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A review examining techniques for enhancing the health and longevity of *Lactobacillus* bacteria within probiotic food items

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Abstract

Given the increasing importance of probiotic products in promoting human health, maintaining the viability of probiotic strains, particularly *Lactobacillus*, throughout the production and storage process is a major challenge in this industry. Probiotics are live microorganisms whose consumption can help regulate and strengthen the digestive and immune systems, as well as be beneficial in the prevention and treatment of certain diseases. Several factors, including environmental conditions, the nature of the product, and the intrinsic characteristics of the strains, affect the survival of these bacteria. This review article provides a comprehensive and detailed examination of various methods for enhancing the viability of *Lactobacillus* strains in probiotic food products. The survival of probiotic bacteria is of paramount importance because their positive effects on human health depend on their survival during processing, storage, and until consumption. This study examines advanced techniques and methods such as microencapsulation, the use of appropriate packaging, optimization of storage temperature, and the application of protective additives like prebiotics. This article aims to evaluate and analyze the effectiveness of these methods in increasing the viability of *Lactobacillus* bacteria when exposed to various industrial conditions and to examine the impact of these strategies on the shelf life of probiotic food products. This study can lead to the development of more efficient probiotic food products with broader health benefits and create new perspectives for future research and the improvement of industrial processes.

Introduction

In recent decades, functional products have gained special popularity due to their properties and positive effects on various diseases. These products have attracted the attention of many consumers and researchers due to their beneficial effects on improving health and reducing the risk of various diseases (Aghajani *et al*., 2011). One of these products is probiotic microorganisms, which are defined as living microorganisms that contribute to human and animal health, especially in maintaining and improving the balance of the microbial flora of the digestive tract and intestines (Irvani *et al*., 2015). The balance of the microbial flora of the digestive tract plays a vital role in digestion, absorption of nutrients, and strengthening the body's immune system. Probiotics can be present in various forms such as dietary supplements, pharmaceutical tablets or in the formulation of food products. (Champagne *et al.,* 2018; Lenfestey *et al*., 2018). Probiotics exert their positive effects on gut health through a range of actions, as Shekh *et al.* (2020) point out. Beyond their core functions, probiotics also exhibit significant antioxidant and bile salt hydrolase activities. Enzymatic activity is also an important factor, influencing the ability of probiotics to metabolize various compounds in the gut and produce beneficial metabolites (Castro *et al.,* 2023; Marcia *et al.,* 2023). This includes the production of short-chain fatty acids (SCFAs), which have various health benefits. These additional properties further enhance their capacity to promote overall well-being. These include their ability to stick to the mucus lining of the intestines, form protective biofilms, and hinder the growth of harmful bacteria. These microorganisms are from the family of lactic acid bacteria (LAB). (Akin *et al*., 2017). Forming biofilms offers a practical way to boost the survival of probiotics. According to Marcia (2023), this creates a protective network that shelters the bacteria from harsh conditions that they might encounter. Biofilms are complex communities of bacteria that are attached to a surface and encased in a self-produced matrix of extracellular polymeric substances (EPS). Biofilms protect against environmental stress and antibiotic residues, enhancing the survival and resilience of *Lactobacillus* strains (Rezaei *et al.,* 2021; Rezaei *et al.,* 2022). Lactic acid-producing bacteria, especially *Lactobacillus* and *Bifidobacteria*, are naturally found in the digestive tract. In 1994, the World Health Organization (WHO) attempted to use probiotics to strengthen the immune system instead of prescribing drug-resistant antibiotics. These efforts aimed to reduce the excessive use of antibiotics and reduce the risk of drug resistance. The benefits of probiotics include helping to treat lactose intolerance, diarrhea, allergies, inflammatory bowel diseases, irritable bowel syndrome, and strengthening the immune system (Abdollahniya *et al*., 2018; Sharma *et al.,* 2019). *Lactobacillus* bacteria are famous for making lactic acid. This acid helps preserve fermented foods like yogurt, cheese, and sauerkraut, and it's also what gives them their distinct tangy flavor (Beshkova and Frengova, 2012). As said in Saadi *et al. (*2024), Kiekens *et al.* (2019), and Byl *et al.,* (2019) strategies for maintaining *Lactobacillus* strains in the product during processes and usage are paramount because the effectiveness of health recovering advantageous probiotic products is contingent upon the adequacy of *Lactobacillus* strains' viability (Tyutkov *et al.,* 2022). These benefits are achieved through modulation of the immune system, production of antimicrobial compounds, and improvement of intestinal wall function. Research has shown that consumption of probiotic products has positive effects in the elderly, especially in people with AIDS (Lollo *et al*., 2013; Lollo *et al*., 2015). Several factors, including environmental conditions (temperature, pH, and water activity), the nature of the food matrix, the presence of antimicrobial compounds, and the intrinsic characteristics of the strains, can affect the survival of these bacteria. Matrix acidity is one of the factors responsible for reducing the viability of probiotics, posing a challenge for their incorporation into acidic food products (Terpou *et al.,* 2018; Terpou *et al.,* 2019). Low pH can damage the bacterial cell membrane and disrupt its metabolic processes, leading to cell death. Reduced viability of probiotic strains in food products not only leads to a decrease in their effectiveness but can also impact the sensory quality of the product (Bernatek *et al.,* 2022). *Lactobacillus* strains must be able to tolerate acidic conditions to survive in food products and the gastrointestinal tract, ensuring their ability to reach the gut and exert their beneficial effects (Mukhtar *et al.,* 2020; Mukhtar, 2020). Acid tolerance is a strain-specific characteristic that is influenced by various factors, such as cell wall composition and the presence of acid-protective enzymes. The addition of ethanolic extract and essential oil of *Ferulagoangulata* can influence pH and acidity in yogurt, potentially affecting the viability of *Lactobacillus* strains (Dini *et al.,* 2013;

Keshavarzi *et al.,* 2021; Maleki *et al.,* 2021). These compounds can have antimicrobial properties and may either enhance or inhibit the growth of *Lactobacillus*, depending on their concentration and the specific strain. Lower water activity influences the survival of *Lactobacillus plantarum*, affecting its ability to maintain viability during storage (Han *et al*., 2015; Ozkan *et al.,* 2021). Water activity refers to the amount of unbound water in a food product, which is available for microbial growth and chemical reactions. Lowering water activity can inhibit microbial growth and extend the shelf life of food products. To ensure probiotics remain alive and effective for consumers to gain their intended health benefits, proper storage is key, as Ren *et al.* (2019) emphasize. This involves carefully managing factors like temperature, moisture, exposure to light, and air. The heat involved in food production presents a significant hurdle for creating foods packed with live probiotics, as de Souza Neves Ellendersen *et al.* (2012) and Chen *et al*. (2019). This is because high temperatures can severely harm and kill probiotic cells by altering their proteins, damaging their outer layers, and disrupting their genetic material. This review study aims to conduct a comprehensive and systematic investigation of various methods for increasing the viability of *Lactobacillus* strains in probiotic food products, focusing on protective methods. This study will first examine the factors affecting the reduction of bacterial viability and then detail methods such as prebiotic fortification, heat treatment, the addition of prebiotics, and protective compounds. Finally, considering the existing findings, suggestions will be provided for improving the viability of *Lactobacillus* strains in food products. The results of this review study can help researchers, producers, and consumers of probiotic products to produce and consume higher quality and more effective probiotic products by being aware of different methods for increasing the viability of *Lactobacillus* strains. Additionally, this study can contribute to the development of production standards for probiotic products.

Factors affecting the viability of probiotic bacteria

Probiotic bacteria are susceptible to several factors that can lead to a reduction in their viability. These factors can also play a significant role in the production, storage, and consumption of functional food products. For instance, the low pH of the stomach, approximately 2, creates an acidic environment that is harmful to many bacteria, including probiotics, and can destroy a large number of them. Additionally, proteases and bile salts, especially bile salts, which are involved in the breakdown of proteins and the weakening of the bacterial cell wall structure, lead to a decrease in the number of live bacteria in the intestine (Fadel *et al.,* 2021; Rasoli *et al.,* 2021). Unfavorable environmental conditions can also reduce the viability of probiotics. These bacteria are sensitive to temperature and humidity, and changes in these conditions can lead to a significant decrease in their survival. Improper processing and storage of probiotic products cause the bacteria to be exposed to oxygen, which can be harmful to some of them and reduce the effectiveness of the probiotics. Furthermore, the use of preservatives in these products may disrupt the function of the bacteria and weaken their positive effects. To increase the viability of probiotic bacteria, their ability to survive under unfavorable processing and consumption conditions should be investigated before adding these microbes to the final product, and the most resistant strain among the available strains should be selected (Ashrafi Yarghanloo *et al*., 2015). In general, it can be said that various factors such as stomach pH, proteases and bile salts, unsuitable environmental conditions, improper processing and storage, and product composition can reduce the viability of probiotic bacteria. By understanding and controlling these factors, it is possible to provide probiotics with higher viability and better efficacy, which can have more beneficial effects on human health.

Encapsulation methods

Extrusion is a simple and widely used technique for microencapsulating *Lactobacillus* cells, involving the expulsion of a *Lactobacillus* suspension through a small nozzle into a hardening solution, forming spherical microcapsules (Foroutan *et al*., 2017; Saadi, 2024; Lina *et al.*, 2025). To solidify the protective layer around the *Lactobacillus* cells, solutions usually include a positively charged ion with a valency of two, like calcium. This ion acts as a bridge, linking the molecules of the encapsulation material, often alginate, to create a gel-like network that holds the bacteria inside. This process of pushing the mixture through a small opening, called extrusion, can be done with simple tools like syringes or more sophisticated automated machines. This flexibility allows for the creation of tiny capsules of different sizes and shapes. Because it's fairly cheap and straightforward to do, extrusion is a go-to method for producing small batches of these protected *Lactobacillus* cells. However, it might not be the most efficient way to trap a high number of cells, and it can sometimes result in capsules that aren't all the same size. Spray drying is a cost-effective and versatile technique for microencapsulating *Lactobacillus* cells, involving the atomization of a *Lactobacillus* suspension into a hot air stream, resulting in the rapid evaporation of water and the formation of dry microcapsules (Soukoulis *et al*., 2013; Anekella, 2020; Pérez-Díaz *et al*., 2021). When preparing *Lactobacillus* for drying, the bacterial liquid usually has a coating substance mixed in, like maltodextrin, whey protein, or gum arabic. This material creates a shield around the *Lactobacillus* cells as they dry. The drying itself is done using a spray dryer, and there are different kinds of these machines, each with its pluses and minuses when it comes to the size and shape of the resulting particles and how well the bacteria are protected. (Hajipour *et al.,* 2019). Spray drying is a good option for making large amounts of these protected *Lactobacillus* cells, and it can create very uniform, tiny capsules. However, because the process involves high heat, it can harm the *Lactobacillus* and reduce how many of survivors. So, it's really important to fine-tune the drying settings to keep cell death to a minimum. Emulsion techniques improve bacterial viability in low pH conditions by dispersing *Lactobacillus* cells in an oil phase, which protects them from the acidic environment of the stomach, and then encapsulating the oil droplets within a protective matrix, forming microcapsules (Ali *et al*., 2024; Shah *et al.,* 2024).

The effect of microencapsulation on probiotic stability

The microencapsulation process, as an innovative method for enhancing the stability of probiotics, plays a significant role in maintaining the viability of these microorganisms in food products (Kazemi-Gorji *et al.,* 2013; Das *et al.,* 2014). In this process, probiotic cells are coated with a layer of hydrocolloids such as alginate, carrageenan, gelatin, chitosan, and cellulose acetate phthalate to protct them from the adverse effects of external environments like temperature changes, pH, oxygen, and antimicrobial compounds (Kavitakia *et al.,* 2018; Yao *et al.,* 2020). Microencapsulation presents a robust approach to safeguard *Lactobacillus* cells against challenging environmental factors, substantially enhancing their survival and effectiveness in probiotic food items (Adeel *et al*., 2023; Saadi *et al*., 2024). This method entails enclosing *Lactobacillus* cells within a defensive structure, creating tiny capsules that insulate the bacteria from damaging conditions encountered during food production, storage, and passage through the digestive system. This protective structure can be made from diverse materials like alginate, chitosan, or tapioca flour, each providing distinct characteristics and advantages. Microencapsulation can be carried out using various techniques, including extrusion, spray drying, and emulsion methods, each appropriate for different uses and offering different degrees of protection (Nemat Shahi *et al.,* 2012; Mohite *et al*., 2020). These coatings act as physical barriers, preventing direct contact of probiotic cells with damaging agents, which results in increased lifespan and stability of these microorganisms. Numerous research findings have demonstrated that microencapsulation increases the survival rates of probiotics under various processing and storage conditions of food products, particularly in the acidic environment of the stomach and the alkaline environment of the intestine (Abbasi *et al.,* 2013; Ehsani *et al.,* 2015). This process also helps to increase the chances of survival and growth of probiotics in the gastrointestinal tract, maximizing their properties and benefits for human health. Furthermore, this process extends the shelf life and effectiveness of probiotic products stored at low temperatures, including ice cream and frozen yogurt (Golbaghi *et al.*, 2012; Martin *et al*., 2015). Under these conditions, microencapsulation prevents damage to probiotic cells caused by ice crystals and temperature fluctuations. For example, research has shown that the use of microencapsulation in probiotic ice cream increases the survival rate of *Lactobacillus casei* during storage (Golbaghi *et al*., 2024). By employing microencapsulation, the survival rate of *Lactobacillus* strains is significantly improved as they undergo food processing, are stored, and travel through the gastrointestinal system. This ensures that enough living cells reach the colon to deliver their health-promoting benefits (Hameed *et al.,* 2020; Hossain *et al.,* 2021). In the course of food production, *Lactobacillus* cells can encounter high temperatures, very acidic or alkaline conditions, and physical forces that can harm their outer layers and diminish their ability to survive. Microencapsulation acts as a physical shield, protecting the *Lactobacillus* cells from these stresses, thus preventing cellular damage and maintaining their viability. Throughout storage, *Lactobacillus* cells can be subjected to changes in temperature, moisture levels, and oxygen exposure, all of which can also decrease their survival. Microencapsulation can establish a small, controlled environment within the microcapsule that lessens these stresses, thereby prolonging the usable life of probiotic food products. When passing through the digestive system, *Lactobacillus* cells face the highly acidic environment of the stomach, bile salts in the small intestine, and competition from other microorganisms. Microencapsulation can defend *Lactobacillus* cells against these challenging conditions, enabling them to survive their journey through the gastrointestinal tract and reach the colon. Once there, they can exert their positive influences on the gut's microbial community and the overall health of the individual (Taghizadeh *et al.*, 2014).

Common encapsulation materials

Alginate, a natural sugar-like substance extracted from brown seaweed, is a popular material for encapsulation because it's compatible with living tissues, breaks down naturally, and can form a gel. These properties make it a great option for protecting *Lactobacillus* cells (Foroutan, 2017). When alginate encounters ions with two positive charges, like calcium, it forms a gel structure in three dimensions. This network traps the *Lactobacillus* cells inside the tiny capsules. The alginate gel can withstand the low acidity of the stomach, shielding the *Lactobacillus* cells from this harsh environment. However, the gel can break down in the less acidic conditions of the small intestine, allowing the *Lactobacillus* cells to be released gradually in the gut. Additionally, alginate is a relatively cheap and easily accessible material, making it an economical choice for microencapsulation (Nasirvand *et al.,* 2022).

Derived from chitin, the primary component of crustacean shells, chitosan, a substance akin to sugar, not only improves the efficiency of cellular enclosure but also imparts adhesive qualities. This stickiness facilitates the attachment of the minuscule capsules to the intestinal lining, thereby promoting the colonization of *Lactobacillus* cells in that environment (Foroutan, 2017; Adeel, 2023). Chitosan has a positive electrical charge, which lets it connect with the negatively charged surfaces of *Lactobacillus* cells, making it easier to trap them inside the microcapsules. Chitosan also fights off microbes, which can further protect *Lactobacillus* cells from being contaminated by unwanted microorganisms. The mucoadhesive quality of chitosan improves how well the microcapsules connect with the intestinal lining, keeping the *Lactobacillus* cells in the gut longer and giving them more chances to interact with the body's defense system. Furthermore, chitosan is compatible with living tissues and breaks down naturally, making it a safe and environmentally sound choice for microencapsulation.

Synbiotic approaches

Definition and rationale

Synbiotics represent a strategic combination of probiotics, which are live microorganisms conferring health benefits, and prebiotics, which are non-digestible food ingredients promoting the growth and activity of beneficial gut bacteria, to enhance probiotic survival and activity in the gastrointestinal tract (Palanivelu *et al.,* 2022). This clever combination strategy tries to tackle the difficulties of getting probiotics to where they need to go. The goal is to make sure enough live and active probiotic cells make it to the colon, where they can do their good work for our health. By giving the probiotic bacteria a better chance to grow, prebiotics can help them settle in and stick around in the gut (Mohammad Hosseini *et al.,* 2019). This can lead to a healthier gut and better overall health. The thinking behind synbiotics is simple: create a more welcoming environment for the probiotic bacteria in our digestive system. This boosts their odds of surviving and thriving, ultimately making their health benefits for us even greater (Markowiak and Śliżewska, 2017). Prebiotics selectively stimulate the growth and/or activity of one or a limited number of beneficial bacteria in the colon, providing them with a competitive advantage over other microorganisms and enhancing their colonization and persistence in the gut (Rosburg *et al.*, 2010). Prebiotics are usually types of carbohydrates that our bodies can't digest, like oligosaccharides and polysaccharides. Instead of being broken down by our own enzymes, these travel to our gut where friendly bacteria, such as *Bifidobacterium*, happily ferment them. This fermentation process creates short-chain fatty acids (SCFAs) think of acetate, propionate, and butyrate, which are super beneficial for our gut and overall health. These SCFAs can make the colon more acidic, which is unfriendly to bacteria that don't like it, hindering their growth. They can also get absorbed into our bloodstream, giving us energy and helping to reduce inflammation (Dehghan-Nairy *et al.,* 2017). So, by specifically feeding the good bacteria in our gut, prebiotics help to bring the microbial balance back into harmony, which in turn improves our digestion, how well we absorb nutrients, and even our immune system. This smart pairing of probiotics and prebiotics is a win-win for gut health and our general well-being. It works by fostering a healthy balance of gut bacteria, strengthening our immune defenses, and making digestion and nutrient uptake more efficient (Rosburg *et al.,* 2010). Having a good mix of gut bacteria is super important for staying healthy. These tiny residents play a big part in breaking down our food, soaking up nutrients, keeping our immune system strong, and fending off harmful invaders. Probiotics lend a hand by introducing more of the good bacteria into our gut, while prebiotics act like food for these beneficial microbes, helping them grow and stick around. When you combine probiotics and prebiotics, they can also give your immune system a boost by encouraging the production of protective substances. Plus, synbiotics can make digestion and nutrient absorption better by helping to break down complex foods. So, by fostering a healthy gut environment, strengthening immunity, and improving digestion, synbiotics contribute to better gut health and overall wellness.

Examples of synbiotic foods

Yogurts containing beta-glucan and *Bifidobacteria* offer combined health benefits, providing both prebiotic and probiotic effects in a single product, promoting gut health and cardiovascular well-being (Rosburg *et al.,* 2010). Putting beta-glucan into yogurt gives a real boost to the growth and activity of *Bifidobacteria*, helping them to settle in and thrive in your gut. These *Bifidobacteria* then do their part in keeping your gut bacteria in good balance, which leads to better digestion, nutrient absorption, and a stronger immune system. On top of that, the beta-glucan itself is good for your heart, helping to lower cholesterol and prevent big swings in blood sugar. This dual benefit the prebiotic action of beta-glucan feeding the probiotic *Bifidobacteria* makes yogurt with this combination a really appealing food for people who want to look after their gut and heart health. Tiny capsules made with tapioca flour and *L. casei* help the bacteria survive longer when stored. The tapioca flour creates a safe environment that protects the bacteria from harsh conditions, and it also acts as a prebiotic, giving them the food they need to stay alive and active (Izadi *et al.,* 2014; Tavares *et al*., 2023). The alginate shell around the *L. casei* acts like a shield, guarding it against changes in temperature, moisture, and air. This helps them stay fresh for longer. Meanwhile, the tapioca flour works as a prebiotic, giving the *L. casei* cells the food they need to stay alive and well while they're stored. This smart combination–the protective alginate and the nourishing tapioca flour-makes these tiny capsules a really good way to deliver probiotic bacteria, ensuring they can do their beneficial work for us. Yogurt that has had *Lactobacillus rhamnosus* added in tiny protective capsules stays alive and well during storage. This means you get a functional food that's extra good for you because the probiotics remain active for longer (Saadi, 2024). The capsule coating shields the *L. rhamnosus* from the tough environment inside the yogurt, like its acidity and high oxygen levels, which can harm the bacteria. The dates in the yogurt also contribute by providing food and protective compounds that help the *L.rhamnosus* survive and stay active. This smart combination-the protective capsules and the beneficial dates makes date yogurt with encapsulated *L. rhamnosus*an effective way to get these good bacteria into your gut, helping to improve your gut health and overall well-being (Meybodi *et al*., 2020).

Protective matrices and carrier agents

Role of matrix composition

The composition of the food matrix plays a critical role in influencing the survival of probiotic bacteria during shelf life and transit through the gastrointestinal tract, affecting their ability to deliver health benefits to the host (Karu *et al.,* 2016; Mantovani *et al.,* 2020). The stuff that makes up a food product creates an environment, both physically and chemically, that can either help or hurt probiotic bacteria, depending on what it's made of. Things like how acidic it is (pH), how much water is available, how much oxygen is present, and whether other tiny organisms are around can all affect whether probiotic bacteria survive and stay active in food. So, picking the right kind of food base is super important to make sure probiotic foods actually work. Things like the pH, how much solid stuff is in there, and how much fat it contains can really change how well probiotic bacteria can handle the harsh conditions in our digestive system. This affects whether they can make it through the stomach and set up shop in the gut. By carefully controlling these factors in the food matrix, it is possible to enhance the survival and functionality of probiotic bacteria, improving their ability to deliver health benefits to the host. Selecting an appropriate food matrix is crucial for ensuring the viability and efficacy of probiotic foods, as the matrix can either protect or harm the probiotic bacteria, depending on its specific characteristics (Mantovani *et al.,* 2020). If the environment isn't very acidic (high pH), it can shield probiotic bacteria from the harsh stomach acid. But if it's too acidic (low pH), it can actually damage their outer layers and reduce how many survive. Lots of solid stuff around them can create a physical shield, protecting them from stressful conditions, whereas not much solid content can leave them more open to harm. A good amount of fat can also protect them from the bile salts in your small intestine, which can act like detergents. If there's not much fat, they're more likely to be vulnerable to these effects. By paying close attention to and adjusting these elements within the food, we can really boost the survival and effectiveness of probiotic bacteria. This, in turn, makes them better at providing health benefits to us. Choosing the right food base is super important for making sure probiotic foods actually work, because that base can either protect or harm the beneficial bacteria, depending on its specific qualities (Mantovani *et al.,* 2020). The ideal food base for probiotic bacteria will act like a safe haven, protecting them from harsh conditions, helping them grow and do their thing, and even encouraging them to stick to the lining of your intestines. Plus, it needs to taste good and be appealing so people will actually eat enough of it to get the health benefits (Table 1).

Skim milk and whey protein

Skim milk provides good protection to probiotic bacteria during freeze-drying and storage, offering a readily available and cost-effective option for preserving probiotic cultures for extended periods (Terpou *et al.,* 2017; Terpou *et al.,* 2019).

Strain selection for enhanced survival

Screening for stress-tolerant strains

Selecting *Lactobacillus* strains that possess inherent tolerance to various environmental stresses is a fundamental strategy for enhancing their survival in probiotic food products (Lebeer *et al.,* 2008; Beshkova *et al.,* 2012). Different *Lactobacillus* strains exhibit varying degrees of resistance to factors such as acid, bile, and osmotic stress, making it crucial to identify and utilize those strains that can withstand these challenges (Beshkova *et al.,* 2012). By carefully checking different types of *Lactobacillus* to see how well they handle stress, we can make them survive better during food production, while they're being stored, and as they travel through our digestive system. This ultimately makes probiotic foods work even better (Lebeer *et al.,* 2008).

The impact of packaging type on enhancing probiotic viability

The survival of probiotics in food products, particularly dairy products, is influenced by the interplay of various factors, including product formulation, processing conditions, and packaging characteristics. Among these factors, the physicochemical properties of packaging materials and packaging techniques play a pivotal role. Numerous studies have demonstrated that the oxygen permeability of packaging materials has a direct impact on probiotic viability. Most probiotics, especially *Bifidobacteria*, are sensitive to oxygen and thrive better in anaerobic environments. Therefore, packaging with low oxygen permeability can provide a suitable environment for maintaining the viability of these bacteria. Miller *et al*. (2013) showed that using packaging with strong oxygen barriers and oxygen scavengers creates an optimal environment for preserving probiotics in yogurt. Dianawati *et al*. (2016), by comparing the survival of *L. acidophilus* in yogurts packaged in glass and plastic containers, confirmed the superiority of glass containers in maintaining the viability of this probiotic. More recent studies, including research by Cruz *et al*. (2013), have also emphasized the importance of low oxygen permeability in packaging materials for increasing probiotic viability. These studies indicate that, in addition to packaging materials, the use of techniques, such as vacuum packaging, active packaging using oxygen scavengers and edible coatings containing antioxidants, can also help improve probiotic survival. Considering the economic and technical importance, the use of alternative packaging, such as multilayer packaging with strong oxygen barriers, can be considered as a suitable solution for increasing the shelf life of probiotic products and maintaining their quality. However, the selection of the appropriate packaging type depends on various factors, including the type of product, processing conditions, cost, and market requirements (Cruz *et al.,* 2019).

The impact of storage temperature on probiotic survival

Storage temperature plays a crucial role in the viability of probiotic bacteria in food products. Temperature influences the enzymatic and metabolic activity of probiotics; increased temperatures lead to higher enzyme activity and consequently the degradation of cellular components. Additionally, high or low temperatures can damage the cell membrane and result in a reduction in the viability of probiotic bacteria. Low temperatures slow down the growth rate of probiotics, while high temperatures rapidly decrease probiotic survival and cause the degradation of nutrients in the culture medium (Dianawati *et al.*, 2016; Dianawati *et al.*, 2017). Research has shown that storage at low temperatures can help maintain probiotic viability. For example, a study by Shahrampour *et al*. (2024) on alginate film containing *L. plantarum* showed that at a temperature of 4°C, the survival rate of this bacterium was over 84%, while at 25°C, this rate decreased to about 50%. The findings of Dini *et al.* (2013) indicated that lower temperatures can increase the survival of probiotic bacteria, such as *L. casei* and *Bifidobacterium lactis*, during storage. These studies emphasize that proper storage conditions, including the use of low storage temperatures, can help maintain the quality and effectiveness of probiotic products. Overall, a temperature of 4°C is recommended as a suitable storage temperature for products containing probiotics, as at this temperature, the growth rate of probiotics is reduced but not completely stopped, and their viability is better preserved.

The impact of adding prebiotics on probiotic viability

Prebiotics help improve the viability of probiotics by creating beneficial changes in the gut environment. These non-digestible or poorly digestible saccharide compounds (oligosaccharides) pass through the small intestine to the large intestine and act as a source of carbon and energy for probiotics. Specifically, prebiotics increase acidity and lower the pH of the environment, which provides favorable conditions for the activity of probiotic bacteria (Djukic *et al.,* 2015; Yousefi *et al.,* 2018; Hosseini *et al*., 2024) also, confirmed in their research that probiotics need sugar for growth. In addition to providing the necessary energy for probiotics, prebiotics also inhibit the growth of harmful bacteria. Prebiotics narrow the competition space and make the environment more conducive to the growth of beneficial probiotic bacteria. Prebiotic compounds stimulate the growth of probiotics. Oligosaccharides or short-chain carbohydrates are among the most important prebiotic compounds. Galacto-oligosaccharides are biotics that have health-promoting effects on human health (Habibi *et al*., 2014; Holz *et al.,* 2015).

Specific compounds, such as fructo-oligosaccharides and galacto-oligosaccharides, have been well demonstrated to help improve probiotic viability. For example, adding fructo-oligosaccharides to probiotic yogurt increases the number of bacteria and lactic acid production, indicating greater activity and better survival of probiotics (Shiran *et al.,* 2022). Synbiotics, which include a combination of probiotics and prebiotics, can improve probiotic survival and enhance human health during processing, storage, and passage through the gastrointestinal tract. These synergistic products have found widespread use in the food and pharmaceutical industries due to the effective interactions between probiotics and prebiotics. With a better understanding of the mechanisms of interaction between these two types of compounds, more efficient synbiotic products can be designed and produced, leading to increased lactic acid production and ultimately strengthening the digestive system. Dehghan Niri *et al*. (2017) stated that enzymatic hydrolysis of lactose had a significant effect on probiotic viability, and a distinct effect due to changes in the initial lactose concentration was also observed on the viability of *L. acidophilus* and *B. lactis*. Therefore, this process can be used to improve probiotic viability (Laličić-Petronijević *et al.,* 2015). Madhu *et al*. (2012) concluded in their studies that adding fructo-oligosaccharides as a prebiotic to probiotic yogurt increases acidity and lowers pH, indicating greater bacterial activity and consequently lactic acid production. The research results of Hosseini *et al*. (2024) showed that adding up to 15% date syrup to probiotic yogurt leads to increased probiotic viability. This increase is likely related to the specific properties of dates. Although after the seventh day, viability decreases due to the reduction of nutrients, the results compared to the control sample showed that date syrup as a prebiotic can significantly increase the viability of *L. acidophilus*. Therefore, the significant difference ($P<0.05$) between the viability of probiotics in date yogurt compared to plain probiotic yogurt (control) highlights the role of dates as a prebiotic in the growth of *L. acidophilus*.

Challenges in maintaining *Lactobacillus* viability

The viability of probiotic *Lactobacillus*-containing food products is greatly impacted by food processing methods, storage conditions, and the extreme environment of the gastrointestinal tract (Amund, 2016; Hossain *et al.,* 2021; Palanivelu *et al*., 2022). These probiotic products entail maintaining microbial proliferation as well as cell functionality, which poses difficulty owing to the sensitivity of the *Lactobacillus* cells. Stress induced by environmental temperatures along with pH levels, and even mechanical stress further complicates the survival of *Lactobacillus* cells, which is already critically low. The difficulty does not cease here; food processing also subjects the cells to extreme pH levels, high temperatures, and mechanical stress, which most certainly reduces those chances of survival. In addition to this stretchable change in temperature, humidity levels, and exposure to oxygen further increase the risk of inactivity. Stepping out of the bounds of storage, the gastrointestinal tract offers a new set of challenges, including the stomach's acidic nature, bile salt content within the small intestine, and other competing microorganisms. However, these cells still require battling their way through to the colon before gaining the ability to exert any benefits for the host. Crawling out from all of these saber-toothed struggles requires a specific strategy designed to deal with environmental changes to guarantee cell growth through the production process. Acidity, high oxygen concentration, and rival bacterial presence within the food matrix can all seriously threaten the viability of *Lactobacillus* in probiotic products (Papadaki *et al*., 2020; Papadaki *et al*., 2021). The acidic environment in which a large number of food products are situated, especially fermented elixirs, is devastating to the survival of *Lactobacillus* because these bacteria flourish in neutral or mildly acidic pH conditions. Oxygen is also capable of compromising the viability of *Lactobacillus*, as many strains are anaerobic or microaerophilic and sensitive to oxidative stress. Other bacteria present in the food matrix can set up competitive interactions in which competing organisms may produce metabolites detrimental to the growth of *Lactobacillus* or even kill the probiotic cells. This emphasizes the negative consequences that miscontrolof the composition and processing parameters of probiotic foods can have on *Lactobacillus* viability. Buffers, proprietary packaging, and the selection of non-arresting starter cultures are suggested alterations to combat these factors. For any health benefits to be actualized (Amund, 2016), probiotic bacteria like *Lactobacillus* must be kept active throughout every stage of food production, storage, and distribution. To guarantee a sufficient number of viable cells survive all of the aforementioned methods, it is critical that these cells eventually reach the colon, where the exercise of their health-promoting functions on the gut microbiota and the host health can begin.

In summary, the study of methods to enhance the viability of probiotic bacteria in food products reveals that the complex interaction between probiotics and the food matrix they reside in necessitates extensive research. Environmental factors, food composition, and processing procedures significantly influence the survival of these beneficial microorganisms. The use of microencapsulation as a promising strategy increases the resistance of probiotics to acidic pH, bile salts, and oxidative stress, and allows for their controlled release in the gastrointestinal tract. Packaging materials and storage conditions also play a crucial role in determining probiotic survival. Low-oxygen packaging and appropriate storage temperatures can extend the shelf life of probiotic products. The synergistic relationship between probiotics and prebiotics also has a significant positive impact. Prebiotics enhance the growth and establishment of probiotics in the gastrointestinal tract, contributing to their better effectiveness. Despite significant advancements in improving probiotic viability, several challenges remain that require a comprehensive and practical approach to develop more effective and stable products. Future research should focus on developing improved microencapsulation systems, advanced packaging technologies, and synbiotic formulations. In conclusion, leveraging combined strategies, including microencapsulation, optimized packaging, and the use of prebiotics, can lead to the production of higher quality and more stable probiotic products that promote the health and well-being of consumers.

Table 1. Techniques to enhance health and longevity of Lactobacillus in probiotic foods.

Category	Technique	Description	Effect on *Lactobacillus*	Citation
Microencapsulation	Alginate, chitosan coating	Encapsulating bacteria in protective matrices (e.g., alginate, chitosan)	Enhances survival in gastric conditions and storage	Anal & Singh, 2007
Microencapsulation	Dairy Matrices	Microencapsulation techniques can be applied in dairy products like pasta and filata cheese to protect probiotics during processing and storage.	Enhanced stability and health benefits in dairy functional foods.	D'Amico et al., 2025.
Prebiotic fortification	Prebiotic Fortification	Addition of substances like inulin or FOS	Promotes *Lactobacillus* growth and viability in the gut	Saad et al., 2013
Low-temperature storage	Low-Temperature Storage	Refrigeration slows metabolic degradation	Maintains viability over shelf life	Ranadheera et al., 2010
reeze-drying (Lyophilization)	Cryoprotectants (e.g., trehalose, skim milk)	Dehydration method preserving microbial structure	Protects bacteria during long-term storage	Carvalho et al., 2004
Stress adaptation	(Sub-lethal stress)	Pre-exposure to mild heat or acid shock	Increases resistance to future harsh conditions	Corcoran et al., 2008
Use of protective carriers	Use of Protective Carriers	Dairy, cereal, or fruit-based carriers with buffering capacities	Provides nutrients and buffers against acid/bile	Shah, 2000
Modified atmosphere packaging (MAP)	Modified atmosphere packaging (MAP), oxygen barrier films	Replacing oxygen with inert gases (e.g., nitrogen)	Reduces oxidative stress on probiotics	Champagne et al., 2005
Controlled release systems	CRS	Development of delivery systems using materials like polymethacrylate-based copolymers (Eudragit) and interpenetrating polymer network hydrogels for targeted release in the intestine.	Sustained release and higher viability during gastrointestinal transit; prolonged shelf-life.	Yan et al., 2021.
Spray drying with protective agents	Whey protein, soy protein	Encapsulation of *Lactobacillus* using spray drying combined with protective agents like whey protein isolate, inulin, or flaxseed mucilage to enhance thermal stability.	Improved survival during processing and storage; enhanced thermal resistance.	Bustamante et al., 2015; Rama et al., 2020; Fritzen-Freire et al., 2013.
High internal phase emulsions (HIPEs)	(HIPEs)	Encapsulation of probiotics in HIPEs stabilized with whey protein isolate microgels to protect against harsh gastrointestinal conditions.	Significant improvement in gastrointestinal survival rates.	Sun et al., 2025.

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