

MICROENCAPSULATION OF PROBIOTICS FOR THERAPEUTIC APPLICATIONS

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1. INTRODUCTION

Probiotics (Greek *pro*, for and *bios*, life), are live bacteria that are administered in order to provide a health benefit to the host. They have beneficial effects on the equilibrium and the physiological functions of the human intestinal micro flora. The concept of ingesting live microorganisms for therapy (Probiotics) can be traced back to the beginning of the 20th century.

There are oligosaccharide polymers called prebiotics, which are processed in the large intestines. The combination of these with probiotic bacteria is called as a synbiotic system. This synbiotic system may play a role in the possible beneficial effects of probiotics on intestinal process.

While a majority of the indigenous flora are benign or exhibit health promoting properties, some possess the potential to cause disease. For example, bifidobacteria and lactobacilli are associated with health, while clostridia are considered detrimental to health. Normally, a balance exists between pro-health and anti-health organisms. However, when this delicate ecological balance is perturbed by environmental or physiological factors, predisposition to infectious and immuno inflammatory diseases are enhanced.

Desirable Characteristics of Probiotic Microorganisms :-

Probiotic microbes are host specific. They should possess the following ideal properties: -

1. Adhere to the intestinal mucosa of the host
2. Be easily cultured
3. Be nontoxic and nonpathogenic to the host
4. Exert a beneficial effect on the host
5. Produce useful enzymes or physiological end products that the host can use
6. Remain viable for a long time
7. Withstand gastric secretion in the host's stomach and bile salts in the small intestine

Mechanism of Action of Probiotics: -

Numerous mechanisms have been proposed for probiotic functionality and many clinical end targets have been measured. It has been recognized that prevention of gastrointestinal tract (GIT) colonization by a variety of pathogens is a primary mechanism of beneficial effects mediated by probiotics. Evidence suggests that probiotic bacteria

attach to enterocytes and thus inhibit the binding of enteric pathogens to the intestinal mucosa by production of inhibitory substances (competitive exclusion of pathogens) like bacteriocins, lactic acid and toxic oxygen metabolites. The important toxic oxygen metabolite worth mentioning is hydrogen peroxide as it exerts a bactericidal effect on most pathogens. Therefore, inclusion of probiotic bacteria in fermented dairy products enhances their value as better therapeutic functional foods. The synbiotic systems produce butyric acid which enhances the beneficial effects of probiotics

The major mechanisms of action of probiotics are:

1. Competition with pathogens for nutrients and adhesion sites
2. Inactivation of pathogenic bacterial toxins or metabolites
3. Production of substances that inhibit pathogen growth
4. Stimulation of non specific immunity

Probiotic Bacteria: -

Bacterial strains selected as probiotics are predominantly from the genera *Lactobacillus* and *Bifidobacterium* which are indigenous to the human gastrointestinal tract

Lactobacillus Species: - *Lactobacillus* species belongs to the genera of nonsporeforming

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Gram-positive bacteria. They exist in long to very short rods, often in chains. These are catalase-negative and usually nonmotile. These are strictly fermentative organisms, but can usually tolerate air. Some strains are anaerobic .

Many different strains and species of lactobacilli (Table 1) have been used commercially as probiotics.

Bifidobacteria: - The genus *Bifidobacterium* can generally be characterized as Gram-positive, non-sporeforming, non-motile anaerobes that are catalase-negative and saccharolytic. Bifidobacteria are a major component of the human and animal gastrointestinal tract

Some strains of bifidobacteria are listed in Table 2.

Apart from these other lactic acid bacteria (LAB) like *Ent. faecalis* , *Ent. faecium*, *Sporolactobacillus inulinus* and non LAB like *Bacillus cereus* (for animals), *Escherichia coli*, *Propionibacterium freudenreichi* (for animals), and *Saccharomyces cerevisiae* worth mentioning as probiotics.

Therapeutic uses of Probiotics: -

The therapeutic applications of free (unencapsulated) Probiotics are reviewed elsewhere . To brief, they are used in: *Diarrhea, Colorectal cancer, Inflammatory bowel disease, Ulceration, Steatorrhea of lipids (malabsorption of lipids), Enhancing immunity, Hypercholesterolemia, Chronic kidney Failure, and to treat Kidney stones.*

Encapsulation of Probiotics: -

Encapsulation of living cells or Bioencapsulation involves putting a living core, for example, live bacterial cells inside a shell or an envelope. Since Microencapsulation includes Bioencapsulation and microencapsulation of living bacteria (probiotics) is the center theme of this article, an attempt was made to present basic concepts and overall picture concerning the usefulness of microencapsulation technology in general and in probiotic research in particular.

General Overview of Microencapsulation: -

Microencapsulation is the packaging of small droplets of liquid or particles with a thin film. Bioactive materials such as drugs and cells are amenable for microencapsulation in a simple and cost-effective way within a semi-permeable polymeric membrane for the purpose of protection and releasing the enclosed substances or their

products in a controlled fashion . Microencapsulation technology has originated both from the paper and the pharmaceutical industries. Large efforts in microencapsulation research were done by the pharmaceutical industry in 70-80's. Microencapsulation is a growing field that is finding application in many technological disciplines, such as pharmaceuticals, food, food additives, cosmetics, adhesives, house hold products, agricultural materials, aerospace and many more.

Microencapsulation Terminology: -

a. Microparticle/Microcapsule/Microsphere

The products obtained in the microencapsulation process fall into two categories called as microspheres or microcapsules. These are collectively referred to as microparticles. "Microspheres" specifically refers to spherical microparticles and the subcategory of "microcapsules" applies to microparticles which have a core surrounded by a material which is distinctly different from that of the core.

b. Wall / Envelope/ Shell/ Membrane:

It is that material which encases the product. The coating material should be capable of forming a film that is adhesive with the core material. Coating material provides the desired coating properties such as strength, permeability, optical properties and stability.

c. Core material:

The material inside the microcapsules is referred to as core material or internal phase or fill. The configuration of the core can be a spherical or irregular particle, liquid-phase suspended solid, solid matrix, dispersed solid and aggregates of solids or liquid forms.

There are several reasons why substances may be encapsulated .

1. Taste masking
2. Selective sorption
3. Separation of incompatible components for functional reasons
4. Conversion of liquid to solid for enhancing stability
5. Reduction of volatility
6. Reduction of gastric irritation
7. Sustained normalization of diabetic condition by encapsulating islet of Langerhans
8. Controlling release of the active components for delayed (timed) release or long-acting (sustained) release

Microencapsulation of several core materials was presented in a recent review . Various techniques of microencapsulation are given in standard text books

Artificial Cells: -

Microencapsulation technology paved the way for the design of artificial cells for possible replacement or supplement of deficient cell functions. Like natural cells, biologically active materials inside the artificial cells are retained and prevented from coming into contact with external materials like leukocytes, antibodies or tryptic enzymes. These artificial cells are, in fact, ultra-thin polymer membrane microcapsules used to encapsulate materials such as transplanted cells, enzymes and absorbents .

Rationale for Microencapsulation of Probiotics:

A prerequisite for any effect of ingested bacteria is a successful implantation in the gastrointestinal tract. So bacteria must remain viable during gastric transit. While existing live bacterial cell therapies show great therapeutic potential, they have several limitations. For instance, when given orally bacterial cells are exposed to difficult gastro intestinal (GI) conditions and experience low survival requiring that a large dosage be given . Also, oral administration of live bacterial cells can cause a host immune response and can be retained in the intestine replacing the natural intestinal flora . Thus, concerns of safety and practicality have prevented the regular use of this promising therapy in clinical practice. Hence, it is important to protect them by encapsulation.

Compared to immobilization/entrapment techniques, microencapsulation has many advantages . The microcapsule is composed of a semi permeable, spherical, thin and strong membranous wall. The polymer membrane can protect encapsulated materials from harsh external environments while at the same time allowing for the metabolism of selected solutes capable of passing into and out of the microcapsule. In this manner, the enclosed material (in our discussion live bacteria) can be retained inside and separated from the external environment, making microencapsulation particularly useful for biomedical and clinical applications.

Microorganisms can be easily engineered to overproduce enzymes and peptides and to metabolize large amounts of unwanted metabolites and others. For these reasons, artificial cells containing bacterial

cells are very good candidates for oral artificial cell therapy . Studies show that artificial cell microcapsules can be used for oral administration of live genetically engineered cells that can be useful for therapeutic functions .

Coat Materials for Probiotic Microencapsulation:

For the encapsulation of viable bacterial cells, the materials used as coat should be gentle and non-toxic. By far, the most commonly used bio gum for microencapsulation of bacterial cells is alginate. The advantages of using alginate as an encapsulating agent includes: non-toxicity, forms gentle matrices with calcium chloride to trap sensitive materials such as probiotic bacteria, the viability of bacteria is not affected during the encapsulated shelf life . Alginates with a high content of guluronic acid blocks (G blocks) are preferable for capsule formation because of their high mechanical stability, high porosity and tolerance to salts and chelating agents . Chitosan, a water-soluble polymer (pH <6) has been used to microencapsulate *Lactococcus lactis* The anti-bacterial property of chitosan, however, limits its use as coating material in encapsulation . Another bio gum that has been used as an encapsulant was gellan gum . Several other substances are being used such as various proteins, polyhemoglobin, and lipids .

The use of different membranes allows for variations in permeability, mass transfer, mechanical stability, buffering capability, biocompatibility, and other characteristics. A balance, however, has to be maintained among the physical properties of capsule membranes so as to support the entrapped cells' survival .

Microencapsulation Techniques: -

The techniques used in probiotic microencapsulation used include Spray Drying, Emulsion and Phase Separation and/or coacervation methods. Most of the reported literature on micro-encapsulation of probiotic bacteria was based on small-scale laboratory procedures involving emulsion, extrusion and/or coacervation. These are discussed in a review by Kailasapathy .

Some Potential Therapeutic Applications of Microencapsulated Probiotics: -

a. In Coronary Heart Diseases (CHD): - The World Health Organization (WHO) predicts that by the year 2020, up to 40% of all deaths will be related to cardiovascular diseases or disease of the heart . Elevated blood cholesterol is a well known major risk factor for CHD . Certain strains of bacteria act

directly on bile acids in the gastrointestinal (GI) tract and are beneficial in reducing serum cholesterol levels. Jones et al examined the potential of artificial cell microencapsulated genetically engineered *Lactobacillus plantarum* 80 (pCBH1) cells for bile acids deconjugation to lower cholesterol. They predicted the oral doses of microencapsulated *Lactobacillus plantarum* 80 (pCBH1) cells required for lowering cholesterol .

b. In Curing Kidney Stones: - Oxalate is major risk factor for kidney stone formation. Oxalate-degrading enzymes produced by microencapsulated *Oxalobacter formigenes* breaks down unwanted oxalate. Thus they can be used to prevent subsequent evolution of kidney stones .

c. In Kidney Dialysis: - Genetically engineered *E coli* DH5 cells cause overexpression of the urease enzyme and the subsequent lowering of elevated blood levels of urea and other metabolites such as ammonia, during renal failure .

d. In Colorectal Cancer: - Bile acids are important to normal human physiology. However, bile acids can be toxic when produced in pathologically high concentrations in hepatobiliary and other diseases. Microencapsulated genetically engineered LP80 (pCBH1) and *L reuteri* were found to deconjugate bile acids and make them less bioavailable for exfoliation of the GI and any potential carcinogenic damage .

e. In Diarrhea and Dehydration: -

Microencapsulated genetically engineered LP80 (pCBH1) and *L reuteri* were found to deconjugate bile acids mitigating the problems associated with excessive electrolyte and water secretion associated clinically with diarrhea and dehydration.

f. In Lowering Elevated blood levels of amino acids: - Microencapsulated genetically engineered cells were shown to lower phenylalanine in phenylketonuria

g. To prevent Decompression Sickness in Divers: -

Microencapsulated H₂ metabolizing (*M smithii*) N₂ fixing (Enterobacteriaceae) live bacteria were found helpful in divers to prevent decompression sickness or reduce decompression time.

2. CONCLUSION

Probiotics have already emerged as alternative treatment of a number of ailments/ medical

disorders. Genetically engineered bacteria are useful to overproduce many enzymes which have wide clinical applications. Microencapsulation of these live bacteria offers a number of advantages like increased survival in GIT and avoiding host immune response. However, more in vivo studies should be conducted using human subjects to confirm the efficacy of microencapsulation in delivering probiotic bacteria and their controlled release in the gastrointestinal system.

Table 1- Lactobacillus Species used as Human Probiotics.

Obligately homofermentative

L. acidophilus

L. crispatus

L. amylovarus

L. gallinarum

L. gasseri

L. johnsonii

L. helveticus

L. delbrueckii subsp. bulgaricus

L. salivarius subsp. Salivarius

Facultatively heterofermentative

L. casei

L. paracasei subsp. paracasei

L. paracasei subsp. tolerans

L. plantarum

L. rhamnosus

Obligately heterofermentative

L. fermentum

L. reuteri

Table 2- List of some Bifidobacteria Strains used as Human Probiotics

Bifidobacterium animalis

B. asteroides

B. bifidum

B. bifidum. breve

B. infantis

B. longum

B. longum 1

B. longum 2

B. pseudolongum

B. pseudolongum

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