CHAPTER 2

EFFECT OF SUPPORTING MATERIAL CONCENTRATIONS ON THE SURVIVAL OF IMMOBILIZED *L. acidophilus* AND *B. bifidum*

INTRODUCTION

In the last 20 years there has been an increase interest in the role of probiotic bacteria in human health. In order for these bacteria to exert positive health effects, they have to reach their site of action alive and establish themselves in certain numbers. However, studies indicate that the bacteria may not survive in high enough numbers when incorporated into dairy products (Dave and Shah, 1996; Hamilton-Miller *et al*., 1999). Many studies have also focused on the survival of these bacteria in dairy products under different product and storage conditions (Kailasapathy and Supriadi, 1996; Kebary *et al.,* 1998; Beal *et al*., 1999; Gardini *et al*., 1999). Prasad *et al.* (1998) have studied the metabolic status of probiotic bacteria in the gastrointestinal tract (GIT). Strain selection approaches and other techniques have been used to improve the survival of bacteria. The addition of growth promoting factors or prebiotics, such as starch, and oligosaccharides (Mituoka, 1992), buffering of yoghurt mixes with whey proteins (Kailasapathy and Supriadi, 1996; Ravula and Shah, 1998), and modulating packaging conditions have improved the survival of bacteria (Tanaka and Hatanaka, 1992).

In addition, microencapsulation methods have been applied to increase the survival and delivery of bacterial cultures. Several methods have been developed for the microencapsulation of bacteria for use in fermentation, as well as for incorporating into products. Microencapsulation helps in segregating the bacterial cell from the adverse environment, for example, of the product, thus potentially reducing cell loss. Several studies have shown successful microencapsulation and coating of bacteria using various encapsulating materials and methods. Calcium alginate has also been used widely for the immobilization of lactic acid bacteria due to its ease of handling, its non-toxic nature, and due to its low cost. Model studies are available

where alginates have been used for the microencapsulation of bacteria for fermentation purposes or for incorporation into products, (Larisch *et al.,* 1994; Kim *et al.,* 1996; Jankowski e*t al*., 1997; Khalil and Mansour, 1998). The objective of this section was to optimize the concentration of supporting material of the probiotic bacteria in order to increase the survival of encapsulated cultures.

2.1 LITERATURE REVIEW

2.1.1 Probiotic Microencapsulation Technology

Viability of probiotic bacteria in a product at the point of consumption is an important consideration for their efficacy, as they have to survive during the processing and shelf life of food and supplements, transit through high acidic conditions of the stomach acids, enzymes and bile salts in the small intestine. The consumption of probiotics at a level of 10^8-10^9 cfu/g per day is a commonly quoted figure for adequate probiotic consumption, equating to 100 g of a food product with 10^6-10^7 cfu/g. Analysis of probiotic products in many different countries has confirmed that probiotic strains exhibit poor survival in traditional fermented dairy products. Probiotic survival in products is affected by a range of factors including pH, post-acidification (during storage) in fermented products, hydrogen peroxide production, oxygen toxicity (oxygen permeation through packaging), storage temperatures, stability in dried or frozen form, poor growth in milk, lack of proteases to break down milk protein to simpler nitrogenous substances and compatibility with traditional starter culture during fermentation. Oxygen plays a major role in the poor survival of probiotic bacteria. Providing probiotic living cells with a physical barrier against adverse external conditions is an approach currently receiving considerable interest. In the past, microorganisms were immobilized or entrapped in polymer matrices for use in bio-technological applications. The physical retention of cells in the matrix facilitated the separation of the cells from their metabolites. As the technique of immobilization or entrapment became refined, the immobilized cell technology has evolved into microencapsulation of cells. Microencapsulation tends to stabilize cells, potentially enhancing their viability and stability in the production, storage and handling of lactic cultures. An immobilized environment also conferred

additional protection to lactobacilli and bifidobacterial cells during rehydration and lyophilization (Krasaekoopt *et al*., 2003).

Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influences of specific conditions. A microcapsule consists of a semi-permeable, spherical, thin and strong membrane surrounding a solid/liquid core, with a diameter varying from a few microns to 1 mm. Microencapsulation can be used for many applications in the food industry, including stabilizing the core material, controlling the oxidative reaction, providing sustained or controlled release (both temporal and time-controlled release), masking flavours, colours or odours, extending the shelf life and protecting components against nutritional loss. Food-grade polymers such as alginate, chitosan, carboxymethyl cellulose (CMC), carrageenan, gelatin and pectin are mainly applied, using various microencapulation technologies (Annan *et al*., 2008).

Microcapsules can be engineered to gradually release active ingredients. A microcapsule may be opened by many different means, including fracture by heat, salvation, diffusion and pressure. A coating may also be designed to open in the specific areas of the body. A microcapsule containing acid-labile core materials that will be consumed by gastrointestinal fluids must not be fractured until after it passes through the stomach. A coating can therefore be used that is able to withstand acidic conditions in the stomach acids and allows those active ingredients to pass through the stomach. These microcapsules were first incubated in simulated gastric fluid (pH 1.2) for 2 h and then transferred into simulated intestinal fluid (pH 7.4) (Anal and Singh, 2007).

Microencapsulation of probiotics in a biodegradable polymer matrix has a number of advantages. Once entrapped or encapsulated in matrix beads or in microcapsules, the cells are easier to handle than in a suspension or in slurry. The number of cells in beads or microparticles can be quantified, allowing the dosage to be readily controlled. Cryo and osmo-protective components can be incorporated into the matrix, enhancing the survival of cells during processing and storage. Finally, once the matrix beads or microcapsules have been dried, a further surface coating can be applied. This outer layer can be used to alter the aesthetic and sensory properties of

the product and may also be functional, providing an extra level of protection to the cells. In addition, the coating layer can have desirable dissolution properties, which permit delayed release of the cells or release upon, for example, a change in pH. Various polymer systems have been used to encapsulate probiotic microorganisms to protect against low pH and high bile concentrations and to enhance physical stability during downstream processing. Microcapsule or bead systems using various biopolymers are very easy to prepare on a lab-scale, and any ingredients can be encapsulated, whether it is hydrophilic, hydrophobic, liquid, or a viscous oil, a solid etc. However, the scaling-up of the process is very difficult and processing costs are very high. Moreover, most of the conventionally produced microcapsules (e.g. calcium alginate beads/microcapsules), tend to be very porous which allows fast and easy diffusion of water and other fluids in and out of the matrix. Spherical polymer beads with diameters ranging from 0.3 to 3.0 mm and immobilizing active biomass are produced using extrusion or emulsification techniques, by thermal (κ-carrageenan, gellan, agarose, gelatin) or ionotropic (alginate, chitosan) gelation of the droplets (Anal and Singh, 2007). Microencapsulation helps to separate a core material from its environment until it is released. Thereby improving its stability, extends the core's shelf life and provides a sustained and controlled release (Figure 3).

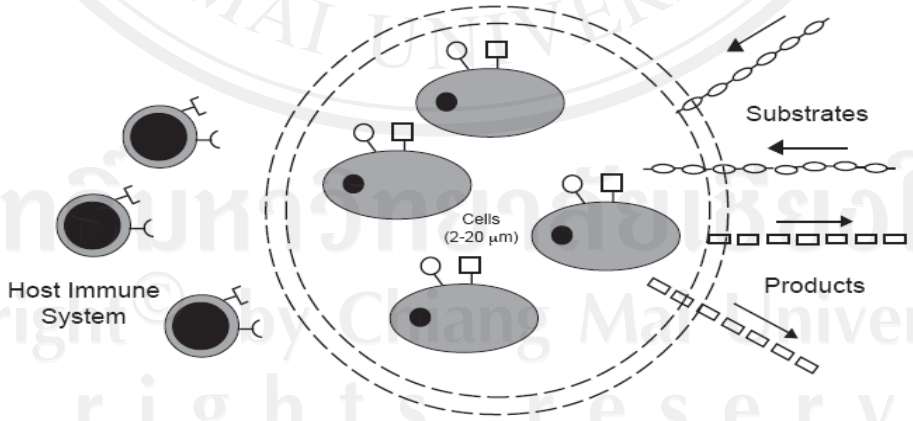

Fig. 3 Principle of Encapsulation: Membrane barrier isolates cells from the host immune system while allowing transport of metabolites and extracellular nutrients. Membrane with size-selective pores (30-70 kDa) (Franjione and Vasishtha, 1995)

Entrapment of cells in a gel matrix of alginates is the most popular system of immobilisation reported. The terms immobilisation and encapsulation were used interchangeably in most reported literature. While encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall as a core of encapsulated material, immobilisation refers to the trapping of material within or throughout a matrix. A small percentage of immobilized material may be exposed at the surface, while this is not the case for encapsulated material. Encapsulation occurs naturally when bacterial cells grow and produce exo-polysaccharides. The microbial cells are entrapped within their own secretions that act as a protective structure or a capsule, reducing the permeability of material through the capsule and therefore, less exposed to adverse environmental factors. Many lactic acid bacteria synthesise exo-polysaccharides, but they produce insufficient exo-polysaccharides to be able to encapsulate themselves fully. The structure formed by the microencapsulation agent around the core substance is known as the wall. The properties of the wall system are designed to protect the core and to release it at controlled rates under specific conditions while allowing small molecules to pass in and out of the membrane. The capsules may range from submicron to several millimeters in size and can be of different shapes. Compared to immobilisation/entrapment techniques, microencapsulation has many advantages. The microcapsule is composed of a semipermeable, spherical, thin and strong membranous wall. Therefore, the bacterial cells are retained within the microcapsules. Moreover, compared to an entrapment matrix, there is no solid or gelled core in the microcapsule and its small diameter helps reduce mass transfer limitations. The nutrients and metabolites can diffuse through the semi-permeable membrane easily. The membrane serves as a barrier to cell release and minimizes contamination (Kailasapathy, 2006).

Microencapsulation of probiotics in hydrocolloid beads has been tested for improving their viability in food products and during gastrointestinal tract transit. Microencapsulation using gelatin or vegetable gum provides protection to acid-sensitive bifidum bacteria; however, the most widely-used matrix for microencapsulation is alginate. Alginate beads have been found to increase the survival of probiotics by up to 80-90% (Mandal *et al*., 2006). Alginate has the

benefits of being non-toxic to the cells being immobilized, and it is an accepted food additive. The reversibility of encapsulation, solubilizing alginate gel by sequestering calcium ions, and the possible release of entrapped cells in the human intestine is another advantage (Chandramouli *et al*., 2004). Microencapsulation of *Lactobacillus rhamnosus* in alginate improved survival at pH 2.0 up to 48 h, while the free cells were destroyed completely. Similarly, the death rate of *Bifidobacterium longum* immobilized in alginate decreased proportionately with increasing alginate concentrations (2-4%) and bead size. Microencapsulation of bifidobacteria also exhibited a lower population reduction during exposure to simulated gastric environment and bile solution (Picot and Lacroix, 2004).

There are several factors affecting bead preparation, such as concentrations of alginate and $CaCl_2$, timing of hardening of the beads and cell concentrations on encapsulation of probiotics. The conventional encapsulation method, with sodium alginate in calcium chloride ($CaCl_2$), has been used to microencapsulate *L. acidophilus* to protect this organism from the harsh acidic conditions in gastric fluid. Studies have shown that calcium alginate-immobilized cell cultures are better protected as shown by an increase in the survival of bacteria under different conditions, than the non-encapsulated state. The results from Anil and Harjinder (2007) indicated that the viability of microencapsulated bacteria in simulated gastric fluid increases with an increase in capsule size. The study also indicated that these bacteria should be microencapsulated within a particular size range. Nine different strains of *Bifidobacterium spp.* were tested for their tolerance to simulated gastrointestinal conditions, and observed some variations among the strains for resistance to gastric fluid (pH 2-3) and bile salts (5 and 10g/l). Among these strains, only a strain *Lactobacillus lactis* Bb-12 was found to be resistant to low pH and bile salts. The worker also microencapsulated some of the strains in alginate microspheres to evaluate their resistance properties in gastric fluid and to bile salts. It was obtained alginate microspheres (20-70 μm) by emulsifying the mixture of cells and sodium alginate in vegetable oil and subsequently cross-linking with $CaCl_2$. Cryo-scanning electron microscopy revealed that these microparticles were densely loaded with probiotic bacteria and were porous (Anal and Singh, 2007).

Chan (2005) developed microencapsulation technique of compression coating which permits the stabilization of lyophilized cells during storage. This technique involves compressing the lyophilized cell powder into a core tablet and then compressing coating materials around this core to form the final compact. Adhikari *et al.* (2000) worked to investigate the use of methacrylic acid copolymer as an enteric coating material for the compression coating of an industrially-sourced strain of *L. acidophilus*. The coating material used was a mixture of sodium alginate and hydroxypropyl cellulose in the weight ratio 9:1. The microencapsulated cells showed a 10^4-10^5 fold increase in cell survival compared with free cells under acidic conditions.

Cui *et al*. (2001) prepared poly-L-lysine–cross-linked alginate microparticles loaded with bifidobacteria. They used an air atomization method to spray the alginate–bacteria culture in a coagulation bath containing $CaCl_2$. The microparticles were further cross-linked with poly-L-lysine. The survival of bifidobacteria from the alginate–poly-L-lysine microparticles was much higher, even in the lower pH media. Due to stability of poly-L-lysine–cross-linked alginate microparticles in gastric fluid, bifidobacteria can be protected without losing their survivability. The survival of bifidobacteria loaded in the particles remained highest (2.67×10^9 cfu/g) at pH 6.8 while the number is reduced at lower pH (1.5, exposure time, 2 h) to 5.0×10^7 cfu/g. However, only 1–3% of the unencapsulated bifidobacteria can survive in lower pH. The stability of the free-flowing bifidobacteria–alginate–poly-L -lysine microparticles was also improved during storage at 4°C in a refrigerator, compared with free cultures.

The microencapsulation of *Bifidobacterium pseudolongum* with cellulose acetate phthalate (CAP) increased the survival of bacteria under simulated gastric acid conditions as compared with the non-encapsulated bacteria (Khalida *et al*., 2000). Khalil and Mansour (1998) demonstrated that calcium alginate-immobilized cultures were better protected, as shown by the increase in survival of bacteria, under different tested conditions, than when bacteria were tested in the non encapsulated state. *Bifidobacterium* survived in higher numbers in frozen ice milk in beads made from alginate than those made from κ-carrageenan. Alginate microencapsulation has been used successfully to immobilize bacterial cultures for incorporation into

mayonnaise. Microencapsulation thus may enhance the shelf life of probiotic cultures in dairy products (Sheu and Marshall, 1993).

2.1.2 Application of microencapsulation

Sheu and Marshall (1993) reported that a product was developed to entrap culture bacteria using a two-phase water/oil system. It consisted of 3% sodium alginate mixed with microbial cell and suspended in an oil bath containing 0.2% Tween 80. While stirring at 200 rpm, calcium chloride 0.05 M solution was added to break the water/oil emulsion and from calcium alginate gel. The calcium alginate beads containing microbial cell had a mean diameter of 25-35 μm, ranged 5-100 μm. The entrapped microbial cells were released completely from the drop-shaped beads by gentle shaking in 0.1 M phosphate solution pH 7.5 for 10 min. About more than 40% of lactobacilli survived freezing of ice milk when they were entrapped in calcium alginate than when they were not entrapped.

Iyer and Kailasapathy (2005) reported that three different complementary prebiotics, inulin, oligofructose and high amylase corn starch (hi-maize starch) were separately be used to co-encapsulate *L. acidophilus* CSCC 2400 or CSCC 2409 and tested for their efficacy in improving the viability of bacteria under in vitro acidic conditions. Additions of hi-maize TM starch to capsules containing *Lactobacillus* spp. provided maximum protection to the encapsulated bacteria after 3 h of incubation at pH 2.0 compared with the other two prebiotics, inulin and oligofructose. Viable counts of *Lactobacillus* spp. increased significantly ($p<0.05$) with hi-maize concentrations for up to 1.0% (w/v). Further increase in hi-maize concentration did not protect the encapsulated bacteria effectively. Effects of three different polymers, chitosan, poly-L-lysine and alginate were also tested for their efficacy in protecting the encapsulated bacteria at pH 2.0 by an extrusion technique. An addition of hi-maize 0.1% 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 condition and also in stored yoghurt compared with alginate encapsulated cells.

Sultana *et al*. (2000) reported that incorporation of hi-maize starch, a prebiotic, improved encapsulation of viable bacteria as compared to when the bacteria

were encapsulated without the starch. The acidification kinetics of encapsulated bacteria showed that the rate of the acid produced was lower than that of free cultures. The encapsulated bacteria, however, did not demonstrate a significant increase in survival when subjected to in vitro high acid and bile salt conditions. The survival of encapsulated cultures of *L. acidophilus* and *Bifidobacterium* spp. showed a decline in viable count of about 0.5 log over a period of 8 weeks while there was a decline of about 1 log in cultures which were incorporated as free cells in yoghurt. The extrusion technique did not result in a uniform bead size.

Resistant starch is starch that is not digested by pancreatic amylase in the small intestine and reaches the colon, but it can be fermented by human and animal gut microflora. In a study where rats were fed with native potato starch, an increase in the intestinal population of bifidobacteria, lactobacilli, streptococci and enterobacteria was demonstrated. The benefit of using resistant starch extends beyond traditional prebiotics, since the resistant starch can be used to ensure the viability of probiotic populations from the food to the large intestine. Resistant starch offers an ideal surface for adherence of the probiotics to the starch granule during processing, storage and transit through the upper regions of the gastrointestinal tract, providing robustness and resilience to environmental stresses. Bacterial adhesion to starch may also provide advantages in new probiotic-technologies to enhance delivery of viable and metabolically-active probiotics to the intestinal tract (Sultana *et al*., 2000).

Hansen Truelstrup *et al.* (2002) reported that *Bifidobacterium adolescentis* 15703, *Bifidobacterium breve* 15700, *Bifidobacterium lactis* Bb-12 and *Bifidobacterium longum* Bb-46 with their best overall resistance, were encapsulated in alginate microspheres, a mean diameter of 20 and 70 μm, by an emulsion technique. *B. lactis* Bb-12 was significantly more resistant to low pH and bile than any other test strains. Preliminary trials revealed that sphere size 1-3 mm which used gellan-xanthan and alginate-starch mixtures was too large to allow direct incorporation in food products such as milk, yoghurt and sour cream, without adversely affecting the feel in the mouth. Reduction of the sphere size to less than 100 μm would be advantageous for texture considerations and allow direct addition of encapsulated probiotics to a multitude of food. Furthermore, survival of probiotics in alginate-starch microspheres in the size range of 0.5-1.0 mm was improved during

refrigerated storage in yoghurt but not affected when exposed to acid and bile solutions.

Lee and Heo (2000) showed that *B. longum* encapsulated in Ca-alginate spheres survived exposure to simulated gastric juice (SGJ) pH 1.55 significantly better than free cell. Survival decreased with decreasing sphere size diameter 1-2.6 mm and increased with increasing alginate concentration 1-3%.

2.2 EXPERIMENTAL

2.2.1 Probiotic bacteria microencapsulation

The mixture solution of alginate with different concentrations of starch was prepared. The solution of 1.8% alginate (Sigma, Australia) was mixed with different levels of hi-maize starch (Sigma, Australia) of 0.5, 1.0, 1.5 and 2.0% (w/v). For each solution, an individual culture of *B. bifidum* or *L. acidophilus* at the level of 10^9cfu/ml was inoculated. After that, the solution was dropped by 0.1 mm syringes (24 G (0.55x25 mm), Nippro Corporation, Japan) into 0.1 M $CaCl_2$ (Merck, Germany) according to the method previously described by Chandramouli *et al*. (2004). The beads were left to be harden for 30 min at room temperature, rinsed with 0.1% of sterile peptone solution (Lab scan Co., Ltd, Thailand) and kept at 4^oC for 12 h before use in any experiment.

2.2.2 Characterization of the probiotic microencapsulated beads

The beads obtained from 2.2.1 were determined for their diameter. A number of 25 randomly selected beads was measured by a vernier caliper (OHAUS Co., Ltd, USA). The bead diameter was then calculated to the bead volume, using a formula of $4/3\pi r^3$. A number of 20 randomly selected beads were weighed by a balance (OHAUS Co., Ltd, USA) to determine for their mass.

2.2.3 The number of probiotic in microencapsulated beads.

An amount of 0.1 g of bead was added into 10 ml of phosphate buffer 0.1 M, pH 7.0 (Krasaekoopt *et al.*, 2004). The solution was shaken for 10 min at room temperature, then used 10-fold serially dilution, using Maximun Recovery Diluent (MRD, Oxoid England) to count the amount of *B. bifidum* and *L. acidophilus*. The numbers of *L. acidophilus* were enumerated using Homofermentative and Heterofermentative Differential (HHD) agar that composed of 2.5% (g/l) of fructose

(Fluka, Switzerland), 2.5% (g/l) of KH_2PO_4 (Fluka, Switzerland), 10.0% (g/l) of soytone (Bacto, France), 1.5% (g/l) of peptone from casein (Merck, Germany), 3% (g/l) of casamino acid (Bacto, France), 1% of Tween 80 (chemical of highest quality, Mark, Japan), 1% (g/l) of yeast extract (Merck, Germany) and 1.5% (g/l) of agar (U&V Holding, Thailand) and incubated anaerobically at 37°C for 72 h (McDonald *et al*., 1987). Enumeration of encapsulated bifidobacteria was determined following the procedure of Sun and Griffiths (2000).

2.2.4 Determination of microstructure of sodium-alginate bead

The determination of microstructure of sodium-alginate beads followed the method of Habib *et al*. (2002) with slight modification. The beads were placed on aluminium mount using double-side Scotch^R tape and stored overnight in tightly sealed glass desiccator. The determination was carried out by scanning electron microscopy (JSM-5910 LV, Jeol Ltd., Japan). Microparticle was placed on a carbon adhesive paper and was coated with 200-Å gold metal with an ion sputtering coater (Hitachi E101, Japan).

2.2.5 Statistical analysis

Data from three repeated experiments was analyzed by a SPSS program (SPSS version 11, SPSS Inc., Chicago, USA). If the significant differences between means were found, the mean comparation with Duncan's multiple range test would be applied. The predetermined acceptable level of probability was 5% ($p<0.05$) for all the comparison (Montgomery, 2001).

2.3 RESULT AND DISCUSSION

This step of experiment studied about the influence of supporting material, hi- maize starch concentrations which were added in beads, on the bead properties, and the survival of probiotic in the beads. The concentrations of sodium alginate and calcium chloride used in this step were 1.8% (w/v) and 0.1 M, respectively. The studied hi-maize starch concentrations were 0.5 to 2.0% (w/v). The result showed that these hi-maize starch concentrations did not significantly affect the bead diameter (Tables 13 and 14) and the bead volume, which was calculated based on the radius of the beads. The bead volume could mainly be affected by the physical parameters

used to prepare the beads, including the syringe size and hi-maize starch concentrations (Krasaekoopt *et al*., 2003; Chandramouli *et al*., 2004).

Table13 The characteristics of sodium alginate hi-maize starch beads containing *L. acidophilus*

Hi-maize starch concentration (%)	Bead volume (ml)	Bead mass (g)	Bead diameter (mm)^{ns}
0	0.01±0.007^{a}	3.8±1.8^{a}	1.2±0.1
1.0	0.03±0.006^{b}	5.6±2.6^{b}	1.3±0.2
1.5	0.07±0.007^{c}	6.1±1.6^{c}	1.2±0.2
2.0	0.12±0.005^{d}	6.8±2.0^{d}	1.2±0.2

Table14 The characteristics of sodium alginate hi-maize starch beads containing *B. bifidum*

Hi-maize starch concentration (%)	Bead volume (ml)	Bead mass (g)	Bead diameter (mm)^{ns}
0	0.01±0.004^{a}	3.5±1.2^{a}	1.3±0.2
1.0	0.03±0.007^{b}	5.4±2.2^{b}	1.2±0.2
1.5	0.06±0.006^{c}	6.0±2.0^{c}	1.2±0.1
2.0	0.10±0.006^{d}	6.5±2.2^{d}	1.2±0.2

Table 15 The enumeration of *L. acidophilus* and *B. bifidum* cells in sodium alginate hi-maize starch beads

Hi -maize starch concentrations (%)	*L. acidophilus* (cfu/g)	*B. bifidum* (cfu/g)
0	$6.2\pm1.4\times10^{7a}$	$7.1\pm2.8\times10^{5a}$
1.0	$3.6\pm2.0\times10^{8b}$	$2.6\pm2.6\times10^{6b}$
1.5	$6.9\pm2.2\times10^{8c}$	$5.4\pm2.0\times10^{6c}$
2.0	$4.8\pm2.4\times10^{9d}$	$9.8\pm2.2\times10^{6d}$

The encapsulation procedures resulted in bead size, bead mass and bead volume of 1.2 – 1.3 mm, 3.5-6.8 g and 0.01 – 0.1 mm^3, respectively. The shape of the bead was generally spherical. The bacteria were distributed randomly in the bead matrix (Figure 4). Hi–maize starch was used at different concentrations in the encapsulation procedure to provide a prebiotic compound for probiotic bacteria. Krasaekoopt *et al.* (2003) reported an average diameter of 2-5 mm for alginate capsule produced by extrusion. In this study, the diameter of beads was smaller than the result of Krasaekoopt *et al.* (2003) because different equipment was used.

McMaster *et al.* (2005) indicated that particles with a diameter below 3 μm are undetected by the tongue. Therefore, above this value, capsule can impart a gritty texture to foods not normally associated with the sensation. Adhikari *et al.* (2000) reported that when microcapsules were added to yoghurt at 10% (w/v), there was an unfavourable consumer response due to the resultant grainy texture. The 10% content of encapsulated *B. longum* was necessary to provide the Recommended Dietary Allowance (RDA) of the organism. Ideally, an uniform capsule diameter is need in such that sensory qualities of the food are not altered, whilst simultaneously delivering therapeutic dose of probiotic. The diameter must not adversely affect viability of the organism. Hansen Truelstrup *et al*. (2002) reported that alginate

capsule should have diameter of at least 100 μm to prevent a reduction in *Bifidobacteria* viability in simulated gastric juice.

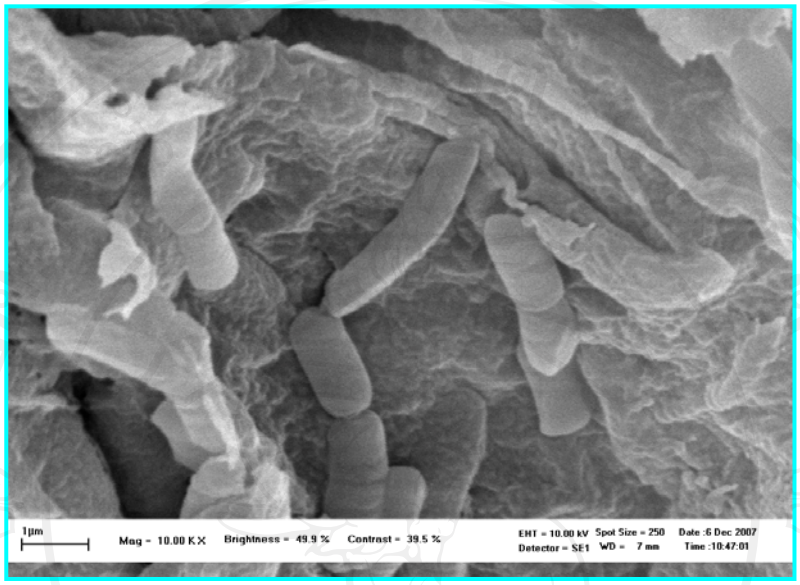

Fig. 4 Scanning Electron Microscope of a sodium alginate-hi-maize starch containing *L. acidophilus*

Different hi-maize starch concentrations were found to significantly affect the amount of *L. acidophilus* and *B. bifidum* cells that were entrapped in the sodium alginate hi-maize starch beads. Increasing the hi–maize starch concentrations to the alginate resulted in an increase in the numbers of *L. acidophilus* and *B. bifidum* cell in the beads which were from 6.2×10^7 to 4.8×10^9 cfu/g and 7.1×10^5 to 9.8×10^6 cfu/g, respectively (Table 15).

For the result of using hi-maize starch in encapsulation process, the prebiotic is necessary for the survival of *L. acidophilus* and *B. bifidum* which agreed with the experiment of Sultana *et al.* (2000) who immobilized *L. acidophilus* and *B. infantis* so that the survival rate of aforesaid microbes increased, because hi-maize starch is oligosaccharides, a prebiotic that support the survival of probiotic in gastrointestinal tract.

For the concentrations hi-maize starch, it was shown that the survival rate of probiotic depended on the concentrations of hi-maize starch. As higher levels of hi-

maize starch were used, the survival rate of probiotic was increased as well. When the concentration of hi-maize starch was raised to a specific level (approximately 4%), the survival rate of probiotic was also increased (Sultana *et al*., 2000 and Kim *et al.,* 1996). Furthermore, the addition of hi-maize starch in encapsulation process of probiotic cells can affect the survival rate more than a probiotic which was encapsulated without the addition of hi-maize starch. It was reported that the probiotic is a microorganism that does not grow well in ordinary environment. Thus, appropriate condition adjustment can make those microbes grow better. As alternate popular method, the addition of oligosaccharide which is prebiotic properties will support the growth of probiotic. The prebiotic substance is popular for use and acceptable (Capela *et al*., 2007).

Sultana *et al*. (2000) indicated that there was great potential for using the probiotic resistant starch with alginate during encapsulation since it could enhance the survival of probiotic bacteria. The viability of encapsulated probiotic bacteria in simulated gastric condition increased with increased alginate capsule size and was dependent on the gel concentration. It may also be noted that *L. acidophilus* and *B. bifidum* have a different response to the gastro-intestinal condition. Mituoka (1992) reported the *L. acidophilus* is most active in the small intestine, while *B. bifidum* is most active in the large intestinal. In order to survive and reach the colon in quantities sufficient to facilitate colonization, a large number of initial cells must be entrapped in the microcapsules. Increase in cell concentration during encapsulation increased the number of bacteria survivors at the end of 3 h incubation in simulated gastric conditions.

Kailasapathy (2005) reported that hi–maize starch used as a filler material in the capsules was a modified starch. It will absorb water but will not gelatinize fully during the heating step in yoghurt making. The swollen starch, therefore, will contribute to increase viscosity and firmness. It is reported that alginate hydro gel is susceptible to disruption in the presence of excess chelating agents such as calcium ions. The porous nature of the sodium alginate capsules may have allowed diffusion of the starch granules from within the capsule into the yoghurt matrix, contributing to increased gritty mouth feel.