprayed has to be minimized as low as possible. Lipsanen (2008) has also suggested that the coated granule size is directly proportional to the coating rate. For successful coating of the cells to occur, melted coating solution has to be sprayed slowly over the circulating cells. When the melted coating solution comes in contact with the cells, agglomeration process will occur between the cells and coating solution. During the agglomeration process, cells that are colliding with one another will be bonded by the coating solution. Over time, the melted coating solution will lose its heat and solidify, resulting in the formation of granules of coated cells.

In the present study, in order to ensure that stearic acid remained liquefied during the coating process, it was melted on a hotplate at a very high temperature of above 150°C and the inlet temperature of the fluidized bed granulator was maintained above 70°C. The coating rate was increased to 80 g/min to ensure a smooth flow of melted stearic acid into the spray nozzle. However, the increased coating rate of melted stearic acid caused excessive coating on the upper portion of the circulating freeze-dried *L. reuteri* C 10 cells. As stearic acid accumulated on the upper portion, the freeze-dried *L. reuteri* C 10 cells became heavier and dropped to the bottom of the bed chamber, affecting the circulation of the lower portion of the freeze-dried *Lactobacillus* cells. This created the formation of a big lump, consisting of stearic acid on the upper portion of the lump [Figure 1 (2a)] and an excessive amount of freeze-dried *Lactobacillus* cells on the lower portion [Figure 1 (2b)], inside the granulator bed chamber. The high inlet temperature and excess heat of the melted stearic acid also prevented the stearic acid from solidifying and coating the cells as soon as the agglomeration process occurred.

The overall results of the coating process of *L. reuteri* C 10 cells with stearic acid using a fluidized bed granulator showed that the use of high inlet temperature and increased coating rate would result in the formation of a big lump of stearic acid and *Lactobacillus* cells, while the use of lower inlet temperature and coating rate would lead to solidification of stearic acid and clogging of the tube and spray nozzle.

The viability of *L. reuteri* C 10 cells in the lump of stearic acid after the coating process was determined, but the results were very inconsistent as the cells were not uniformly coated or evenly distributed in the stearic acid lump, and were, thus, not reported here.

Stearic acid was chosen as the coating material in this study because of its hydrophobic characteristic which could act as a barrier to protect the *Lactobacillus* cells from exposure to adverse environmental conditions as well as to prevent easy diffusion of moisture in and out of free saturated fatty acid, and melting point within the range of 40 to 75°C. Although, the coating process of *Lactobacillus* cells with stearic acid was not successful in this study, stearic acid has been successfully used by others to coat *Lactobacillus* cells or other bacterial cells. Durand and Panes (2003) had successfully coated *L. acidophilus* R052, *L. casei* EQ 85 and *Saccharomyces cerevisiae* I 1079 with stearic acid, palmitic acid,

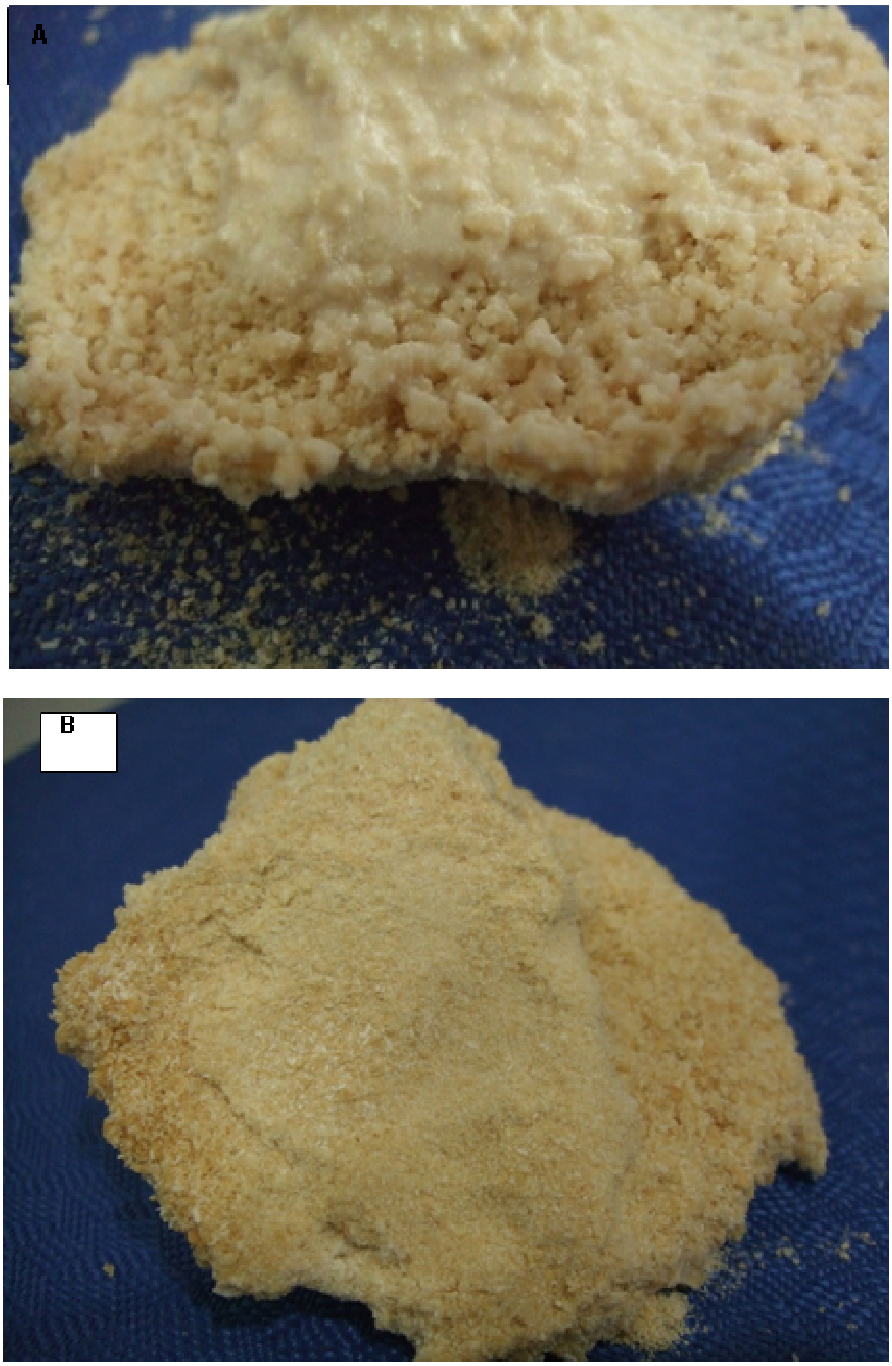

Figure 2. A lump of stearic acid with cells of freeze-dried *L. reuteri* C 10 formed after the coating process using a fluidized bed granulator. (A) vast amount of hardened stearic acid on the top portion of the lump; (B) accumulation of freeze-dried *L. reuteri* C 10 cells on the lower portion of the lump.

vegetable wax and a mixture of them using a modified fluidized bed dryer granulator that had a temperature-controlled receptacle to maintain the temperature of the coating material. They reported a reduction of less than 1 log cfu/g during the coating process. Rutherford et al. (1992) also had successfully coated freeze-dried *Enterococcus faecium* with stearic acid using a rotary disc. They installed a moisture absorbing column for the molten stearic acid and a dehumidifier to increase the recovery of the viable microorganisms. They reported

that the coated cells were able to maintain excellent viability during storage for as long as 70 days.

Conclusion

In conclusion, the results of this study showed that cells of *L. reuteri* C 10 with cryoprotectants were able to survive high temperatures and acidic conditions better than those without cryoprotectants, and could be used for coating with stearic acid. However, the use of a fluidized bed granulator with stearic acid as a coating material to coat freeze-dried cells of *L. reuteri* C 10 was not successful in this study because the fluidized bed granulator could not maintain the temperature of stearic acid above its melting point. Installation of a temperature jacket on the fluidized bed granulator may be necessary to control the temperature of stearic acid in the tube and spray nozzle above its melting point.

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