The Andhra Agric. J 70 (2): 268-272, 2023
10.61657/aaj.2023.40

Optimisation of Growth Conditions of Probiotics (*L. acidophilus*) Encapsulated with Calcium Alginate Beads

K H Ajisha, C L Sharon, K A Athira and Simla Thomas
Department of Community Science, College of Agriculture, K A U, Thrissur, Kerala

ABSTRACT

In the present study optimisation of growth conditions of *L. acidophilus* MTCC 10307 in alginate beads was carried out with regard to substrate (growth media constituents- sorbitol, cocoa powder and corn starch), temperature (36, 38 and 40!) and inoculum concentration (100, 200 and 300µl). The study revealed that the maximum number of probiotic cells was found in sorbitol containing beads with the viability of 50.66×10^8 CFU/g (9.70 log CFU/g) than cocoa powder and corn starch. The optimum temperature reported was 38! (9.70 log CFU/g) and maximum probiotic count of 85.33×10^8 CFU/g (9.93 log CFU/g) was observed in encapsulated bead inoculated with 300µl of bacterial suspension. Microcapsule prepared with these conditions may help to protect, isolate and control the release of probiotics which is of growing interest in many sectors of food product development.

Key words: *L. acidophilus, microencapsulation, probiotics, sorbitol and sodium alginate*

The term 'probiotic' refers to a preparation of defined microorganisms, in sufficient numbers to alter the microflora in the intestinal compartment of the host and bring beneficial effects. Probiotics have been associated with mankind ever since people started consuming fermented milk and fermented foods. Probiotics aid in breakdown of proteins and fats in food and help to absorb vitamins, minerals and amino acids, efficiently. In addition to these, probiotic bacteria boost immune system and prevent or limit the growth of harmful bacteria like *Salmonella* and *E.coli*. Under natural conditions, the protective gut microflora is sufficient and there is no much need for bacterial supplements. Various factors that call for the need for probiotics are change in food habits, fast life, unhealthy living conditions and excessive consumption of antibacterial substances like antibiotics. The most widely used probiotic strains are lactobacilli, bifidobacterium and streptococci (Wolfe *et al.*, 2023).

A new approach to improve the probiotic survival is by physical protection by encapsulation which can protect the bacterial cells from the hostile conditions such as those present within gastrointestinal tract, thus potentially assuring viable cells. Microencapsulation can be done for a number of reasons like protection of reactive material from their environment and safe and convenient handling of the materials. The physical and chemical characteristics of the resulting microcapsules are determined by the choice of the right coating material. The coating polymer plays barrier between the core and the external environment. This prevents inadequate exposure to the core. The polymers form a membrane, which dissolves in reaction to specific stimuli (Han *et al.*, 2008).

The type of material chosen greatly affects the chemical and physical properties of the microcapsules. The polymer should be able to create a coherent film around the core substance. The coating should have the desired coating properties such as strength, flexibility, permeability, optical properties, and stability, as well as be chemically compatible and non-reactive with the core material (Kiyoyama *et al*., 2003). There are so many polymers that are preferred including ethyl cellulose, polyvinyl alcohol, gelatin, cellulose acetate phthalate and styrene maleic anhydride etc. The polymers could be hydrophobic, hydrophilic or both in nature. The film thickness depends completely on the core material and its physical properties (Singh *et al*., 2010).

Microencapsulation technologies work by protecting the probiotics from harsh environmental conditions, and increasing their mucoadhesive properties. Typically, the probiotics are either embedded inside or coated with food grade materials like biopolymers or lipids. In addition, protein free starch and gums are also suitable to produce hypoallergenic products that are targeted for people with protein allergy. In certain cases, additional components can be co-encapsulated to enhance the probiotic viability such as nutrients or protective agents (Yao *et al*., 2020). In any microencapsulation process, the medium and condition plays a critical role because they effect the viability of probiotics, product quality and yield and thus effecting the overall process. Hence, the present study was attempted to optimise the growth conditions to attain the maximum number of viable probiotic cells in the encapsulated form with regard to substrate (growth media constituents), temperature and rate of inoculum.

MATERIAL AND METHODS

Preparation of cells for encapsulation

Lactobacillus acidophilus maintained in the MRS agar slant was activated by transferring to MRS broth and incubating overnight at 37!. The activated cells obtained were then inoculated at two per cent level to 50 ml MRS broth and incubated at 37^{0}C for 19 h.

Encapsulation of bacterial cell

Encapsulation of bacterial cells with sodium alginate was carried out by extrusion technique (Malmo *et al*., 2013). The highly concentrated *L. acidophilus* suspension was mixed with 1ml of 2 per cent sodium alginate solution. Thorough vortex mixing of the alginate cell suspension was done to ensure complete embedment of cells into the gel matrix. This mixture was then carefully added drop by drop to 0.05M calcium chloride solution having a temperature of 29! using a sterile dropper with a diameter of 2 mm. Upon contact with the calcium chloride solution, the drops immediately formed spherical gels. The gel balls were retained in the same solution for 30 minutes to make it more rigid and stable. The beads containing probiotic cells were collected, washed with 0.1 per cent peptone water and stored in sterile containers at 4! till further assays.

Enumeration of viable cells in the bead

One gram of beads were initially homogenized in nine millimetres of normal saline using sterile mortar and pistle to ensure complete release of the cells. Appropriate dilutions of this were pour plated in MRS (De Man Rogosa and Sharpe) agar and incubated at 37^{o}C for 48 h. Colonies were counted and expressed as CFU/gram of beads.

Optimisation of substrate (growth media constituents)

One gram of either substrate (cocoa powder, sorbitol and corn starch) was added to 100 ml distilled water and boiled until it formed a gel, then sodium alginate was added and stirred until they were dissolved. The viability of probiotic organism in the encapsulated beads were assessed using MRS medium. One gram of the beads measured and transferred to a test tube containing 9ml sterile distilled water (10^{-1} dilution). This was then serially diluted up to 10^{-9} dilutions. The microbial enumeration was done by pour plate method using MRS agar and the results are expressed as CFU/g. The most suitable substrate was selected based on the viability.

Optimisation of temperature

The encapsulated beads of *L. acidophilus* with suitable substrate was taken and incubated at varying temperatures of 36^{0}C, 38^{0}C and 40^{0}C. Viability of *L. acidophilus* was tested after incubation and expressed as CFU/g.

Optimisation of rate of inoculum

From the highly concentrated *L. acidophilus* suspension, 100µl, 200µl and 300µl was taken and mixed with 1ml of 2 per cent sodium alginate solution. Encapsulation of probiotic *L. acidophilus* was carried out. After this, the viability of probiotic organism was enumerated and expressed as CFU/g.

RESULTS AND DISCUSSION

Optimisation of substrate (growth media constituents)

The viability of probiotic organism in the encapsulated beads were assessed by adding different growth media constituents (cocoa powder, sorbitol and corn starch). It is clear from Table 1 that, the sorbitol containing beads exhibited maximum growth

followed by cocoa powder and their probiotic counts were 50.66 and 44×10^8 CFU/g respectively. Several studies have reported the optimisation of growth conditions for probiotic encapsulation. Corn syrup, sorbitol and maltodextrin are commonly used substrates for encapsulation process. When an appropriate mix of sorbitol and maltodextrin was used as carrier media to encapsulate *L. paracasei* by spray cooling, high bacterial viability was obtained due to its high osmotic pressure (Semyonov *et al*., 2010). Two potential probiotic yeasts *Saccharomyces cerevisiae* (strain KTP) and *Issatchenkia occidentalis* (ApC) were microencapsulated using maltodextrin and sorbitol with an aim to improve its effectiveness by spray drying. The study provided an evidence for incorporation of sorbitol to enhance the encapsulation efficiency (35 to 45%) by spray drying (Suryabhana *et al*., 2019). Khodadadi *et al*. (2022) studied the effects of sorbitol, skim milk and yeast powder on survival of *Lactobacillus rhamnosus* encapsulated with alginate during one week storage at room conditions. The use of yeast powder and sorbitol with alginate increased the encapsulation efficiency of *Lactobacillus rhamnosus* from 51 to 97 per cent respectively. The skim milk with alginate and sucrose resulted in the highest survival rate after one week of storage at room conditions. Therefore, encapsulation of *Lactobacillus rhamnosus* with sorbitol and skim milk can be effective for survival of these bacteria.

Table 1. Viable count of microencapsulated *L. acidophilus* at various substrates

Sl. No	Substrates	Viable counts (×10^{8}CFU/g)
1	Cocoa powder	44 -9.64
2	Sorbitol	50.66 -9.7
3	Corn starch	42.33 -9.62

All values are means of three independent enumerations
Figures in parenthesis indicates log CFU/g

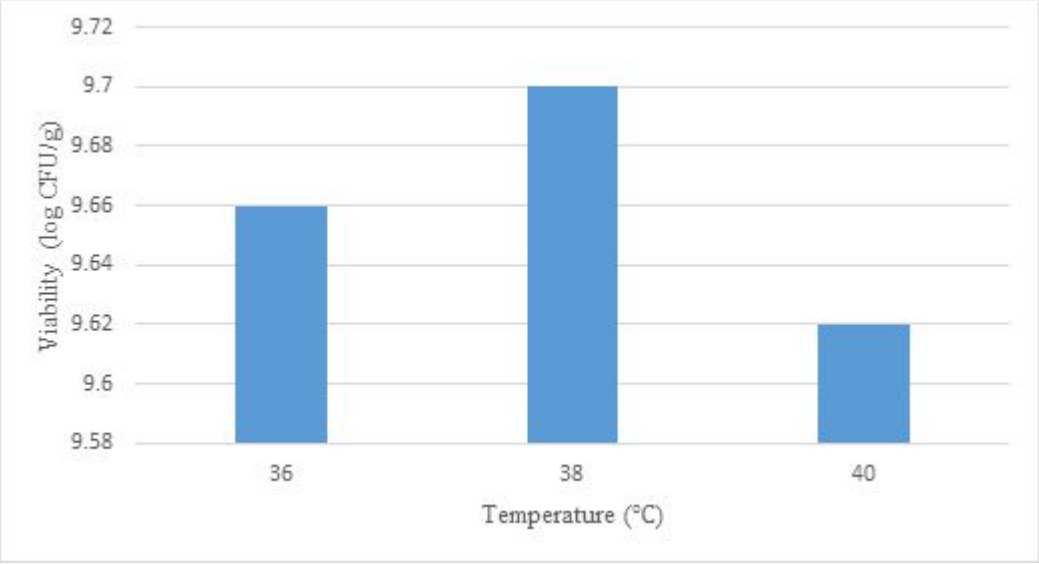

Figure 1. Viability of encapsulated *L. acidophilus* at different temperatures

Optimisation of temperature

The probiotic activity of encapsulated *L. acidophilus* with different temperature (36, 38 and 40 !) were carried out and tabulated. The results are represented in Figure 1. From the data, it is clear that the optimum temperature was found to be 38! (9.70 log CFU/g) followed by 36! (9.66 log CFU/g) respectively. Praepanitchai *et al.* (2019) standardised

the optimum temperature of 37! to enhance the survival of the encapsulated probiotics (*Lactobacillus plantarum*) in alginate-soy protein isolate-based hydrogel beads. Probiotic bacteria, namely *L. casei* (DSM 20011), *L. reuteri* (DSM 20016) and *L. delbrueckii* (DSM 20081) were microencapsulated with the temperature of 37! in sodium alginate and the beads had a probiotic viability greater than 8 log CFU/g in fruit based beverages (Olivares *et al*., 2017). Ashrafuzzaman *et al*. (2015) optimised the temperature of growth for *L. acidophilus* and they reported the maximum activity of the bacteria at 37! and no growth at 45!. Rojnik *et al*. (2005) evaluated the inûuence of various preparation temperatures on particle size and morphology of capsule. According to them, at optimum temperature (between 30 to 40!), average size of microsphere was found to increase, surface become smooth and particle size distribution widen signiûcantly.

Optimisation of inoculum concentrations

The table 3 shows the viable count of encapsulated *L. acidophilus* at various inoculum concentrations (100 µl, 200 µl and 300 µl). The viability of encapsulated *L. acidophilus* ranged from 48.66 to 85.33×10^8 CFU/g. The probiotic count tend to increase from 100 µl to 300 µl and the maximum growth was observed in 300 µl concentration (9.93 log CFU/g). Sharon *et al*. (2015) optimised the growth conditions for *L. acidophilus* (MTCC 447) in banana based food mixture. The study reported that maximum cell count observed at temperature of 37! and an inoculum concentration of 300µl. The study conducted by Wardani *et al*. (2017) to know the effect of inoculum concentration and incubation temperature on the growth of *L. plantarum*. The results show that increase inoculum concentration from 1 to 5% increased the viable cells whereas higher incubation temperature beyond 37°C decrease the cell growth. A similar result was stated by Remya (2020) who developed jackfruit based food probiotic mixture, the maximum viable numbers of *L. acidophilus* was observed at 37! (85×10^9 CFU/ml). The maximum probiotic count was observed when 50g substrate was inoculated with 300µl of *L. acidophilus* MTCC 10307.

The study concluded that maximum viability of *L. acidophilus* was observed in microcapsule containing sorbitol, with 300µl of bacterial suspension inoculated at temperature of 38!. Microencapsulation of probiotics has been applied in a wide variety of products from different areas, and studies have shown an enormous potential to provide the core with advantageous features, resulting in superior quality products.

Table 3. Viable count of encapsulated *L. acidophilus* at various inoculum concentrations

Sl. No	Concentration of inoculum (µl)	Viable counts (×108CFU/g)
1	**100**	**48.66** **-9.68**
2	**200**	**67** **-9.82**
3	**300**	**85.33** **-9.93**

All values are means of three independent enumerations
Figures in parenthesis indicates log CFU/g

LITERATURE CITED

Ashrafuzzaman M, Momtaz F, Foysal M J, Ali M H and Rahman M M 2015. Optimization of probiotic activity of Lactobacillus acidophilus characterized from commercial yoghurt *International Journal of Biological Science*, 7:94-99

Han B, Shen B, Wang Z, Shi M, Li H, Peng C, Zhao Q and Gao C 2008. Layered microcapsules for daunorubicin loading and release as well as *in vitro* and *in vivo* studies *Polymers of Advanced Technology*, 19: 36-46

Khodadadi P, Tabandeh F, Alemzadeh I and Soltani M 2022. Effects of sucrose, skim milk and yeast powder on survival of Lactobacillus rhamnosus GG encapsulated with alginate during one-week storage at room conditions *Applied Food Biotechnology*, 9: 251-259

Kiyoyama S, Shiomori K E A, Kawano Y and Hatate, Y 2003. Preparation of microcapsules and control of their morphology *Journal of Microencapsulation*, 20: 497-508

Malmo C, Storia A L and Mauriello G 2013. Microencapsulation of *Lactobacillus reuteri* DSM 17938 cells coated in alginate beads with chitosan by spray drying to use as a probiotic cell in a chocolate soufflé *Food Bioprocessing Technology*, 6: 795-805

Olivares A, Silvaa P and Altamirano C 2017. Microencapsulation of probiotics by efficient vibration technology *Journal of Microencapsulation*, 34: 667–674

Praepanitchai O A, Noomhorm A and Anal A K 2019. Survival and behaviour of encapsulated probiotics (*lactobacillus plantarum*) in calcium-alginate-soy protein isolate-based hydrogel beads in different processing conditions (pH and temperature) and in pasteurized mango juice *Biomed Research International*, 27:1-8

Remya P R 2020. Process optimisation and quality evaluation of jackfruit based probiotic food products. PhD Thesis, Kerala Agricultural University, Thrissur, Kerala

Rojnik T A, Frlan R, Bogataj M, Bukovec P and Mrhar A 2005. Effect of preparation temperature in solvent evaporation process on eudragit RS microsphere properties *Chemical and Pharmaceutical Bulletin*, 53: 143-146

Semyonov D, Ramon O, Kaplun Z, Levin-Brener L, Gurevich, N and Shimoni E 2010. Microencapsulation of *Lactobacillus paracasei* by spray freeze drying *Food Research International*, 43: 109-203

Sharon C L, Aneena E R and Lakshmy P S 2015. Effect of probiotic fermentation on the *in vitro* digestibility of starch and protein of banana based food mixtures *International Journal* of *Development Research*, 1: 58-82

Singh M N, Hemant K S Y and Shivakumar H G 2010. Microencapsulation: A promising technique for controlled drug delivery *Research in Pharmaceutical Science*, 5: 65-77

Suryabhana P, Kunyeit L and Appaiah A 2019. Sucrose and sorbitol supplementation on maltodextrin encapsulation enhance the potential probiotic yeast survival by spray drying *Food Science and Technology*, 107:243-248

Wardani S K, Muhammad C, Endang R and Tyas U 2017. The effect of inoculum size and incubation temperature on cell growth, acid production and curd formation during milk fermentation by *Lactobacillus plantarum* Dad *13 International Food Research Journal*, 24:921-926

Wolfe W, Xiang Z, Yu X and Li P 2013. The Challenge of applications of probiotics in gastrointestinal diseases *Advanced Gut and Microbiome Research,* 2023:1-10

Yao M, Xie J, Du H, Clements D J, Xiao H and Li L 2020. Progress in microencapsulation of probiotics: A review *Comprehensive Review of Food Science and Food Safety*, 19:857-874

Received on 13.03.2023 and Accepted on 21.05.2023