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Probiotics for treating disorders: microencapsulation a boon to potentiate their therapeutic applications

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ABSTRACT
Probiotics are nutraceutical products which have been used substantially for supplementation of the intestinal microbiota. Probiotics have been indicated in the therapy of various disorders such as gastric ulcer, diarrhoea, gastroenteritis, inflammatory bowel disease, colon cancer, urogenital infection, allergy, respiratory infection and to promote the individual's well-being. The present review article focuses on the mechanism followed by probiotics for inactivating the harmful antigens. The various microencapsulation techniques useful in improving the processing, transit and storage stability of probiotics have also been described. Microencapsulation process linked protection and increased survival rate of probiotics have been discussed systematically.
Keywords: *Probiotics; microencapsulation; gastric disorders; viability.*

1. INTRODUCTION
Probiotics are referred to as viable microorganisms that show a beneficial impact on the consumer's health when consumed in an appropriate amount. To enhance their effectiveness in the host, probiotics should withstand the strong acidic nature of the stomach and must reach the large intestine in satisfactory amounts so as to initiate colonization and proliferation The foods containing probiotic bacteria need to be in the range of 10^8-10^9 colony forming unit (cfu)/g prior to administration in order to obtain sufficient curative effect of 10^6-10^7cfu/g in the colon [1]. Probiotics trigger the activation of certain genes in the host which is useful in stimulating, modulating and regulating the host's immune system as well as release the gastrointestinal hormone.

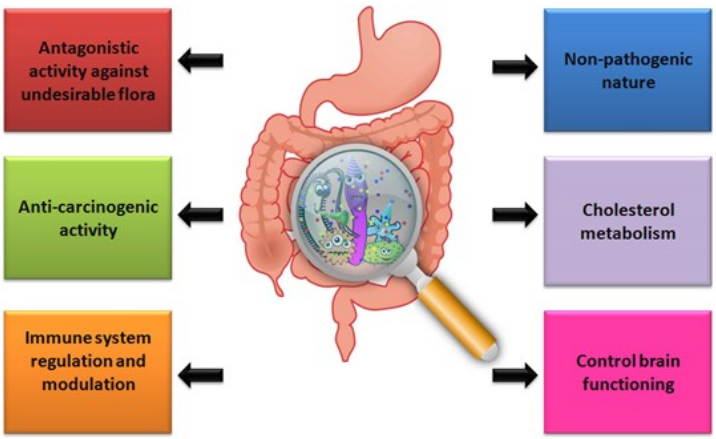

Figure 1.Biological and therapeutic functions of probiotics.

They even control the functioning of the brain through bidirectional signaling of neurons. Acute and chronic inflammation in the intestinal mucosal tissue induced due to inflammatory bowel disease (IBD) can also be regulated by probiotics [2].

Furthermore, probiotics make a significant contribution in decreasing the occurrence of cancer. Evidence have shown that microorganisms belonging to the *Bifidobacterium* and *Lactobacillus* species lower the levels of colonic flora produced carcinogenic enzymes.

The proposed mechanism includes normalization of the intestinal penetrability as well as the microbial community balance. Various studies demonstrated that the presence of probiotic bacteria in food products has proved to be beneficial in decreasing the level of serum cholesterol in the body as well as controlling blood pressure, thereby preventing coronary heart diseases [3]. Figure 1 represents the biological and therapeutic functions of probiotics.

1.1. Mechanism of action of Probiotics.
The mechanisms of action of probiotics include barrier function, production of antimicrobial substances and interference with quorum sensing signaling.

1.2. Barrier function.
Probiotics alter the functioning of the epithelial layer by raising the production of mucin or by reducing the programmed death of the intestinal cells. *Lactobacillus rhamnosus* GG showed a reduction in inflammation as well as apoptosis of the intestinal epithelial cells and led to an enhancement in the regeneration of mucosal lining.

1.3. Production of antimicrobial substances.
Probiotics are capable of preventing pathogenic invasion to epithelial cells by releasing host cell antimicrobial peptides such as defensins and cathelicidins along with antimicrobial factors like bateriocins, short fatty acids (lactic acid, acetic acid) which obstruct the entry of different species of virus, fungi and bacteria. Probiotic strains like *Escherichia coli* DSM 17252 G2 and Lactobacilli have been shown to express defensins.

1.4. Interference with quorum sensing signalling.
Bacteria utilize chemical signalling molecules (auto-inducers) to interact with one another and the surrounding environment. This process is known as quorum sensing which encourages the successful colonization of bacteria and therefore initiates infection

in the host. Probiotics hinder this communication between the pathogenic bacteria [4]. Medellin-Pena *et al.* stated that *Lactobacillus acidophilus* secretes a substance which either restricts the quorum sensing phenomenon or interacts directly with the colonization process linked with the transcription of *Escherichia coli* O157 gene [5]. The beneficial effects of probiotics to the host along with their mechanism of action have been discussed in Table 1 [6].

Table 1. Proposed mechanism and therapeutic benefits of probiotic microorganisms.

Beneficial Effects to Host	Proposed Mechanism of Action
Anticolon cancer effect	❖ Activation of immune system ❖ Detoxification of cancer causing metabolites ❖ Altering the bile salt concentration ❖ Influencing the pro-cancerous action of the microorganisms in the colon
Resistance to enteric pathogenic microorganisms	❖ Enhanced production of antibodies ❖ Resistance to colonization ❖ Obstructing the entry of pathogens (toxic oxygen metabolite, antimicrobial peptides, defensins/ bacteriocins, lactic acid production)
Immune system modulation	❖ Improved protective action (non-specific and antigen-specific) against tumors and infection ❖ Production of anti-inflammatory cytokines. ❖ Reduced release of toxic N-metabolites.
Urogential infection	❖ Adheres to the cells of vagina and urinary tract ❖ Competitive inhibition ❖ Inhibitor production such as biosurfactant, H_2O_2.
Blood lipids, heart disease	❖ Assimilation of cholesterol by bacterial cell ❖ Influencing the functioning of BSH enzyme ❖ Antioxidant action
Infection due to Helicobacter pylori	❖ Preventing adherence to mucosal cells as well as limiting the growth of *Helicobacter pylori* ❖ Reducing the concentration of *Helicobacter pylori* in the stomach
Inflammatory bowel diseases, type I diabetes	❖ Increased mucosal barrier function
Assist in lactose digestion	❖ Bacterial lactase breaks down lactose present in the small intestine
Crohn's disease	❖ Decrease in the CD_4 cells as well as pro-inflammatory cytokinins (TNRα)
Allergy	❖ Prevents the transfer of antigen in the blood stream.
Rotaviral gastroenteritis	❖ Enhanced production of IgA in response to virus

2. PROBIOTICS IN VARIOUS DISORDERS

Probiotics have attracted the interest of various clinicians and cell biologists due to their increasing therapeutic importance. The therapeutic effects of probiotics on various diseases including gastrointestinal disorders, respiratory infections, colon cancer and allergic reactions have been summarized below. Table 2 enlists the probiotics useful in the treatment of various disorders.

2.1. Gastric Ulcer.

Gastric ulcer is amongst the severe chronic disorders associated with the upper gastrointestinal tract which results from the lack of balance between the mucosal defensive system and destructive elements at the luminal surface of the stomach. It is a localized deep necrotic lesion that involves complete mucosal thickness and the muscularis mucosa. Ulcerogenesis begins with the destruction of the protective mucosal epithelial layer. An increase in the secretion of acid and pepsin by parietal and zymogenic cells may lead to mucus layer deterioration. Several studies have shown that probiotics can be used to treat stomach ulcers with *Lactobacillus* and *Bifidobacterium* species being the most widely investigated probiotics. These microorganisms have non-pathogenic nature and have the capability of resisting the severe acidic environment of the gastrointestinal tract. The administration of Lactobacillus strain in a rat model having acetic acid-induced ulcers showed improvement in the recovery from ulcer. Besides bacteria, some yeast like *Saccharomyces boulardii* has also exhibited prospective curative impact in a rat model with gastric ulcer induced by ibuprofen [7].

2.2. Antibiotic- Associated Diarrhea.

Antibiotic therapy is usually accompanied by diarrhoea as anadverse effect. Around 5-39% patients fall victim to antibiotic-associated diarrhoea (AAD). AAD can be caused by any category of antibiotics but clindamycin, cephalosporins and aminopenicilins particularly increase the susceptibility of a patient towards AAD [8]. *Clostridium difficile* and *Klebsiella oxytoca* are responsible for severe cases of AAD as they contribute to the progression of colonic lesions. Certain tests have revealed that *Saccharomyces boulardii* considerably reduced the time period of AAD [9].

2.3. Gastroenteritis.

Gastroenteritis is a frequent disorder that occurs due to parasites, bacterial pathogens or viral pathogens. Rotavirus infection is considered to be the most common cause in children. Gastroenteritis is the major reason for occurrence of acute diarrhoea and heals spontaneously in a few days. The main treatment for this is administration of oral rehydration solutions, but this therapy doesn't decrease the duration of diarrhoea. *Lactobacillus rhamnosus* GG has shown effective results against infant rotavirus diarrhoea whereas *Enterococcus faecium* SF68 was useful in treating Gastroenteritis in adults [9]. Another report demonstrated that administration of *Bifidobacterium bifidum*and *Streptococcus thermophilus* considerably decreased the susceptibility of infants towards diarrhoea [10].

2.4. Traveller's diarrhoea.

Around 50% of the travellers visiting high-risk regions are affected by acute diarrhoea. There is significant morbidity even if the cases are mild. Antibiotics can be used as a preventive measure but are not recommended for widespread use [9]. Various researchhas stated that probiotics including *Lactobacillus bulgaricus, Lactobacillus acidophilus* and *Saccharomyces boulardii* were useful in treating Traveller's diarrhoea due to their

effective results on the suppression of harmful microorganisms present in the intestine [11].

2.5. Inflammatory bowel disease.

Inflammatory bowel disease refers to a multifactorial condition accompanying chronic or recurring inflammation in the intestine. These disorders include Crohn's disease, indeterminate colitis and ulcerative colitis. Even if the mechanism responsible for the onset of inflammatory process remains unidentified, the major theory states that defective microbial balance or abnormal host response to the intestinal microflora may result in inflammatory bowel diseases [12, 13]. It may be difficult to treat and therefore new therapies are required for reducing the symptoms as well as preventing recurrence of the disease. Various experiments have shown significant curative effects of probiotics in inflammatory bowel diseases of different animal models. In a mice model suffering from dextran sulphate sodium-induced colitis, intra-colonic administration of *Lactobacillus reuteri* R2LC resulted in remarkable reduction of the disease [14]. In another study, *Faecalibacterium prausnitzii* showed protective action by suppressing inflammatory bowel disease. The results concluded an induction of IL-10 in dendritic cells of murine and human origin and thus, halt the occurrence of chronic inflammation [15].

2.6. Colon cancer.

The endogenous microbial community as well as the immune system contribute a significant part in the regulation of carcinogenesis. Modulation of these factors by probiotics leads to trials assessing the efficiency of probiotics in prevention and elimination of animal tumours. Researchers have shown that certain probiotics can reduce enzymes that are associated with colon carcinogenesis. In a rat model, preneoplastic aberrant crypt foci were suppressed by using a mixture of *Lactobacilus rhamnosus* and *Lactobacillus acidophilus* [16]. Some of the epidemiologic studies also recommend that fermented dairy food containing probiotics seemed to have positive results in colorectal cancer [17].

2.7. Urogenital infection.

Each year billions of women are affected by urinary infecti ons like vulvovaginal candidiasis(VVC), bacterial vaginosis (BV) and urinary tract infections (UTI) leading to significant healthcare costs and morbidity. Women with recurrent infections have limited treatment choices [18].

The use of probiotics is one possible approach. Various studies have demonstrated that *Lactobacillus* strains including *Lactobacillus fermentum RC-14* and *Lactobacillus rhamnosus GR-1* metabolize the carbohydrates present in the vaginal epithelium and release lactic acid. This leads to a reduction in pH, thereby creating unfavorable conditions for the survival of the pathogens. They also have the ability to produce antibacterial metabolites such as bacteriocins and hydrogen peroxide, therefore decrease the occurrence of UTI [19].

2.8. Respiratory infection.

Upper respiratory tract infections (URTIs) refer to the diseases which occur due to the infection of the mucous lining present in the nose, pharynx, larynx and sinuses. The pathogens initiate the infection by directly invading the mucosal surface of the upper airways.

They then have to fight with the healthy microflora of the airways as well as beat the immune system of the host. Generally, these infections are resolved immediately by the body. But in case of negligence, the symptoms can worsen leading to breathing and swallowing problems.

Most of the therapies are meant to relieve the symptoms but not eliminate the cause of the infection. Administration of antibiotics requires caution due to the promotion of secondary infections and bacterial resistance as well as the side effects associated with them. An alternate to this is the use of probiotics. On the administration of food products containing probiotics, temporary colonization of the bacterial strains occurs in the upper respiratory tract. This inhibits the pathogens present in the upper respiratory tract as well as prevents them from reaching the inaccessible areas such as sinuses and middle ear, thereby protecting and restoring the healthy microflora. Two strains, *Streptococcus oralis*89a and *Lactobacillus rhamnosus*LB21 have the ability to obstruct the harmful action of URTI related pathogens like *Haemophilus influenza, Streptococcus pyogenes, Streptococcus pneumonia* and *Moraxella catarrhalis*. Moreover they are useful in the prevention of ear and throat infection as proved by clinical trials [20].

2.9. Probiotics in allergy.

An allergy refers to a hypersensitivity reaction caused due to immunological mechanisms. Probiotics modulate allergic disorders by stimulating the levels of IgA and the responses of B and T cells. Various studies have concluded that LactobacillusGG reduced the recurrence frequency of atopic dermatitis in the newborns to 50% when administered in pregnant females suffering from asthma, allergic rhinitis and eczema. It also finds application as an oral vaccine for rotaviruses. The probiotic strain of *Lactobacillus casei* activates the immune system and protects the patient from enterobacterial infections [21].

Table 2. Probiotics useful in various disorders

Disorders	Probiotics
Gastric Ulcers	Lactobacillus, Bifidobacterium, *Streptococcus boulardii*
Antibiotic Associated Diarrhea	*Streptococcus boulardii*
Gastroenteritis	*Lactobacillus rhamnosus, Enterococcus faecium*SF68, *Bifidobacterium bifidum, Streptococcus thermophilus*
Traveller's Diarrhea	*Lactobacillus acidophilus, Lactobacillus bulgaricus, Saccharomyces boulardii*
Inflammatory Bowel Disease	*Lactobacillus reutri*R2LC, *Faecalibacterium prausnitzii*
Colon Cancer	*Lactobacilus rhamnosus*, *Lactobacillus acidophilus*
Urogential Infection	*Lactobacillus fermentum*B-54, *Lactobacillus rhamnosus* GR-1
Respiratory Infection	*Streptococcus oralis*89a, *Lactobacillus rhamnosus* LB21
Allergic Reactions	*Lactobacillus* GG, *Lactobacillus casei*

3. MICROENCAPSULATION OF PROBIOTICS

The intake of most bacteria via oral route leads to reduction in viability due to the harsh acidic conditions and high bile salt concentrations encountered by it while passing through the stomach. This reduction of viability results in decreased efficiency of the administered product. Microencapsulation of the probiotics is a new approach to minimize the cell death while passage through the gastrointestinal tract along with their release across the intestinal tract in a controlled rate [22]. Table 3 enlists the various pros and cons of microencapsulation techniques.

Microencapsulation is a process by which continuous film of the polymeric coating material is deposited on the solid or liquid core with size ranging in micrometers. It aids in the protection of the unstable core from the external environmental stresses like gastric acidity, heat and oxygen, thereby augmenting the shelf life of the core, enhancing its stability as well as providing controlled and sustained release [23]. The advantages related to microencapsulation of probiotics are depicted in Figure 2.

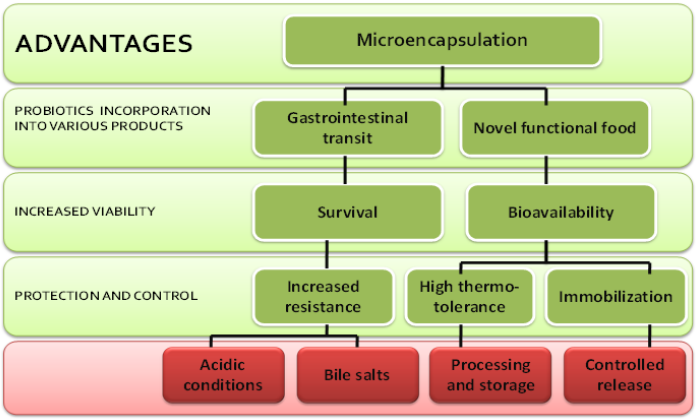

Figure 2. Advantages of microencapsulated probiotics.

Different techniques for the microencapsulation of probiotic microorganism have been discussed in detail hereunder.

3.1. Spray Drying.

Spray drying is the persistently employed technique because of low production cost and readily accessible equipment. It is seen as a solution to the prevailing drying issues as the process not only proves to be efficient but is also economically beneficial. In this technique, the active core ingredient is dispersed into the suitable coating solvent with continuous heating and homogenization of the dispersion. The preparation is then atomised by streaming through a nozzle into heated air. The solvent evaporates when exposed to the heated air stream, resulting in the formation of microcapsules [24]. Various researchers have utilized this technique for encapsulating different probiotics in order to enhance their therapeutic effectiveness. In a research conducted by Dos santos *et al.*, gastrointestinal survival of *Lactobacillus acidophilus* (La-5) was enhanced by spray coating with inulin. The factors responsible for the survival of microencapsulated probiotic strains include low solubility of inulin and resistance of inulin to hydrolytic degradation caused by gastrointestinal enzymes [25]. Tao *et al.* encapsulated the probiotic strain, *Lactobacillus paracasei* Lpc-37 with the help of spray drying method. The experiment concluded that polysaccharide encapsulated strains showed tolerance towards acidic conditions, followed by their release in the intestine. Amongst the different polysaccharides, best results were shown by carboxymethylcelluose and sodium alginate when used in combination with skim milk [26]. In another report, Arslan *et al.* improved the survivability of *Saccharomyces boulardii* in simulated gastric media by spray coating it with diverse wall materials like gum Arabic, pea protein isolate and gelatin. *Saccharomyces boulardii* provides health benefits including destruction of the pathogens and supporting the immune system. The study also revealed that drying temperature as well as exposure time had an impact on the survival rate of the microcapsules. Higher tolerance towards the gastric media was shown by microcapsules which were prepared at higher drying temperature (125 °C) when compared with those prepared at low drying temperatures (80 °C). Furthermore, survival was decreased as time of exposure to simulated gastric media was increased [27]. Gul *et al.* utilized spray drying technique for encapsulating *Lactobacillus casei* Shirota with various combinations of coating materials such as gum Arabic, reconstituted skim milk and maltodextrin. The inclusion of gum arabic into maltodextrin or reconstituted skim milk led to an enhancement in the encapsulation efficiency as well as protected the probiotic strain from the gastrointestinal juices [28].

3.2. Spray Cooling

In spray cooling technique, mixture of the active ingredient and coating material (molten matrix) is sprayed with the help of an atomiser into a chamber through which chilled air current is passed. The introduction of cold air results in rigidization of the droplets, thereby forming microcapsules [29]. Bampi *et al.* prepared microparticles by encapsulating *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. Lactis with the help of spray cooling method to inculcate them in savory cereal bars. The resulting product was in the form of smooth spheres have low water activity as well as low moisture content. On evaluating the viability of the microencapsulated probiotics it was found that they could be stored for atleast 90 days at -18 °C. Also, the microparticles prepared by spray cooling method showed the potential of releasing the probiotic in the intestine of consumer with the help of fat digestion [30]. In another experiment performed by Silva *et al.* on the same strains, encapsulation was done with molten vegetable fat. The survivability of probiotics increased to 75% under different pH conditions as well as simulated gastrointestinal environment, indicating that encapsulation of probiotics shielded them from destruction when compared to non-encapsuated cells [31].

3.3. Fluid-Bed Coating

Fluid bed coating involves the introduction of core material into the air stream. The coating solvent is injected into the chamber with the help of a nozzle and sprayed upon the core material. The circulation of particles leads to uniform covering of the core particles by the coating material, improves the drying rate

as well as decreases the chances of agglomeration [32]. In an experiment performed by Schell *et al.*, *Lactobacillus reuteri* DSM 20016 was coated with dietary shellac and sweet whey powder by top-spraying fluidized bed coating technique. The encapsulation of the bacteria leads to an enhancement in the survival rate of the bacteria under low pH and allows its release from the core directly in the intestine [33]. Semyonov *et al.* employed the technique of air-suspension fluidized-bed coating for preparing multi-layered microcapsules of probiotics. Firstly, different solutions consisting of probiotic *Lactobacillus paracasei*, trehalose and maltodextrin in varying ratios were sprayed and adsorbed onto microcrystalline cellulose. In the second step, the prepared particles were layered with wax and ethyl cellulose (ETHOCEL). The results concluded that trehalose and maltodextrin led to an enhancement in the cell viability as well as stability of probiotic bacteria during storage and the additional coating of ETHOCEL showed protective effect against high acid content in the stomach [34]. Albadran *et al.* utilized the fluid bed drying technique for the preparation of microcapsules of alginate and probiotic bacteria, Lactobacillus plantarum, with chitosan as the coating material. As a result, an increase in the survivability of the dried encapsulated cells during storage was observed, thereby considering fluid bed drying a proficient technique for manufacturing probiotic containing capsules [35].

3.4. Freeze Drying

Freeze drying is a popular technology useful in drying as well as enhancing the stability of different pharmaceuticals. It involves 3 stages: solidification by freezing, sublimation by primary drying and removal of unfrozen water by secondary drying. This technique is also termed as lyophilisation [36]. Thermally sensitive materials which get degraded at high temperatures can be efficiently dried by this technique [37]. Heidebach *et al.* produced casein microcapsules consisting of *Bifidobacterium lactis* Bb12 by freeze drying process. On investigating their subsequent storage, it was found that encapsulation led to an improvement in the viability of *Bifidobacterium lactis* Bb12 upto 90 days of storage period [38]. Dhewa*et al.* loaded prebiotics such as inulin and acacia gum along with the probiotic strain, *Lactobacillus plantarum* and prepared a synbiotic formulation with the help of freeze drying method. The addition of prebiotics modified the microbiota of the gut, provided protection from the harmful pathogens and enhanced gastrointestinal immunity [39]. Alehosseini *et al.* prepared alginate-based microcapsules consisting of sensitive strain *Bifidobacterium pseudocatenulatum* CECT 7765. The probiotic containing bend was further dipped into biphasic oil/aqueous solution and freeze dried. This method proves to be useful for protecting oxygen-sensitive microorganisms as the capsules were prepared in lipid medium, thereby restricting the interference by oxygen. The probiotic cells were even safeguarded in the acidic conditions of the stomach and an improvement in viability was observed when compared to uncoated probiotic strains [40].

3.5. Emulsion Based Technique

Emulsion is a dispersion in which both the phases (core and coating material) are immiscible with each other. The core material can be aqueous or hydrophobic. If it is aqueous, the emulsion prepared is considered to be water-in-oil (w/o) emulsion and if it is hydrophobic, the emulsion is considered to be oil-in-water (o/w) emulsion. Several combinations of double emulsions can also be prepared. The probiotic microbes get entrapped into the emulsion droplets which protect them from the harsh environment [41]. According to Song *et al.* probiotic yeast Y235 cells were entrapped in calcium alginate beads with the help of emulsification technique. The beads produced were further dipped into chitosan to form alginate-chitosan (AC) microcapsules. External gelation method led to the formation of irregular-shaped beads with poor encapsulation efficiency whereas internal gelation method produced spherical beads that provided sufficient protection to the encapsulated bacteria. The cell viability was found to be near about 80% for both the emulsification processes [42]. Singh *et al.* prepared carboxymethylcellulose -gelatin water-in-water emulsions incorporating *Lactobacillus rhamnosus* GG. Carboxy methyl celluose-in-gelatin dispersions protected the bacteria in the simulated digestive fluids thereby increasing their proliferation and viability as compared to the naked bacteria [43]. Zou *et al.* utilized emulsification ⁄ internal gelation technique for producing polymer-blended (pectin and starch) and polymer-coated (chitosan) alginate microspheres. The results concluded that amongst the 3 types of alginate microspheres, chitosan-coated alginate microspheres ensured a higher survival in the simulated gastric environment and was also useful in the delivery of the bacteria to intestine [44]. Calligaris *et al.* demonstrated the use of monoglyceride structured emulsions (MSEs) for entrapping *Lactobacillus rhamnosus* cells. An improvement in the survival rate of the bacterial cells during freezing was observed on comparison with the non-encapsulated cells. Moreover, MSEs showed sufficient ability in protecting the probiotic from stresses encountered during processing and storage. [45].

3.6. Coacervation Phase Separation

Coacervation phase separation technique basically involves three stages. In the first stage, 3 immiscible phases: a) core material phase, b) coating material phase, c) liquid manufacturing phase (solvent for the coating material) This separation of phases can be carried out by induction of polymer-polymer interaction, varying the temperature of polymer solution or by addition of a solvent. The second stage involves the deposition of coating material onto the core material and the last stage involves rigidization of the coating material with the help of desolvation or thermal cross-linking technique, resulting in the formation of microcapsules [46]. Da silva *et al.* enhanced the protection from the simulated gastric environment as well as improved viability of the probiotic bacteria *Lactobacillus acidophilus* by encapsulating it with the coacervation technique and subsequent crosslinking by transglutaminase [47]. Bosnea *et al.* utilized whey protein isolate and gum Arabic for microencapsulating *Lactobacillus paracasei* subsp. paracasei (E6) and *Lactobacillus paraplantarum* (B1) by coacervation method. The probiotic coacervates showed increased encapsulation efficiency as well as high viability of probiotic strains in low pH conditions and storage in refrigerator for 60 days when compared with non-encapsulated cells. With the help of this technique, probiotics can be incorporated in fermented dairy products and juices [48]. Oliveira et al. prepared coacervates of *Bifidobacterium lactis* (BI 01) and *Lactobacillus acidophilus* (LAC 4) by using casein/pectin complex. The coacervated product was further atomized by spray drying process for ensuring an improvement in the stability. The wall material is chosen and the

process followed wasuseful in efficiently shielding the probiotics from the spray drying process as well as the simulated gastric conditions. *Bifidobacterium lactis* lost its viability prior to the end of storage period whereas *Lactobacillus acidophilus* showed longer storage viability (120 days) [49].

3.7. Extrusion Technique

In extrusion technique, the core material which is to be encapsulated is loaded in the solution of sodium alginate. Then this mixture is allowed to extrude drop-wise from a syringe into the hardening solution [50]. This technique imparts the advantage of long shelf life to oxygen-sensitive compounds as it provides a barrier against oxygen [51]. In a study conducted by Mirzaei*et al.,* calcium alginate gel and resistant starch were used for encapsulating *Lactobacillus acidophilus* (La5) by extrusion method. The results concluded increased survivability of the strain in Iranian white-brined cheese when stored for a period of 182 days [52]. Lee *et al.* prepared microspheres of *Lactobacillus acidophilus* KBL409 with the help of alginate (Al) and alginate-chitosan (Al/Chi) by extrusion technique. The mucoadhesion shown by non-encapsulated cells was 88.1% whereas that shown by encapsulated cells was greater than 94%. Moreover, the highest survival rate was observed in the case of Al/Chi-encapsulated cells in the simulated body fluids (gastric as well as intestinal) [53]. Phoem*et al.* suggested that microencapsulation of *Bifidobacterium longum* with *Eleutherine americana* extract by extrusion method showed superior viability and survival in food products than the free bacterial cells. Moreover, the microencapsulated form of *Bifidobacterium longum* enhanced the functional properties as well as the sensory quality of food products. Its addition to the pineapple juice led to a lowering of the rate of post-acidification during storage period [54]. Chavarri*et al.* used chitosan as a coating material to improve the stability of probiotics, *Lactobacillus gasseri* and *Bifidobacterium bifidum* in alginate beads. The complex formed between chitosan and alginate decreased the voids in the alginate beads, thereby reducing the leakage of the encapsulated bacteria. The microencapsulation technique proves to be beneficial for improving their viability in the simulated gastrointestinal fluids as well as delivering the bacterial cells to the colon in an appropriate amount [55].

Table 3. The pros and cons of different microencapsulation techniques.

Technique	Pros	Cons
Spray Drying	• Continuous process • Material monodispersity • Economical	• High temperatures lead to reduction in viability. • Predominantly used with aqueous suspension (coating material needs to be water-soluble)
Spray Cooling	• Mild temperature setup • Continuous process • Mass production	• The load is lower (10-20%) when compared with spray drying process (5-50%) • Problem in delaying the release for more than 30 minutes for water-soluble ingredient
Fluid Bed Coating	• Different coatings can be used for modifying the drug release. • Enhanced stability on storage	• Cells might be damaged by certain forces • Although temperature is lower, the exposure could be longer along with oxygen exposure.
Freeze Drying	• Perfectly dried finished product • Ideal for sensitive substances	• Expensive • If not done properly, cell damage with subsequent crystal formation occurs
Emulsification	• Simple process • Increased survivability of bacteria	• Scale-up is expensive • Variation in the shape of the material
Coacervation	• Preparation conditions are mild • Enhanced shell integrity • High payloads (around 99%)	• Costly technique • Quality issues
Extrusion	• Potential to scale-up potential • Mild working conditions	• Larger capsules produced • Usually other techniques are also used in combination for the final drying of the product

4. CONCLUSIONS

Oral administration of probiotic microorganism bears the major challenge of significant loss of viability leading to a reduction in efficacy. Microencapsulation is an effective method to increase the protection and hence the survival rate of the entrapped probiotic microorganisms. Therefore, there is a widespread interest of researchers to develop sustainable and effective microencapsulation technique for the delivery of probiotics. Improvement in the physicochemical properties (physical, mechanical) of polymers is yet another challenging area for developing microencapsulation method for probiotics. Future challenges in this field include development and optimization of appropriate technique, development of customized materials and bacterial strains, exploring newer and diverse applications of microencapsulated probiotics, cost minimization of processes and development of in-vitro/ in-silico evaluation techniques. Issues related to large scale production and regulatory compliance must also be addressed to access commercial viabilities of microencapsulation of probiotic microorganism.

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