



Probiotics: A Review on Formulation Aspects of Probiotics

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ABSTRACT

Probiotics are live microbial food supplements or components of bacteria, which have been shown to have beneficial effects on human health. They are also called "friendly bacteria" or "good bacteria." Experts have debated how to define Probiotics. One widely used definition, developed by the World Health Organization and the Food and Agriculture Organization of the United Nations, is that Probiotics are "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host." Several probiotic preparations seem to have promise in prevention or treatment of various conditions. There currently exists good evidence for the therapeutic use of Probiotics in infectious diarrhoea in children, recurrent *Clostridium difficile* induced infections and postoperative pouchitis. The possible benefit in other gastrointestinal infections, prevention of postoperative bacterial translocation, irritable bowel syndrome and inflammatory bowel disease continues to emerge. This review addresses the different microencapsulation methods such as spray-drying, spray-coating, spray-chilling, extrusion, emulsion and phase separation coacervation and different polymers that have been used for production of probiotics.

Keywords: Gastrointestinal infections, Inflammatory bowel disease and Microencapsulation, Probiotics, Pouchitis.

INTRODUCTION

Probiotics are microbially derived factors that stimulate the growth of other microorganisms. The term "Probiotics" was first introduced in 1953 by Kollath. In 1989 Roy Fuller defined Probiotics are live microbial feed supplement which beneficially affects the host animal and physiological functions of the human by improving the intestinal microbial balance. Fuller suggested that viability is an important parameter for Probiotics to produce health benefits in host. According to World Health Organization and the Food and Agriculture Organization of the United Nations, Probiotics were defined as "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host."

Recently Probiotics considered as "live microbes which transit the gastro-intestinal tract and in doing so benefit the health of the host differing from the earlier statement's on Probiotics which focused on interactions of Probiotics with indigenous intestinal microbes to improve physiological function of host.¹

Now a days Probiotics are consumed world widely as food supplement such as soft-, semi-hard and hard cheeses, ice cream, frozen fermented dairy desserts, yogurt and soy juices etc. Probiotics also used for their therapeutic purpose to treat antibiotic induced diarrhoea, Irritable bowel syndrome, *Helicobacter* infection, vaginal infection and colon cancer etc.²⁻⁴

Probiotics are bacterial organism similar to those naturally found in human intestinal flora. Most often, *Lactobacillus* or *Bifidobacterium* are common examples of bacteria whereas *Saccharomyces boulardii* is common

example of yeasts used for probiotics production. There are different species (for example *L.bulgaricus*, *L.lactis*, *B.infantis*, *B.longum* etc.) and within each species, different strains are available used for production.^{5,6,7} Probiotic cultures to increase the viability or to maintain the survival of organism are frequently supplied in frozen or dried form, as either freeze-dried or spray-dried powders.⁸⁻¹⁰ The well established technology for production of probiotic bacterial cells evolved from the immobilized cell culture technology that is microencapsulation technique. Different microencapsulation methods have been used for production of probiotics such as spray-drying, spray-coating, spray-chilling, extrusion, emulsion and phase separation coacervation.

Functions of Probiotics

- Act as nutritional supplement as well as improved absorption of vitamins and minerals.
- Improve lactose tolerance in lactic acid deficient individuals.
- Treat constipation, irritable bowel syndrome by maintaining gut motility.
- Prevent incidence of diarrhoea due to (antibiotics, *Clostridium difficile*, rotaviral) or either travellers associated diarrhoea.
- Improve immune system by increasing IgA type of antibody.
- Decrease inflammation by regulating cytokines and desensitizing lymphokines.



- Prevent occurrence of colon cancer and bladder cancer.
- For treating pouchitis (a condition arises after colon removal).
- Decrease serum cholesterol level by inhibiting reabsorption of cholesterol and by efficient bile salt deconjugation.
- Important role in decreasing blood pressure by inhibiting A.C.E like peptides.
- For treatment of urogenital infection (antibiotics and radiation induced colitis, yeast infections and vaginitis in women).
- To prevent intestinal tract infections caused by *Helicobacter Pylori*.
- Block adhesion and movement of pathogens inside the body in case of burn, shock, and trauma.

Disadvantages of Probiotics

- Probiotic supplements are very costly and are difficult to process.
- The viability of probiotics is difficult to achieve.
- Probiotics safety data has not been established much scientifically that how safe they are for healthy people and people with compromised immune systems.
- Probiotic products such as yogurts could be a cause for obesity.
- Probiotics sometimes produces digestive side effects such as gas or bloating.
- Probiotics could cause unhealthy metabolic activities, too much stimulation of the immune system, or gene transfer (insertion of genetic material into a cell).
- Probiotics are able to produce severe acute pancreatitis.
- Physiological effects obtained from one species or strain of probiotics may not be able to obtain the same effects from different preparations of the same species or strain.

Steps involved in manufacturing process of Probiotics

1. Selection of bacterial strain
2. Isolation of bacterial strain
3. Identification of isolated bacterial strain
4. Mass production of selected bacterial strain through fermentation
5. Encapsulation

The Manufacturing Process of Probiotics

1. Selection criteria of bacterial strain

The strains of microorganisms used for probiotic production should be generally recognized as Safe (GRAS microorganisms) for human use.

The microorganisms should not be killed by the defence mechanisms of the host and they should be resistant to the pH, bile and pancreatic juice conditions.

The selected microorganism should not be able to cause too much stimulation of immune response in the host.

The microbial strain must have high viability and survival during processing (e.g. water, oxygen, heat etc).

2. Isolation of bacterial strain

Various technologies successfully employed for isolation of bacterial cell for probiotic production such as 16S rRNA-targeted oligonucleotide probes, Dot blot hybridization (DNA-DNA hybridization) and Fluorescent in situ hybridization (FISH).¹¹

3. Identification of isolated bacterial strain

PCR in combination with 16S r RNA analysis

PCR with 16S rRNA gene-targeted species/group-specific primers has recently been introduced to analyze LAB in intestine and faeces of human host. PCR assay for rapid and sensitive detection of a novel thermo tolerant bacterium, *Lactobacillus thermotolerans*, in chicken faeces was developed and assessed by Selim *et al.*

Multiplex polymerase chain reaction (PCR)

Primer set for the identification of a number of probiotic *Lactobacillus* species has been developed. It uses the primer sets comprising of specific and two conserved primers derived from the integrated sequences of 16S and 23S rRNA genes and their rRNA intergenic spacer region of each species with 93.6 % accuracy.¹¹

SDS PAGE (Whole-cell protein profiling method): This technique has been used to identify a new thermo tolerant species of LAB isolated from chicken faeces.

Other methods generally used for identification are Transmission electron microscopy, Scanning electron microscopy, ELISA, Radio-labeling and Fluorescent Staining (carboxy fluorescein diacetate).^{11,18}

4. Mass production of selected bacterial strain through fermentation

Fermentation technology is used for the biomass production of beneficial micro-organism, isolated from human, plant or animal that act as probiotic. The various fermentation methods used are Liquid fermentation, submerged fermentation, solid state fermentation, and two-phase fermentation.



Liquid Fermentation

In liquid fermentation, the soluble substrate such as sugars used as source of energy for inoculated organism. High volume of air is required for aeration. It is a continuous process. The substrate to product ratio produced is 30:80 gm/l. The fermentation broth is directly bottled. A modification in liquid fermentation technique carried out which includes air lift type fermentation known as submerged fermentation to overcome the limitation of liquid fermentation. In submerged fermentation, bacterial contamination is less and it is easy for scale up production.

Solid state fermentation

In solid state fermentation, the polymer insoluble substrates such as starch, cellulose, pectins, lignin used as source of carbon for the inoculated organism. It provides easy aeration due to high surface exchange and static condition for growth. It is a batch process. The solid state fermentation product was better in comparison to submerged fermentation, more resistant to drought condition, more stable. But the drawback is easily contaminated, low output, difficult to scale up and used only for aerobic microbial fermentation. The substrate to product ratio produced is 100:300 gm.¹¹

Liquid-solid two- phase fermentation process

This technique combines the use of submerged and solid state fermentation so as to avoid the disadvantages of both. First of all submerged fermentation carried out by rapid production of large number of bacterial cell, and then inoculation into the fermentation tank done to carry out solid state fermentation. In this way product processing done at low temperature with good aeration for biomass production of microorganism.

Microencapsulation

As a process it is a means of applying relatively thin coatings on core material, the core material may be small particles of solids or droplets of liquids and dispersion. In view of microencapsulation of probiotics the bacterial cell either in form of solid or dispersion state employed for encapsulation. Encapsulation tends to stabilise cells, potentially enhancing their viability and stability in the production, storage and handling. It also regulate the release of the bacterial cell from the preparation. The various method used for microencapsulation of bacterial cell are Extrusion, Emulsion, Spray process, Direct compression, Electrostatic method etc.

Extrusion method (droplet system)

Extrusion is the method where hydrocolloid solution of polymer is prepared. The bacterial cell's used for the probiotic production than mix with the hydrocolloid solution of polymer to form a suspension. The prepared suspension is extruded through syringe needle to form droplets which was taken into hardening solution of calcium chloride to harden the cell coat. The obtained

bead than dried by suitable drying technique to produce hydrocolloid capsule. This method generally produces beads of 2-5 mm diameter.¹²

Krasaekoopt et.al in 2013 generated bead of 2-3 mm diameter by using alginate as polymer of 1-2 % and calcium chloride as hardening solution of 0.05- 1 M.

This method is simple, cheap and producing minimal cell injuries. But the extrusion process is very slow, difficult to scale up and produces larger beads in compare to other methods.

Emulsion method

In compare to extrusion method, this method is easy to scale up and producing bead of smaller diameter of 25 µm to 2mm. In emulsion method, the bacterial cell polymer slurry is prepared which act as dispersed phase added into continuous oil phase, Eg. (Soya-sun flower oil, corn, millet, liq. Paraffin etc.) with continuous agitation to form water in oil emulsion. The formed water in oil emulsion is solidified either by addition of gelling agent (polyacryl amide gel) or cross linking agent (calcium chloride). After cross linking the water soluble polymer get insoluble thus makes gel particle to move towards oil phase to form an outer coat. The prepared beads can be isolated by membrane filtration or simply by decantation followed by drying.

However this method has several disadvantage of having high cost and the residual oil in the preparation, which is not suitable for low fat food product.

Electrostatic method

This method used where two oppositely charged polymer was used for encapsulating the bacterial cells. It includes mixing of bacterial cell and coating material followed by its extrusion into oppositely charged polymer solution to produce microcapsules, followed by drying of micro capsule either by using freeze or oven drier. In 1995, King prepared microencapsulated cells by using gelatin as it is positively charged below its isoelectric point and anionic gel forming poly saccharide gellan gum which is negatively charged for encapsulation.¹³

Compression coating

In Compression coating lyophilized bacterial cell powder compressed into a core tablet followed by compression of coating material around the core contain bacterial cell to form a final compact.

Coacervation/phase separation

This technique is employed for water soluble polymer coating only. It includes process which is carried out under continuous agitation, addition of bacterial cell suspension into polymer solution, deposition of polymer coating, followed by rigidizing the coating usually by thermal, cross linking or desolvation techniques to form microcapsule.



Table 1: Polymers used for microencapsulation of microorganism^{18,19}

Polymers	Properties
Alginates	It is a natural polymer, composed of 1-4 linked β -D- mannuronic acid (M) & α -L-guluronic acid (G) arranged as block copolymer either as homo-polymer block (MM/GG) mixed block (MG/GM). GG block responsible for preferential binding to counter ion calcium. GGCalcium-GG It is used in concentration range of (0.5-4%), produce diameter of bead 1-3 μ m having pore size of <7 nm. As alginates are non toxic, cheap and form gentle matrices most widely used for encapsulation. But the matrices formed by alginates are susceptible to acidic environment and having reduced barrier properties.
K-carrageenan	A natural polysaccharide, extracted from marine micro algae. Used in concentration range of (2-5%) dissolved at 60° to 80°C. A ratio of 2:1 of K-carrageenan to locust bean gum produces stronger gel beads due to interaction of galacto-manannan chain of gum with carrageenan.
Cellulose acetate phthalate	It is an enteric coating polymer which is soluble above pH 6. It is hygroscopic and relatively permeable to moisture and gastric fluids in comparison to other enteric polymer. Ghassi K and Vandamme T (2012), encapsulate B.lactis and L.acidophilus in cellulose acetate polymer by using spray drying technology. ¹⁵
Gelatin	It is a type of thermally reversible gelling agent, amphoteric in nature. Hyndaman, Groboillot, Poncelet, Champagne and Nufeld in 1993 reported that in concentration of 24% w/v gelatin by cross linking with toluene-2-4, diisocyanate can be used to encapsulate L.lactis. ¹⁶
Chitosan	N-deacetylated product of polysaccharide chitin, cationic in nature. Isolated from crustacean shells, insect cuticles and membrane fungi, used for encapsulating bacterial cells. To increase the stability of microcapsule chitosan can be cross link with polyphosphates or sodium alginate.
Starch	High amylose corn starch (HACS) by hydrothermal and retrogradation process used to produce resistant starch (not digested by pancreatic amylase). Haralampu, (2000) reported that HACS with resistant starch used to encapsulate bacterial cell for enteric delivery purpose. ¹⁷

Table 2: Example of probiotics produced through micro Encapsulation^{20,21}

Carrier Employed	Bacterial strain	Method of Production
Alginate	B.bifidum, B.infantis, B.longum, L.casei, L.acidophilus, B.breive.	Gel bead Direct compression Emulsification
Alginate/Glycerol /Chitosan/pectin	Bifidobacterium.	Gel bead
Alginate/SLS	L.delbruceki.	Gel bead
K-carrageenan	Lactobacillus, S.thermophilus.	Gel bead
K-carrageenan/Locust bean gum	L.bulgaricus, L.lactis, B.infantis, Bifidobacterium, L.casei.	Gel bead Emulsification
Cellulose acetate phthalate	Bifidobacterium pseudolongum.	Gel bead
Gelatin/Toulene 2-4-diisocyanate	L.lactis.	Gel bead
Starch	Bifidobacterium, Lactobacillus	Gel bead

Fluidized bed processing

This process consist of fluidization of bacterial cell suspension in a supporting air stream and than giving the

spray coating of the air suspended bacterial cell. It is applicable for both water soluble or insoluble polymer. But it is a less widely used technique due to the injuries produced to bacterial cell, and difficulty in maintaining viability of bacterial cell.

Encapsulation in Super critical fluid CO₂

This method used for probiotics preparations which are sensitive towards exposure to water or oxygen and heat. It is based on principle of inter polymer complex formation. This technique has been used to encapsulate Indomethacin & Bifidobacterium longum in PVP-PVA cocrotonic acid.

Spray process

Spray drying

In this method water soluble polymer solution is mixed with bacterial cell atomized to form a fine mist, followed by introduction of hot air to allow rapid evaporation of solvent in which the polymer material is dissolved. The dried coated microcapsule separated based upon the size.

Spray coating

In this method the bacterial cell in solid form not previously mixed with polymer solution, maintain in motion either by injection of air or by rotary action. Upon this coating material sprayed (polymer solution) followed by drying.¹⁴



O’Riordan et al;2001 used this technique to coat Bifidobacterium cell by using gelatinised modified starch keeping inlet and outlet temperature 100°C and 45°C respectively.

Spray congealing

This method quite similar to spray drying method, except the bacterial cell is dispersed in a coating material melt rather than a coating solution, and use of cold air instead of hot air. The coating material used in this method are waxes or fatty acids, which forms molten matrix at 32–42°C. The microcapsule can be solidified by congealing either by adding non solvent or by injecting cold air.

Though spray congealing is a continuous process, it has several disadvantage due to high temperature used for drying, which decreases the viability of bacterial cell, poor drying rate and sometimes causes sticking of the product and also accumulates in cyclone or receiving flask.

Table 3: Marketed product of Probiotics²²

Carrier Employed	Bacterial Strain	Marketed Product
Calcium alginate	B.bifidium & B.infantis	Mayonnaise
	B.bifidium, B.infantis & B.longum	Crescenza cheese
K-carrageenan/Locust bean gum	L.lactis	Fresh cheese
	L.casei	Yoghurt

CONCLUSION

Even so, the various technologies used for probiotic productions are well developed, but the major challenges for all those techniques is to maintain the viability of probiotic product at (low pH, high bile concentration) and during the manufacturing processes particularly heat processing.

Considering microencapsulation process selection of appropriate microencapsulation method is an important parameter for encapsulation of probiotic. All microencapsulation methods could not be able for large-scale production of food-grade probiotic products. One important challenge for cell encapsulation is the large size of microbial cells (typically 14 mm) or particles of freeze-dried culture (more than 100 mm) Due to this characteristic the cell loading for small capsules is difficult, lead to larger size capsule production

There is also need of conducting studies to understand the effect of encapsulation on the safety of probiotic bacteria.

As far as fermentation process employed for biomass production, sometimes the product quality obtained is unstable. In addition there is not any quality testing standards available for the obtained biomass. One important challenge in front of drying process employed for probiotic cell such as, is the removal of bacterial cell

bound water lead to damage of (cell protein, cell membrane, cell wall).Hence, lead to loss of functional integrity of RNA and DNA and decrease in biological activity of bacterial strain.

The technology of micro-encapsulation, however, needs to be developed with more precise machinery and better delivery systems. In the future multiple-delivery may be developed, such as co-encapsulating prebiotics and probiotics. Nanoencapsulation may assume importance in the near future to develop probiotic bacteria for their controlled release in the gastro-intestinal system, immune modulation etc.

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