MILK BASED EXTRUSION METHOD FOR MICROENCAPSULATION OF PROBIOTICS

Project report submitted in partial fulfillment of

Degree of Masters of Technology

in

BIOTECHNOLOGY

Submitted By:
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Under the Supervision
of
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2018

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Certificate of Originality

This is to certify that the work entitled **"Milk based extrusion method for microencapsulation of probiotics"** submitted by **Manisha Singh** in partial completion of the requirements for the award of degree of Masters of Technology in Biotechnology, of Jaypee University of Information Technology, Solan, has been carried out under the supervision of **Dr. Gunjan Goel**. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of Supervisor

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Acknowledgements

All praise belong **the almighty lord** to whom we thank for the strength, courage and perseverance bestowed upon me to undertake the course of the study. I feel privileged to express my deep sense of gratitude to my revered mentor, **Dr. Gunjan Goel**, Associate professor, biotechnology, for valuable guidance, suggestions, support, constant encouragement, cooperation and providing requisite facilities to carry my project which otherwise would have remained incomplete. I gratefully acknowledge with deep gratitude, the cooperation and help given by Mr. Sampan Attri, Ms. Kavita Sharma, Mrs. Rajni Kashyap, Mr. Deepak Sharma and all members of Jaypee University in helping with my project. I feel indebted to Dr. Sudhir Syal, Head of Department of Biotechnology and Bioinformatics, Jaypee University of Information and Technology for his support and guidance. I would like to thank my institution and members of technical and non-technical staff of the department without whom this project will be a distant reality.

Manisha Singh

Abstract

Probiotics are the live microorganism which when administered in adequate amount imparts health and nutritional benefits to the host. When they are ingested orally they have to go through low gastric pH and bile salts that decrease their survival to reach to the colon. Also when free probiotics are incorporated into product then they have to go through different physiological and chemical conditions during food processing and storage as a result of which their viability count decreases. Therefore, to improve the viability count of probiotics they are microencapsulated which provide them a suitable microenvironment helping them to maintain their viability count. In the present study microencapsulation using extrusion with sodium alginate and skimmed milk (coating material) in the 1:1 ratio was conducted for already reported proboitic *Lactobacillus gastricus BTM7*. Calcium Chloride was used as a hardening solution. Free and microencapsulated bacteria were evaluated for their viability count in gastrointestinal condition and during storage. The encapsulation increased the survival of probiotics under simulated gastric and pancreatic conditions as compared to free cells. During the storage in skimmed milk based media, a higher survival of 96.1% was obtained for microencapsulated probiotics as compared to 84.1% survival of free cells. Therefore, these results indicate that microencapsulation provides protection to probiotics against gastrointestinal conditions and during storage of the probiotic food products.

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List of Abbreviations

PBS	Phosphate buffer saline
LAB	Lactic acid bacteria
SCFA	Short chain fatty acid
GRAS	Generally regarded as safe
GI	Gastro intestinal
CFU	Colony forming unit
µl	Microlitre
G	Gram
mL	Millilitre
M	Molar
L	Litre
MRS	deMan Rogosa Sharpe
SA	Sodium Alginate
SASM	Sodium Alginate-Skimmed milk
Rpm	Revolution per minute
$CaCl_2$	Calcium Chloride
NaCl	Sodium Chloride
SIJ	Simulated intestinal juice
SGJ	Simulated gastric juice

1.Introduction

Probiotics are the live microorganism whose recommended intake imparts various health and nutritional benefits to the host. These probiotics are projected to colonize and proliferate our gut microflora to fight disease caused by pathogens by competing with them for adherence to the gut lining and also by producing or aiding to our immune system to produce substances that are toxic to these disease causing pathogens. All the lactic acid bacteria are considered probiotic. They have the capability of fermentation, so when they are incorporated into food product they break down complex food components into simpler structure that makes its digestion and absorption easier. Different strains of bacteria used as probiotics are *Lactobacillus bulgaricus, Lactobacillus gastricus, Lactobacillus paracasei, Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactococcus lactis, Streptococcus thermophilus,* etc.

When probiotics are incorporated into food product, then during food processing and storage their viability gets affected. The do not withstand different physiological and chemical conditions through which the food in which they are incorporated as a result of which their viability gets decreased and also their sensory properties are also lost to same extant. When they incorporated into free state, they may grow and proliferate in the food product itself and thus the quality of food gets affected, as while proliferation they may produce lactic acid and other secondary metabolites giving product a bitter taste. When these probiotics are administered orally they may not be able to reach the target in recommended amount or even they reach to target place in ample amount but during passage through GI tract they may loose their sensory properties as they have to pass through varied pH range and bile salt and thus are unable to produce desired health and nutrional effect to the host. Drawbacks linked with probiotics when they are incorporated into the food product for losing their viability

count during food manufacturing and storage, lack of targeted delivery when ingested orally so that they reach the target in ample amount made it necessary to provide these probiotics a microenvironment which can give protection to them against different physiological and chemical conditions during food processing and storage, protection against harsh acidic and salt conditions in the GI tract and also facilitate in the targeted delivery of these probiotics. Microencapsulation of probiotics gives it a microenvironment which protects the probiotic against harsh condition during food processing and also aid in the targeted delivery of probiotics by using matrices which are pH sensitive and thus leads to release of probiotics at targeted place in the gastrointestinal tract. On the basis of these drawbacks my project was designed with three objectives as follows:

a) To microencapsulate the probiotic bacteria in milk based matrix.
b) To compare the survivability of free and microencapsulated probiotics in simulated gastrointestinal condition.
c) To evaluate the viability of probiotics during storage at 4˚C.

2.Literature Review

Around 400 bacterial species that has been identified that have powerful metabolic achievements and are of huge significance and useful for human health. This characteristics of bacteria have make them of great importance and really helpful accept those which are pathogenic and are not good for human health. Their ecology gets reduced or affected in a bad way when come in contact with different toxins or exposed to them in the form of polluted water or food as well as use of antibiotics as a result of which their microbial count gets reduced adversely [1,2]. Awareness of complex correlation between food and health has challenged researches to propose functional food that not only provide basic nutrition but also has health benefit properties [3]. As these days due to change in eating habits, increased pollution our body have become very susceptible to different disease as a result of which it has got huge attention towards the utilization of foods and beverages containing these health benefiting probiotic microorganisms which is currently growing worldwide on huge scale [4].

2.1 Probiotics

Probiotics are the live microorganisms which when administered in configuered amount shows health benefits to cosumer. Thes probiotics colonize our gut microflora and impart various health benefits. Probiotic bacteria should be able to survive at varied low and high pH and stay alive in GI pH and enzymes. They should be able to maintain their sensory properties in the intestinal tract that contains bile salts in specific concentrations, they should be able to adhere on to the intestinal lining cells, should have long shelf life, should proliferate, substitute and restore the intestinal microflora and they should provide

clinically proven benefits to which they were intended. Probiotics used as food additives should be of human origin.

2.2 Health benefits of probiotics

Anti-atherogenic and cholesterol-lowering attributes of probiotics makes it beneficial for daily intact as different cardiovascular diseases is a situation in which the main arteries supplying heart with blood and oxygen are no longer able to performance efficiently, this happens due to increase of cholesterol level into the blood. Probiotics have potential to decrease the cholesterol level in the blood [5]. It also helps with diabetes and obesity- Improving the gut microflora helps to fight obesity and prevention and treatment of diabetes [6].

Health benefits imparted by probiotics to the host include antimicrobial properties of probiotics. Antimicrobial activity of probiotic is one of the most effective mode of action which includes production of bacteriocins, hydrogen peroxide, diacetyl by probiotics which are anti-microbial components that is used as defense against pathogens, competitive inhibition of probiotics with disease causing pathogenic bacteria, inhibition of bacterial adherence or translocation on the GI tract by competing with harmful bacteria and thus eliminating them from the GI track, reduction of luminal pH by the production of lactic acid, citric acid and hippuric acid thus generating unfavourable environment for disease causing bacteria as a result they are not able to colonize and proliferate in the GI track. Probiotic bacteria can also enhance intestinal barrier function by increasing the production of mucus in the lining of GI track making

it difficult for disease causing bacteria to enter the cell lining [3].

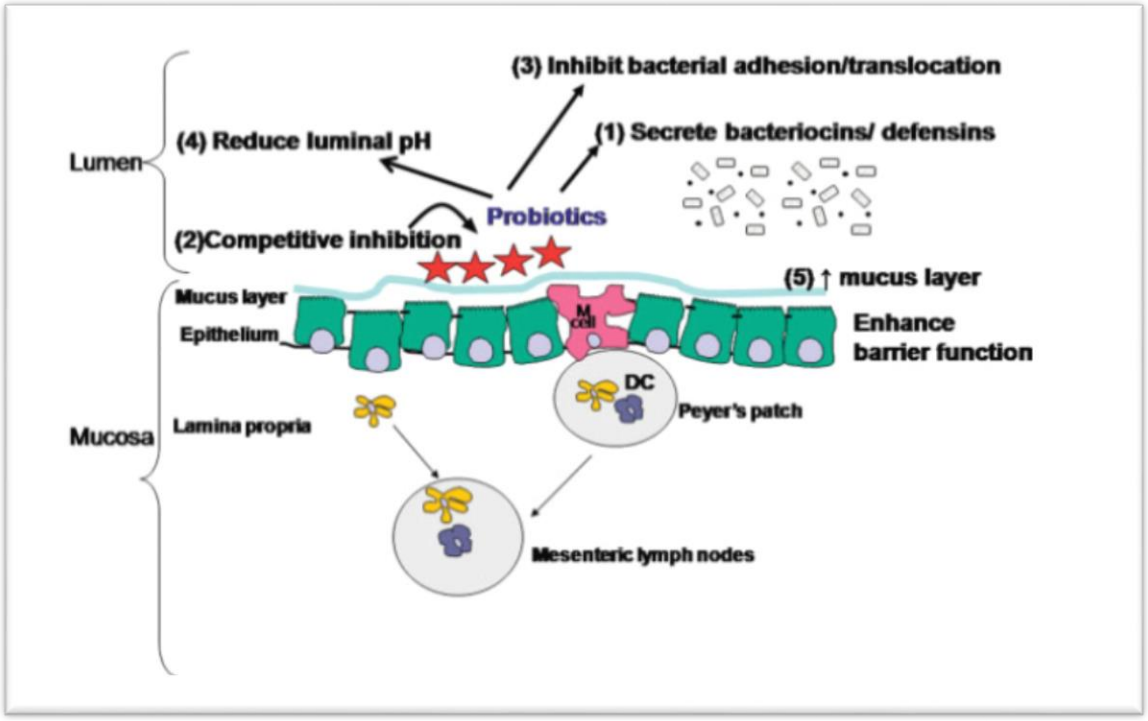


Fig.1. Mode of action of probiotics by antimicrobial activity

Figure.1 represents the antimicrobial activity mechanism of probiotics in which (1) shows the secretion of bacteriocin against disease causing bacteria, (2) shows competitive inhibition of probiotics with pathogenic bacteria, (3) shows inhibition of probiotic competes with pathogens and disease causing bacteria to eliminate them from the GI track, (4) depicts reduction of intestinal pH by the production of different acids and finally (5) increasing mucus production and thus acting as intestinal barrier by preventing the entry of disease causing bacteria.

Anticarcinogenic properties is another property of probiotics as they have the ability to inhibit the growth of organisms that are usually found in the gut microflora that have high activities of enzymes such as β-glucuronidase nitroreductase, azoreductase, and β-

glycosidase or the capability for nitrosation that converts procarcinogens into carcinogens [4]. They help in impoving digestive health. They are considered very helpful in treatment and preventing different GI diseases. The strain of probiotic used in the treatment varies depending on the type of disease that has to be treated. Including foods rich in good bacteria and using probiotic supplements may help to provide protection from inflammatory bowel diseases, including ulcerative colitis and Crohn's disease.

They may also helpful in treatment of mental illness. Probiotics have shown its affect on reduction in depression symptoms, according to a study that has shown clear evidence [23]. Another study also showed a very suprising observation including the impact of probiotic on the symptoms of autism. It was observed that a large number of patients with the disorder had number of digestive issues based on which it was concluded that altering and improving the quality of gut bacteria may not only benefit the digestive system but also the symptoms of autism [24].

They also affect the expression of tumor suppression genes and various other that are involved in boosting immunity. They also play an important role in cell signaling, apoptosis, cell growth, cell differentiation by aiding to cyclins, caspases, oncogens , etc which regulate these processes. They enhance short-chain fatty acid production as probiotics are mainly involved in the incentive of intestinal fermentation, the stimulation of SCFA assembly is one of the essential factors for the beneficial property exerted by probiotics.

3.3 Parameters for probiotics

Now before probiotics are considered safe for intake they have to under different test and clinical trials to ensure that it does not have any health hazards that it might cause to host after consumption. There are different properties essential for successful probiotics that includes that it has to be GRAS certified. Probiotics used should be listed on the probiotic list certified by GRAS after testing different strains only after which it is considered safe for consumption. The other one is adhesion property i.e. probiotic should have ability to adhere the gut. The next important parameter is that it should have bile and acid stability. Tolerability to GI salts and varied pH is important because sometimes even if the probiotic is able to survive throughout the GI tract, but they lose their sensory properties and as result they are not able to effectively show up the health benefits to host. Colonization and immunogenicity is another important parameter as for probiotics to show their health benefit it is very important that they are able to colonize our gut and proliferate. If they are not able to maintain their population then they won't be able to show the required health benefits for which it was intended. Also after its intake it should not be immunogenic to body or otherwise it might be considered as foreign microbe as a result it the body will have an immune response towards it.

3.3 Issues arised due to incorporation of free probiotics in food product

From application point of view it is very necessary that it should be compatible with food. When probiotic is incorporated into food product it should be able to survive and retain its sensory properties without spoiling the food. Their viable count in the food product should be atleast 10^7 cells per g or mL of a food so that the food is considered as

probiotic product. It should also be able to survive during storage. It should be able to withstand different chemical and physiological conditions during food manufacturing and storage.

2.5 Properties of LAB

LABs are used widely in industries as these organisms have fermentative ability and imparts numerous health and nutritional benefits to host. Some studies have shown in past that they give fermented foods distinctive flavour and texture which may vary from strain to strain depending upon which strain has been used in the implemented application for example in production of yougurt *Strpticoccus thermophilus* and *Lactobacillus bulgaricus* is used together which give yogurt the required flavor and texture. The fermentation using LAB improves nutritional value of food products in which they are incorporated by increasing the quality, availability for absorption through lining of intestine, digestibility and assimilability of nutrients as these LABs break the complex form of food into simpler as a result it reduces the burden of stomach for digestion. The essential role of LAB is growth inhibition of food spoilage and pathogenic bacteria that are not good for health and thus improves safety of food for human consumption and food products shelf life. Many beneficial aspects are atributed to LAB regarding human health, such as on gut microbial ecology, lactose digestion, mineral absorption and some other beneficial effects. Since the Metchnikow thesis (1908) LAB have been considered to posses probiotic properties. The parameters that make LAB a

probiotic and how these parameters helps to show its effect on the host is shown in Fig.2.

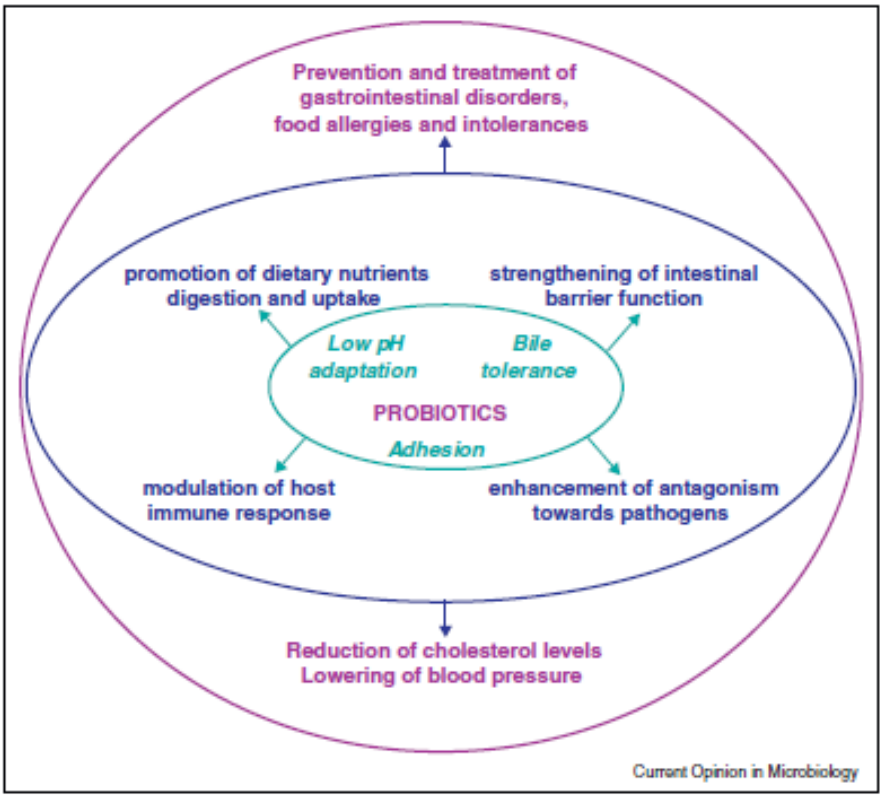


Fig. 2. Parameters and effects of probiotics

2.6 Need of probiotics and associated limitation

In today's scenario due to changing life style with time that also includes eating habits, has led to decline in gut microbiota of human body which makes our body susceptible to various diseases. Microbiota of gut helps our body to fight disease causing pathogens invading the gastrointestinal tract by inducing defence mechanism of our body. They compete with the pathogenic bacteria for adherence and their multiplication in the intestinal lining and not only this, they either themselves or assist our immunity to produce substances that are harmful for pathogens which leads to their death. Microbiota population can be elevated by adequate intake of probiotics via probiotic loaded food

products or various probiotic calsules available in market. To confer the health benefits for which it is intended, probiotics must survive in the product during manufacture and storage of food in which it is incorporated as they have to go under different physiological and chemical conditions like low pH, hydrogen peroxide, dissolved oxygen content, storage temperature, species and strains which may affect the viability of probiotic bacteria in food products [12-14]. They should also able to survive transit through the harsh conditions of gastric environment and different salts that are released in the gut to which these probiotic may be sensitive and affect their sensory property. They should also be able to reach the large intestine in adequate amounts to enable colonization and proliferation [7]. It is only when they will impart the desired health nutritional benefit if they reach their target and also in required amount. The standard for any food sold with health claims from the addition of probiotics is that it must contain per gram at least 10^6-10^7 log CFU of viable probiotic bacteria [8]. If the foods containing these probiotics do not meet the parameter of carrying a desired or minimum load it would not be considered a probiotic food product. To ensure this different quality control process are being adapted. A considerable number loss of cell number occurs in stomach and duodenum due to acidic conditions and presence of bile. Therefore, in order to ensure that these probiotics are able to maintain their viable count and sensory property throughout the manufacturing process and storage, to facilitate their targeted delivery and give them a microenvironment for their protection throughout the gastrointestinal tract microencapsulation of probiotics has been suggested.

2.7 Microencapsulation

The bacteria may not survive in sufficient numbers when incorporated into dairy products and also during their passage through the gastro-intestinal tract [9-11] as discussed earlier. Additionally, different studies have reported that most of the probiotics which are not microencapsulated do not survive in fermented dairy products during their storage [15-16]. Also free probiotics incorporated in the food product can multiply within the product and may effects the sensory properties of the product including bitterness in taste. For a product to be successful it is very important that it meets all the specification that is on label and if the probiotic are not encapsulated then the parameters may get varied during the storage affecting the quality of probiotic food product. In this context, microencapsulation has been widely researched to create a physical barrier protecting the bacteria from adverse conditions during different physiological and chemical conditions during processing and storage of food and also protecting against acidic condition in the GI tract [17]. ME is the technique of encapsulating solid, liquid and gaseous materials in microspheres that release or deliver their contents at optimized rates over prolonged periods of time under the influence of certain processing and environmental triggers as desired(e.g. shear, temperature, enzyme, pH, fermentation etc.) [28]. There are many microencapsulation techniques which have been used with probiotics such as emulsion, extrusion, spray drying, freeze drying, coacervation, fluidized bed coating and phase separation [18].

2.8 Extrusion method

Amongst all the techniques mentioned above, extrusion method is the most popular for microencapsulation of probiotics because it has several advantages over other techniques including its ease, simplicity, low cost, and gentle formulation conditions ensuring high retention of cell viability [19]. These advantages gives extrusion method an upper hand over other methods and also because of its low cost it is highly adapted in the industrial firms who are involved in the production of probiotic product to reduce down their overall cost to get the maximum profit. Among the available techniques for immobilizing living cells, entrapment in calcium alginate beads has been frequently used for the immobilization of lactic acid bacteria [20].

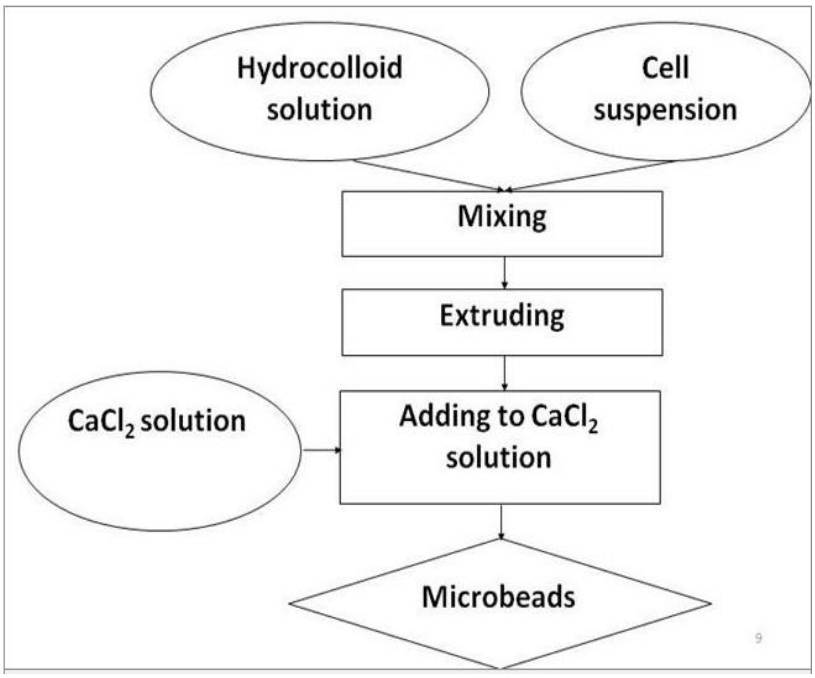


Fig. 3. Extrusion Method

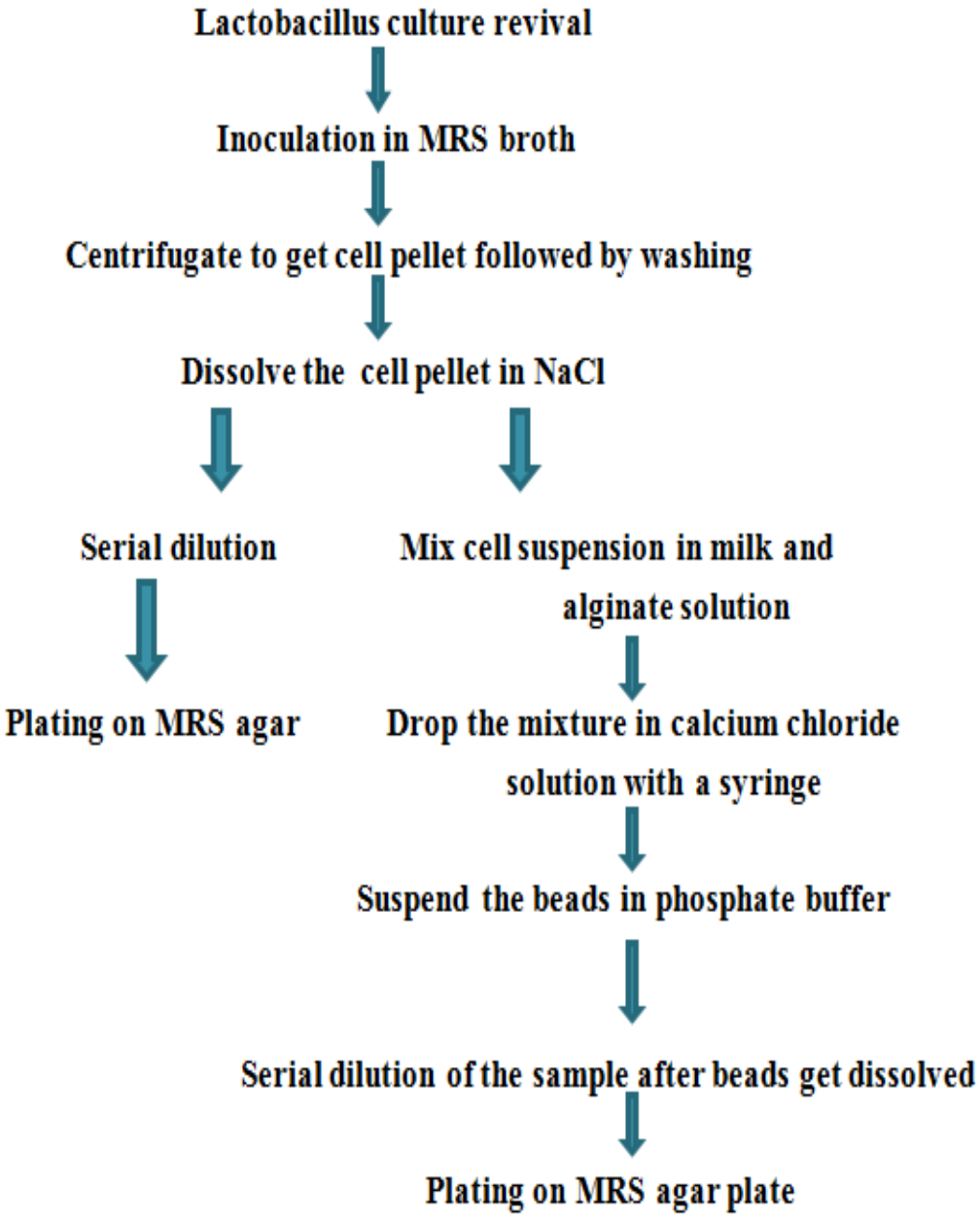
2.9 Polymer and coating material used for microencapsulation

Various gel matrices such as alginate, dextran and k-carrageenan have been successfully reported for encapsulation of microbial cells.Alginate has the benefits of being non-toxic to the cells being immobilized, and it is an accepted food additive [21]. Alginate is an anionic polysaccharide scattered extensively in the cell walls of brown algae which is a sea weed, where through binding with water it forms a gel. It is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. Solubilization of alginate gel by sequestering calcium ions and the possible release of entrapped cells, cheapness, gentle environment and biocompatibility are other advantages that makes it highly acceptable for microencapsulation of probiotics. They are compatible with different lactic strains. But alginate microspheres with porous structure permit the diffusion of acid in and out of microspheres effecting viability of microencapsulated probiotics as gastric and intestinal juice of gastrointestinal tract having low pH and bile salts affect the viability of encapsulated probiotics as they can seep into the capsule and also when added in some food product different salts added to food may affect the viability and sensory properties of the probiotics. The large size of the produced beads may induce contrary effects on the sensorial quality of the enriched product. Therefore, a coating material is used to decrease the porosity of the alginate beads ultimately decreasing the diffusion of acid inside the microspheres and also providing the probiotic a favourable microenvironment. Biocompatibility of milk with lactic cultures, buffering capacity in microspheres that makes it excellent pH tolerance makes it good coating material. Other properties such as

binding small molecules, self-assembly, excellent gelation that helps in forming the encapsulation matrics easily, pH-responsive gel swelling behavior that aisds in targeted delivery of probiotics in the gastrointestinal tract and ability to interact with other polymers for the formation of complexes [22] makes it a great coating material for microencapsulation adding to the quality of probiotic products and optimized delivery of probiotics in GI tract.

3.Materials and Methods

Basic experimental design



Thus harvested beads are tested for survival of probiotics in different conditions-

- Gastric condition
- Pancreatic condition
- During storage in skimmed milk

3.1 Different chemicals and equipments:

MRS Broth, test tubes, centrifuge, sodium chloride, calcium chloride, potassium dihydrogen phosphate, di-potassium hydrogen phosphate, food grade sodium alginate, skimmed milk, vernier caliper.

3.2 Bacterial cultures:

The lactic acid bacteria isolated from different traditional fermented foods and dairy products identified as probiotic viz., *Lactobacilus gastricus BTM7* from our laboratory (Sharma et al., 2017). All the strains were revived from their glycerol stock in MRS broth and routinely subcultured and checked for purity. The cultures were activated in MRS broth before the start of any experiment.

3.3 Microencapsulation of probiotics:

Inoculated culture was incubated in shaker for 24 hrs at 37˚C. 1.5 ml of overnight grown culture of probiotic bacteria in MRS broth was transferred in 4 autoclaved eppendorfs and centrifuged at 10000 rpm for 10 min at 4˚C. The cell pellet obtained was washed twice with normal saline solution. The cells were suspended in normal saline for further encapsulation method. Two different encapsulation methods were tested SA and SASM.

3.4 Preparation of microencapsulation matrix:

A 200 mg each of Sodium alginate and skimmed milk powder was added to 5ml sterilized water to obtain 1:1 ratio. This mixture was extruded in 0.2 M $CaCl_2$ solution as hardening solution. The sodium alginate-skimmed milk cell matrix was dripped in $CaCl_2$

using 21 G needle with a dropping height of 10cm, leading to the formation of calcium alginate beads containing probiotic cultures. The hardening of calcium alginate microcapsules was done for 30 min. The microcapsules were collected, washed with distilled water and suspended in saline solution. To observe the effect of addition of skimmed milk, separate alginate microcapsules were also prepared and checked for efficiency. The size of different microcapsules was measured using a vernier caliper.

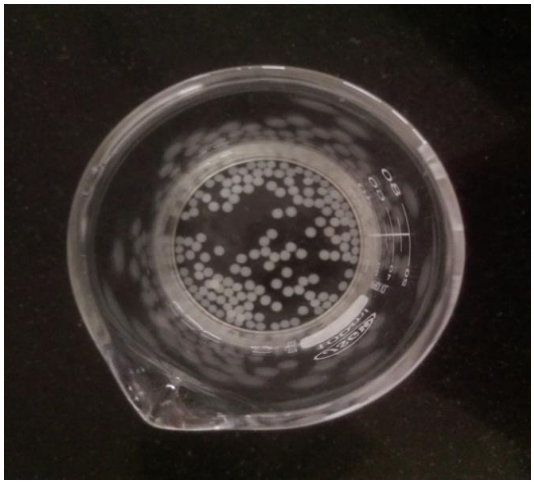


Fig. 4. Microspheres containing probiotics

Encapsulation efficiency: The probiotic bacteria from microencapsulated cells were released in phosphate buffer (pH 7). The solublized suspension was serially diluted andplated on MRS agar plates. The plates were incubated at 37˚C for 24 - 48 h and the number of colonies was counted to determine the microencapsulation efficiency.

$$\text{Encapulation efficiency} = \frac{\text{Number of colonies from the beads}}{\text{Number of colonies in cell suspension}} \text{x}100$$

Fig. 5. Microcapsules before release of cells in phosphate buffer

Fig. 6. Dissolved microcapsules in phosphate buffer after incubation

3.5 Survival of free and encapsulated bacteria in simulated gastrointestinal conditions:

Simulated gastric juice (SGJ) was prepared by dissolving 0.01 g/L pepsin and 0.08 g/L NaCl to 10ml phosphate buffer before which the pH was adjusted to pH 3.0 using HCl [27]. The free cell suspension and microencapsulated beads were added to SGJ. The mixture was kept at 37 C at 100 rpm for 1 hr. After incubation, spreading for the suspension from each flask was done on MRS agar. For the free cells, the samples were taken and centrifuged at 10,000 rpm for 10 min, at 4 °C. The pellet was dissolved in PBS and used for cell enumeration. In the case of microencapsulated bacterial cells, the microcapsules were separated from the samples and dissolved in PBS before plating. For enumeration, all samples were serially diluted in PBS (Oxoid, UK) and viable cells were

enumerated The plates were incubated at 37˚C for 24 - 48 hr and viable cell counts were determined to measure percent survival of probiotic under simulated GI conditions.

Simulated intestinal juice (SIJ) was prepared by dissolving 0.01 g/L pepsin, 0.08 g/L NaCl, 0.08 g/L bile salt, 0.01g/L pancreatin in 10ml phosphate buffer with pH of 8.0 [27]. The effect of pancreatin conditions on survival of free and encapsulated bacteria was determined as discussed above.

3.6 Survival of encapsulated probiotic under storage:

For determining the survival of encapsulated probiotics, the beads were stored in 10 ml of sterilized reconstituted 10% skimmed milk. Approximate 20 beads were added to the skimmed milk vials. The vials were stored under refrigerated conditions for 21 days. At each interval of seven days, 10 capsules was withdrawn washed in PBS. The cells were released in PBS and plated on MRS agar plates after serial dilutions. The survival was determined as the number of cells recovered during storage.

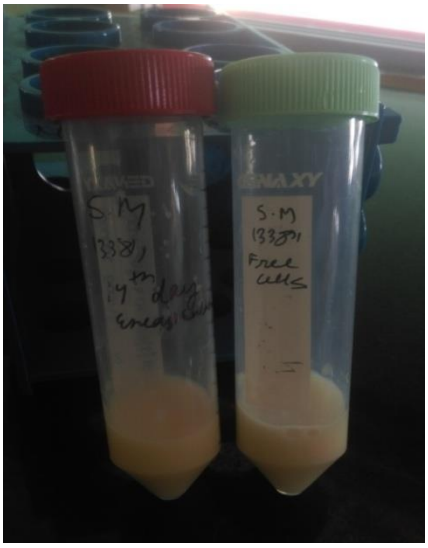


Fig. 7. Free and microencapsulated probiotics in skimmed milk

4.Results and Discussion

4.1 Formation of microcapsules:

Microcapsules were formed as soon as mixture of sodium alginate and skimmed milk was dripped in calcium chloride solution due to cross linking of of calcium which is divalent ion with the alginate replacing sodium which is monovalent ion and thus forming more stable and efficient matrix in the form of microsphere.

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4.2 Release of encapsulated cell:

It was found that the microcapsules started to dissolve in phosphate buffer whose pH was set at 7. This showed that presence of potassium which is a monovalent ion hindered the cross linking of calcium with alginate and therefore destabilizing the microspere structure leading to release of cells. pH responsive gel swelling due to presence of milk protein has led to optimized release of core material. By altering the concentration of skimmed milk in the matrix the porosity can be decreased or increased according to the requirement.

4.3 Encapsulation and coating:

Microencapsulation was done in two steps. First step involved preparation of milk based microencapsulation matrix using skimmed milk and sodium alginate in the concentration ratio of 1:1 and the second step involved dripping of microencapsulation matrix into calcium chloride solution which lead to formation of skimmed milk coated calcium alginate microcapsules. The use of skimmed milk as coating material lead to decrease in the porosity makes the microsphere more efficient for usage.

4.4 Survival of free and microencapsulated *Lactobacillus gastricus BTM7* in simulated gastric juice:

Microencapsulation increased the survivability of cells in simulated gastric condition by providing protection to cell from damaging by not allowing the gastric juice to seep in the matrix. The viable cell count of free *Lactobacillus gastricus BTM7* decreased during incubation of 120 min but there was detectable cell count. But the viable cell count of microencapsulated *Lactobacillus gastricus BTM7* was comparatively higher then the viable cell count of free cells in simulated gastric condition. The bacterial survival of free cells during incubation in simulated gastric juice at 1^{st} and 2^{nd} hr was 0.7% and 0.6% respectively, and where as survivability of microencapsulated cells at 1^{st} and 2^{nd} hr during incubation was 1.1% and 0.8% respectively(as shown in Table 1). The present study also demonstrated that skimmed milk as coating material was efficient in maintaining the microencapsulated beads as it effeciently interacts with other polymers for the formation of complexes and has excellent buffering capacity that makes the microsphere complex pH tolerant. Studies show that in the presence of pepsin there does breakdown of peptide chains of α-lactalbumin and bovine serum albumin which are secondary proteins comprise the whey solution [25].

Table 1: Survival % of free and encapsulated probiotic under simulated gastric conditions

Incubation Period	Free Cells	Encapsulated cells
1 hr	1.7	2.1
2 hr	0.6	1.8

4.5 Survival of free and encapsulated *Lactobacillus gastricus BTM7* in simulated pancreatic juice:

It was observed the beads got dissolved in the pancreatic juice during incubation of 120 min indicating that alginate and milk based matrix for microencapsulation provides an additional benefit of targeted delivery in intestine as alkaline pH of pancreatic juice facilitated pH-responsive gel swelling of milk that was used as a coating material.

The viability count of free cells decreased during incubation whereas the viability count of microencapsulated cells was higher as microencapsulation provided protection to the *Lactobacillus gastricus BTM7* to some extent before the microsphere matrix got solubilised during the period of incubation. The bacterial survival of free cells during incubation in simulated pancreatic juice at 1^{st} and 2^{nd} hr was 1.81% and 0.5% respectively, and where as survivability of microencapsulated cells at 1^{st} and 2^{nd} hr during incubation was 4.51% and 1.93% respectively(as shown in Table 2).

Table 2: Survival % of free and encapsulated probiotic under simulated pancreatic conditions

Incubation Period	Free Cells	Encapsulated cells
1 hr	1.81	4.51
2 hr	0.5	1.93

4.6 Stability of free and microencapsulated *Lactobacillus gastricus BTM7* under refrigerated condition in skimmed milk

The viable count of free *Lactobacillus gastricus BTM7* was low when compared to viable count of microencapsulated cells during the storage of 21 days at 4˚C and the readings were taken at 0^{th}, 7^{th}, 14^{th} and 21^{st} day. In case of microencapsulated *Lactobacillus gastricus BTM7* microencapsulation aided the increased survivability of microencapsulated *Lactobacillus gastricus BTM7* when compared to free during storage at 4˚C for 28 days (as shown in Table 3). This result indicated that free cells showed lower ability of free cells to maintain their viability count that is which is lower than the recommended count of 6 CFU cfu g^{-1} and where as microencapsulation provided stable microenvironment for the cells because of which cells were able to survive and maintain their viable recommended count of 6 log CFU g^{-1}. During the storage of microencapsulated and free probiotics for 21 days it was observed that microencapsulation enhanced the survival percentage of the probiotics when it was compared with the survival % of free probiotics during storage. The survival of free

probiotics reduced to 84.1% from 100% after 20 days of storage while survival of microencapsulated probiotics got reduced just by 4% after 20 days. The survival percentage of microencapsulated probiotics on 21st day was 96.1%(Fig 10). This result showed that microencapsulation increased the survivability of probiotics during storage. Similarly some studies also shows that alginate aid to survival of probiotic bacteria in providing stable microenvironment to the cells during their storage [26].

Table 3: Log$_{10}$ CFU/ml count (% survival) of free and encapsulated probiotic under refrigerated storage in skimmed milk

	0 day	**7 days**	**14 days**	**21 days**
Free cells	6.75	6.05(89.6%)	5.84(86.5%)	5.68(84.1%)
Encapsulated cells	7.11	7.02(98.7%)	6.86(96.4%)	6.83(96.1%)

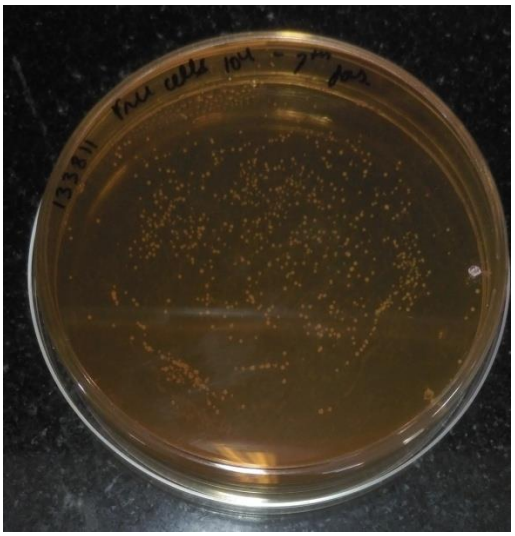

Fig. 8. Culture plate of free cells during storage

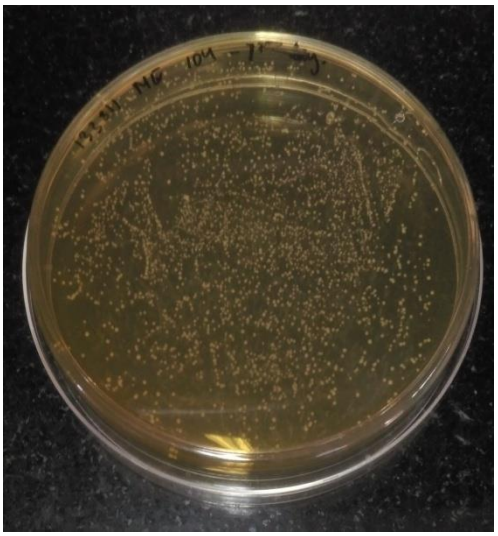

Fig. 9. Culture plate of microencapsulated cells during storage

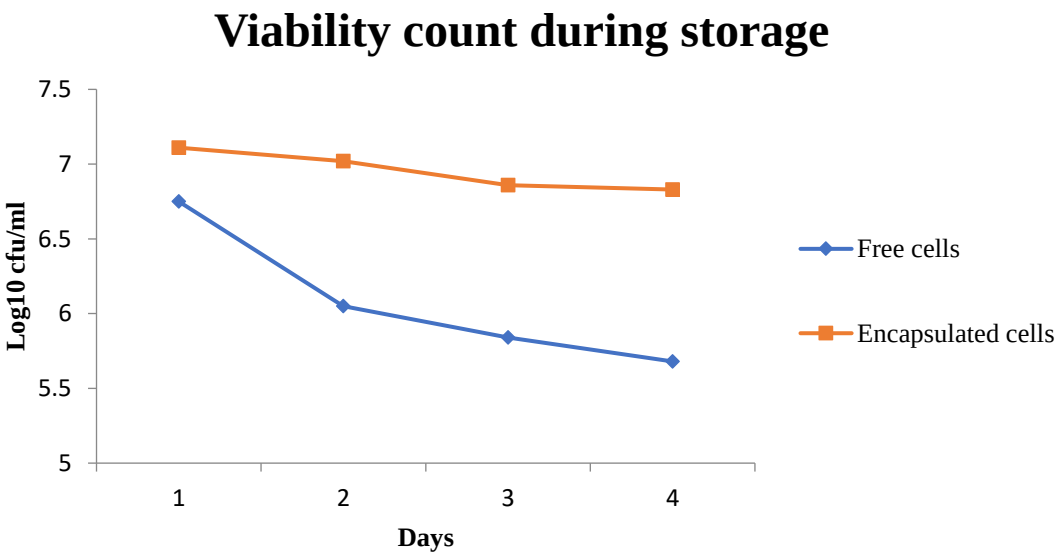

Fig. 10. Viability count of free and microencapsulated bacteria during storage

5.Conclusions

When microencapsulated lactobacillius are treated in gastric condition it was observed that their survival per cent was enhanced as compared to when the free cells were suspended in gastrointestinal conditions.

Thus problems such as process stability, Gastro-Intenstinal (GI) residence time, bitter taste etc associated with the food industry and utilisation of food products by human beings can overcome by food encapsulation. There is pressing for edible encapsulation system for probiotics by protecting them throughout their shelf life without compromising their sensory properties and and the quality of food at the same time. It also increases the bioefficacy.

Also microencapsulation improves the survivability during storage of probiotic products by providing a suitable microenvironment to probiotics which efficiently help them to maintain their viability count.

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