Effect of Co-encapsulation of Probiotics with Prebiotics on Increasing the Viability of Encapsulated Bacteria under In Vitro Acidic and Bile Salt Conditions and in Yogurt

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ABSTRACT: Three different complementary prebiotics (selected by in vitro fermentation) were separately co-encapsulated with *Lactobacillus acidophilus* CSCC 2400 or CSCC 2409 and tested for their efficacy in improving the viability of bacteria under in vitro acidic conditions. Addition of Hi-maize™ starch to capsules containing *Lactobacillus* spp. provided maximum protection to the encapsulated bacteria after 3 h of incubation at pH 2.0 compared with other 2 prebiotics, Raftiline® and Raftilose®. Viable counts of *Lactobacillus* spp. increased significantly ($P < 0.05$) with Hi-maize concentration of up to 1.0% (w/v). Further increase in Hi-maize concentration did not protect the encapsulated bacteria effectively. Effects of 3 different polymers (chitosan, poly-L-lysine, and alginate) were also tested for their efficacy in protecting the encapsulated bacteria at pH 2.0. Addition of Hi-maize (1.0% w/v) to capsules containing *Lactobacillus* spp. and further coating with chitosan significantly increased ($P < 0.05$) the survival of encapsulated bacteria under in vitro acidic and bile salt conditions and also in stored yogurt compared with alginate encapsulated cells.

Keywords: co-encapsulation, *Lactobacillus* spp, prebiotics, viability, yogurt

Introduction

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer health benefits to host" (Reid and others 2003). Bacteria belonging to genera *Bifidobacterium* and *Lactobacillus* are often used as probiotic supplements. Over the past 20 y, there has been an increased interest in the role of probiotic bacteria in human health. Health advantages associated with the probiotic intake include alleviation of symptoms of lactose malabsorption, increase in natural resistance to infectious diseases of the intestinal tract, suppression of cancer, reduction in serum cholesterol concentrations, improved digestion, and stimulation of gastrointestinal immunity (Kailasapathy and Chin 2000). It is generally accepted that successful delivery and colonization of viable probiotic cells in the intestine are essential for probiotics to be efficacious (Conway 1996).

As a guide, the Intl. Dairy Federation has recommended that the bacteria be viable and abundant in the product and be present at a population of at least 10^7 colony-forming units (CFU)/g until the date of consumption (Ouwehand and Salminen 1998). However, studies indicate that the bacteria may not survive in sufficient numbers when incorporated into dairy products and during their passage through the gastrointestinal tract (Dave and Shah 1996; Kailasapathy and Rybka 1997; Hamilton-Miller and others 1999). Several factors influence the survival and colonization of these bacteria, including resistance to low pH, bile acids, and digestive enzymes (Conway 1996). Although a number of approaches have been adopted to improve survival of probiotic bacteria, limited success has been achieved (O'Riordan and others 2001). Providing probiotic living cells with a physical barrier against adverse environmental conditions is an approach currently receiving considerable interest (Krasaekoopt 2003).

Among the available techniques for immobilizing living cells, entrapment in calcium alginate beads has been frequently used for the immobilization of lactic acid bacteria (Sheu and Marshal 1993). Alginate has the benefits of being nontoxic to the cells being immobilized, and it is an accepted food additive (Prevost and Divies 1992). Although calcium-induced alginate encapsulation has been widely used for probiotic bacteria, there is no uniformity in the literature as to the protective nature of capsule against adverse gastrointestinal conditions and in products' shelf life (Chandramouli and others 2004). Lee and Heo (2000) reported that survival of calcium alginate–entrapped *Bifidobacteria* was dependent on several factors. Earlier we investigated the effect of different encapsulation parameters (capsule size, alginate concentration, cell load, and hardening time in calcium chloride solution) and reported the effect of these parameters in increasing the survival of probiotic bacteria in simulated gastric conditions (Chandramouli and others 2004). Gibson and Roberfroid (1995) reported that prebiotics (nondigestible carbohydrates) selectively stimulate probiotic strains. Some authors (Fooks and others 1999; Roberfroid 2000) suggest that prebiotics may improve the survival of bacteria crossing the upper part of the gastrointestinal tract, thereby enhancing their effects in the large bowel. To further increase the viability of encapsulated bacteria, the effect of adding complementary prebiotics (selected by in vitro fermentation) was assessed. The aim of this study was to select a complementary prebiotic for the selected probiotic strain and to investigate its effect in protecting the encapsulated bacteria under

MS 20040363 Submitted 6/2/04, Revised 7/8/04, Accepted 9/20/04. *The authors are with the Centre for Advanced Food Research, Univ. of Western Sydney, Locked Bag 1797, NSW 1797, Australia. Direct inquiries to author Kailasapathy (E-mail: k.kailasapathy@uws.edu.au).*

in vitro acidic and bile salt conditions. In addition, the effect of different polymers (chitosan, poly-L-lysine, and alginate) as a coating material for encapsulation on the viability of encapsulated bacteria was also examined. The term *co-encapsulation* in this article refers to encapsulation of both probiotic bacteria and prebiotics together.

Materials and Methods

Bacterial strains, growth conditions, and preparation of cell suspensions

Pure cultures of probiotic bacteria *Lactobacillus acidophilus* CSCC 2400 and CSCC 2409 were procured from the Australian Starter Culture Research Centre's Collection (Werribee, Australia). The bacteria were cultured at 37 °C for 24 h under anaerobic conditions in de man Rogosa and Sharpe (MRS) broth (Oxoid, Basingstoke, U.K.) reconstituted with 1.0% salicin (Sigma Chemical Co., St. Louis, Mo., U.S.A.). Anaerobic conditions were achieved using an anaerobic glove box (95% N_2 and 5% H_2, Coy Laboratory Products, Grass Lake, Mich., U.S.A.). Cells were harvested by centrifugation at 3000 × *g* for 10 min at 4 °C and washed twice with phosphate buffer (pH 7.0). The cell suspensions were subsequently used either directly (free cells) in assays or subjected to microencapsulation as described subsequently.

Selection of complementary prebiotics by in vitro fermentation

A broth medium free of fermentable carbon source was used to investigate the ability of the *L. acidophilus* strains to grow on selected prebiotic oligosaccharides. The growth medium (pH 6.8) contained 1.0% beef extract, 0.3% yeast extract, 1.0% pancreatic digest of casein, and 0.5% NaCl. The tested carbohydrates were added aseptically to the medium at 1.0% (w/v). The prebiotics investigated were inulin (Raftiline® ST; Orafti Pty., Tienen, Belgium); oligofructose (Raftilose® P95; Orafti Pty.), and high amylose corn starch (Himaize™; Starch Australasia Ltd., Lane Cove, Australia). Additionally, growth of *Lactobacillus* was examined on glucose, fructose, lactose, and also in a carbon-free medium, which acted as the control. Criteria for the evaluation of the use of prebiotics were the growth of *Lactobacillus* and acidification rate compared against the results obtained in the control medium. Anaerobic fermentation was carried out in a 50-mL shake flask at 37 °C for 48 h in a shaker (100 rpm). Duplicate flasks of each treatment were prepared. The flasks were then inoculated separately with approximately 1.0×10^6 colony forming units (CFU)/mL of active *L. acidophilus* cultures and incubated at 37 °C for 48 h. An aliquot from each treatment was taken at 0 h and 48 h, diluted (1:10, v/v) with 0.1% (w/v) sterile buffered peptone water (Amyl Media Pty. Ltd., Dandenong, Australia), and mixed uniformly with a vortex mixer. Serial dilutions were prepared and viable numbers enumerated using spread plating on MRS-salicin agar, and colonies were counted after 48 h of anaerobic incubation at 37 °C. Rate of acidification by these strains was determined as changes in pH using a pH meter (Model PH 215; Denver Instrument Co., Arvada, Colo., U.S.A.).

Co-encapsulation of complementary prebiotics with probiotic bacteria

The capsules were prepared aseptically using an Inotech Encapsulator® (Inotech AG, Dottikon, Switzerland) with a 300-μm nozzle size, as described by Chandramouli and others (2004), modified by the addition of prebiotics. The standard conditions used for encapsulation were 1.8% (w/v) alginate (Sigma Chemical Co., St. Louis, Mo., U.S.A.), bacterial culture (approximately 10^{10} CFU/mL), 1.0% (w/v) prebiotic and 30 min hardening in 0.1 *M* calcium chloride solution.

Coating with different polymers

Three different coating materials, poly-L-lysine, chitosan, and alginate (Sigma Chemical Co.) were tested for their efficacy in protecting the viability of encapsulated bacteria at low pH (pH 2.0). Alginate capsules were coated by immersion in the selected polymer as described by Champagne and others (1992) and Zhou and others (1998).

Examination of alginate capsules

The dimensions of the alginate capsule were determined using an objective micrometer on an optical microscope at a 400× magnification. The dispersion of Hi-maize starch in the alginate matrix was examined by staining with iodine and observing under light microscopy (Model BX 60; Olympus, Tokyo, Japan).

Survival of free and encapsulated bacteria under in vitro acidic and bile salt conditions

The encapsulated and free bacteria were added to Non-fat milk, glucose, yeast extract and cysteine medium (12% non-fat skim milk, 2.0% glucose, 1.0% yeast extract, and 0.05% cysteine) (NGYC) that had been adjusted to pH 2.0, pH 3.0, or 6.5 (control) with 5 M HCl or 1 M NaOH in 10-mL aliquots (Lankaputhra and Shah 1995). The samples were incubated in 37 °C for 3 h. An aliquot from each treatment was taken hourly for determination of the viable cell counts, diluted (1:10, v/v) with 0.1% (w/v) sterile buffered peptone water (Amyl Media Pty. Ltd., Dandenong, Australia), and mixed uniformly with a vortex mixer. Serial dilutions were prepared and viable numbers enumerated using spread plating on MRS-salicin agar and colonies counted after 48 h incubation anaerobically at 37 °C. The resistance to bile salts was determined by inoculating free, encapsulated, and co-encapsulated cells in milk-yeast extract medium pH 6.9 (Truelstrup and others 2002) containing 0% (control), 0.5%, and 1.0% bile salts (Oxgall; Sigma Chemical Co.). Duplicate samples were withdrawn after incubation at 37 °C for 0, 3, and 6 h and cell counts of free, encapsulated, and co-encapsulated bacteria were enumerated on MRS-salicin agar as described previously. To determine the viable counts of the encapsulated bacteria, test sample contents were centrifuged (3000 × *g* for 10 min at 4 °C) and the alginate capsules dissolved by re-suspending in 9.0 mL of phosphate buffer (0.1 *M*, pH 7.0) followed by gentle shaking at room temperature for 15 min. The number of released cells was determined by spread plate count using MRS-salicin agar as described previously.

Co-encapsulated synbiotic yogurt

A set-type yogurt was prepared for this experiment. Homogenized whole milk (5 L) containing 4.0% fat, 9.0% solid-not-fat was heated to 45 °C, and skim milk powder (SMP) 5.0%, was added with high-speed stirring, to make 18% total solid in yogurt. This was then heated to 85 °C for 20 min and allowed to cool. Once the standardized milk had cooled to 45 °C, a commercial yogurt starter culture (YoFlex, Chr. Hansen, Bayswater, Australia) was inoculated (0.1% w/v) into it. The probiotic cultures were added as free, encapsulated, or co-encapsulated cultures. The acidification profile was recorded hourly until a pH of 4.6 was reached. Fermentation was stopped by quickly cooling the yogurt. The filled yogurt cups (200 mL) were stored at 5 °C, and the CFU/g of yogurt was determined at 2-wk intervals for up to 6 wk.

Statistical analysis

Each experiment was independently replicated 3 times in a completely randomized design. All the analysis and enumerations were done in duplicate. Statistical analysis was conducted using Student

t test using the software package SPSS (Version 11.5. SPSS Inc., Chicago, Ill., U.S.A.) and $P < 0.05$ was considered statistically significant.

Results and Discussion

Selection of complementary prebiotics

Growth of the test strains of *Lactobacilli* in a shake flask containing media with different prebiotics was variable (Figure 1). Acidification of *L. acidophilus* strains (CSCC 2400 and 2409) with prebiotics and with control medium after 48 h fermentation are given in Table 1. *L. acidophilus* strains were able to grow in the presence of all the 3 prebiotics tested indicating that these strains used the prebiotics as a carbon source to sustain their growth. There was no significant difference among the prebiotics tested on bacterial growth. There was a large mortality rate in the control treatment with no carbon source with cell counts decreasing from10^6 to 10^1 CFU/mL during incubation, confirming the base medium was carbon limiting to the growth of this strain. The bacterium grew very poorly on the monosaccharide fructose in comparison with its growth on oligosaccharides composed predominantly of fructose moieties. This phenomenon was earlier observed for a number of *Bifidobacterium* strains (Gibson and Wang 1994; Crittenden and others 2001) and suggests that these organisms have a specific substrate transport mechanism that is most efficient at transporting indigestible oligosaccharides than simple sugars. Results from this study show that all the 3 different prebiotics (Raftiline, Raftilose, and Hi-maize) can be potentially used as a complementary prebiotics for the *L. acidophilus* CSCC 2400 and CSCC 2409.

Table 1—Acidification of *Lactobacillus acidophilus* in minimal nutrition media (after 48 h fermentation) containing different prebiotics[a]

	pH	
Prebiotics	*L. acidophilus* CSCC 2400	*L. acidophilus* CSCC 2409
Hi-maize™ starch	4.66 ± 0.11	4.71 ± 0.05
Raftiline®	4.62 ± 0.08	4.66 ± 0.14
Raftilose®	4.52 ± 0.12	4.48 ± 0.06
Glucose	4.21 ± 0.10	4.24 ± 0.15
Fructose	4.65 ± 0.09	4.60 ± 0.11
Lactose	4.51 ± 0.2	4.55 ± 0.04

[a]Measurements are mean ± SD of 3 replicates for all treatments.

Co-encapsulation and survival under in vitro acid conditions

As the *Lactobacillus* strains were able to grow in all the test prebiotics, individual prebiotics were further examined as a co-encapsulant for its efficacy in protecting the probiotic bacteria under in vitro acidic conditions (Figure 2). The co-encapsulated bacteria survived very well after exposure to in vitro acidic conditions (pH 2.0) for 3 h compared with the free and encapsulated bacteria. There was a significant increase ($P < 0.05$) in the viability of cells co-encapsulated with Hi-maize compared with the one encapsulated with Raftilose or Raftiline. This could be because there is Hi-maize inside the capsular matrix blocking the pores of the capsule, thereby preventing the diffusion of acidic contents in to the capsule. The protective effect of Hi-maize to probiotic bacteria against gastric conditions has been earlier reported by Crittenden and others (2001). Hi-maize was used at different concentrations in the co-encapsulation procedure to test its efficacy in increasing the viability of encapsulated bacteria under in vitro acidic conditions (Figure 3). Addition of this prebiotic at 1.0% (w/v) was effective in protecting the co-encapsulated bacteria at pH 2.0 compared with 0.5% (w/v). Concentration of 1.5% (w/v) of Hi-maize did not significantly improve the survival of encapsulated bacteria at pH 2.0 after 3 h of incubation compared with 1.0% (w/v). This could be due to disruption of the alginate gel matrix with excess Hi-maize, which in turn resulted in decreasing the efficacy of capsule to protect the bacteria in acidic conditions. The addition of Hi-maize at the concentration of 2.0% (w/v) and greater made uniform spherical capsule formation difficult because of increased viscosity.

Effect of different coating material

There was an almost 0.6-log increase in the viable cell counts of co-encapsulated capsules coated with chitosan after 3 h anaerobic incubation under in vitro acidic conditions compared with the one coated with alginate or poly-L-lysine (Figure 4). Yu and others (2001)

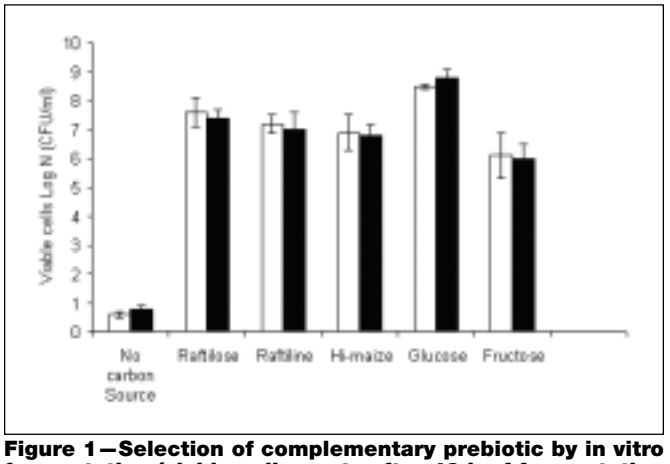

Figure 1—Selection of complementary prebiotic by in vitro fermentation (viable cell counts after 48 h of fermentation in anaerobic condition at 37 °C). □ = *Lactobacillus acidophilus* CSCC 2400; ■ = *L. acidophilus* CSCC 2409. The error bars represent mean ± standard deviation of 3 replicates for all treatments.

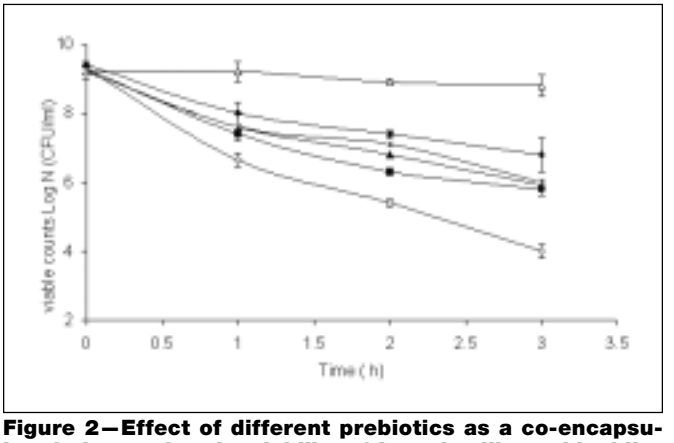

Figure 2—Effect of different prebiotics as a co-encapsulant in increasing the viability of *Lactobacillus acidophilus* CSCC 2400 under in vitro acidic conditions (pH 2 for 3 h at 37 °C). The error bars represent mean ± standard deviation of 3 replicates for all treatments. ◇ = free cells; ■ = enc. cells; x = co-enc. cells with Raftiline®; ▲ = co-enc. cells with Raftilose®; ◆ = co-enc. cells with Hi-maize™; △ = free cells pH 6.5 (control).

and Koo and others (2001) reported that *Bifidobacteria* and *Lactobacillus casei* entrapped in alginate beads containing chitosan had higher viability than in alginate beads without chitosan. They also suggested that it may be because of the favorable internal and surface gel structure formed as a result of strong ionic binding of chitosan and alginate interior, even at low pH values.

The co-encapsulation procedure used in this study resulted in 450-μm capsules. Capsule size increased to nearly 500 μm with chitosan coating. The shape of the alginate bead was uniformly spherical. The bacteria were distributed randomly in the alginate matrix, and Hi-maize appeared as granules inside the capsule at 400× magnification under light microscopy (Figure 5).

Survival of *Lactobacillus* under optimized co-encapsulation conditions

The survivals of free, encapsulated, and co-encapsulated bacteria (optimized conditions of Hi-maize concentration and chitosan coating) under in vitro acid and bile conditions are given in Figure 6 and 7, respectively. There was only a 2.2-log decrease in viable cells of co-encapsulated *L. acidophilus* CSCC 2400 after 3 h of incubation at low pH (pH 2.0) compared with a 3.3-log and 5-log decrease in the encapsulated and nonencapsulated free cells, respectively (Figure 6a). At pH 2.0, there was a 1.7-log and 2.3-log decrease in co-encapsulated and encapsulated cell number of *L. acidophilus* CSCC 2409, respectively, after 3 h incubation, compared with a 4.2-log decrease in the free cells under similar conditions (Figure 6b).

Survival of *L. acidophilus* (CSCC 2400 and 2409) was monitored up to 6 h after exposing them to milk-yeast medium containing 0.5% and 1% bile extract (Figure 7). There was a 1.0-log and 0.8-log decrease in viable cells of free nonencapsulated *L. acidophilus* CSCC 2400 and CSCC 2409, respectively, at 1.0% bile salt concentration after 6 h of incubation at 37 °C. There was only 0.5-log and 0.3-log decrease of viable cells of encapsulated *L. acidophilus* CSCC 2400 and *L. acidophilus* CSCC 2409, respectively, under similar conditions. There was no decrease ($P > 0.05$) in viability of co-encapsulated bacteria (coated with chitosan) under any of these bile salt conditions.

The effect of encapsulation on the survival of bacteria under adverse conditions has been reported with variable results. Sheu and Marshall (1993), Lee and Heo (2000), and Chandramouli and others (2004) reported the advantage of encapsulating lactic acid bacteria over that of free cells under in vitro gastric conditions, whereas Rao and others (1989), Sultana and others (2000), Koo and others (2001), and Trulestrup and others (2002) indicated that encapsulation of bacteria does not effectively protect them from strong acidic conditions. Sultana and others (2000) reported that survival of probiotics in alginate-starch microspheres in the size range of 1.0 mm did not improve after exposure to acidic and bile salt solutions. In contrary, results from this study show that co-encapsulated probiotic bacteria with Hi-maize survived well at pH 2.0 compared with encapsulated bacteria. This result is in agreement

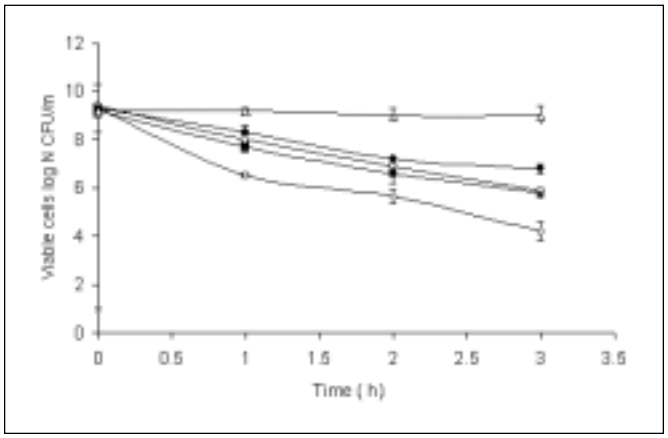

Figure 3—Effect of different prebiotic concentration (Hi-maize™) in increasing the viability of *Lactobacillus acidophilus* CSCC 2400 under in vitro acidic conditions (pH 2 for 3 h at 37 °C). The error bars represent mean ± SD of 3 replicates for all treatments. ◇ = free cells; ○ = co-enc. cells (0.5% Hi-maize); ■ = co-enc. cells (1.5% Hi-maize); ◆ = co-enc. cells (1.0% Hi-maize); △ = free cells pH 6.5 (control).

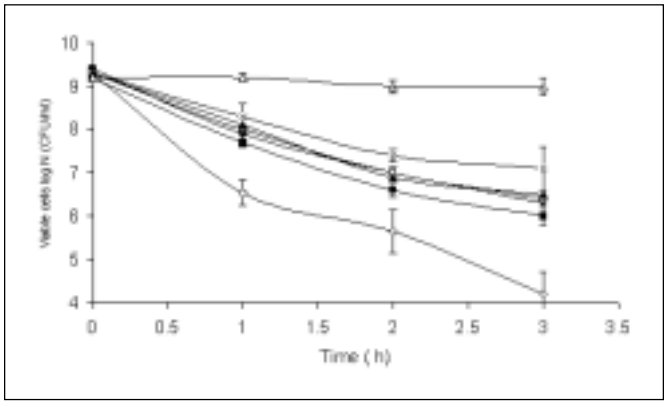

Figure 4—Effect of different coating materials on survival of *Lactobacillus acidophilus* CSCC 2400 under in vitro acidic conditions. The error bars represent mean ± SD of 3 replicates for all treatments. ◇ = free cells; ■ = enc. cells ; ◆ = co-encapsulated cells; ▲ = co-encapsulated cells with alginate coating; □ = co-encapsulated cells with Poly-L-Lysine (PLL) coating; x = co-encapsulated cells with chitosan coating; △ = free cells pH 6.5 (control).

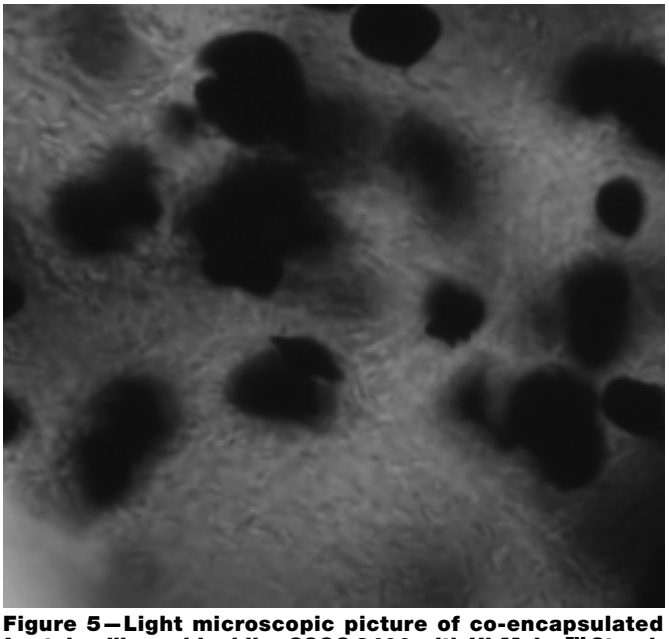

Figure 5—Light microscopic picture of co-encapsulated *Lactobacillus acidophilus* CSCC 2400 with Hi-Maize™ Starch (400×magnification). The dark areas are Hi-Maize granules.

with Jankowski and others (1997) who reported that alginate/starch liquid core capsules offer the ability to encapsulate *L. acidophilus* without loss of viability and fermentation ability.

Resistant starch is largely undegraded in the small intestine and is a major part of the carbohydrate available in the human colon for bacterial fermentation, which results in the production of short-chain fatty acids and gases (Cummings and others 1990; Brown 1996). These end products of fermentation can exert significant positive health effects on the host (Kritchevsky 1995). Thus, besides increasing the protection of probiotic bacteria inside the capsules from adverse acidic conditions, Hi-maize also confers health benefits.

Co-encapsulated synbiotic yogurt

The yogurts were monitored over a storage period of 6 wk for change in the viable cell count (Table 2). There was a decline of about 4.0 log over a period of 6 wk in both the cell numbers of strains of *L. acidophilus* CSCC 2400 and CSCC 2409 when present as free cultures, whereas there was only a 2.0-log and 1.0-log cycle decrease in cell numbers in both encapsulated and co-encapsulated cells (chitosan coated), respectively. The pH in the yogurts decreased from 4.5 at the start of storage to 4.0 after 6 wk of storage. The presence of lactic acid combined with the low pH of yogurt might be responsible for the low viability of free probiotic cultures in yogurt. There was a significant increase ($P < 0.05$) in the viable counts of *Lactobacillus* spp. in chitosan coated co-encapsulated beads compared with the encapsulated cells. Survival of probiotics in alginate-starch beads (in the size range of 1.0 mm) was improved during refrigerated storage in yogurt (Sultana and others 2000). We have shown the positive role of incorporation of prebiotic (Hi-maize starch) into the alginate mix during encapsulation.

Conclusions

The concept of co-encapsulation offers the potential for increased efficacy of functional foods by exploiting the synergy between prebiotic and probiotic ingredients. Addition of Hi-maize as a co-encapsulant and further coating the capsule with chitosan appears to improve the survival of encapsulated probiotic bacteria significantly under in vitro acidic condition and in yogurt compared with alginate encapsulated cells.

Future studies need to be carried out to monitor the efficacy of co-encapsulated bacteria in the gut, using animal models. Also, the sensory evaluation of yogurt with microencapsulated prebiotics and probiotic bacteria (study in progress) will reveal the consumer response to the texture and the changes in organoleptic characteristics of the yogurt.

Acknowledgments

The authors gratefully acknowledge Dr. Candy Briggs (School of Food Science and Horticulture, Univ. of Western Sydney, Australia) for her technical assistance with microscopic studies.

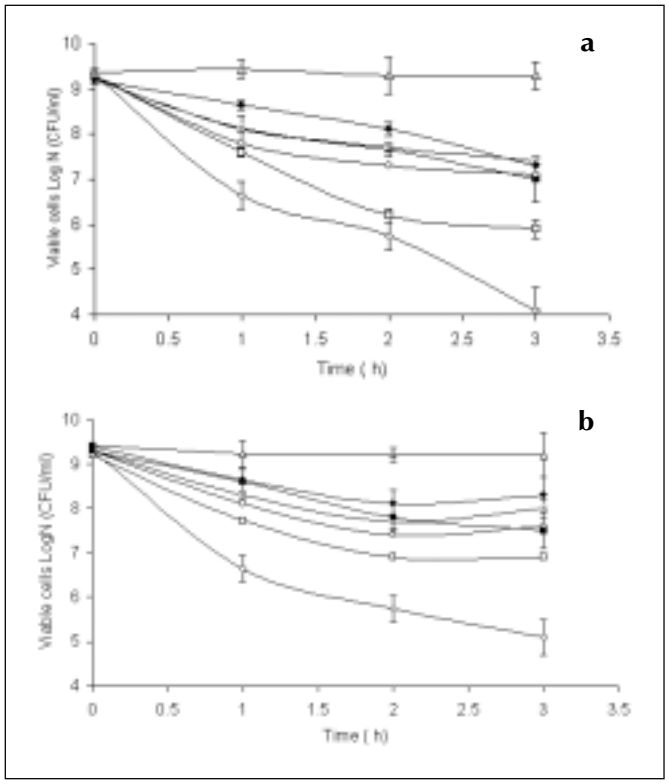

Figure 6—Survival of free, encapsulated, and co-encapsulated bacteria (chitosan coated) in vitro acidic conditions. (a) Viable *Lactobacillus acidophilus* CSCC 2400 and (b) *L. acidophilus* CSCC 2409 after exposure to in vitro acidic conditions at pH 6.5 (control), pH 3.0 or pH 2 for 3 h at 37 °C. The error bars represent mean ± SD of 3 replicates for all treatments. ◇ = free cells pH 2; ○ = free cells pH 3; □ = enc. cells pH 2; x = enc. cells pH 3; ■ = co-encapsulated pH 2; ● = co-encapsulated pH 3; △ = free cells pH 6.5 (control).

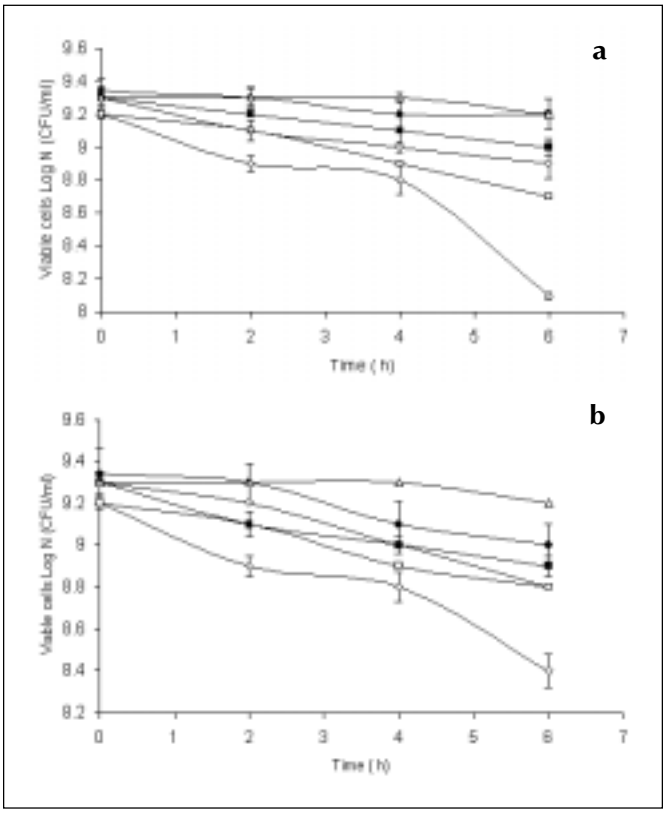

Figure 7—Survival of free, encapsulated, and co-encapsulated bacteria (chitosan coated) in milk-yeast medium with Oxgall (bile). (a) Viable *Lactobacillus acidophilus* CSCC 2400 and (b) *L. acidophilus* CSCC 2409 after exposure to 0% (control), 0.5%, or 1% bile for 6 h at 37 °C. The error bars represent mean ± SD of 3 replicates for all treatments. ◇ = free cells 1.0% bile; ○ = free cells 0.5% bile; □ = enc. cells 1.0% bile; x = enc. cells 0.5% bile; ■ = co-encapsulated 1.0% bile; ● = co-encapsulated 0.5% bile; △ = free cells 0% bile (control).

Table 2—Survival of free, encapsulated, and co-encapsulated bacteria (chitosan coated) in yogurt over a shelf life period of 6 wk[a]

Lactobacillus cultures in different states	Storage period (wk)			
	0	2	4	6
Free a*	$4.8 \pm 2.0 \times 10^8$	$2.5 \pm 0.3 \times 10^6$	$4.4 \pm 0.6 \times 10^5$	$2.3 \pm 0.7 \times 10^4$
Free b*	$4.6 \pm 1.0 \times 10^8$	$3.1 \pm 0.1 \times 10^6$	$4.8 \pm 0.2 \times 10^5$	$3.3 \pm 0.1 \times 10^4$
Encapsulated a	$3.9 \pm 0.6 \times 10^8$	$3.6 \pm 1.0 \times 10^7$	$2.8 \pm 1.1 \times 10^6$	$1.5 \pm 2.0 \times 10^6$
Encapsulated b	$3.5 \pm 0.2 \times 10^8$	$4.5 \pm 1.1 \times 10^7$	$5.4 \pm 0.3 \times 10^6$	$2.5 \pm 2.4 \times 10^6$
Co-encapsulated a	$3.8 \pm 1.1 \times 10^8$	$5.8 \pm 2.2 \times 10^7$	$3.3 \pm 0.4 \times 10^7$	$2.6 \pm 0.6 \times 10^7$
Co-encapsulated b	$4.0 \pm 0.9 \times 10^8$	$6.7 \pm 1.2 \times 10^7$	$4.2 \pm 0.6 \times 10^7$	$1.2 \pm 0.8 \times 10^7$

[a]Measurements are mean ± SD of 3 replicates for all treatments. a* = *Lactobacillus acidophilus* CSCC 2400; b* = *L. acidophilus* CSCC 2409.

References

Brown IL. 1996. Complex carbohydrates and resistant starch. Nutr Rev 54:115–9.

Champagne CP, Gaudy C, Poncelet D, Neufeld RJ. 1992. *Lactococcus lactis* release from calcium alginate beads. Appl Environ Microbiol 58:1429–34.

Chandramouli V, Kailasapathy K, Peiris P, Jones M. 2004. An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp in simulated gastric conditions. J Microbiol Methods 57:27–35.

Conway PL. 1996. Selection criteria for probiotic microorganisms. Asia Pacific J Clin Nutr 5:10–4.

Crittenden RG, Morris LF, Harvey ML, Tran LT, Mitchell HL, Playne MJ. 2001. Selection of a *Bifidobactrium* strain to complement resistant starch in a synbiotic yoghurt. J Appl Microbiol 90:268–78.

Cummings JH, Banwell JG, Englyst HN, Coleman N, Segal I, Bersohn D. 1990. The amount and composition of large bowel contents. Gastroenterology 98:A408.

Dave RI, Shah NP. 1996. Evaluation of media for selective enumeration of *Streptococcus*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacteria*. J Dairy Sci 79:1529–36.

Fooks LJ, Fuller R, Gibson GR. 1999. Prebiotics, probiotics and human gut microbiology. Int Dairy J 9:53–61.

Gibson GR, Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–12.

Gibson GR, Wang X. 1994. Bifidogenic properties of different types of fructooligosaccharides. Food Microbiol 11:491–8

Hamilton-Miller JMT, Shah S, Winkler JT. 1999. Public health issues arising from microbiological and labeling quality of foods and supplements containing probiotic microorganisms. Public Health Nutr 2:223–9.

Jankowski T, Zielinska M, Wysakowska A. 1997. Encapsulation of lactic acid bacteria with alginate/starch capsules. Biotechnol Techniq 11:31–4.

Kailasapathy K, Chin JC. 2000. Survival and therapeutic potential of probiotics organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol 78:80–8.

Kailasapathy K, Rybka S. 1997. *Lactobacillus acidophilus* and *Bifidobacterium* spp.: their therapeutic potential and survival in yoghurt. Aust J Dairy Technol 52:28–35.

Koo SM, Cho YH, Huh CS, Baek YJ, Park J. 2001. Improvement of stability of *Lactobacillus casei* YIT 9018 by microencapsulation using alginate and chitosan. J Microbiol Biotechnol 11:376–83.

Krasaekoopt W, Bhandari B, Deeth H. 2003. Evaluation of encapsulation techniques of probiotics for yoghurt: a review. Int Dairy J 13:3–13.

Kritchevsky D. 1995. Epidemiology of fiber, resistant starch and colorectal cancer. Eur J Cancer Prevent 4:345–52.

Lankaputhra WEV, Shah NP. 1995. Survival of *L. acidophilus* and *Bifidobacterium* spp. in the presence of acid and bile salts. Cult Dairy Prod J 30:2–7.

Lee K, Heo T. 2000. Survival of *Bifidobacterium longum* immobilized in calcium alginate beads in simulated gastric juices and bile salt solution. Appl Environ Microbiol 66:869–73.

O'Riordan K, Andrews D, Buckle K, Conway P. 2001. Evaluation of microencapsulation of a *Bifidobactrium* strain with starch as an approach to prolonging viability during storage. J Appl Microbiol 91:1059–66.

Ouwehand AC, Salminen SJ. 1998. The health effects of cultured milk products with viable and non-viable bacteria. Int Dairy J 8:749–58.

Prevost H, Divies C. 1992. Cream fermentation by a mixed culture of lactococci entrapped in two-layer calcium alginate gel beads. Biotechnol Lett 14:583–8.

Rao AV, Shiwnarain N, Maharaj I. 1989. Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices. Can Inst Sci Technol J 22:345–9.

Reid GG, Sanders ME, Gaskins H R. 2003. New scientific paradigms for probiotics and prebiotics. J Clin Gastroenterol 37:105–18.

Roberfroid MB. 2000. Prebiotics and probiotics: are they functional food? American J Clin Nutr 71:1682–7.

Sheu TY, Marshall RT, 1993. Micro-encapsulation of *Lactobacilli* in calcium alginate gels. J Food Sci 54:557–61.

Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K. 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastro-intestinal conditions and in yoghurt. Int J Food Microbiol 62:47–55.

Truelstrup Hansen L, Allan-Wojtas PM, Jin YL, Paulson AT. 2002. Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. Food Microbiol 19:35–45.

Yu W, Yim T, Lee K, Heo T. 2001. Effect of skim milk-alginate beads on survival rate of *Bifidobacteria*. Biotechnol Bioproc Eng 6:133–8.

Zhou Y, Martins E, Groboillot A, Champagne CP, Neufeld RJ. 1998. Spectrophotometric quantification of lactic bacteria in alginate and control of cell release with chitosan coating. J Appl Microbiol 84:342–8.