



Probiotics with an emphasis on their encapsulation

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Abstract

Probiotics, live microorganisms that confer health benefits when consumed in adequate amounts, have gained significant attention for their potential to improve human health. The challenges that probiotics face in the gastrointestinal tract are exposure to acidic pH, bile salts, and enzymatic degradation and functionality of probiotics during their passage through the harsh conditions of the gastrointestinal tract. In recent years, researchers have turned their focus toward innovative strategies to improve probiotic delivery and efficacy, with an emphasis on coating techniques. The significant role of coating probiotics is a profitable strategy to enhance their viability, resilience, and targeted delivery.

Keywords:coating, Beneficial health, live microorganisms

Introduction

The term “probiotic” is derived from the Latin word pro meaning “for,” and the Greek word βιωτικός, meaning “of” “or pertaining to life”(Liddell and Robert, 1889)(1). And can be determined as “live microorganisms (bacteria and yeast)(2).which, when administered in adequate amounts, confer a health benefit to the host” (FAO/WHO, 2001)(3). Probiotics were first associated with maintaining health over a century ago when Metchnikoff recommended that the consumption of fermented milk products was responsible for the long life of Bulgarian peasants (Metchnikoff, 1908)(4). Over the decades, evidence pointing to the beneficial effects of probiotics on human health has continued to expand. The increased use of antibiotics, radiation, and immunosuppressive treatments has been linked to changes in the composition of gut flora (Gupta and Garg, 2009)(5). The introduction of beneficial bacterial species into the gastrointestinal tract represents a strategy to revive the microbial equilibrium and prevent disease (Gupta and Garg, 2009)(5). Probiotics are live microorganisms, if distributed sufficiently enhance the host’s health. They play a fundamental role in intestinal health by restoring gut microbiome composition and providing a favorable environment for the commensal bacteria that results in the treatment of many infections (Anselmo et al., 2016; Wang et al., 2021) (6)(7).when probiotics are ingested, they need to face harsh environmental complexity in the gastrointestinal(GI) tract. Naturally, they can persist at the pH range of about 6–7 (Yeung et al., 2016) (8)but, gastric fluids are highly acidic (pH around 1–3) that can be harmful to probiotic species (Sarao & Arora, 2017)(9).In addition, ionic strength and enzyme (pepsin) activity in the stomach (Yao et al., 2020; Yeung et al., 2016)(10), yellow bile acid, and digestive enzymes (lipases, proteases, and amylases)in the small intestine furthermore declined the chance of the viability of probiotics (Han et al., 2021)(11).

Health benefits of probiotic consumption

Fig. 1. Beneficial health effects of probiotic bacteria on human health. Data from (Markowiak and Slizewska, 2017)(12).

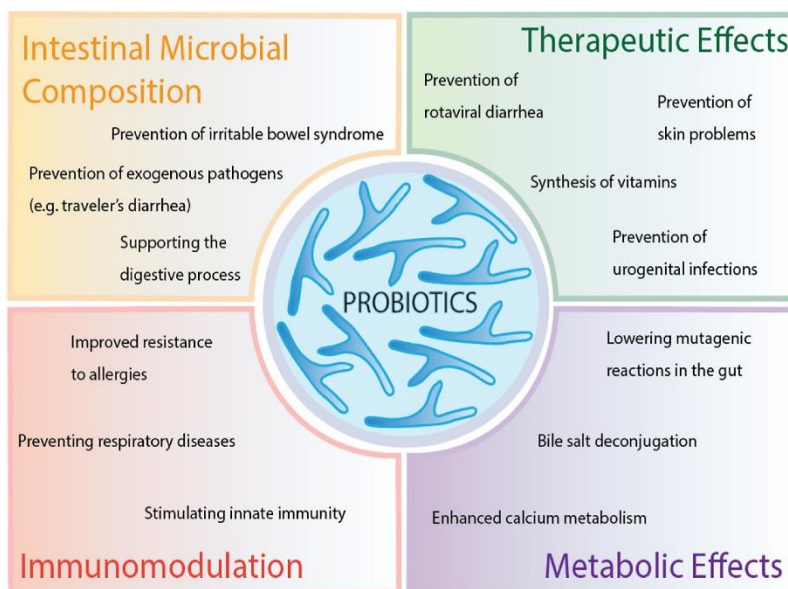


Fig. 1 summarizes several health benefits of probiotic consumption. The attractive properties of probiotics have directed a boosted interest in the development of probiotic formulations, containing super-functional foods, which can confer powerful health advantages to consumers. The most typically used probiotics include strains of the *Bifidobacterium* and *Lactobacillus* genera (Vlasova et al., 2016)(13). Some fungal strains belonging to *Saccharomyces* have also been used in probiotic formulations (Ansari et al., 2021)(14). Probiotics are traditionally

added to dairy products, such as cheeses (Sharifi et al., 2021)(15), yogurts (Afzaal et al., 2019)(16), and milk (Abesinghe et al., 2020)(17), but recently non-dairy products, for instance, meats, bread, juices, and chocolates have also been explored as probiotic delivery matrices (Aspri et al., 2020)(1). Nevertheless, despite the growing market for probiotic formulations, maintaining the viability of these products through processing, storage, and passage through the gastrointestinal tract remains a challenge (Rajam and Subramanian, 2022)(19).

Born a new generation of probiotics

Probiotics when consumed in appropriate amounts, which can provide many health benefits for humans. In recent years, have seen significant growth of probiotics in production due to increasing public awareness and their unique properties such as detoxification, cholesterol reduction, normalization of the microbiome, and stimulation of the immune system (Hu et al., 2019)(20). This group of bacteria is a valuable microorganism. Scattered settling them in the areas of the intestine and changing that to correct microbial balance, enhances usefulness. (Kaushik et al., 2009; Rezaei et al., 2021)(21). Efforts to generate probiotic products over the past few decades have resulted in the generation of primary probiotics. Initially, lyophilized planktonic bacteria were employed to make dairy products like yogurt(22). Later, to address the trouble of declining probiotic populations in food processing, storage, and gastrointestinal conditions, the second generation of probiotics was made in which bacteria are coated with natural or artificial polymers before freeze-drying (Salas-Jara et al., 2016)(23). To solve the problem of second-generation probiotics was used a method of encapsulating called third-generation probiotics. This method applies the entrapment of probiotics by mechanical or physicochemical operations **for instance extrusion, emulsification, coacervation, and spray drying into certain polymeric materials**(22). This method is based on the encapsulation of microbes with nanometer to millimeter-sized biopolymers to level up probiotic survival and promote controlled release in the gastrointestinal tract (Burgain Et al., 2011)(Cheow & Hadinoto, 2013)(24)(25).

Coating material

Different polymeric materials such as polysaccharides (alginate, starch, chitosan, cellulose acetate phthalate, k-carrageenan, plant gum), protein (gelatin, milk protein, whey protein), and fat are used to encapsulate probiotic bacteria and other bioactive components(26•27•28). Sodium alginate is also a widely used polymer as it forms a nontoxic, biocompatible, and highly versatile matrix for the protection of microorganisms against adverse conditions during the processing and storage of materials in the digestive system. (Misra, S et al., 2022)(29). The application of prebiotics such as resistant starch in the formulation can overcome weakness and enhance the stability of probiotic bacterial cells (Chen et al., 2005)(30). Nami et al. (2020) observed that *L.lactis* encapsulated with alginate, Persian gum, and inulin have a viability increase, while the unencapsulated cells showed a reduction in viability during the digestive system. Also, Probiotics can be encapsulated using various methods such as Spray cooling/chilling, Spray freeze drying, Coacervation, Ionic gelation, Emulsification, Spray freeze drying, Vacuum drying, Extrusion-dripping technique, Whey protein coating (31•32 •33).

Novel technologies for microencapsulation of probiotics

Spray cooling/chilling

The spray cooling/chilling process is mainly used for the entrapment of textural ingredients, This encapsulation technology is the least inexpensive method typically referred to as a “matrix” type method. Fat-based hydrophobic materials such as fatty acids, phospholipids, fatty alcohols, hydrogenated fat, polyethylene glycol, waxes, triacylglycerol, and Their mixture are commonly employed in the spray chilling process (Chambi et al., 2008; Sillick & Gregson, 2012) (34). In the matrix encapsulation process, the coated materials generally release the entire contents shortly. Factors such as osmotic forces, diffusion of water through the shell material, and mechanical dispersion play a significant role in the release mechanism. Spray chilling technology has been applied to microencapsulate different probiotic strains for improving viability. This technique was applied to encapsulate different bacterial

Cells such as *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, and *Saccharomyces boulardii* in a single and double layer with the addition of hydrogenated palm oil. Single-layered microcapsules showed better survivability (Arslan-Tontul & Erbas, 2017) (35). The application of vegetable fat as carrier material can be considered in the spray chilling technology for better protection, application, and delivery of probiotic strains such as *Bifidobacterium lactic* and *Lactobacillus acidophilus* (de Lara Pedroso et al., 2012) (36).

Spray freeze drying

This is an advanced method of encapsulation of probiotics that involves the processing steps of both spray and freeze drying (Amin et al., 2020) (37). This process is advantageous due to the formation of microcapsules with a larger size than spray-dried microcapsules and the coating of additional shell materials protects the core ingredients against adverse conditions (Semyonov et al., 2010) (38). But this process consumes high energy due to the long processing time with a requirement of high cost. (Zuidam & Shimoni, 2010) (39). The spray-chilling method and freeze-drying process are similar because of achieve encapsulation efficiency through the use of lower temperatures, resulting in lower operating costs (Dianawati et al., 2013) (40).

Vacuum drying

Vacuum drying is similar to freeze drying with a difference in the removal of water vapor through the evaporation process rather than sublimation. Generally, vacuum dryers operate at a higher temperature and higher pressure as compared to freeze dryers. However, the process parameters are lower than spray drying. Loss of viability of heat-sensitive probiotics is less in vacuum drying as the process can handle the thermal stress gently. Oxygen-sensitive probiotics such as *Bifidobacteria* can be effectively handled by this drying process but severe viability loss can also happen due to dehydration stress. Misra S, Pandey P, Dalbhat, etc 2022) (41).

The application of protective agents such as sugars or polyalcohol is beneficial for bacterial viability. Different sugars such as trehalose, lactose, and polyalcohol such as sorbitol have been used in the vacuum drying of probiotics (Crowe, 2007; Foerst et al., 2012) (42). With the addition of trehalose or sorbitol, the survivability of vacuum-dried *L.paracasei* enhanced (Foerst et al., 2012). Conrad et al) (43).

Bacterial viability and water activity were greatly influenced by the process parameters of vacuum dryers such as drying time and temperature. To protect against the chances the bacterial damage before drying different protectants like saccharides, skim milk, or alginate matrix have been used with probiotics to encapsulate probiotic culture *Lactobacillus brevis* RK03. And found that The highest survival was found at the processing time by employing casein and whey protein isolate as carrier materials (Wu et al. 2021) (44).

Extrusion-dripping technique

Extrusion-dripping technique The extrusion-dripping technique involves the mixing of active agents with a wall material/polymeric solution and the further formation of droplets by passing the solution through a nozzle. The droplets are rapidly transformed into solid particles in the presence of a crosslinking agent/gelation bath (Chan et al., 2009)(45). The interaction of gravitational, surface tension, impulse, and frictional forces results in various mechanisms of droplet generation (Saifullah et al., 2019)(46).

Protein hydrogels probiotic encapsulation with milk proteins includes the confinement of probiotic cells in an interior microenvironment that originates from milk proteins. As encapsulating agents, milk proteins have good solubility, gelling, and film-forming properties (47). These features make the proteins (e.g., caseins) a versatile platform to stabilize different emulsion systems. Compared to polysaccharide systems, milk proteins are also nutritive and bioactive (48). In particular, dense milk proteins with buffering capacities are suitable for producing microbeads that can resist gastric conditions.

Encapsulation by using whey proteins produces different microbead sizes, depending on the preparation method. Relatively large microbeads are created by extrusion, with particle size determined by the extrusion conditions. Emulsion techniques produce capsules with a relatively smaller size than extrusion methods. More recent approaches include the use of an enzyme that can induce the gelation of proteins (e.g., transglutaminase). The ability of these enzymes to cross-link proteins such as casein under mild conditions makes encapsulation of living cells inside a gel matrix possible (49)(50).

In summary, the use of milk proteins for probiotic encapsulation has indicated strong protective effects in acidic conditions. However, multiple optimization processes are needed, including heating conditions for the denaturation of milk proteins, pH, the effect of conformational behavior of milk protein (fibrillar and capsular), and interactions between the surface components of probiotic cells and milk proteins (51).

Whey protein coating

Whey protein is a mixture of globular proteins isolated from whey and shows good performance by protecting active substances such as probiotics before their targeted release in the host (Razavi et al., 2021)(52). High temperature leads to the deterioration of probiotic bacteria, so denatured whey proteins are used to enclose probiotics. Sweet whey acts as an efficient carrier material for probiotics and enables the protection of microbes in simulated GI conditions (Maciel et al., 2014)(53). Many current studies have demonstrated microencapsulation of probiotics using whey proteins. Such as protein isolate (WPI), whey concentrate (WPC), and sweet whey (a product containing casein and whey proteins due to its better gelling and emulsifying activities, excellent solubilization properties, and thermal stability (De Castro-Cislaghi et al., 2012)(54).

Conclusion

Coating probiotics presents an intriguing avenue to overcome challenges associated with their viability, enhancing their efficacy and potential health benefits. A comprehensive overview in this field contributes to the growing body of knowledge on probiotics coatings and further exploration is promising. In the future, it is important to continue to investigate the efficacy and safety of probiotic coatings in human clinical trials. It is also necessary to develop new and innovative coating materials that can further enhance the delivery and viability of probiotics. With research and

development, probiotic coatings have the potential to revolutionize the field of probiotic delivery and offer a range of new and stimulating opportunities for improving human health.

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