



# DEVELOPMENT OF PROBIOTIC DELIVERY SYSTEM

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## ABSTRACT

Over the past decades, the administration of probiotic bacteria as a nutraceuticals has gained much attention. Probiotics are live microorganisms which confer a health benefit on the host when administered in an adequate amount. The health benefits of probiotics are dependent on the viability and sufficient number of probiotics in the target intestine. Due to probiotic's vulnerability to several environmental factors such as temperature and PH, maintaining the viability of probiotics has long been a hurdle to develop successful probiotic delivery system. In this review, we provide an overview of health benefits of probiotics, hurdles in probiotic delivery, commonly used encapsulating materials and recent probiotic delivery technologies.

**Keywords:** probiotic delivery, Microencapsulation, Thermo-tolerance, Acid resistance

## Introduction:

In recent years, there has been increasing interest in food products containing probiotic bacteria. The addition of probiotic bacteria as functional food supplements has become popular due to the health benefits of these bacteria. The probiotics segment dominates the functional food ingredients market. Evidence from scientific studies suggests that probiotic strains exert a beneficial effect against various disorders, such as gastrointestinal diseases, bacterial vaginosis and urinary tract infections. Probiotics have been implicated in inhibiting enteric pathogens, maintaining gut permeability, modulating the immune system, reducing inflammation, alleviating lactose intolerance, enhancing bowel motility and reducing cholesterol concentration. To confer these health benefits to the host, a sufficient number of live cells is required to adhere to the host colon. As defined by the Food and Agriculture Organization of the United Nations and the World Health Organization (2002), probiotics are living microorganisms which, when administered in sufficient amounts, confer health benefits to the host.

However, the viability of probiotic bacteria is questionable when they are exposed to harsh environments during processing (i.e., dehydration), storage and delivery to their site of action (i.e., the gastrointestinal tract). Several studies have reported that oral doses higher than 10<sup>9</sup> colony-forming units (CFUs) per day are required to restore and maintain the balance of bacteria. Thus, probiotic bacteria should maintain high levels of viability during processing and remain alive during storage and delivery; for example, as they pass through the GIT. The survivability and dose levels of probiotics during storage and delivery are important parameters for probiotic efficacy.

During storage and delivery by oral administration, probiotics are exposed to water, oxygen, heat, strong acid and bile. To overcome these adverse factors, various dosage forms, such as capsules, tablets, powders and liquids, have been used. Moreover, some special forms, such as vaginal suppositories and eye drops are also used. These forms were first designed to maintain the viability of the probiotic bacteria during storage and delivery. With the development of new technologies, such as materials and embedding technologies, targeted release and directional delivery have become important research directions.

## **Bacterial Probiotics**

### **Microorganisms used probiotics**

The composition of the gastro intestinal tract flora varies between individuals and also within the same individual during life. The tract contains both 'friendly' and pathogenic bacteria that exist in a complex symbiosis. Various factors such as diet, climate, ageing, medication (particularly antibiotic consumption), illness, stress and lifestyle can upset this balance leading to diarrhea, mucosal inflammation or other serious illnesses.

The crucial event in the development of the probiotic approach to animal health was the finding that the newly hatched chicken could be protected against salmonella colonization of the gut by dosing it with a suspension of gut contents prepared from healthy adult chickens. Microorganisms used in probiotics include those derived from the *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Clostridium*, *Bifidobacterium* species and *E. coli*. Bacterial probiotics have been effective in chickens, pigs and pre ruminant calves.

Species belonging to *Bifidobacterium* and *Lactobacillus* are largely used as probiotics. Most of the probiotic bacteria are lactic acid producing bacteria (LAB). It has been shown that lactic acid inhibited the growth of coliforms in the gastro-intestinal tract. Acidic environments are detrimental to many pathogens. The most commonly used probiotic strains include the lactic acid bacteria (LAB) and Gram-positive microbes that have been used for centuries in food production processes (yogurt, cheese, pickles). Members of the LAB such as *Lactococcus* and *Streptococcus* are also important components of the endogenous microbiota in the human ileum and jejunum administered to neonates to prevent the colonization of their digestive tract by multi-drug resistant pathogens.

## Probiotic selection chart

- Source
- Isolation and reliable identification
- *In vitro* and *In vivo* screening probiotic use based on technological and beneficial criteria
- *In vitro and in vivo* studies of beneficial mechanisms
- Development of formulation containing adequate levels of live cells maintaining viability
- During storage
- *In vivo* preclinical trial (acute and chronic toxicity in animals)
- Double blind, randomized, placebo or formula controlled clinical trial in humans
- Governmental agency approval
- Probiotic commercialization

## Mechanism of action of prebiotics

Probiotic bacteria counteract inflammatory processes by stabilizing a healthy microbiota and thus improving the intestine's permeability barrier. In addition to influencing gut microbiota and immune system, other mechanisms of probiotic action have been proposed, such as inhibition of pathogens by competition for nutrients and attachment sites or by production of antimicrobial substances, reduction of cholesterol levels through deconjugation of bile salts or binding of toxins and preventing their absorption.

The potential mechanisms by which probiotic agents might exert their protective effect include: antagonism by the production of substances that inhibit or kill the pathogen, competition with the pathogen for adhesion sites or nutritional sources; immunomodulation of the host; and inactivation of microbial toxin (Other mechanisms by which probiotics may exert protection is through a recuperation of mucosal barrier function when disturbed, trapping pathogens on their surface and stimulating mucus production, specific probiotic bacteria have been reported to modulate local and systemic immune responses.

## Dosage forms containing probiotics

### Liquids

The first probiotic products available were mostly liquid formulations . Probiotics in liquid form are commercially available in various food matrices. Fresh dairy products are the most common products used for probiotic delivery. During the past few years, the diversity of probiotic foods on the market has increased. Probiotics can now be found in non-fermented milk, fruit and berry juices and cereal-based products. As an example, a functional probiotic drink using rice and soy as the fermentation substrates produces unique flavor substances and bioactive substances through the combination of the two ingredients, which can be developed as a new type of plant-based drink.

## Powder

Powder The low transport and storage temperature requirements are the main commercial disadvantage of these liquid preparations, since the environmental stresses such as pH, water activity and oxygen in liquid preparations can affect the viability of probiotics. To minimize the costs, it is important to produce probiotics in dry form. Orally administered powders are dry, solid granules made from a homogeneous mixture of a drug and its excipients. Probiotic powder, in the form of a dry powder, exhibits various advantages, such as convenient handling, storage and transportation and it can be used individually as a dosage form or as an intermediate in many other probiotic dosage forms. Manufacturing dehydrated probiotic powders is challenging because it involves maintaining a large bacterial population and high viability after dehydration to prolong the storage time in complex environments.

## Capsules

Capsules Delivering viable probiotic cells to the GIT is challenging, especially when the probiotic product is in liquid or powder forms. Capsules are considered to be one of the ways to address this challenge. Capsules are solid dosage forms with either a hard or soft soluble container or shell made of a suitable form of gelatin. Most dietary supplements are Foods sold in the form of capsules. Hard capsules are generally preferred to administer probiotics. These capsules are available in different sizes and varieties and contain probiotics in the form of powders or microcapsules. They may also contain excipients, such as diluents, glidants, disintegrants or fillers. These excipients maintain the physiology of the selected probiotic.

## Microcapsule

Microcapsules are very small capsules containing a material (such as an adhesive or a medicine) that is released when the capsule is broken, melted or dissolved. They range from nano meters to milli meters in diameter. The microencapsulation of probiotic bacteria is a promising technology to ensure bacterial stability during the drying process and to preserve their viability during storage, without a significant loss of functional properties, such acid tolerance, bile tolerance, surface hydrophobicity and enzyme activities. The encapsulation of probiotics is used to increase the resistance of bacteria to freezing and freeze drying. In most studies, probiotic bacteria have been entrapped in a gel matrix of biological materials, such as alginate,  $\beta$ -carrageenan and gellan /xanthan.

## Tablet

The tablet, a dosage form with a high share of the global market, provides many advantages, such as physicochemical stability, a simple manufacturing process, low manufacturing cost and a high level of acceptance by consumers. Although tablets are not the preferred dosage form for probiotic preparations, the properties of tablets make them an important direction for probiotic drug development. In view of the adverse effects on the bioactivity of probiotics caused by compression and wet granulation methods, the general process for probiotic tablet formulation is to mix the powder with an excipient after a drying procedure and then press the tablets directly into shape . However, processes such as drying, mixing and compression inevitably destroy

a wide range of cellular and biologically active components of probiotics, which is a challenge that needs to be addressed in the design of probiotic tablets.

## Suppositories

A suppository is a solid drug delivery system that typically dissolves and releases its components at normal body temperature. These delivery systems include rectal, vaginal and urethral suppositories. Suppositories are capable of preserving probiotic viability to a considerable degree and they are suitable for mass production and molding. The vast majority of probiotic suppositories are vaginal suppositories, which maintain dosage uniformity, can provide less irritation to the vagina than other forms, such as effervescent Foods tablets, and eliminate the need for large amounts of solution to dissolve the drug, such that it is more likely to be accepted by the user.

## Recent trends of probiotic delivery system

As described in the previous section, encapsulating probiotics into carrier materials had been a common strategy for probiotic delivery until recently. However, challenges still exist for effective protection of probiotics from tough conditions during a manufacturing process, a long-term storage and a transit in the GI tract in order to obtain a sufficient number of viable bacteria in the target site. This has propelled development of new strategies in probiotic delivery.

In this section, we describe recent advancement in probiotic delivery systems. Alginate has been the most extensively studied encapsulating material; however, a protective effect of bare alginate is not enough to obtain a sufficient number of viable probiotics in target sites due to a porous nature and an uncontrollable swelling behavior, which could allow H ion penetration and make the alginate system susceptible to acids. In addition, cell leakage by low mechanical durability in storage is a potential problem of alginate. Recent studies have employed various coating technologies to overcome the limitation by providing an additional protection to the surface of alginate microparticles or beads. Chitosan coating on alginate beads has been used to provide probiotics for protection from acids by reducing pore size of alginate beads.

Overview of recent probiotic delivery systems Delivery system Materials Probiotic strain Key purposes References Multi-layer coating Chitosan, alginate Bifidobacterium breve Elucidate pH protective effect due to alginate-chitosan multilayers Cook et al. Core-shell Protamine, chitosan, alginate Lactobacillus casei Rapid release in target area Effective Inhibition of H ion permeation. Enteric coating Eudragit Lactobacillus casei Target delivery of encapsulated probiotics de Barros et al. Multi particulate PLGA, chitosan, alginate Bifidobacterium breve Co-delivery with prebiotics to maximize health promoting effect Increased pH-protective effect due to increased hydrophobicity by PLGA Cook et al. Composite Bacterial nanocellulose, pectin Bacillus coagulans Maintain stability to thermal drying and Long-term storage Improve viability to GI tract condition Khorasani Cell surface engineering Carboxymethyl cellulose, chitosan Lactobacillus acidophilus Prevent from large molecular weight enzyme penetration Priya et al. Probiotic delivery systems: a brief overview 123 viability of probiotics from chitosan-coated alginate beads and compared to bare alginate beads.

After 60 min of incubation in an acidic pH, chitosan-coated alginate beads showed a higher viability by delayed H<sup>+</sup> penetration as compared to bare alginate beads. In another work, chitosan-coated alginate beads were used for encapsulating *Bifidobacterium breve*, resulting in over 6 log CFU/ml of cells survived, while no viable cells were observed with non-coated in detectable range. As the number of chitosan-alginate coating increased, the protective effect for probiotics were also enhanced.

survival while the viability of free cells decreased to 77 % after 35 day of storage In another paper, *Bifidobacterium BB-12* was encapsulated in milk proteins blended with oligofructose-enriched inulin. In the study, more than 10.5 log CFU/g of cells were survived after 180 days at 4 C storage Since many of probiotics can be exposed to high temperatures for pasteurization and spray-drying process and low temperatures for a freeze-drying process, maintaining viability during the manufacturing processes is also of importance.

Various technologies have been incorporated into formulations to improve the survival rate of probiotic bacteria during manufacturing processes and in storage a large molecular weight which is present in gastric juice. Inhibition of pepsin penetration to a probiotic wall is of important for probiotic viability. By introducing chitosan and carboxymethyl cellulose onto bacterial surface, the penetration of large molecular weight enzyme was effectively inhibited while leaving a small molecular nutrition freely flow in and out. As a result, more than 8 log CFU/g of *Lactobacillus*

### **Importance of probiotics:**

Many health benefits have been related to human and animal intake of probiotic. Several studies have supplied clinical evidences of the benefits generated by probiotics, as for example in diarrhea treatment, lactose intolerance and irritable bowel syndrome (IBS). In the meantime probiotics are applied as feed supplements, pharmaceuticals, dairy products, of Nutrition and Health Sciences. It is necessary to remember that more than 2000 years ago ancient Greek physician Hippocrates, considered as the father of Western medicine, said, "Let food be thy medicine and medicine be thy food." The use of foods for their medical value as for their nutritional benefits is an ancient tradition in China, India, Egypt and Japan. Besides their desired health and clinical properties, probiotics must meet several basic requirements for the development of marketable probiotic products. The most important requirements are that probiotic bacteria survive in sufficient numbers in the product, that their physical and genetic stability during storage of the product be guaranteed, and that all of their properties essential for expressing their health benefits after consumption be maintained during manufacture and storage of the product. Preparation of viable microorganisms that is consumed by humans or other animals with the aim of inducing beneficial effects by qualitatively or quantitatively influencing their gut microflora and/or modifying their immune status. Public health importance meat products. The conventional use of probiotics to modulate gastrointestinal health, such as improving lactose intolerance, increasing natural resistance to infectious diseases in the GI tract, suppressing traveler's diarrhea and reducing bloating, has been well investigated and documented. Clinical trials have evaluated their use in the prevention and treatment of GI diseases caused by pathogenic microorganisms or by disturbances in the normal micro-flora . Recent research on the molecular biology and genomics of *Lactobacillus* has focused on the interaction with the immune system,

anti-cancer potential, and potential as a biotherapeutic agent in cases of antibiotic-associated diarrhea, travelers' diarrhea, pediatric diarrhea, inflammatory bowel disease and irritable bowel syndrome.

### **Nutritional importance**

Fermentation is a process in which a microorganism transforms food into other products, usually through the production of lactic acid, ethanol and other metabolic end-products. Probiotic bacteria are sold mainly in fermented foods, and dairy products play a predominant role as carriers of probiotics. These foods are well suited for promoting the positive health image of probiotics for several reasons: fermented foods and dairy products in particular, already have a positive health image; consumers are familiar with the fact that fermented foods contain living microorganisms (bacteria); probiotics used as starter organisms combine the positive images of fermentation and probiotic culture. For centuries it has been considered that a diet based on fermented milk products has a beneficial effect, controlling the development of pathogenic bacteria in the thin intestine, leading to improved overall health, and having as main effect increase life period. Metchnikoff proposed that consumption of fermented milk would "seed" the intestine with harmless lactic-acid bacteria and decrease the intestinal pH and that this would suppress the growth of pathogenic bacteria.

Lactic acid fermentation is the most common method and one of the easiest to experiment with. It is a process where lactic acid bacteria (LAB), mainly the *Lactobacillus* species, convert sugar into lactic acid, which acts as a preservative. Prior to refrigeration and pasteurization, fermentation allowed food to be stored and preserved for later use, preventing spoilage by the natural defenses of lactic acid producing bacteria. Lactic acid producing bacteria (LAB) are functional classification of non-pathogenic, nontoxicogenic, Gram-positive, fermentative bacteria that are associated with the production of lactic acid from carbohydrates, making them useful for food fermentation.

### **Safety aspects of probiotics**

Most of the micro-organisms used as a probiotics in animal are safe, although some have problems particularly the enterococci, which may harbor transmissible antibiotic resistance determinants and *Bacillus cereus* group that are known to produce enterotoxins and an emetic toxins. Particular attention for safety assessment is focused on the presence of transmissible antibiotic resistance markers and the potential for production of harmful metabolites. Thus the appealing properties of probiotics include the ability to reduce antibiotic use, the apparently high index of safety and the public's positive perception about natural or alternative therapies. The main currently used tests for the study of probiotic strains are resistance to gastric acidity and bile acid, adhesion to gut epithelial tissue, antimicrobial activity against potentially pathogenic bacteria and ability to modulate immune responses.

There are numerous advantages and health benefits associated with probiotics or probiotic food products, there are risks associated with probiotic therapy. These risks are mainly concerned with respect to safety in vulnerable target groups such as immune compromised individuals (pregnant women, babies and the elderly) or critically ill or hospitalized patients. Probiotics can interact with commensal bacteria and can also have a direct impact on the host. Understanding these interactions is one of the key challenges for future research. Other key challenges are to understand their mechanisms of action, to elucidate more specifically which probiotic strains can offer

which health benefits and to define the intake levels needed to achieve those effects. Some species of lactobacilli and bifidobacteria are normal residents of or common transients through the human digestive system and as such do not display infectivity or toxicity.

Traditional lactic acid bacteria (LAB), long associated with food fermentation, are generally considered safe for oral consumption as part of foods and supplements for the generally healthy population and at levels traditionally used. Regulations for dietary supplements are nonexistent in many countries, or much less strict than those that apply for prescription drugs. There is concern over the use in foods of probiotic bacteria that contain specific drug resistance genes. Bacteria, which contain transmissible drug resistance genes, should not be used in foods. To formalize and underwrite this concept, a system for a pre-market safety assessment was proposed that leads to a 'Qualified Presumption of Safety (QPS)' in the European Community. In summary, a safety assessment of selected groups of micro-organisms from a defined taxonomic group (e.g. genus or group of related species) can be made on the basis of four pillars of information (identity, body of knowledge, possible pathogenicity and end use) actococcus, and Streptococcus.

### **Nanotechnology for Probiotics**

With advances in nanotechnology, nanomaterials have been widely used for formulation since they have useful physicochemical properties including biocompatibility and the capability to respond to environmental stimuli for controlled drug release. Particularly, some nanomaterials-based formulations such as nanofibers, nanoparticles, and nanocomposites exhibit many advantages as delivery systems for viable probiotics, which include efficient encapsulation, site specific delivery, controlled release, and improved stability of bacterial cells during manufacturing, storage, and in vivo applications.

### **Nanofibers**

Nanofibers are produced by an electrospinning of polymer solutions under strong electric fields. Natural polymers have better biocompatibility and lower immunogenicity but it is easier to modify synthetic polymers to obtain the desired physicochemical properties; therefore, polymer blends are commonly used. Polyvinyl alcohol, polyethylene oxide, cellulose, and chitosan are the most commonly used polymers for incorporating probiotic bacteria. Nanofibers exhibit uniform morphology and composition, nanometric diameter, large surface areas, and high porosity, offering many advantages for the efficient delivery of biological substances. Consequently, nanofibers can be used to encapsulate various microorganisms, cells, genes, and proteins. The incorporation of probiotics into nanofibers can improve the stability of probiotic bacterial cells and also allow their site-specific delivery. López-Rubio et al prepared ultrathin PVA-based electrospun fibers encapsulating *Bifidobacterium animalis* Bb12 which had a mean diameter of 150 nm and were effective in retaining the viability of encapsulated probiotics for 40 days of storage at room temperature and for 130 days under the refrigerated conditions. Silva et al also fabricated PVA-nanofibers containing *L. rhamnosus* CRL1332, which had a mean diameter of 95 nm and maintained the viability of the probiotic bacteria over 360 days of oxygen-excluding storage at 4°C. Furthermore, nanofiber-immobilized *L. rhamnosus* cells retained with their inhibitory effect against urogenital pathogens.



## Nanoparticles

Active ingredients can be encapsulated in nanocontainers to address various issues related to stability, solubility, safety, and target specificity. Accordingly, probiotics can be formulated as polymeric nanoparticles, lipid-based nanoparticles, and inorganic nanoparticles. In recent years, hybrid nanoparticles using two different materials (eg organic/inorganic, lipid/ polymer nanoparticles) have been actively pursued to combine the advantages of each single system. Furthermore, the surface of nanoparticles can be decorated with ligands or functional materials for the targeted delivery or stimuli-responsive controlled release of probiotics. These nanoparticles-based drug delivery systems can protect the probiotics from harsh environments, increase the residence time at the target site, and enhance the bioavailability of probiotics. Ebrahimnejad et al fabricated chitosan nanoparticles loaded with *Lactobacillus acidophilus* that had a particle size of 146 nm and significantly improved viability in simulated gastric and intestinal fluids. Ghibaudo et al also developed iron-pectin nanoparticles loaded with *L. plantarum* CIDCA 83114 with enhanced stability during freeze drying and storage. Moreover, these nanoparticles protected the entrapped probiotics against the gastric acidity. Gained their inhibitory effect against urogenital pathogens.

## Conclusion

There are evidences from well-conducted clinical trials of beneficial health effects from probiotics in a range of clinical conditions. The concept of 'synbiotics' has recently been proposed to characterize health-enhancing food and supplements used as functional food ingredients in humans, and with the advent of the functional food concept, it is clear that there is an important niche for these probiotic-based approaches. Although from the ongoing research, more of promising potential health effects of probiotics are being observed, more standardized and verifiable clinical studies are needed to demonstrate the safety, efficacy, and limitations of a putative probiotic, to determine effects on the immune system in healthy and diseased individuals and effects of long-term consumption, and to resolve whether it is superior to existing therapies. Also, the prospect of GM probiotics targeted for clinical conditions demands a rigorous safety strategy to prevent spread into the environment and dissemination of the genetic modification.

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