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Encapsulation of Probiotics and its Optimization

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Abstract
This study reports the morphology and survivability of the probiotics Bacillus subtilis, Pediococcusacidilactici, Saccharomyces boulardii encapsulated in different alginate concentrations (1%, 2%, 2.5%, 3%, 3.5%, 4%) The release of encapsulated cells when exposed to various pH levels (2, 4, 7, 9, 11) and salt solutions of Sodium Chloride (NaCl) (10ppt, 15ppt, 20ppt, 25ppt, 30ppt) was also assessed. The survivability increased proportionately with increased alginate concentrations. The survival of the encapsulated probiotics was slightly better at moderate pH and low salt concentrations.

Key Word*: probiotics, Sodium Chloride, survivability, encapsulated probiotics, concentration.*

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I. Introduction

Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance. Correspondingly, in feed regulation, probiotics are included in the group of feed additives for stabilizing the microbial communities of the digestive tract in monogastric animals and ruminants. They are also known as digestive bioregulators or direct-fed microbials (DFMs). In a narrower sense, the term probiotics is confined to products which consist of one, or a few, well-defined strains of microorganisms.

Since the viability and activity of probiotics are needed at the site of action, these should withstand the host's natural barriers, the gastrointestinal tract (GIT) transit and the conditions in which they are fed, like the pond environments for aquaculture. However, the stabilization of probiotics using a carrier may improve survival of these microbes in products, both during feeding and GIT transit.

Encapsulation is a process in which the cells are retained within an encapsulating matrix or membrane. Encapsulating of probiotics has been investigated for improving their viability in feed products and the intestinal tract. The most widely used encapsulating material is alginate, a linear heteropolysaccharide of D-mannuronic acid and L-guluronic acid extracted from various species of algae. The use of alginate is favoured because of its cheapness, simplicity and biocompatibility. Alginate beads have been found to increase the survival of probiotics up to 80-95%.This paper reports the effect of varying concentration of alginate onsurvival of encapsulated probiotic bacteria after exposing to pH ranges and salt concentrations.

II. Materials and methods

Microorganisms

2 mg each of freeze dried and lyophilized pure samples of *Bacillus subtilis, Pediococcusacidilactici and Saccharomycesboulardii*were taken [needs editing]

Encapsulation

All glassware and solutions used in the protocols were prepared in aseptic conditions.

Sodium alginate solutions (1%, 2%, 2.5%, 3%, 3.5% or 4%) were prepared, sterilized by autoclaving (120^0C for 15 min) and cooled to 38-40^0C. Five hundred milliliters of this solution and 0.3g of each of the lyophilized probiotic bacteria samples were transferred into a sterile beaker and stirred to homogeneity using a magnetic stirrer for 20 min.

0.1 M (500 mL) calcium chloride solution was prepared using distilled water and was transferred into a wide vessel placed over a magnetic stirrer. The alginate solution along with the probiotics was dropped into the calcium chloride solution via a sterile burette and the nozzle was adjusted so that the solution falls drop wise. A peristaltic pump was used to transfer the alginate solution from the beaker into the burette. The magnetic stirrer prevented the accumulation of the beads in the solution. The beads were separated by filtration using a sieve and subsequently transferred into a tray and maintained in the incubator adjusted to 35-37^0C.

Alginate bead morphology

The shape, size, rigidity and texture of the alginate beads were physically examined. The diameters of 10-15 randomly selected beads of each concentration were measured using a metal ruler under a magnifying glass. The same were examined for two days consecutively as the size reduced due to the considerable drying of the beads. The texture and rigidity of each variation was also measured by physically examining them.

Release of entrapped cells in varying concentrations of alginate

The capsules containing probiotic cells were released by phosphate buffer (pH 7.0 0.1 M). Freshly prepared capsules (1g) of each concentration were placed separately in test tubes containing 10 mL buffer. They were incubated at room temperature for a period of one day and vortexed in a shaker before being used for the enumeration of viable cells over a hemocytomer after depolymerization of the capsules in the buffer.

Survival and release of encapsulated cells invaryingpH and salt levels

Freshly prepared capsules (1g) of each concentration were placed separately in test tubes containing 10 mL solutions of pH values of 2, 4, 7, 9, and 11. The same was repeated for 10 mL salt solutions prepared from sodium chloride (NaCl) i.e., 10 ppt (parts per thousand), 15 ppt, 20 ppt, 25 ppt, and 30 ppt. Each of the separate test tubes were incubated at room temperature for a period of one day and vortexed in a shaker before being used for the enumeration of viable cells over a hemocytometer after depolymerization of the capsules in the different solutions releasing the encapsulated cells up to a certain degree in each.

Analysis of data

All experiments and analyses were run in triplicate. Data were recorded as mean ± standard deviation (SD).

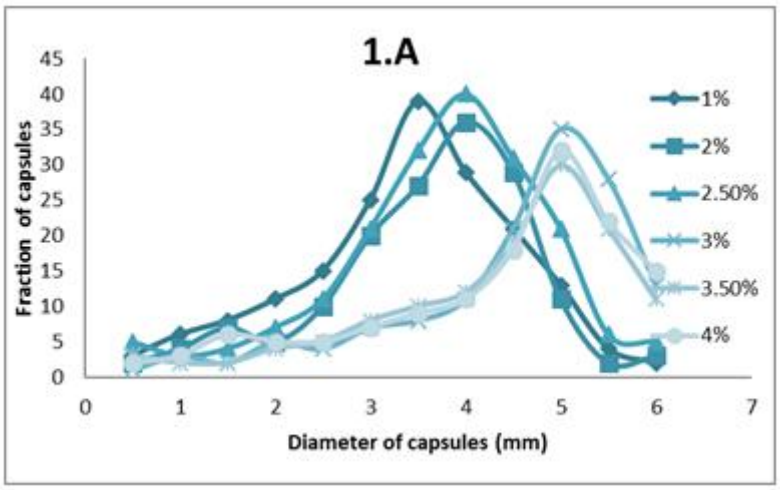
III. Results and Discussions

Capsules characteristics and size

Calcium alginate encapsulation can be affected by various factors such as capsule size, alginate concentrations, probiotic cell load and hardening time in calcium chloride solution. In the present study however, only the alginate concentrations have been altered. Table 1shows results for average diameters of the capsules under different alginate concentrations for different time intervals.

Table 1: Size of the capsules under varying alginate concentrations

Alginate concentration	Diameters of the capsules (mm)		
	0 h	24 h	48 h
1%	3.4 ±0.3	3.1±0.3	1.5±0.2
2%	4.2±0.4	3.2±0.4	2.0±0.5
2.5%	4.4±0.2	3.5±0.1	1.8±0.6
3%	5.0±0.3	3.5±0.4	2.3±0.3
3.5%	5.1±0.2	3.8±0.2	2.4±0.2
4%	5.2±0.1	4.1±0.3	2.5±0.4



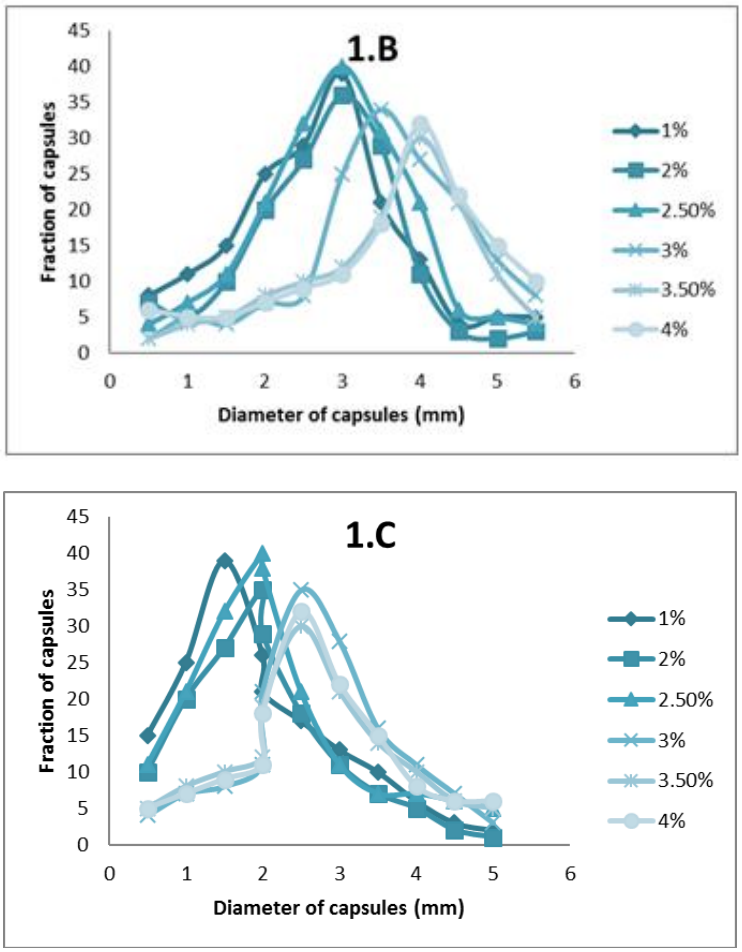


Figure 1: Size of capsules under varying alginate concentrations: (1.A) 0 h (1.B) 24 h (1.C) 48 h. The figures demonstrate the distribution of the capsules over different size ranges taken at different time intervals. The diameters of the capsules considerably reduced with time.

Survival of encapsulated cells in varying concentrations of alginate

The highest survival of cells was recorded in 4% alginate beads, followed by 3.5%, 3%, 2.5%, 2% and 1% when exposed to phosphate buffer (pH 7.0 0.1 M). Thus, the viability of encapsulated probiotic cells improved with increasing alginate concentration. The death rate of the cells entrapped in alginate beads decreased proportionately with increased capsule size and alginate concentration. More number of cellswas released as the time of exposure increased, probably due to the depolymerization of the alginate beads gradually.

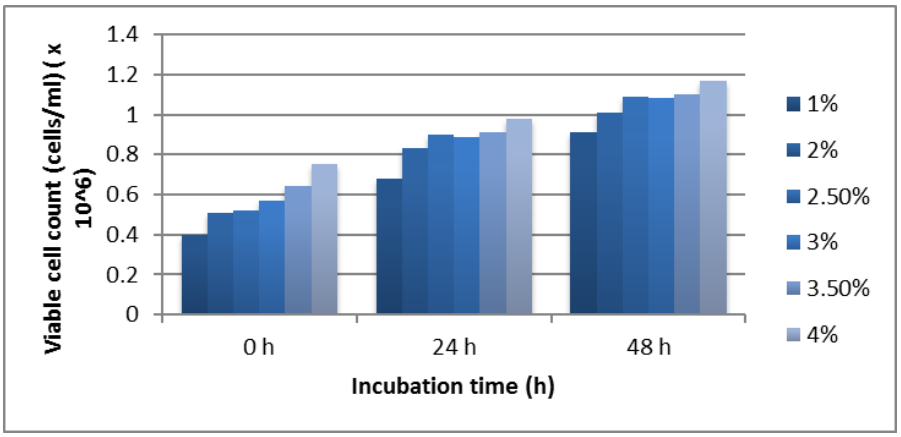


Figure 2*:* Survival of cells in varying concentrations of alginate, kept in phosphate buffer (pH 7.0 0.1M): The figure demonstrates the viable cell counts of encapsulated probiotics in varying alginate concentrations kept in phosphate buffer solution for different time intervals.

Survival of encapsulated cells in varying pH levels

Encapsulation with alginate concentration increasing from 1% to 4% improved the viability of cells at similar pH levels. Also, at any one particular concentration of alginate, at low pH (2, 4) the viable cell count was low, increased at pH 7, but however decreased again at higher pH (9, 11). The viability of probiotic cells decreased proportionately with the time of exposure to pH solutions.

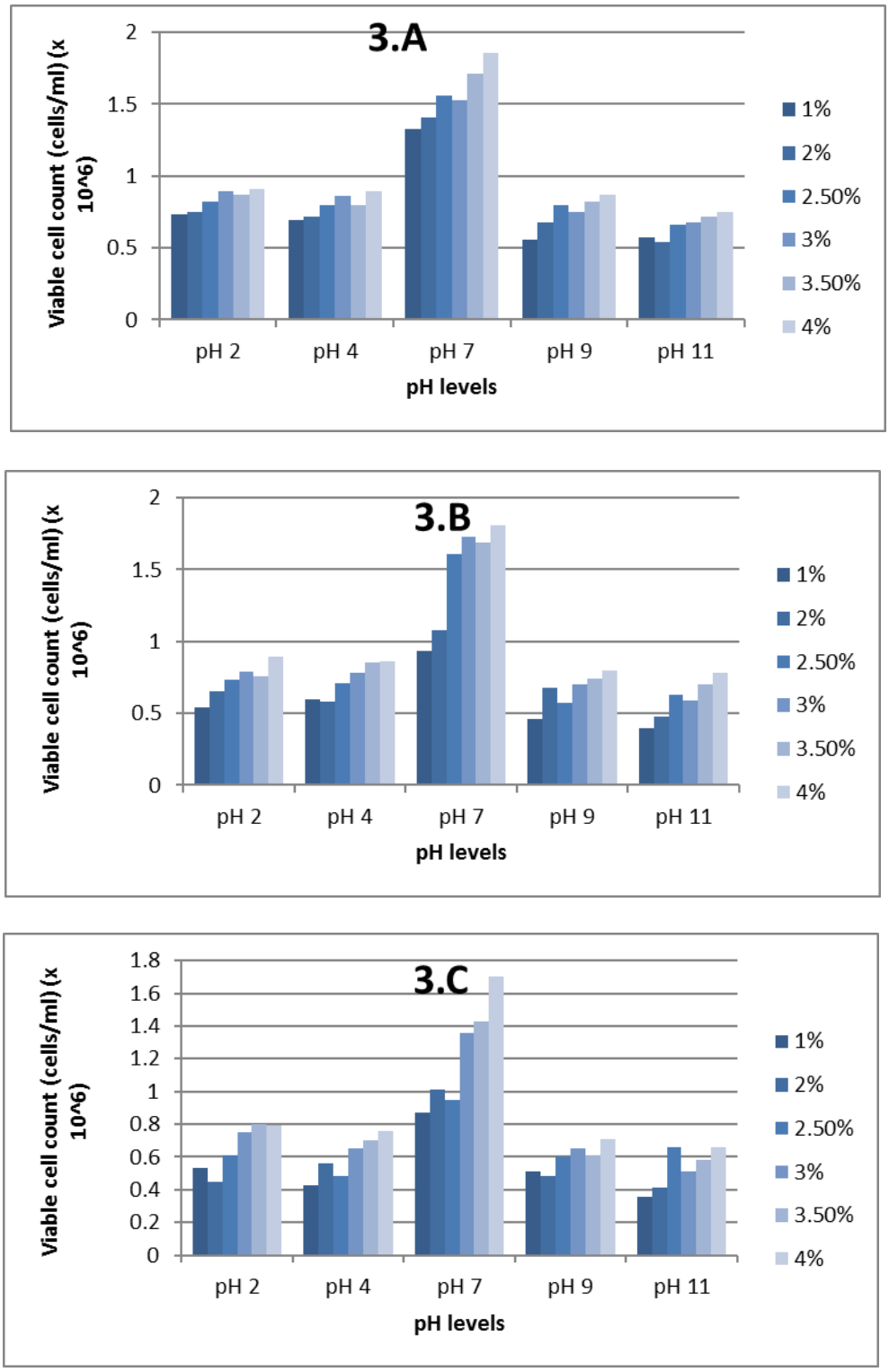
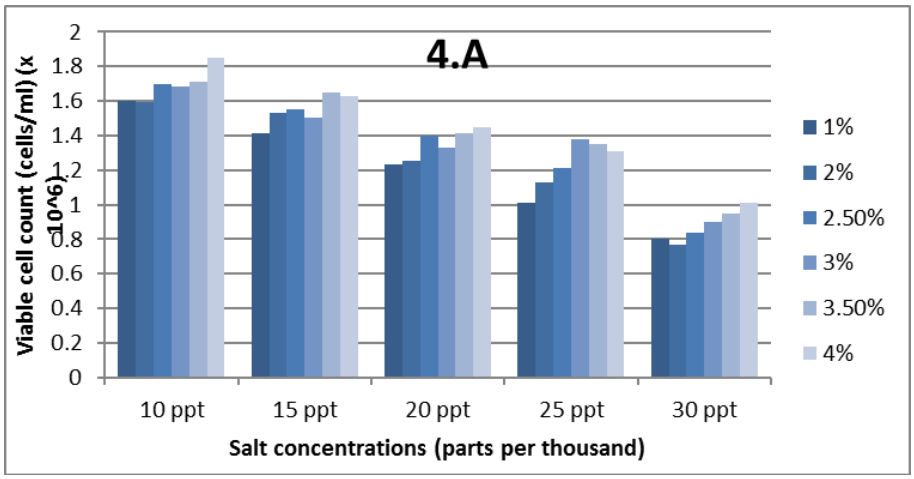
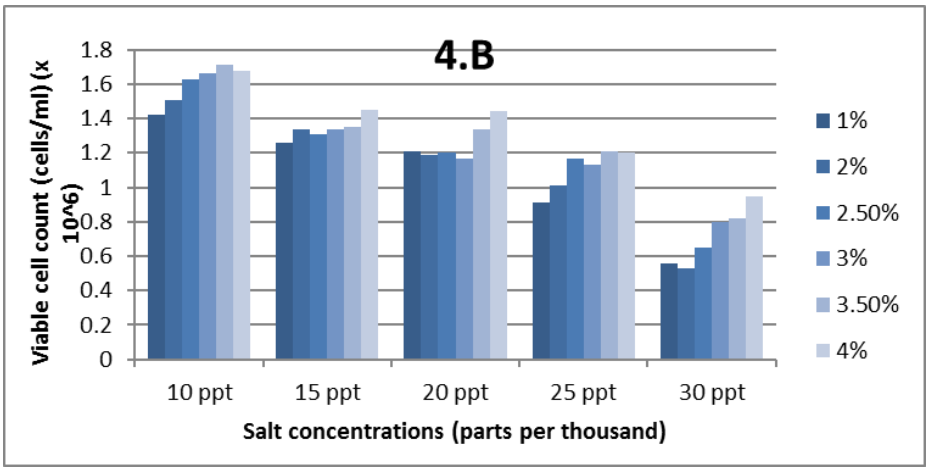


Figure 3: Survival of encapsulated cells in varying pH levels: (1.A) 3 h (1.B) 24 h (1.C) 48 h. The figures demonstrate the viable cell count of encapsulated probiotics in cells/mL. Higher cell count is seen at a moderate pH and also it decreases with time.

Survival of encapsulated cells in varying salt levels

Encapsulation with alginate concentration increasing from 1% to 4% improved the viability of cells at similar salt concentrations. Higher mortality of the probiotic cells was observed as the salt concentration increased from 10 ppt of NaCl to 30 ppt of NaCl, at a constant alginate concentration. The viability of probiotic cells however, decreased proportionately with the time of exposure to salt solutions.





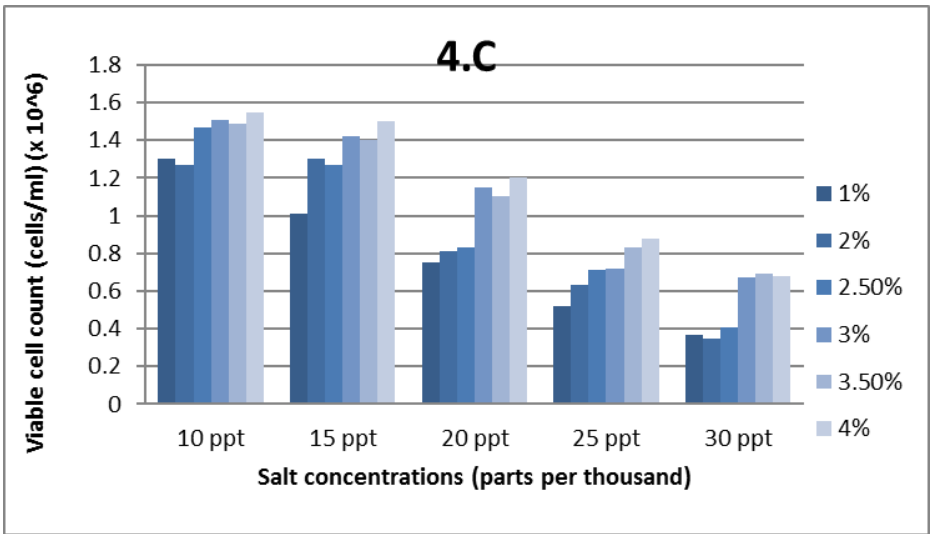


Figure 4: Survival of encapsulated cells in varying salt levels: (1.A) 3 h (1.B) 24 h (1.C) 48 h. The figures demonstrate the viable cell count of encapsulated probiotics in cells/mL. Higher cell count is seen at a lower salt concentration and also it decreases with time.

Release of encapsulated cells

The release of cells from capsules in the gut is essential for growth and colonization of probiotics; otherwise the microorganisms in the beads will be washed out from the body without exerting any beneficial effect. An efficient release of viable and metabolically active cells in the intestine is one of the aims of encapsulation.

IV. Conclusions

Encapsulation of probiotics in alginate beads resulted in its better survival. Increasing alginate concentrations had a positive effect on the survival of the probiotics in the harsh conditions of pH levels and salt solutions without significantly affecting the release of viable cells from capsules. They survived better at moderate pH and low salt conditions.

Further studies need to be carried out in order to monitor the effect of encapsulation on bacteria in the gut, using cells animal models, as well as studying other parameters such as initial cell numbers and cell type. Several parameters may determine the extent to which probiotic strains survive passage through the gastrointestinal tract, *viz.*, the degree of stomach acidity and the period of exposure to bile salts. However, *in vitro* studies, *viz.*, microbiological analyzes of fecal samples after feeding of the inoculated products are required, as other factors also play a role. Before reaching the intestine, probiotic bacteria must first survive the deleterious action of gastric juice during passage through the stomach. The studies on its survival and the efficacy in delivering the viable cells in vivo are needed for better application of probiotics in the development of functional foods.

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