Original article

Microencapsulation of *Lactobacillus acidophilus* ATCC 43121 with prebiotic substrates using a hybridisation system

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Summary   The objective of this study was to evaluate the effects of prebiotic substrates on the growth of *Lactobacillus acidophilus* ATCC 43121 and to investigate the utilisation of these prebiotic substrates as coating materials for microencapsulation. The cell growth of *L. acidophilus* ATCC 43121 was significantly increased in the presence of fructooligosaccharide, lactulose and raffinose. The microencapsulation of *L. acidophilus* ATCC 43121 cells was carried out by dry surface reforming process (hybridisation) using the selected prebiotic substrates and the enteric coating material, Sureteric^TM *sans*. Scanning and transmission electron microscopy revealed that the double-microencapsulated bacteria exhibited smooth, rounded external surfaces, with a thick external coating composed of the prebiotic substrates and the Sureteric. The acid (artificial gastric juice) or heat tolerance (55 °C) of the double-microencapsulated preparations (prebiotic and enteric coating) was significantly higher than that of the uncoated and single-coated (enteric coating) preparation at prolonged acid (5 h) or heat exposure (3 h). On the contrary, no significant differences were found in salt tolerance. During the storage up to 20 days at 25 and 37 °C, the stability of *L. acidophilus* ATCC 43121 was significantly improved by double-microencapsulation.

Introduction

Microencapsulation techniques have been widely employed in the food, medical and cosmetic industries (Bakan, 1973; Putney, 1998). In dairy industry, microencapsulation has been applied to improve survival and delivery of bacterial cultures (Sultana *et al.*, 2000). Teixeira *et al.* (1995a) reported that microencapsulation of lactic acid bacteria (LAB) may enhance stability and viability of LAB during storage, especially under harsh conditions such as acid, alkali, heat and salt stresses in food processing. Recently, several studies have shown successful application of microencapsulated LAB using various encapsulating methods (Rao *et al.*, 1989; Sheu & Marshall, 1993; Sheu *et al.*, 1993; Teixeira *et al.*, 1995b; Koo *et al.*, 2001; Favaro-Trindade & Grosso, 2002). However, currently used wet coating techniques such as spray-drying method may impose some limitations on the viability to bacteria associated with high-temperature treatments and generation of uniform-sized

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capsules (Porubcan & Sellars, 1979; Brennan *et al.*, 1986; Park *et al.*, 2002).

A dry encapsulation technique, also referred to as the hybridisation system, has been developed to overcome these limitations. The hybridisation system consists of a high-speed rotating rotor with six blades, a stator and a powder recirculation circuit. The powder mixture (host and guest particles) placed in the vessel is subjected to high impaction in air stream generated by the blade rotating at high speed. During the process, the particles form ordered mixture by embedding or filming of the guest particles onto the surface of the host particles (Takafumi *et al.*, 1993).

When compared with other microencapsulation techniques, including spray-drying, the hybridisation system results in high yields of microcapsules and minimises heat-induced bacterial damage using a cooling system that maintains temperatures below 30 °C (Thiel *et al.*, 1986; Takafumi *et al.*, 1993).

For the microencapsulation of LAB, polysaccharides such as starch, alginate, carrageenan and chitosan have been extensively studied (Koo *et al.*, 2001), but only few studies have been reported on the

application of functional oligosaccharide as a source of coating materials. Recently, prebiotic oligosaccharides have been developed to improve survival of probiotics in the upper gastrointestinal tract (Corcoran *et al.*, 2005). All prebiotic substrates probably have different functionality as the chemical composition, bond types and degree of purity for prebiotic preparations are different. These oligosaccharides are selectively used by beneficial indigenous gut microbiota such as bifidobacteria and lactobacilli and do not promote the growth of potential pathogens, such as toxin-producing clostridia, bacteroides and pathogenic *Escherichia coli* (de Vaux *et al.*, 2002; Manning & Gibson, 2004). To assure the prolonged survival of probiotic LAB strains in the host, the bacteria should be able to colonise the intestine. Colonisation of an introduced probiotic strain in an established microbial ecosystem requires more than adherence to intestinal epithelium. The administration of probiotics with prebiotics may enhance their viability owing to the ability of the probiotic strains to use the prebiotic oligosaccharides as a carbohydrate source.

Lactobacillus acidophilus ATCC 43121, formerly known as *L. acidophilus* RP32, has excellent acid resistance and also exerts a cholesterol-lowering effect in the host (Gilliland *et al.*, 1985). The objective of this study was to determine the effects of seven prebiotic substrates on the viability of *L. acidophilus* ATCC 43121 and the efficacy of the hybridisation system for the production of prebiotic-encapsulated LAB. In addition, resistance of the prebiotic-encapsulated *L. acidophilus* ATCC 43121 towards various stresses including acid and heat were evaluated.

Materials and methods

Bacteria and media

Lactobacillus acidophilus ATCC 43121 was obtained from a frozen stock collection obtained from the Dairy Food Microbiology Laboratory at Korea University (Seoul, South Korea) and was subcultured three times in MRS broth (Difco, Sparks, MD, USA) at 37 °C for 18 h prior to use. The stock cultures were maintained at −80 °C, using 20% glycerol as a cryoprotectant. For the dry-coating process, *L. acidophilus* ATCC 43121 was cultured in a milk medium (10% skim milk, 0.3% glucose, 0.3% Bacto[TM] peptone, 0.3% Bacto[TM] proteose peptone No. 3 (Difco, Franklin Lakes, NJ, USA), 0.3% yeast extract; pH 6.5) for 24 h, during which pH controller (Jenco model 3671; Whatman Lab Sales, Hillsboro, OR, USA) was used to maintain a pH of 6.0. After 30 min of centrifugation at 4 °C, the pellet was suspended with 10% skim milk containing 10% lactose, lyophilised and stored at −80 °C until use.

Effects of prebiotic substrates on the growth of *Lactobacillus acidophilus* ATCC 43121

The commercial preparations of prebiotic substrates: sorbitol, mannitol, lactulose, xylitol (all obtained from the Sigma Chemical Co., St. Louis, MO, USA), inulin (RAFTILOSE[TM]*serif* HP; Orafti, Tienen, Belgium), fructooligosaccharide (FOS; RAFTILOSE[TM]*serif* P95; Orafti) and raffinose (Difco) were used. Overnight cultures of *L. acidophilus* ATCC 43121 were centrifuged at $3000 \times g$ for 30 min. The pellets were then washed twice in 0.85% NaCl, and 10^4 CFU mL^{-1} was inoculated into basal MRS medium without glucose and beef extract containing various concentrations (0%, 0.50%, 0.75%, 1.00% and 1.50%) of each prebiotic substrate. Positive and negative control cultures, containing glucose and no prebiotic substrates, respectively, were also prepared. The cultures were incubated under anaerobic conditions (BBL Gas-Pak system; Difco, Sparks, MD, USA) at 37 °C for 24 h. The viable cell counts were then determined by the standard plate method on MRS agar, and changes in the pH of the culture broth were assessed with a pH meter (Beckman Instruments Inc., Fullerton, CA, USA).

Two-layer dry-coating process using the hybridisation system

The two-layer dry-coating process was conducted according to the hybridisation system techniques described by Park *et al.* (2002), with slight modifications, using a hybridisation apparatus (Nara Hybridization System, Model NHS-0 & NSH-1; Nara Machinery Co. Ltd, Tokyo, Japan; Takafumi *et al.*, 1993). A poly (vinylacetate) phthalate-based aqueous enteric coating system (Sureteric[TM]*sans*; Colorcon, Dartford Kent, UK) was used as the coating material in all experiments.

The selected prebiotics (FOS, lactulose and raffinose) were applied to 100–200 μm bacteria (the 'core' particles) in the primary coating process, under 6 kg cm^{-2} of air pressure; core:coating material ratios of 9:1, 4:1 and 2:1 (w/w) and a rotor speed of 15 000 r.p.m., for 3 min. The temperature was maintained at below 30 °C throughout the process. A secondary coat, which consisted of the Sureteric enteric coating, was then applied under the same conditions, using a 9:1 core:coating material ratio. Particle size was then evaluated with a particle size analyser (CILAS 106; CILAS, Marcoussin, France) after dispersion in ethyl alcohol. Single-layer microencapsulated bacteria were also prepared, using either prebiotics only or Sureteric only, and these were also applied as described earlier. After coating process, the stability of microencapsulated cells was determined by the number of viable cells under anaerobic incubation at 37 °C for 72 h using standard plating technique. Uncoated bacteria used as controls were

prepared by same coating process without coating materials.

Microscopic observation

The morphology of the microcapsules was observed by electron microscopy. For scanning electron microscopy (SEM), the prebiotic substrates, uncoated bacteria and microencapsulated samples were affixed to 'stubs', using double-sided adhesive metallic tape, then sprayed with a layer of gold–palladium for 60 s, using an ion-sputter coater. Morphology was observed on a scanning electron microscope (S-2380N; Hitachi, Tokyo, Japan), with a 15 kV accelerating voltage. For transmission electron microscopy (TEM), the uncoated and microencapsulated samples were prefixed at 4 °C for 4 h in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M glycine–HCl buffer (pH 2.0), washed, then postfixed for 2 h at 4 °C in 1% osmium tetroxide. After washing, the samples were dehydrated in a graded series of ethanol and embedded in Poly/Bed 812 resin (Polyscience, Warrington, PA, USA). Ultrathin 60-nm sections were cut, stained with uranyl acetate and lead citrate, then examined with a Zeiss EM 912 TEM (LEO Electron Microscope Ltd, Oberkochen, Germany).

Measurement of resistance to acid, salt and heat conditions

Acid tolerance
Artificial gastric juice was prepared from 0.25 M KCl–HCl buffer (pH 1.5) supplemented with 1000 U mL^{-1} of pepsin (Sigma Chemical Co) according to a previously described method (Kobayashi *et al.*, 1974) with slight modifications. The uncoated and microencapsulated *L. acidophilus* ATCC 43121 (10^9 CFU g^{-1}) were inoculated into the artificial gastric juice and then incubated at 37 °C for 0, 150 and 300 min. Serial dilutions (in 0.1% sterile peptone water) of these suspensions were plated onto MRS agar, and the surviving bacteria were counted after 48 h of anaerobic incubation at 37 °C.

Salt tolerance
The evaluation of salt resistance was conducted according to a previously described method (Gardiner *et al.*, 2000) with slight modifications. The uncoated and microencapsulated *L. acidophilus* ATCC 43121 (10^9 CFU g^{-1}) were inoculated into glycine–HCl buffer (pH 1.5), supplemented with 15% NaCl and incubated at 37 °C for 0, 60 and 180 min. The bacterial counts were enumerated as detailed earlier.

Heat tolerance
The uncoated and microencapsulated *L. acidophilus* ATCC 43121 (10^9 CFU g^{-1}) were inoculated into glycine–HCl buffer (pH 3.0) and incubated at 55 °C for 0, 40, 60, 120, 180 and 240 min in a water bath with agitation. The coating materials were removed with 0.1 M phosphate buffer (pH 7.0), and the surviving bacteria were then enumerated, as described earlier.

Assessment of survival during storage

In order to determine the stability of the uncoated and microencapsulated *L. acidophilus* ATCC 43121 during storage, the samples were placed in polyethylene tubes and were stored at 4, 25 and 37 °C for 20 days, respectively. After the designated storage periods (0, 3, 7, 12, 16 and 20 days), samples were taken and cell survival rates were determined using standard plating techniques on MRS agar.

Statistical analysis

All experiments were carried out at least in triplicate. The effects of each treatment were analysed by ANOVA, followed by Duncan's test in SAS software package (ver. 9.1; SAS Inc., Cary, NC, USA). The level of significance was defined at $P < 0.05$.

Results and discussion

Effects of prebiotic substrates on the growth of *Lactobacillus acidophilus* ATCC 43121

Lactobacillus acidophilus preferentially degraded short- or medium-chain oligosaccharides, such as oligofructose, and used for cell growth, rather than long-chain inulin (Makras *et al.*, 2005). Therefore, we evaluate the *in vitro* growth of *L. acidophilus* ATCC 43121 in various concentrations of seven different oligosaccharide substrates in order to characterise their potential prebiotic effects when used as coating materials for microencapsulation. No differences were found in the growth of *L. acidophilus* ATCC 43121 in the presence of mannitol, inulin, xylitol, sorbitol, FOS, lactulose or raffinose until 12 h postinoculation (p.i.); however, at 24 h p.i., cell growth was significantly elevated in the cultures containing 1.5% FOS, lactulose and raffinose, when compared with the growth of the controls ($P < 0.05$; Table 1). This indicates that *L. acidophilus* ATCC 43121 is able to effectively utilise FOS, lactulose and raffinose as a source of carbohydrates to promote growth but is unable to process the other tested substrates.

The production of acid by LAB is also an important consideration in the selection of prebiotic substrates (Laura & Gibson, 2001). The pH of the culture media that contained 1.5% FOS, lactulose or raffinose decreased pH values as low as 4.0 after 24 h p.i. (data not shown). However, no such significant pH changes were observed in the cultures that contained the other tested prebiotic substrates. Therefore, supplementation of the culture medium with a suitable concentration

Table 1 Cell growth of *Lactobacillus acidophilus* ATCC 43121 incubated at 37 °C for 24 h in MRS broth containing various prebiotic substrates

Concentration* (%)	Cell growth† in prebiotic substrate (No. of viable cells ± SD)‡						
	Inulin	Xylitol	Sorbitol	Mannitol	FOS	Lactulose	Raffinose
0	2.249 ± 0.030[a]	2.249 ± 0.030[a]	2.249 ± 0.030[a]	2.249 ± 0.030[a]	2.249 ± 0.030[a]	2.249 ± 0.030[a]	2.249 ± 0.031[a]
0.25	2.202 ± 0.108[cd]	1.189 ± 0.012[e]	2.119 ± 0.141[d]	2.300 ± 0.066[bc]	2.368 ± 0.039[ab]	2.451 ± 0.036[ab]	2.498 ± 0.099[a]
0.50	2.379 ± 0.034[a]	1.309 ± 0.558[b]	2.478 ± 0.044[a]	2.106 ± 0.046[a]	2.515 ± 0.060[a]	2.216 ± 0.053[a]	2.313 ± 0.043[a]
0.75	2.297 ± 0.108[d]	1.318 ± 0.039[e]	2.253 ± 0.081[d]	2.281 ± 0.016[d]	3.118 ± 0.115[a]	2.816 ± 0.168[b]	2.466 ± 0.019[c]
1.00	2.190 ± 0.000[d]	2.568 ± 0.006[c]	2.306 ± 0.015[d]	2.269 ± 0.084[d]	3.058 ± 0.092[b]	3.228 ± 0.163[a]	2.318 ± 0.051[d]
1.50	2.299 ± 0.047[d]	1.640 ± 0.117[e]	2.388 ± 0.011[d]	2.316 ± 0.031[d]	3.702 ± 0.090[a]	3.254 ± 0.076[b]	3.098 ± 0.024[c]

Superscripts letters within the same row indicate significant differences ($P < 0.05$).
*Concentration of prebiotic substrates.
†Cell growth = log (incubated no. of viable cells) − log (initial no. of viable cells).
‡Data are expressed as the mean ± SD of three experiments.

(1.5%) of FOS, lactulose or raffinose resulted in the stimulation of both growth and acid production of *L. acidophilus* ATCC 43121. These three prebiotic substrates were consequently selected for further study, as candidates for the two-layer microencapsulation of *L. acidophilus* ATCC 43121.

Effects of two-layer microencapsulation on the survival of *Lactobacillus acidophilus*

Based on our preliminary experiments, the optimal conditions for the microencapsulation of 100–200 μm *L. acidophilus* ATCC 43121 with Sureteric using a hybridisation system were 6 kg cm^{-2} of air pressure, a 9:1 core:coating material ratio (w/w) and a rotor speed of 15 000 r.p.m., for a duration of 3 min. We selected Sureteric as a wall material, as it showed better attachment to the surface of *L. acidophilus* ATCC 43121 without causing fragmentation of particles than other tested wall materials including Eudragit S100, Eudragit L100–55 (Rohm Pharma, Weiterstadt, Germany), Compritol 888 (Gattefosse, Saint-Priest, France) and carboxylmethylethylcellulose (Park *et al.*, 2002).

We initially attempted to assess the effects of a single coating of each prebiotic (single microencapsulation) on the viability of encapsulated *L. acidophilus* ATCC 43121, using various LAB:prebiotic substrate ratios. Contrary to our expectations, however, we obtained the highest viable cell counts with a 9:1 bacteria:prebiotic ratio; lower ratios resulted in decreased viability (Table 2). We speculated that excess prebiotic substrate may have compromised the viability of the microencapsulated *L. acidophilus* ATCC 43121 cells by causing an increase in friction between the bacteria and the coating materials during processing. These results were consistent with the observations of Espina & Packard (1979) and Lian *et al.* (2002), both of whom noted reductions in the viability of microencapsulated cells when excess coating materials were used during processing.

Table 2 Viability of microencapsulated *Lactobacillus acidophilus* ATCC 43121 prepared at various probiotic to prebiotic ratios

Treatment		
Prebiotic substrate	Formulation ratio (probiotic:prebiotic, w/w)	No. of viable cells (CFU g $^{-1}$)
FOS	2:1	5.52×10^9
	4:1	7.21×10^9
	9:1	3.60×10^{10}
Lactulose	2:1	3.60×10^9
	4:1	9.17×10^9
	9:1	3.20×10^{10}
Raffinose	2:1	6.32×10^9
	4:1	6.83×10^9
	9:1	3.80×10^{10}
Single microencapsulation with Sureteric		6.30×10^{10}
Control		1.47×10^{11}

The effects of two-layer ('double') microencapsulation were examined by applying an initial layer of prebiotic substrate and a second layer of enteric coating, Sureteric according to the conditions and methods described by Park *et al.* (2002). Double-microencapsulation induced a slight reduction in cell viability, when compared with that observed for the uncoated controls and the cells microencapsulated with only a single layer of Sureteric (Table 2). However, we detected no significant differences in the viability of the double-microencapsulated bacteria among the three prebiotic substrates. To our knowledge, this study constitutes the first report of the use of prebiotic substrates as microencapsulation coating materials.

Morphology of single- and double-microencapsulated *Lactobacillus acidophilus*

It was reported that the surface characteristics of bacteria were partially changed by microencapsulation

process (Gardiner *et al.*, 2000; Lian *et al.*, 2000). The morphology of *L. acidophilus* ATCC 43121 obtained after coating process with prebiotic substrates and Sureteric by hybridisation system was observed using SEM. The microcapsules of *L. acidophilus* ATCC 43121, which had been microencapsulated with a single layer of FOS exhibited rounded external surfaces with some surface concavities (Fig. 1e), whereas the microencapsules coated with a single lactulose layer displayed irregular surfaces that formed convex lattices (Fig. 1f). The microencapsules that had been double-coated with prebiotics and Sureteric appeared uniformly smooth and rounded (Fig. 1g, h). These results are similar to those obtained by Lian *et al.* (2002) and Alvarez-Olmos &
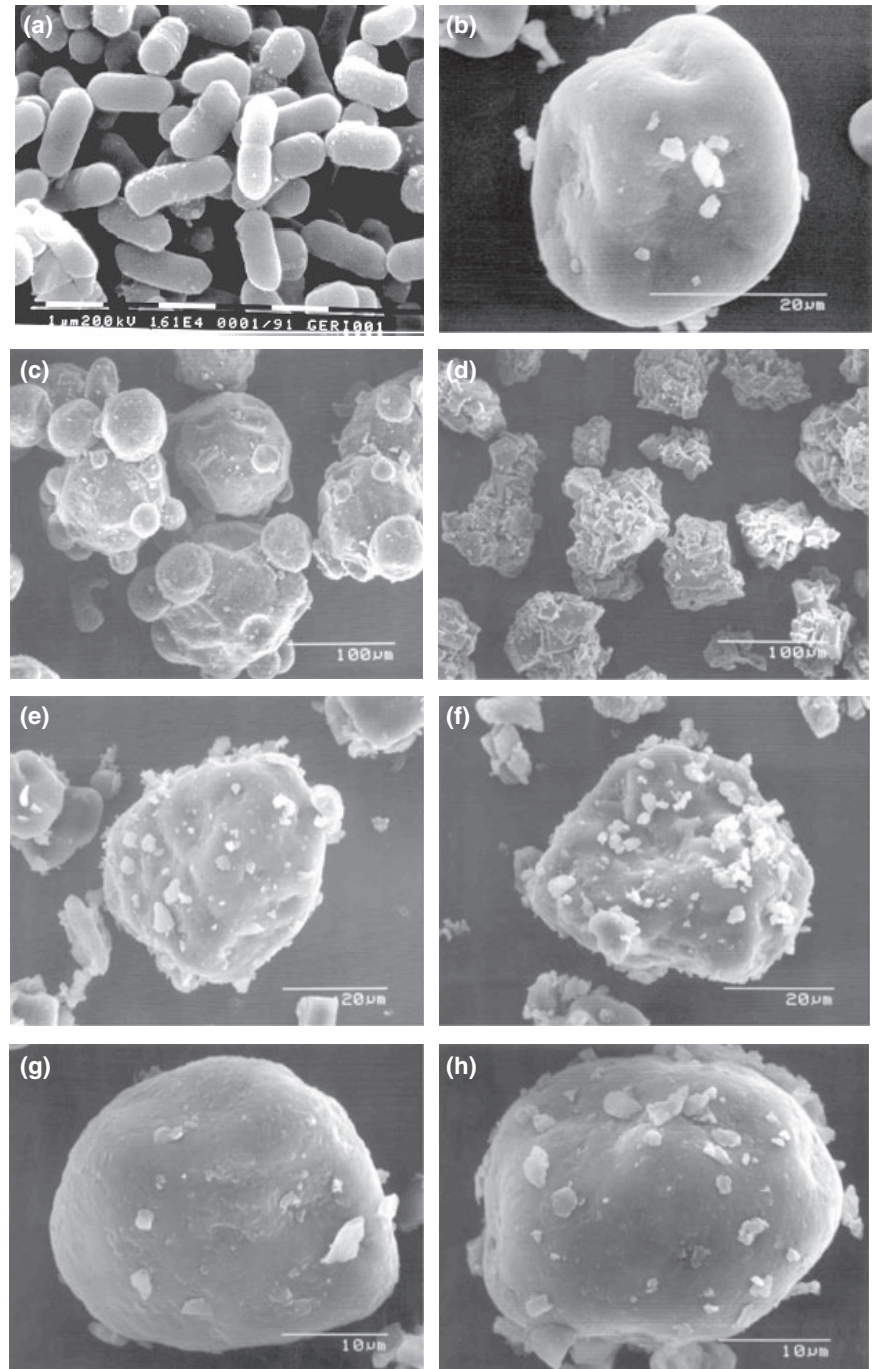
Figure 1 Scanning electron microscopy (SEM) of single- and double-microencapsulated *Lactobacillus acidophilus* ATCC 43121. SEM images of uncoated *L. acidophilus* ATCC 43121 (a) and bacteria that were single-microencapsulated with Sureteric (b); the prebiotic substrates fructooligosaccharide (FOS) (c) and lactulose (d) and bacteria microencapsulated with FOS (e), lactulose (f), FOS and Sureteric (g) and lactulose and Sureteric (h).

Oberhelman (2001)) who reported that their microencapsulated bacteria were variable in size and exhibited smooth, rounded surfaces.

We also visualised our microencapsulated bacteria with TEM. In comparison to the control and single-microencapsulated cells (Fig. 2a, b), double-coated cells had markedly thick and uniform outer surfaces, which have been observed from the SEM pictures (Fig. 2c). These results strongly suggested that the cells were successfully covered with FOS by double-microencapsulation using a hybridisation system. Ongoing study is currently underway to characterise the surface modifications of double-microencapsulated cells using atomic force microscopy. To our knowledge, this study is the first report to have used TEM to determine the morphology of microencapsulated bacteria coated with prebiotic substrates.

Survival of uncoated and microencapsulated bacteria under acid, salt and heat conditions

The resistance of the microencapsulated bacteria to various durations of acid, salt and heat exposure was evaluated. In acid tolerance, there were no significant differences between the uncoated and the microencapsulated preparations after 2.5 h of exposure to acid conditions. Interestingly, *L. acidophilus* ATCC 43121, when double-microencapsulated with selected prebiotic substrates, exhibited a significantly improved survival after 5 h of acid exposure, when compared with the uncoated control and single microencapsulation preparations (Fig. 3; $P < 0.05$). It was previously reported that the microencapsulation of *Bifidobacterium lactis* and *L. acidophilus* with spray-dried cellulose acetate phthalate showed better protection for both microorganisms against exposure to acidic conditions similar to those inherent in the human stomach (Favaro-Trindade & Grosso, 2002). Similarly, our findings suggested that, when compared with uncoated cells, the single- and double-microencapsulated *L. acidophilus* ATCC 43121 cells were effectively protected from exposure to artificial gastric juice, which was designed to closely approximate conditions within the human stomach.

In a study conducted by Gardiner *et al.* (2000), the salt sensitivity of *Lactobacillus paracasei* NFBC 338 was increased from 4% to 70% after spray-dry microencapsulation. In the present study, there were no significant differences on tolerance to high-salt conditions (glycine–HCl buffer containing 15% NaCl) (Fig. 4).

In terms of application of probiotic as a feed supplement, probiotic bacteria may have the potential to enhance intestinal health and improve immune function in animals (Gibson *et al.*, 1997). Thus, heat tolerance can be a critical factor for feed processing such as pelleting. In terms of heat tolerance, there were no significant differences in viability between the uncoated and microencap-
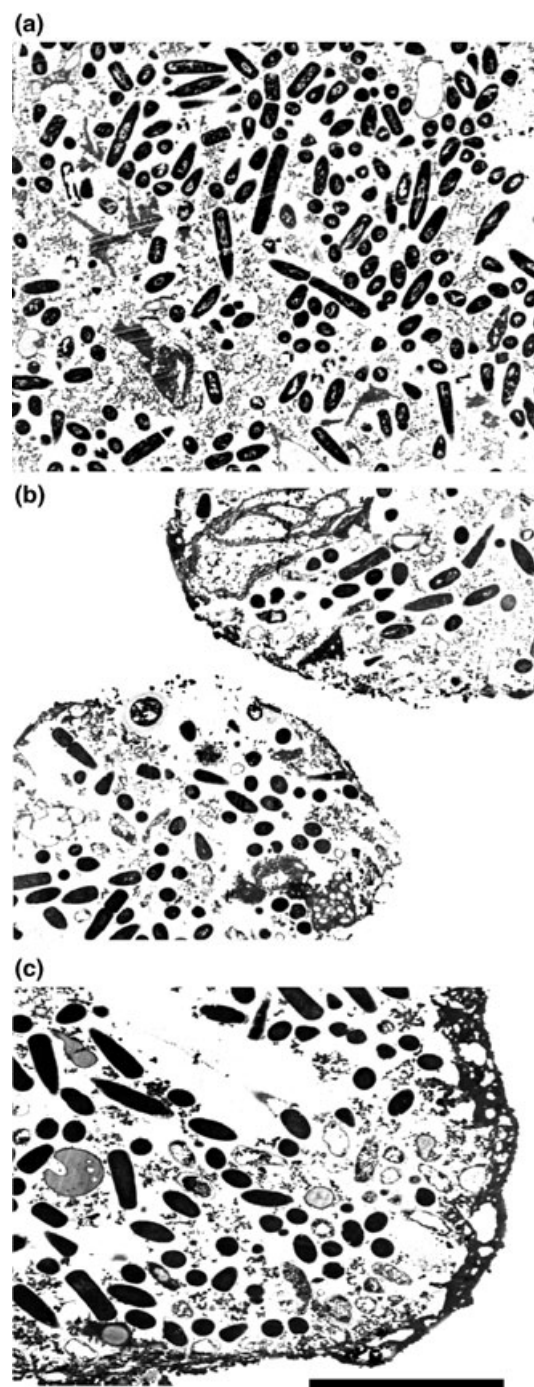

Figure 2 Transmission electron microscopy of microencapsulated *Lactobacillus acidophilus* ATCC 43121. *Lactobacillus acidophilus* ATCC 43121 without coating (a), single-microencapsulated with Sureteric (b) and double-microencapsulated with FOS and Sureteric (c). Scale bar: 5 μm.

sulated *L. acidophilus* ATCC 43121, after 1 h of exposure to a temperature of 55 °C. However, single- and double-microencapsulation processes resulted in
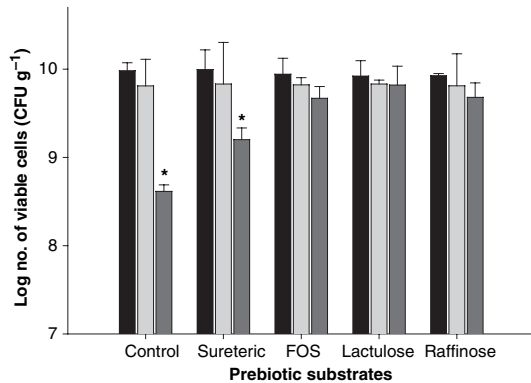
Figure 3 Viability of microencapsulated *Lactobacillus acidophilus* ATCC 43121 after exposure to acid conditions for various durations (black bar, 0 h; grey bar, 2.5 h; dark grey bar, 5 h). Data are expressed as the mean ± SD of three experiments. *Significantly different values between treatments ($P < 0.05$).
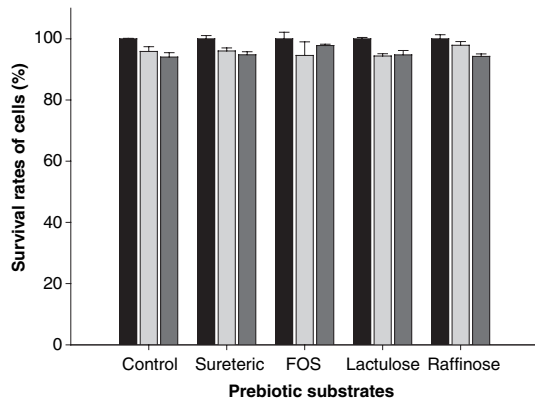

Figure 4 Viability of microencapsulated *Lactobacillus acidophilus* ATCC 43121 after exposure to various durations of high-salt conditions (black bar, 0 h; grey bar, 1 h; dark grey bar, 3 h). Data are expressed as the mean ± SD of three experiments. No significant differences observed between experiments.

Figure 5 Viability of microencapsulated *Lactobacillus acidophilus* ATCC 43121 after exposure to 55 °C heat conditions (-●-: uncoated cells, -○-: single microencapsulated cells, -▼-: double-microencapsulated cells with FOS, -□-: double-microencapsulated cells with lactulose and -■-: double-microencapsulated cells with raffinose). Data are expressed as the mean ± SD of three experiments. *Significantly different values between treatments ($P < 0.05$).

to obtain optimal activity of prebiotic substrates and evaluating the beneficial effects of these microencapsules using animal models.

Survival of uncoated and microencapsulated bacteria during storage

Both uncoated and microencapsulated *L. acidophilus* ATCC 43121 cells were stored at different temperatures (4, 25 and 37 °C), and their viability over a 20-day period was determined (Fig. 6). The survival rates of the uncoated cells declined by as much as four orders of magnitude after 16 days of storage, at all storage temperatures (data not shown). After 20 days of storage, the survival rates were the highest in the samples that had been stored at 4 °C. After storage at temperatures 25 and 37 °C, the bacteria that had been double-microencapsulated with lactulose and raffinose were found to exhibit higher survival rates than those that had been single-microencapsulated with Sureteric and those that had been double-microencapsulated with FOS (Fig. 6). The survival rates of all bacterial preparations in this study declined more rapidly during storage at 37 °C than during storage at 25 °C. Among the bacterial preparations, the highest survival rates at 25 and 37 °C were observed for the bacteria double-microencapsulated with FOS and lactulose, respectively. Interestingly, after 36 days of storage at 25 °C, the bacteria that were double-microencapsulated with FOS maintained a cell count of 9.023×10^9 CFU g^{-1} (data not shown). Previous studies have also shown that temperature is critical for microbial survival during storage, and higher survival rates have been maintained

increased heat tolerance when exposures of heat were increased to 180 and 240 min (Fig. 5). These results demonstrate that heat tolerance might be improved in LAB by encapsulation with prebiotic substrates.

Unfortunately, there was no significant difference on tested stress tolerance between single- and double-microencapsulation preparations except acid tolerance. However, the use of prebiotic substrates as coating materials may promote more beneficial effects, including the reduction of diarrhoea and the inactivation of pathogens in the gastrointestinal tracts of both humans and animals (Hidaka *et al.*, 1991; Hopkins & Macfarlane, 2003) when compared with single microencapsulation using only Sureteric. At present, we are investigating the various microencapsulation conditions
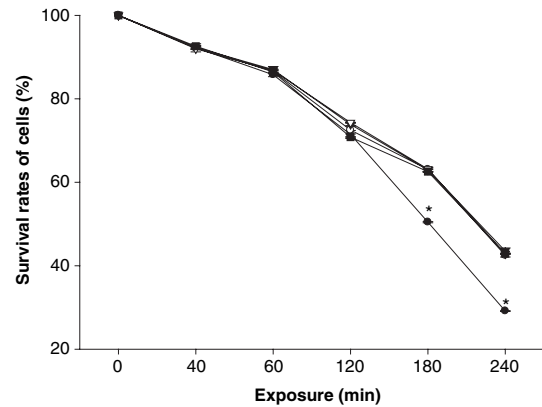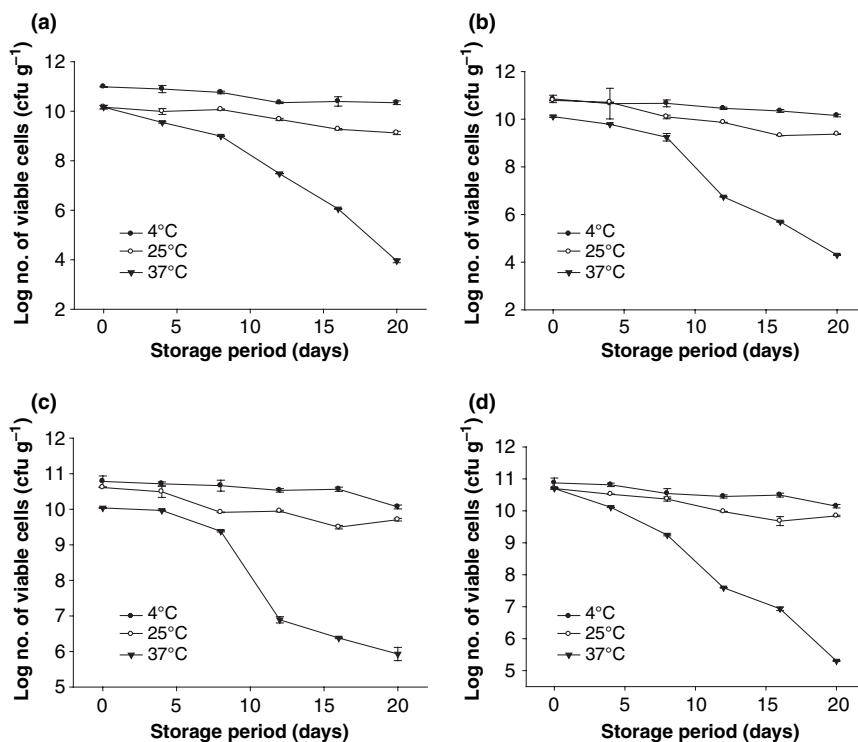
Figure 6 Effects of various prebiotic substrates on the survival of double-microencapsulated *Lactobacillus acidophilus* ATCC 43121 during storage at various temperatures. Survival curves for *Lactobacillus acidophilus* ATCC 43121 single-microencapsulated with Sureteric (a) and double-microencapsulated with Sureteric and FOS (b), lactulose (c) or raffinose (d). Data are expressed as the mean ± SD of three experiments.

at lower storage temperatures (Teixeira *et al.*, 1995a), which restricts the possible applications for many probiotic products (Gardiner *et al.*, 2000). Therefore, the improved survival rates observed in the bacteria double-microencapsulated with prebiotic substrates suggest that these preparations may be used to improve cell viability during ambient storage conditions.

Conclusions

In the present study, the growth of *L. acidophilus* ATCC 43121 was significantly increased in the presence of three selected prebiotic substrates, FOS, lactulose and raffinose. The acid and heat tolerance of the double-microencapsulated preparations by selected prebiotic and Sureteric were greater than that of the uncoated ones. During storage periods, the stability of *L. acidophilus* ATCC 43121 was improved as a result of double-microencapsulation. These results indicate that double-microencapsulation of *L. acidophilus* ATCC 43121 by hybridisation is useful to effectively provide beneficial effects of probiotic bacteria for the host.

Acknowledgments

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