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Review

Application of Probiotic Bacteria in Active Packaging for the Food Industry: Review

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Abstract

The incorporation of probiotic bacteria into active packaging systems represents an innovative strategy to enhance food preservation while delivering health benefits to consumers. This review discusses the selection criteria for probiotic strains focusing on their resistance to environmental stressors, antimicrobial activity, and viability in different food matrices and their integration into edible films and coatings. Polysaccharides, proteins, and hydrocolloids are widely used as biopolymeric matrices due to their biocompatibility and functional properties. The efficiency of probiotic packaging largely depends on three factors: the choice of strain, the encapsulation technique (such as spray drying, emulsification, or electrospinning), and the properties of the matrix material. These packaging systems demonstrate strong antimicrobial activity through multiple mechanisms, including bacteriocin production, competition for adhesion sites, and acidification. Applications in dairy, meat, fish, and fresh produce reveal the potential of these technologies to delay spoilage, reduce pathogenic microorganisms, inhibit lipid oxidation, and maintain nutritional and sensory qualities. Moreover, studies emphasize that combining probiotics with prebiotic compounds can improve both microbial stability and functional performance. Despite promising results, challenges remain regarding the industrial scalability and long-term stability of these systems under varied storage conditions. Future research should focus on optimizing formulation parameters, expanding applications across diverse food categories, and integrating smart packaging technologies. Altogether, probiotic-based edible packaging aligns with current demands for sustainable, health-oriented food solutions.

Keywords: biodegradable materials; edible films; probiotic packaging; shelf life

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1. Introduction

The growing concern among consumers regarding health and environmental sustainability has encouraged the search for functional and eco-friendly alternatives within the food industry [1]. In this context, active and bioactive packaging has emerged as a promising solution by combining the protective role of conventional packaging with extended shelf life and added health benefits for consumers [2]. These packaging technologies are developed using materials that can interact with both the food product and its surrounding environment, enabling, for instance, the controlled release of antimicrobial and antioxidant compounds, as well as the regulation of gases such as oxygen and ethylene [3].

The replacement of conventional plastics by natural biopolymers including polysaccharides, proteins, and lipids has been a key strategy to meet the demand for biodegradable materials free from toxic residues [4]. Additionally, there is a growing interest in the use of natural ingredients in packaging formulations, such as essential oils, plant extracts, and beneficial microorganisms, which enhance the functional value of packaged foods [5,6].

Among these approaches, probiotics have stood out as innovative components in edible packaging systems. According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [7]. Traditionally incorporated into dairy products, probiotics have shown the ability to improve gut health, modulate the immune system, and contribute to disease prevention, thus expanding their potential use beyond conventional foods [2,8].

The application of edible films and coatings enriched with probiotics has gained prominence as an effective strategy for the biofortification of fresh and minimally processed fruits and vegetables. These systems act as natural antimicrobial barriers and simultaneously serve as carriers for delivering beneficial microorganisms, helping maintain the sensory and nutritional quality of foods while reducing postharvest losses [9,10].

Therefore, incorporating probiotics into functional packaging represents a convergence of food technology, sustainability, and public health, positioning itself as one of the most innovative areas in the food sector [2]. The objective of this article is to review and analyze the use of probiotic bacteria in active food packaging, addressing incorporation techniques, functional benefits to the packaged products, and impacts on preservation and shelf-life extension, as well as the technological and market challenges involved in the implementation of this innovation.

2. Probiotic Bacteria: Selection and Mechanisms

2.1. Criteria for Selection of Probiotic Strains

The selection of probiotic strains for application in active packaging systems must consider several critical factors to ensure their viability and functionality during processing, storage, and application to food products. The key criteria for this selection are detailed in Figure 1, which summarizes the main aspects to be considered when choosing suitable probiotic strains for active packaging systems.

According to several authors, the selection of probiotic strains must be based on a set of criteria that ensure both their efficacy and safety. One of the primary factors is resistance to pH variations, as probiotic strains intended for food applications must be able to tolerate acidic conditions commonly found in food systems, such as fermented or acidified products, where pH values may range from approximately 3.0 to 5.0. This tolerance is essential to maintain cell viability and antimicrobial functionality during food processing and storage [11]. Additionally, they should tolerate possible pH fluctuations within the food matrix to which they are applied [11]. Another essential criterion is tolerance to bile salts, since the ability of probiotics to survive in the presence of these substances is crucial for their viability upon reaching the intestine, where they exert their health-promoting effects [12].

Thermal stability is also a relevant criterion, particularly in applications that do not rely on cold chain logistics. Probiotic strains intended for use in active packaging must be able to withstand moderate temperature variations associated with processing steps, such as hot-air drying, polymer extrusion, or the formation of edible films and coatings, as well as food storage conditions [13]. For instance, strains capable of maintaining a high viability after exposure to temperatures ranging from 40 to 60 °C for short periods show greater potential for industrial application. This characteristic is essential to ensure the maintenance of cellular viability throughout the product's shelf life, preserving its functionality until the moment of consumption [13].

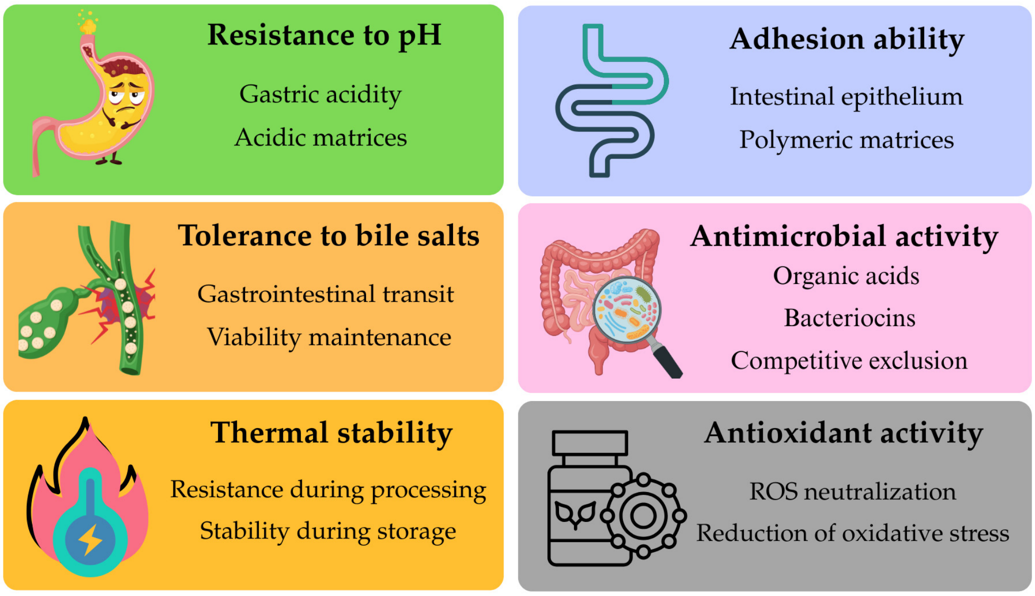

Figure 1. Main functional criteria for selecting probiotic strains intended for active packaging applications.

In addition, the ability of probiotic strains to adhere to mucosal surfaces is considered highly desirable, as it promotes the prolonged colonization of the gastrointestinal tract and enhances interactions with the host's intestinal microbiota [14]. Studies have shown that this property is associated with the presence of surface proteins, exopolysaccharides, and other cellular structures involved in adhesion to the intestinal epithelium. Similarly, these same factors may favor interactions between the strains and polymeric matrices used in edible films and coatings, contributing to their stability and controlled release [14].

Antimicrobial activity represents another functional property of major importance, as many probiotic strains exhibit an intrinsic ability to inhibit pathogenic and spoilage microorganisms through different mechanisms [15]. Commonly reported examples include the production of organic acids, such as lactic and acetic acids, the synthesis of bacteriocins and other antimicrobial metabolites, as well as competitive exclusion for nutrients and adhesion sites. This characteristic is particularly advantageous when probiotics are applied in food preservation strategies, contributing to enhanced microbiological safety and extended product shelf life [15].

More recently, antioxidant activity has been recognized as an additional relevant criterion in the selection of probiotic strains. Some strains are able to neutralize reactive oxygen species through the production of antioxidant enzymes or bioactive metabolites, thereby reducing oxidative stress. This property may contribute both to host cellular protection and to the improvement of food oxidative stability, especially in active packaging systems aimed at preserving food quality and nutritional value [16].

2.2. Antimicrobial Mechanisms of Action

Probiotic bacteria play a crucial role in the antimicrobial properties of active packaging by employing multiple mechanisms to inhibit the growth of pathogenic and spoilage microorganisms. These mechanisms involve a range of biochemical and ecological strategies through which probiotic strains exert their antimicrobial activity.

One such mechanism is the production of bacteriocins, which are ribosomally synthesized antimicrobial peptides produced by probiotics [17]. Bacteriocins can be classified into different types based on their structure and mode of action, including pore-forming peptides that disrupt cell membrane integrity and peptides that inhibit essential cellular processes, such as cell wall synthesis. Therefore, bacteriocins do not share a single antimicrobial mechanism, but rather exhibit diverse modes of action depending on their class and target microorganisms [17]. These molecules are capable of inhibiting a broad spectrum of undesirable microorganisms, offering potential for applications in food preservation and health protection [17]. Another important mechanism involves the competition for nutrients and adhesion sites, whereby probiotics outcompete harmful microbes for essential resources and physical space, effectively preventing their colonization and the formation of biofilms [18].

Additionally, probiotics contribute to microbial control through the production of organic acids, such as lactic acid and acetic acid, as well as other acids, including propionic, butyric, and formic acids, depending on the probiotic strain and metabolic pathway [19]. These compounds lower the pH of the surrounding environment, creating conditions that are unfavorable for the proliferation of pathogenic bacteria [20]. Furthermore, probiotics can also stimulate host defense mechanisms. When incorporated into edible coatings or consumed through functional foods, they may interact with the host's immune system, enhancing its natural ability to defend against infections [21]. Together, these mechanisms illustrate the multifaceted antimicrobial potential of probiotics, highlighting their relevance in both clinical and food-related contexts.

The synergistic action of these mechanisms (described in Figure 1) highlights the potential of probiotics for active packaging applications. Their ability to enhance food safety while preserving quality makes them particularly valuable for innovative packaging solutions. Table 1 presents the most promising probiotic strains for this purpose, along with their specific applications in food preservation.

Overall, the studies demonstrate that the effectiveness of probiotic-based active packaging depends on three key factors: the probiotic strain selection, encapsulation method, and choice of biopolymer matrix [22–24]. The viability and antimicrobial performance of probiotics have been shown to vary significantly depending on the protective biopolymer system used, as observed by Da Silva et al. [24], where alginate–chitosan systems outperformed plant mucilage and konjac glucomannan matrices in maintaining probiotic viability.

Several studies emphasize that microencapsulation is crucial for protecting probiotic cells during storage, with encapsulated strains showing significantly higher survival rates (>8 Log CFU/mL) compared to free cells after 28 days at 4 °C [22,24]. The data also reveal that different probiotic strains exhibit varying efficacy against specific spoilage microorganisms, with *L. casei* LC03 showing broad-spectrum activity against aerobic mesophiles and yeasts/molds, while *L. plantarum* A6 demonstrated particular effectiveness against *Fusarium* and *Rhizopus* species [22,24].

Rahmati-Joneidabad et al. [25] and Azarakhsh et al. [23] found that the antimicrobial performance improved when probiotics were combined with prebiotic compounds (e.g., inulin) in the biopolymer matrix, suggesting synergistic effects between these components. However, the studies collectively indicate that probiotic-based systems are more effective as preventive measures against microbial spoilage rather than solutions for already heavily contaminated products, with maximum reductions of 3.1 Log CFU/g against fungal growth [25].

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Table 1. Main probiotic strains used in active packaging systems.

Strain	Resistance	Associated Biopolymer	Microbial Target	Ref
Lactobacillus plantarum LP3	Survival > 6–7 Log_{10} CFU/g after 5 days at 4 °C	KGM	Fungi—molds/yeasts (Reduced fungal growth by ~1.0 Log_{10} CFU/g)	[26]
Lactiplantibacillus plantarum A6	Survival > 5 Log_{10} CFU/g for 28 days at 30 °C	EPS from *Weissella confusa*	*Fusarium* sp.; *Rhizopus stolonifer* (Complete reduction—elimination)	[22]
Lactiplantibacillus pentosus v390	Survival > 6 Log_{10} CFU/g after 10 days at 4 °C	*Ocimum basilicum* seed gum	Fungi—molds/yeasts (Reduced fungal growth by 3.1 Log_{10} CFU/g)	[25]
Lactobacillus rhamnosus	Remained viable during refrigerated storage (12 days at 4 °C)	Gelatin + inulin	Fungi—molds/yeasts (Reduction of 1.2 to 2.0 Log_{10} CFU/g in total microbial load yeasts and fungi)	[23]
Lacticaseibacillus casei XN18	Survival > 6 Log_{10} CFU/g after 35 days at 4 °C	LRM	Fungi—molds/yeasts (Reduced fungal growth by 2.7 Log_{10} CFU/g)	[27]
Lacticaseibacillus casei LC03	Survival > 8 Log_{10} CFU/mL after 28 days at 4 °C	Alginate + fungal chitosan	Total aerobic mesophiles, yeasts/molds (~2 Log_{10} CFU/g reduction in total aerobic mesophilic bacteria and yeasts/molds)	[24]
Lactobacillus casei and *Bacillus coagulans* GBI-30, 6086	Survival ~6.01 Log_{10} CFU/g after 14 days at 4 °C	NCH–NCL–gelatin nanocomposite film	*Listeria monocytogenes* (Final count: 1.71 Log_{10} CFU/g after 14 days)	[28]
Lactobacillus curvatus 54M16	Survival ~7.5–7.6 Log_{10} CFU/cm^2 after 28 days at 4 °C	Whey protein/gelatin/inulin	*Listeria innocua* C6 (~2 Log_{10} CFU/cm^2 reduction within 24 h; complete inhibition, <1 log CFU/cm^2)	[29]
Lactobacillus pentosus	Maintained viability at ~6 Log_{10} CFU/g in gelatin-based emulsion films during storage	Gelatin/polydextrose emulsion film with camellia oil	*Escherichia coli*—antibacterial activity (inhibition zone assay) *Staphylococcus aureus*—antibacterial activity (larger inhibition zone)	[30]
Lactobacillus sakei NRRL B-1917	Retained antimicrobial activity after incorporation into WPI films (pH 8.0, thermal and enzymatic treatments)	WPI-based film with alginate and glycerol	*E. coli* (reduction of 2.3 Log_{10} CFU/g after 36 h—undetectable: <10 CFU/g) *L. monocytogenes* (reduction of 1.4 Log_{10} CFU/g after 120 h)	[31]
Lactobacillus kefiri MM5	Maintained viability and growth in both protein-based and polysaccharide-based films/coatings during storage at 4 °C and room temperature	Polysaccharide-based: Sodium alginate (2–6% *w*/*v*) + glycerol Protein-based: Pea protein (6.25% *w*/*v*) + glycerol + xanthan gum (coating)	*L. monocytogenes* NCTC 10888 (~3.5–4.5 Log_{10} after 20 days)	[32]

CFU/g (colony-forming units per gram), CFU/mL (colony-forming units per milliliter), Log (refers to the logarithmic scale used to quantify microbial counts), ObM (*Ocimum basilicum* seed mucilage), KGM (Konjac glucomannan), EPS (exopolysaccharide), LRM (*Lallemantia royleana* seed mucilage), NCH (Nanochitosan), NCL (Nanocellulose), WPI (Whey Protein Isolate).

3. Edible Films/Coatings: Materials and Properties

Probiotic Edible Films and Coatings for Food Packaging

The incorporation of probiotic bacteria into edible films and coatings has emerged as a promising strategy to deliver health benefits beyond traditional dairy-based applications. While probiotics have been predominantly associated with fermented dairy products, extending their use to non-dairy foods such as fruits, meats, and baked goods presents unique challenges. Edible films and coatings offer a novel solution by serving as protective carriers that help maintain the viability and stability of probiotics on the food surface. Ensuring the survival of these microorganisms depends on several critical factors, including the composition of the film matrix, the method of film preparation, as well as storage temperature and duration. Optimizing these parameters is essential for enhancing the effectiveness of probiotic delivery through edible packaging systems [33].

Edible packaging materials can be made from three main raw materials: polysaccharides, proteins, and lipids. To improve their mechanical properties, such as durability, elasticity, flexibility, resistance, and reduced brittleness, additives like emulsifiers and plasticizers are commonly incorporated [34]. The use of natural materials such as polysaccharides, proteins, and cellulose enables the creation of biodegradable, non-toxic, and effective carriers for probiotics [35].

Studies have shown that biopolymer-based films and coatings can also serve as effective barriers against oil, odors, and oxygen, while also offering strong mechanical properties [36,37]. An ideal edible film or coating should exhibit several key characteristics, including acceptable sensory qualities, strong barrier and mechanical properties, high microbial stability, absence of toxins, safety for human consumption, production feasibility, and cost-effectiveness to apply in the food field [38,39].

Edible packaging systems are typically prepared using two main approaches: the wet process and the dry process. The wet process, also known as casting or solvent casting, is commonly used in the laboratory and pilot scales. It involves three primary steps: (i) dispersing biopolymers in an appropriate solvent, (ii) pouring the resulting dispersion into a mold, and (iii) drying the cast dispersion within the mold [40,41]. In contrast, the dry process, primarily extrusion, is widely employed on a commercial scale. This method is more time-efficient, has a chemical-free operation, and is cleaner. The extrusion process is divided into three key zones: (i) the feeding zone, where raw materials are introduced; (ii) the kneading zone, where they are mixed and plasticized; (iii) the heating and molding zone, where the material is kneaded and processed into its final forms [42].

In the study conducted by [32], bioactive packaging solutions were developed by incorporating a bacteriocin-producing lactic acid bacterium (LAB) strain into edible films and coatings of varying compositions. The inclusion of *Lactobacillus kefiri* MM5 resulted in packaging materials with enhanced biological value, attributed to the presence of the probiotic LAB. The study reported the sustained growth of *L. kefiri* MM5 within both protein-based and polysaccharide-based films and coatings, with no initial reduction in viable cell count, indicating that the processing conditions did not adversely impact bacterial viability. Moreover, the probiotic strain maintained its viability during storage at 4 °C, contrasting with previous findings reported by other authors; [43,44]. Both types of edible packaging exhibited notable anti-Listeria activity, particularly after 10 and 20 days of storage. Among the polysaccharide-based materials, the coatings demonstrated superior antibacterial performance compared to the films [45].

Beyond their antimicrobial activity, probiotics incorporated into edible films and coatings can influence key packaging properties such as mechanical strength and barrier performance. Soukoulis et al. [46] reported that the inclusion of probiotic bacteria within edible films altered film microstructure and affected mechanical properties, depending on the polymer matrix and probiotic concentration. Similarly, Gagliarini et al. [47] observed that probiotic-loaded whey protein films maintained acceptable tensile strength and flexibility, although slight changes in water vapor permeability were noted due to the presence of bacterial cells acting as dispersed fillers within the matrix. A recent review further confirmed that probiotic incorporation may increase permeability and modify mechanical behavior by creating microvoids and altering polymer chain mobility, particularly at higher cell loadings [48]. Therefore, we need to balance probiotic functionality with the mechanical and barrier requirements of edible packaging.

4. Microencapsulation Approaches for Probiotic-Active Packaging

The encapsulation or microencapsulation of probiotics has emerged as a highly relevant technological approach in the food industry, especially in the development of active and functional packaging. This strategy aims to protect viable microorganisms from adverse environmental conditions while enabling their controlled release into food matrices or during consumption. Compared to proteins, the use of lipids for probiotic encapsulation, aside from emulsions, has been less extensively explored. However, polysaccharides such as cellulose, alginate, chitosan, and gellan gum are widely used as encapsulating materials for probiotics due to their excellent biocompatibility, affordability, availability, ease of modification, and responsiveness to pH. These materials can be applied individually or in combination with others to enhance the survival and colonization of probiotics in the intestinal tract, enable targeted release within the gut, and prolong the retention of probiotics in the digestive system [49].

The selection of wall materials for probiotic microencapsulation is primarily based on their ability to protect microorganisms during processing, storage, and gastrointestinal transit while maintaining cell viability and enabling controlled release [50]. Although chitosan possesses intrinsic antimicrobial properties, it is commonly employed in probiotic encapsulation because its antimicrobial activity is concentration-dependent and its strong film-forming and protective characteristics can enhance probiotic stability when appropriately formulated [51]. Protein-based wall materials, including whey protein and gelatin, are also widely applied because of their high encapsulation efficiency and good nutritional compatibility [52]. Lipid-based systems, while less extensively studied, offer improved protection against oxygen and moisture and are particularly suitable for the co-encapsulation of hydrophobic bioactive compounds alongside probiotics [53].

In the study by Yang et al. [38], cellulose-encapsulated probiotics demonstrate enhanced stability in fermented milk, even after multiple reuses of the starter culture, supporting recyclability, sustained fermentation, and efficient biotransformation over time [38]. Moreover, studies have shown that co-encapsulating probiotics with bioactive compounds in a single matrix can significantly enhance probiotic viability [38,54]. For example, the co-encapsulation of *Lactobacillus casei* with coconut oil and omega-3-rich tuna oil has been found to improve the viability of the probiotic [55].

Among the techniques for microorganism encapsulation, spray drying, electrospinning, and emulsification are the most studied due to their efficiency, manageable process complexity, and operational cost [56]. Spray drying is widely used in industrial settings due to its scalability and relatively low cost. The technique involves atomizing a suspension containing probiotics and encapsulating materials into a chamber of hot air, where the solvent rapidly evaporates, forming dry microcapsules. Although thermal exposure can compromise microbial viability, the selection of wall materials and optimization of operational parameters, such as inlet and outlet air temperature, feed rate, air flow rate, atomization pressure, and solids concentration, can ensure high survival rates [57].

On the other hand, electrospinning has emerged as a promising technique for the encapsulation of probiotic microorganisms, particularly due to its ability to produce nanofibers with high surface area, which, when incorporated into film matrices, contribute to enhanced barrier properties by improving structural compactness and reducing permeability pathways. This technique offers effective protection against environmental stressors and enables controlled release. However, it requires more sophisticated equipment and specific operational conditions, such as controlling the electric voltage, tip-to-collector distance, feed flow rate, ambient humidity and temperature, as well as the viscosity and conductivity of the polymer–probiotic suspension. These factors may influence fiber formation and probiotic viability, and can result in higher production costs [58,59].

Emulsification allows for encapsulation using various polymeric matrices, including alginate, chitosan, pectin, gelatin, cellulose derivatives (e.g., methylcellulose, carboxymethylcellulose), whey protein isolate, and starch-based materials, resulting in capsules with tunable physicochemical properties. It is characterized by the formation of stable colloidal systems in which probiotics are dispersed in a continuous phase, typically with the aid of natural emulsifiers (such as lecithin and proteins) or synthetic emulsifiers like polysorbates (e.g., Tween 80), polyethylene glycol (PEG), Span, and Pluronics. Survival rates of encapsulated probiotics can vary significantly depending on the formulation, the nature of the biopolymers used, and the processing conditions [60]. A comparative summary of the main features of these microencapsulation techniques is presented in Table 2.

Table 2. Comparative analysis of probiotic microencapsulation techniques.

Technique	Main Parameters	Survival Rate (for Probiotic Bacteria) Target (%)	Target Microorganisms	Main Encapsulating Materials	Cost Type
Spray Drying	Inlet/outlet temperature (140–180 °C/60–80 °C); feed flow rate; air flow rate; solids concentration; atomization pressure	~60–85	*Lactobacillus*, *Bifidobacterium*, *Lacticaseibacillus*	Alginate, whey protein, maltodextrin, gum arabic	Process cost (equipment + energy)
Electrospinning	Voltage (10–30 kV); tip-to-collector distance (10–20 cm); solution viscosity and conductivity; flow rate; ambient temperature/humidity	~40–70	*Lactobacillus*, *Bifidobacterium*	Polyvinyl alcohol, gelatin, zein, chitosan	Equipment and operational cost
Emulsification	Emulsifier type/concentration; stirring speed; droplet size; solid content; crosslinking agents	~70–90	*Lactobacillus*, *Bifidobacterium*	Alginate, chitosan, pectin, gelatin	Materials and formulation cost
Freeze-drying	Freezing rate; chamber pressure; drying time; cryoprotectants	~80–95	*Lactobacillus*, *Bifidobacterium*	Skim milk, trehalose, sucrose, whey protein	High survival; porous particles; limitation: high energy/time
Extrusion	Nozzle diameter; $CaCl_2$ concentration; flow rate; gelation time	~70–90	*Lactobacillus*, *Bifidobacterium*	Alginate, carrageenan, pectin	Strong GI protection; beads 0.5–3 mm; limitation: large size

The incorporation of probiotics into edible films presents a promising approach to enhancing both the stability and functionality of probiotics in food systems. In one study, methylcellulose (MC) films were formulated with two probiotic strains, *Lactobacillus delbrueckii* subsp. *bulgaricus* CIDCA 333 and *Lactobacillus plantarum* CIDCA 83114, alongside fructooligosaccharides (FOS) as a prebiotic. The probiotic cells were fully embedded within the film matrix without compromising its structural integrity. FOS played a dual role: it provided a protective effect, particularly for *L. delbrueckii*, but also reduced the glass transition temperature (Tg) of the films; however, a decrease in the glass transition temperature may lead to a more plasticized polymer matrix, which, although increasing film flexibility, may compromise its mechanical and barrier properties, thereby directly influencing the structural stability and functional protection of probiotics during storage. To balance these effects, FOS concentrations were optimized to 3% (*w*/*v*) for *L. delbrueckii* and 1% for *L. plantarum*. Among the two, *L. plantarum* showed a greater viability over time, especially at a higher relative humidity (RH), suggesting its better suitability for practical applications [61].

Building on this concept, other prebiotic compounds, such as inulin, chitosan, chitooligosaccharide (COS), galacto-oligosaccharide (GtOS), and additional FOS, have also been incorporated into maize starch-based edible films. These compounds promoted the growth of probiotic strains like *Bifidobacterium infantis* ATCC 15697 and *Lactobacillus fermentum* ATCC 9398 and also altered the physical properties of the films. Compared to control films without prebiotics, the enriched films demonstrated reduced tensile strength but increased extensibility. These findings highlight the multifunctional role of prebiotics in edible film matrices, not only supporting probiotic viability but also tailoring the mechanical properties of the films, underscoring their potential in developing innovative, functional food packaging systems [62,63].

According to Yang et al. [38], gelatin-based (GN) films demonstrate significantly enhanced antibacterial properties when enriched with *L. lactis* (LA) and cranberry extract (CE). While pure GN films exhibited minimal inhibition against *Listeria monocytogenes*, the addition of either *L. lactis* or CE pristine leads to a dose-dependent increase in antibacterial activity. Notably, combining 1.0–2.0% *L. lactis* with 0.5% CE produced a synergistic effect, resulting in a markedly improved antibacterial performance; the synergistic effect observed between LA and CE [30] is a result of their complementary actions: while CE's proanthocyanidins target the integrity of pathogenic membranes, LA remains viable due to its intrinsic tolerance to phenolic stress, continuing to produce bacteriocins that further destabilize the target cells. This enhanced effect is likely due to the ability of *L. lactis* to produce antimicrobial peptides such as nisin and bacteriocins, which can disrupt the structural integrity of pathogenic bacteria. These findings highlight the importance of incorporating probiotic cultures and plant-based extracts into biopolymer films to enhance their antimicrobial properties.

Recent studies have explored the use of starch–protein-based edible films as a strategy to preserve the viability of *Lactobacillus rhamnosus*. These films were formulated using rice or corn starch combined with different protein sources, including sodium caseinate, concentrated soy protein, and type II bovine gelatin. All formulations were subjected to the same drying conditions (37 °C for 15 h), without individual optimization of parameters for each protein type, to allow a direct comparison of the effects of starch–protein composition on probiotic viability and film properties [64].

Rice starch-based films exhibited superior protective properties, characterized by a more compact and less elastic structure, as well as lower water vapor and oxygen permeability, which contributed to greater survival of *L. rhamnosus* compared to corn starch films. Among the proteins evaluated, sodium caseinate provided the highest stability of the probiotic during drying and storage processes, followed by concentrated soy protein and gelatin [64]. Thus, the enhanced performance observed for films containing sodium caseinate is primarily associated with the physicochemical properties of the polymer matrix, rather than the application of specific drying conditions for this protein.

5. Application in Food Products

The application of active packaging with probiotics has gained significant attention due to its potential to improve both the shelf life and safety of food products, showing promising results across several food matrices, particularly in dairy products, meats, fish, fruits, and vegetables. These packaging technologies focus not only on preservation but also on contributing to microbial stability by mitigating the presence of pathogenic and spoilage microorganisms. This dual functionality makes them highly relevant to the current

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demands of the food industry, which seeks innovative, health-promoting, and sustainable packaging solutions. As evidenced in Table 3, various active packaging systems have shown effectiveness in preserving perishable foods through the incorporation of specific probiotic strains into biopolymeric matrices, resulting in antimicrobial effects and microbial stability during storage.

Table 3. Biopolymers and probiotic strains in functional food packaging systems.

Food Product	Polymer/Biomaterial	Probiotic Strain Used	Ref
Sheep cheese	Flaxseed mucilage	*Lactobacillus rhamnosus* LGG; *Lactobacillus acidophilus* LA-5	[65]
Block cheese	Sodium caseinate; prebiotic fibers: inulin and FOS	*Bifidobacterium animalis* subsp. *lactis* BB-12	[66]
Kashar cheese	Aquafava; *Psyllium* husk powder; PEG 400	*Lactiplantibacillus plantarum* 299v	[67]
Fresh pork meat	Arabic gum; whey protein isolate; isomalt; glycerol	*Lactobacillus rhamnosus* HN001	[68]
Beef	*Malva neglecta* seed mucilage (MNSM)	*Lactobacillus brevis* TD4 (In the form of a postbiotic—PLB)	[69]
Lamb meat	*Malva sylvestris* seed mucilage (MSM)	*Saccharomyces cerevisiae* var. *boulardii* ATCC MYA-796	[70]
Trout filets	Carboxymethylcellulose; sodium caseinate; glycerol	*L. acidophilus*; *L. reuteri*; *L. casei*; *L. rhamnosus*; *Bifidobacterium bifidum*	[71]
Strawberries	Garden cress seed mucilage (*Lepidium sativum* L.)	*Limosilactobacillus reuteri* VTC 864	[72]
Apples	Gellan gum; cranberry extract	*Lactococcus lactis* (LA)	[38]
Fresh potatoes	Gellan gum; cranberry extract	*Lactococcus lactis* (LA)	[38]
Carrots	Sodium alginate; glycerol; sunflower oil; Tween	*Lactobacillus acidophilus* La-14	[73]
Cherry tomatoes	Whey protein isolate	*Lactobacillus casei* 01	[74]
Thompson grapes	Whey protein isolate	*Lactobacillus casei* 01	[74]

Moreover, Table 4 summarizes recent studies on probiotic-based active packaging systems, highlighting their antimicrobial performance, the initial concentrations of inoculated probiotic microorganisms, and changes in viability over storage time. The compiled data provide a comparative overview of how different probiotic strains, biopolymeric matrices, and food applications influence microbial stability and antimicrobial effectiveness. This synthesis allows for the identification of key trends and critical parameters affecting the performance of probiotic-active packaging systems, supporting the evaluation of their potential for food preservation applications.

Table 4. Antimicrobial performance and viability of probiotics in active food packaging.

Antimicrobial Effect	[Probiotics] Inoculated	Probiotic Viability Time (Days)	Ref
L. monocytogenes (12.8 mm); *S. aureus* (12.6 mm); *E. coli* (8.61 mm); *Salmonella typhimurium* (8.7 mm) inhibition zones	10^9 CFU/mL	Viability maintained for 10 days at 4 °C	[65]
Pathogen/microorganism reduction not investigated	10^9 CFU/mL	Viability monitored up to 30 days; probiotic concentration considered insufficient afterwards	[66]

Table 4. *Cont.*

Antimicrobial Effect	[Probiotics] Inoculated	Probiotic Viability Time (Days)	Ref
Coliforms: After 30 days, control cheese (uncoated) had 4.35 Log CFU/g; coated cheeses had lower counts: 3.35 Log CFU/g (with probiotic), 1.35 Log CFU/g (without)	10^{11} CFU/mL	10^8 CFU/g maintained for 60 days at 4 °C	[67]
Fermented film: 2.4 Log CFU/g reduction in *S. aureus*, 1.5 Log CFU/g in *Salmonella* spp.	9.5 Log CFU/mL	Stored 79 days at 4 °C; 30 days at 25 °C; counts remained above 10^6 CFU/g (recommended minimum)	[68]
Total count reduction: MNSM + 15% PLB: <7 Log CFU/g; psychrotrophs: 4.54 Log CFU/g; fungi & yeasts: 1.83 Log CFU/g	Used PLB concentrations: 0%, 5%, 10%, 15% (*v*/*v*)	Not applicable—used postbiotics (non-viable cells)	[69]
Total count: MSM + 10% PSB: 3.53 Log CFU/g; psychrotrophs: 5.25 Log CFU/g; *E. coli*: 1.63 Log CFU/g; *S. aureus*: 1.80 Log CFU/g	PSB concentrations: 0%, 2%, 4%, 6%, 8%, 10% (*v*/*v*)	Not applicable—used postbiotics (non-viable cells)	[70]
Total count reduction: 8.21 Log CFU/g (*L. acidophilus*); Enterobacteriaceae: 3.37 Log CFU/g; psychrotrophs: 6.72 Log CFU/g	9 Log CFU/mL	14 days at 4 °C; all strains stayed above 7 Log CFU/g, except B. bifidum (6.11–6.68 Log CFU/g)	[71]
Fungi/yeast reduction: 13.81% spoiled fruits (vs. 42.45% in control)	Two levels: 1.5 × 10^8 and 3 × 10^9 CFU/mL	10 days at 4 °C; counts remained above 6 Log CFU/g	[72]
Best antibacterial film (GN/2.0% LA/0.5% CE): inhibition zone of 14.9 ± 0.6 mm	1.0% and 2.0% (*w*/*w*)	Viability over 24 days at 25 °C; stayed above 6 Log CFU/g	[38]
Best antibacterial film (GN/2.0% LA/0.5% CE): inhibition zone of 14.9 ± 0.6 mm	1.0% and 2.0% (*w*/*w*)	Viability over 24 days at 25 °C; stayed above 6 Log CFU/g	[38]
No growth of pathogens (*Salmonella*, total coliforms, 45 °C coliforms, coagulase-positive *Staphylococcus*, or *B. cereus*)	7.36 Log CFU/g (film), ~2.3 × 10^7 CFU/g	19 days at 8 °C; final count: 7.11 Log CFU/g	[73]
Pathogen reduction not investigated	0.5% (*w*/*v*)	Viable for 28 days (5.70–7.77 Log CFU/g), but >6 Log CFU/g only until day 14	[74]
Pathogen reduction not investigated	0.5% (*w*/*v*)	Viable for 28 days (5.70–7.77 Log CFU/g), but >6 Log CFU/g only until day 14	[74]

5.1. Dairy Products

Among products, cheese represents a highly suitable matrix for the incorporation of probiotic bacteria due to its favorable physicochemical characteristics, such as pH and solid structure, which contribute to the survival of microorganisms. The application of active edible films in dairy products, especially in cheese, has been widely studied to improve microbiological safety, extend shelf life, and simultaneously maintain or enhance sensory attributes, a factor increasingly sought by consumers not only for taste but also for the overall experience.

A recent study by Mahmoudi et al. [65] evaluated the use of probiotic films based on flaxseed mucilage containing *L. rhamnosus* and *L. acidophilus* applied to fresh Sicilian-style sheep cheese, with the incorporation of 5% (*w*/*w*) of probiotic strains, in the form of active biomass incorporated into the mucilage solution. The results demonstrated that the probiotic coating promoted significant improvements in product preservation, reducing mass loss from 22% to 8% and contributing to pH stability throughout the storage period, indicating a direct influence of the active system on the intrinsic physicochemical properties of the cheese.

In addition, the films exhibited antimicrobial activity against *Listeria monocytogenes*, with inhibition zones of up to 12.8 mm, as well as a relevant antioxidant effect (51%

inhibition of the DPPH radical), suggesting additional potential in reducing oxidative processes. Importantly, these effects were achieved without compromising the sensory acceptability of the product, demonstrating that the incorporation of probiotics into edible films can simultaneously contribute to microbiological preservation and the maintenance of the sensory quality of the cheese [47].

In another study, Ceylan and Atasoy [66] developed edible films based on sodium caseinate containing *B. animalis* ssp. *lactis* BB-12®, combined with inulin and fructooligosaccharides (FOS) as prebiotics, with the incorporation of 150 mL of probiotic cells at a final concentration of 10^9 CFU/mL. These films were applied to sliced processed cheese, reducing weight loss and improving color preservation and softness over 30 days of storage.

The results demonstrated that the symbiotic films acted significantly in preserving the physicochemical and structural properties of the product, promoting a reduction in mass loss as well as greater color and softness stability when compared to the uncoated control. These effects indicate that the active system directly influenced intrinsic food parameters, possibly through the modulation of moisture transfer and the formation of a more efficient surface barrier.

A study conducted by Kahraman Ilıkkan et al. [67] investigated the application of an innovative synbiotic edible film formulated with aquafaba, psyllium husk powder, and polyethylene glycol 400, containing the probiotic strain *L. plantarum* 299v. The probiotic culture was previously cultivated, concentrated by centrifugation, and directly incorporated into the film-forming solution, presenting an initial concentration of 10^{11} CFU/mL. The film was applied by immersion to Kashar-type cheeses, and the results showed that probiotic viability remained above 10^8 CFU/g during 60 days of refrigerated storage. Additionally, the cheeses coated with the probiotic film showed better microbiological performance, with lower counts of filamentous fungi and coliforms at the end of storage compared to uncoated cheeses. Sensory analyses indicated that the addition of probiotics did not compromise attributes such as flavor, texture, or appearance. Physicochemically, the coating contributed to reduced moisture loss and maintained pH stability, reinforcing its potential as a functional preservation technology for dairy products.

5.2. Meat and Fish Products

Meat and fish are highly perishable foods, particularly vulnerable to microbiological spoilage, lipid oxidation, and physicochemical changes during refrigerated storage. The incorporation of probiotics into edible films or coatings has proven effective in preserving quality and extending the shelf life of these foods [75,76].

Lu et al. [68] developed a fermented probiotic edible film based on a mixture of gum arabic, whey protein isolate, isomalt, and glycerol, inoculated with *L. rhamnosus* HN001 with a concentration of approximately 9.5 Log CFU/mL. The fermentation of the film-forming solution significantly improved the film's physical and functional properties, including mechanical strength, barrier performance, and antioxidant activity. When applied to fresh pork, the fermented probiotic system not only delayed microbiological spoilage, with significant reductions in *Staphylococcus aureus* and *Salmonella* spp. counts, but also exerted a direct influence on intrinsic food properties that are fundamental for freshness and quality assessment. The film contributed to the maintenance of pH stability, preservation of color throughout storage, and reduction in total volatile basic nitrogen (TVB-N) values during 12 days of refrigerated storage, indicating lower protein degradation and delayed spoilage processes [50].

Abbasi et al. [69] developed an edible coating based on *Malva neglecta* seed mucilage (MNSM) enriched with postbiotics from *L. brevis* TD4 (PLB) at different concentrations (0%, 5%, 10%, and 15% v/v). The coatings containing 10% and 15% PLB preserved the meat up to the 12th day, maintaining a total viable count below the threshold of 7 Log CFU/g, whereas the uncoated meat reached 8.95 Log CFU/g. In addition to the significant reduction in total microbial load, these same formulations promoted marked decreases in the counts of spoilage and pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, coliforms, and fungi, which was attributed to the presence of bioactive compounds in the postbiotics. From the perspective of intrinsic meat properties, the MNSM–10% PLB and MNSM–15% PLB coatings also showed a superior performance in inhibiting lipid oxidation, resulting in the lowest peroxide values at the end of the storage period (7.00 and 6.37 meq O_2/kg, respectively) compared to the control (11.25 meq O_2/kg) [51].

In the study by Mozaffarzogh et al. [71], films based on carboxymethylcellulose–sodium caseinate (CMC–SC) were formulated by incorporating 1 mL of multiple probiotic strains (*L. acidophilus*, *L. reuteri*, *L. casei*, *L. rhamnosus*, and *B. bifidum*) at a final concentration of 9 Log CFU/mL and applied to rainbow trout filets. Probiotic systems enabled the release of approximately 6.8 log CFU/g of viable bacteria into the fish after 6 h of storage, demonstrating the film's ability to act as a functional vehicle for active microorganisms. Among the evaluated strains, the film containing *L. acidophilus* showed the best performance in inhibiting lipid oxidation, resulting in the lowest peroxide values (0.50 ± 0.01 meq/1000 g) and thiobarbituric acid reactive substances (TBARS) (1.11 ± 0.02 mg malondialdehyde/1000 g) at the end of 14 days of refrigerated storage

Additionally, this same treatment promoted a significant reduction in the formation of biogenic amines, important indicators of fish spoilage, with cadaverine and putrescine levels of 6.65 mg/kg and 3.85 mg/kg, respectively, which were lower than those observed in the control and other treatments. From a microbiological standpoint, probiotic films also contributed to reducing microbial growth, particularly the formulations containing *L. rhamnosus* and *L. acidophilus*, which resulted in final Enterobacteriaceae counts of 3.52 ± 0.01 and 3.37 ± 0.06 log CFU/g, respectively. Psychrotrophic bacteria were also significantly reduced in the probiotic treatments, reaching values of approximately 6.71 log CFU/g in films containing *L. rhamnosus* and *L. reuteri*, and 6.72 log CFU/g in films with *L. acidophilus*. These results demonstrate that the incorporation of probiotic strains into edible films can effectively preserve key intrinsic properties of fish, including oxidative stability, control of biogenic amine formation, and reduction in microbial load, going beyond mere shelf-life extension [71].

5.3. Fresh Fruits and Vegetables

Fresh fruits and vegetables are often affected by fungal contamination and rapid spoilage. The incorporation of probiotics into edible coatings has emerged as a promising approach to extending the shelf life of highly perishable fruits and vegetables such as strawberries, apples, and potatoes.

Rahmati-Joneidabad et al. [72] developed an edible coating using garden cress seed mucilage enriched with *Limosilactobacillus reuteri* VTC 864. The probiotic granules were prepared by centrifuging 100 mL of De Man–Rogosa–Sharpe (MRS) broth containing the bacterial culture, which was then mixed with each coating solution for 15 min. Two probiotic concentrations were evaluated: mucilage containing 1.5 × 10^8 CFU/mL of *L. reuteri* VTC 864 and mucilage incorporating 3 × 10^9 CFU/mL of *L. reuteri* VTC 864. The strawberries coated with probiotics inhibited yeast and mold growth, remaining within the acceptable limit of 10^6 CFU/g. Additionally, the study reported a delayed increase in pH and a decrease in titratable acidity, with smaller variations observed in the probiotic-coated samples. Sensory attributes such as flavor, texture, and aroma were also positively preserved.

In the study conducted by Yang et al. [38], a probiotic film based on gellan gum (GN) was developed, incorporating the probiotic *L. lactis* (LA) at two concentrations (1.0% and

2.0% *w*/*w* relative to GN) and cranberry extract (CE) at levels of 0.5% and 1.0%. The films were applied to fresh-cut apples and potatoes, aiming to enhance preservation properties. Film containing 2.0% LA and 0.5% CE exhibited the highest antibacterial activity, with an inhibition zone of 14.9 ± 0.6 mm against *L. monocytogenes*. Regarding antioxidant activity, the incorporation of 1.0% CE significantly increased the DPPH radical scavenging capacity, reaching values up to 80–100%, whereas the control film without CE showed only 2.4 ± 0.2%. Additionally, the film with 1.0% LA and 1.0% CE presented the highest tensile strength (TS), reaching 32.52 ± 1.2 MPa, with an elongation at break (EB) of 17.91 ± 2.1%, improving the mechanical properties compared to the control film (17.92 ± 0.8 MPa and 12.32 ± 1.3%, respectively) [38].

Shigematsu et al. [73] developed an edible coating based on sodium alginate enriched with a lyophilized probiotic culture of *L. acidophilus* La-14, incorporating 7.36 Log CFU/g of the probiotic. The probiotic-containing coating was effective in partially inhibiting the increase in titratable acidity (from 0.58 ± 0.00 to 0.77 ± 0.00) compared to the control (from 0.77 ± 0.00 to 1.13 ± 0.02) over 19 days of storage, while also helping to preserve moisture content. Color changes showed an increase of 12.5% in a* and 18.1% in b* values by day 18, indicating a better retention of the orange color due to the presence of the sodium alginate coating. These results suggest that alginate-based coatings can serve as suitable carriers for probiotics, while enhancing the freshness and safety of minimally processed carrots.

Dianin et al. [74] studied edible biofilms produced from whey protein isolate (WPI), with or without *L. casei*, applied to cherry tomatoes and Thompson grapes. The films containing probiotics (0.5% *w*/*v*) exhibited a higher tensile strength (23.3 N vs. 12.6 N) and lower flexibility, while maintaining probiotic viability above 6 Log CFU/g for 14 days at 25 °C. The coating reduced weight loss by 20.58% compared to the uncoated control (25.92%) and decreased total soluble solids (2.63 °Brix vs. 4.25 °Brix), indicating a ripening control effect. In grapes, the probiotic film also reduced the increase in TSS (27.25 °Brix); however, it led to greater weight loss, possibly due to the higher porosity of the film. Nevertheless, the coating demonstrated potential effectiveness in delaying ripening and dehydration in certain fruits.

Beyond the individual case studies discussed previously, a broader comparison with conventional active packaging systems is necessary to contextualize the preservation effects of probiotic-based materials.

When compared to conventional active packaging systems based on antimicrobial agents, antioxidants, or plant extracts, probiotic-based materials present distinct advantages. While traditional active agents primarily act through passive release mechanisms, probiotics and their metabolites may continuously interact with the food matrix, contributing to the dynamic modulation of physicochemical properties. In addition, probiotic systems can simultaneously provide antimicrobial, antioxidant, and functional effects, whereas non-biologically active materials generally target a single deterioration pathway [77,78].

However, it should be noted that probiotic-based systems may exhibit a greater sensitivity to processing and storage conditions compared to non-biologically active materials, highlighting the importance of formulation strategies capable of ensuring microbial viability and functional stability.

6. Final Considerations

This study explored the application of probiotic bacteria in active packaging for the food industry, highlighting their ability to improve safety, extend shelf life, and offer health benefits to consumers. The research demonstrated that the effectiveness of probiotic-based active packaging is influenced by the selection of the probiotic strain, encapsulation method, and choice of biopolymeric matrix.

Applications in various food matrices, including dairy products, meats, fish, fruits, and vegetables, demonstrate the potential of probiotic packaging to delay microbial spoilage, inhibit lipid oxidation, and preserve sensory and nutritional attributes. Although effective as preventive measures against microbial deterioration, probiotic-based systems have limitations in products that are already heavily contaminated.

Future research on antimicrobial packaging should focus on developing multifunctional systems that incorporate natural bioactive agents, such as probiotics, postbiotics, bacteriocins, and plant-derived compounds, to enhance food safety and shelf life. Additionally, greater attention should be given to industrial scalability, cost-effectiveness, and compatibility with existing manufacturing processes.

In addition to the aspects discussed, several challenges still limit the large-scale implementation of probiotic-based active packaging systems. Maintaining probiotic viability throughout processing, storage, and distribution remains a major technological barrier, particularly under non-refrigerated conditions. Furthermore, interactions between probiotics and different biopolymeric matrices may affect both microbial stability and packaging performance, requiring further optimization. Regulatory frameworks and consumer acceptance also pose significant hurdles, particularly regarding the classification of probiotic packaging as functional or bioactive materials.

From this perspective, future research should prioritize the development of robust and multifunctional packaging systems that integrate probiotics with complementary bioactive compounds, such as prebiotics, postbiotics, bacteriocins, and plant-derived extracts. Advances in microencapsulation technologies, scalable manufacturing processes, and the integration of smart packaging are expected to play a key role in improving stability, functionality, and industrial feasibility. In addition, comprehensive life cycle assessments and cost–benefit analyses will be essential to support commercial adoption. Overall, probiotic-based active packaging holds strong potential to contribute to sustainable and health-oriented food systems, provided that these technical and regulatory challenges are systematically addressed.

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