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Research Article

MICROENCAPSULATION OF PROBIOTIC *LACTOBACILLUS PLANTRAUM CM25*

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ABSTRACT
Research regarding the use of live bacterial cells for therapeutic purposes has been rapidly growing over the years and has generated considerable interest to scientists and health professionals. Probiotics are defined as essential live micro-organisms that, when administrated in adequate amounts, confer a health benefit on the host. Various health benefits have been attributed to probiotics such as anti-mutagenic, anti-carcinogenic, anti- infection properties, immune system stimulation and nutritional enhancement. Further the survival of these bacteria in the human gastro intestinal tract is questionable. To overcome these problems, the aim of this study to microencapsulate the live probiotic bacterial strain namely *Lactobacillus plantarum* CM 25 was isolated from cow milk, on MRS medium supplemented with 0.2 % of sodium taurcholate and sodium glucocolate and was already characterized using PCR assay and biochemical activities aided with PIB Bryant software. The microencapsulation was done using an alignate, extrusion, coacervation microencapsulate methods. In the alginate method of microencapsulate production approx. 130-150g (contained 108- 109 cfu/g probiotic bacteria), semi hard, white, spherical wet microencapsules of diameter approx. 500-700 um were obtained and by the extrusion and coacervation method hard, white, spherical microencapsulates were obtained respectively.

Key Words: *Probiotic, Encapsulation, Lactobacillus Plantarum Cm 25*

INTRODUCTION
Modern consumers expect their food to be healthy and to prevent illness as they are increasingly interested in their personnel health (Kailasapathy, 2009). This explains the reason for a rising interest in a probiotic health based products. Probiotic live bacteria are recognized as good or friendly bacteria and thought to be reducing potentially harmful bacteria from the intestine. Therefore, these live bacterial micro-organisms can improve microbial balances in intestine and exert positive health effects on the host. Probiotic is a term that means “for life” and defined as “Live microorganism that beneficially affects the host health by improving its microbial balance” (Fuller, 1989). More recently probiotics have been defined as “Live microorganisms that when administrated in adequate amounts, confer a health on the host” FAO\WHO, 2002). Species of *Lactobacillus acidophilus, L caesei, Bifidobacterium bifidum, B longum. B breve*s are the most popular bacteria applied food probiotics products (Daly and Davis, 1998, Macfarlane and Cummuigs, 1998, Champagne and Fustier, 2007). The probiotic health benefits may be due to as these bacteria causes proteolysis breakdown of protein into easily assailable compounds. These bacteria also produce bacteriocin\acid to inhibit the growth of similar or closely related bacterial strains (Chen and Chen 2007). Probiotics have been recorporated into a wide varities of food including dairy products (such as yogurt, cheese, ice creams, dairy desserts), but also in non dairy products (such as chocolates, cereals, juices) (Anal and Singh, 2007). Viability loss of probiotics in food products and acidic bile condition of gastrointestinal tract has always encouraged researches to find new efficient methods of viabiality improvement. Microencapsulation is one of the newest and most efficient methods, has recently been used especial consideration and investigation. This is a defined as the process of entrapment/enclosure of micro-organisms cells by means of coating them with proper hydrocolloids in order to segregate the cells from surrounding environment. In the previous study *Lactobacillus plantarum*

CM25 has been isolated from cow milk and molecular characterization up to species level (Singhal *et al.*, 2009). We aim of this study to obtain the encapsules of this isolate.

MATERIALS AND METHODS

Bacterial Strain and Culture Condition

Lactobacillus Plantarum: CM 25 isolated from the cow milk sample and was previously identified on the basis of morphological, biochemical and upto genus using 16S rRNA sequencing PCR. The selected culture was subjected to characterization for functional and probiotic attributes. *Lactobacillus plantarum* CM 25 was grow in MRS broth (De *et al.*, 1960) and maintained in litmus milk at 4^0C.

Techniques of Microencapsulation

Alginate Method: Alginate (3%w\v) was added to double distilled water, autoclaved and allowed to cool to 25°C. To this material add suspension of *Lactobacillus plantarum* CM25 cells (in 0.1% peptone and 250 mM glycerol) at number of 10 log cfu\ml was added in ratio of about 1.5 (v\v) to obtain microcapsules containing about 9 log cfu\ml. Add this to 0.5M CaCl2 solution through syringe needle drop by drop. Alginate hydrogels are extensively used for cell encapsulation (Rowley *et al.*, 1999).

Extrusion Method: Lactobacillus plantarum CM25 that was cultivated on MRS broth for 40 hours at 37oC anaerobically. After 2 days cells were harvested by centrifugation at 1500g for 15 minutes at 25°C and washed twice with sterile saline solution. The pellet was resuspended into the MRS broth and was divided into two parts. One part is used for microencapsulation and other was added as free cells without capsules. This mixture was infused with a magnetic bar and placed through a syringe and sprayed through a flask containing 1000 ml of 0.5 M CaCl2 solution under gentle stirring with a magnetic flask Gouin (2004).

Coacervation/Phase Seperation Method: In this method first the core material (usually culture of *Lactobacillus plantarum* CM25) is dispersed into a polymer solution (e.g. Gelatine). The second polymer (e.g. Canada balsam) solution is then added to the prepared dispersion. Deposition of shell material onto the core particles occurs when the two polymers form a complex. This process is triggered by the addition of salt or by changing pH, temperature or by dilution of the solution (Gouin, 2004).

RESULTS AND DISCUSSION

Microencapsulation (ME) is a physicochemical or mechanical process to entrap a substance in a material in order to produce particles with diameters of a few nanometers to few millimetres (Chen and Chen, 007). Probiotic encapsulation is used to protect the cells against an adverse environment more than controlled release (Champagne and Kailaspatty 2008, Zuidam and Shimoni, 2009). Encapsulation gives a structure and allows creating new function and innovative systems (Poncelet *et al.*, 2007) for probiotic products. The reasons for microencapsulation are countless. In some cases, the core must be isolated from its surroundings, as isolating vitamins from the deteriorating effects of oxygen, retarding evaporation of a

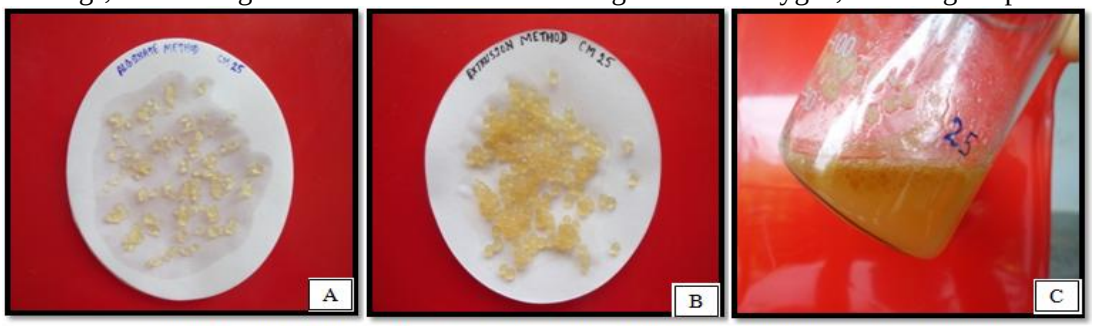

Figure 1-A, 1-B and 1-C: Microencapsules of isolate *Lactobacillus plantarum* CM 25obtained by Alginate method, Extrusion method and by Coacervation method respectively.

volatile core, improving the handling properties of a sticky material or isolating a reactive core from chemical attack. In other cases, the objective is not to isolate the core completely but to control the rate at

which it leaves the microcapsule, as in the controlled release of drugs or pesticides. The problem may be as simple as masking the taste or odour of the core, or as complex as increasing the selectivity of an adsorption or extraction process. The various methods are available at industrial and laboratory level to obtain the microencapsule but at present, at laboratory level some techniques are viable like alginate method extrusion method and coacervation/Phase separation Method. Alginate method is the most important method of microencapsulation production. After applying this method approx. 130-150g (contained 108-109 cfu/g probiotic bacteria), semi hard, white, spherical wet microencapsules of diameter approx. 500-700 um were obtained. In Extrusion Method and Coacervation/Phase Separation Method, hard, white, spherical microencapsulates were obtained successfully.

CONCLUSION

Probiotics have been defined as ‘‘live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host’. Probiotics taken orally can destroy the acidic condition of the stomach so number of micro-encapsulation technique has been developed to overcome this problem. Microencapsulation is the process by which individual particles or droplets of solid or liquid materials (the core) are surrounded or coated with a continuous film of polymeric material (the shell) to produce capsules in the micrometer to millimeter range known as microcapsules. Use of different encapsulation technologies for protection of health ingredients achieved high ingredients efficiency. It not only depends upon developing or choosing the right encapsulation technique but also requires expertise in food processing. Probiotic therapy is based on the concept of healthy gut microflora. The delivery of viable micro encapsulated probiotic bacteria will become important in the future. Future research could be concentrated on the aspects such as applying more efficient encapsulation materials or improving the common used ones; consideration of probiotic encapsulation in the products which have still been investigated or few evidences are present about them.

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