

Review

Probiotics and Beneficial Microorganisms in Biopreservation of Plant-Based Foods and Beverages

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Abstract: Maintaining the overall quality and shelf life of plant-based food and beverages is particularly important yet challenging to the food industry. Demand for natural preservation techniques has increased with the rising concerns over food safety and consumer awareness, e.g., health consciousness and food trends such as veganism and the demand for clean, labelled foods. Thus, a technique such as biopreservation has the potential to enhance food safety while fostering the quality, originality and naturalness of food. The application of probiotic microorganisms to foods and beverages provides various health benefits in addition to improved shelf life, stability and microbial safety of the food. The provision of probiotics is known to deliver various health benefits for the host's gut health. Therefore, this review aims to investigate the importance of biopreservation and the role of probiotics in the food industry. An attempt was made to explore the various possibilities of shelf-life enhancement through the use of probiotic microorganisms as biopreservatives. Noticeable improvements in the shelf life of plant-based foods and beverages were observed due to the antimicrobial effects exerted by probiotics and potential probiotic strains which make them useful alternatives to artificially synthesized chemical preservatives.

Keywords: biopreservation; biopreservatives; plant-based food; beverages; probiotics



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1. Introduction

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host [1]. Probiotics have been associated with improved digestive health and enhanced immune systems. Probiotics restore the composition of the gut microbiome and give beneficial functions to gut microbial communities, such as preventing gut inflammation and other intestinal diseases. Probiotics initiate β -defensin and immunoglobulin A (IgA) production in the host's body to suppress the growth of pathogens [2]. The potential antimicrobial activity and the ability of probiotics to render health benefits have created opportunities to explore options for probiotic sources, formulations, and delivery methods into food products. Suitable strains are selected for food product development involving probiotic bacteria in industrial production. Modern food production technology aims to maintain the high viability of probiotics during the post-harvest or storage phase of food commodities. Attempts have been made to introduce non-viable microorganisms to obtain health benefits as well [3].

Probiotic formulations must contain sufficient quantities of viable microorganisms ($>10^6$ cfu/mL or g of food) at the time of consumption to deliver any claimed health

benefits [4]. Many approaches like encapsulation ensure that the required number of probiotics are delivered. Probiotics are one of the most widely incorporated ingredients into functional foods where significant investments have been attracted to develop novel technologies and food formulations [4]. Novel probiotic delivery strategies include the production of capsules, powders, liquids and conventional food forms incorporated into dairy products, baked products, confectioneries and drinks. These are proliferating at a rapid rate, with a recent focus on non-dairy matrices [4].

Food spoilage leads to unnecessary waste and adversely impacts the economy and brand reputation of the manufacturers. Meantime, increased shelf life can be a reliable indication of a microbiologically safe and good-quality food product. Thus, the food industry is constantly working to protect the nutritive value, appearance, sensory attributes, and microbial safety of the food throughout the supply chain. The crucial aspects of food safety and quality can be effectively managed by various preservation techniques, among which biopreservation is gaining high levels interest. The food industry focuses on the development of healthy and safe food products, which can be achieved through probiotic microorganisms [5]. Replacing artificially synthesized chemical preservatives with biopreservatives benefits both consumers' health and the environment. Among different means available for biopreservation, probiotics are promising candidates as biopreservative agents for plant-based foods, in addition to their known multiple benefits for the host. The involvement of probiotics helps in maintaining a balance between beneficial and harmful bacteria, thus making it an efficient biopreservative. The preservative action of probiotics allows an interesting opportunity to utilize as a biopreservative agent to prevent spoilage of a variety of plant-based food products, including those which originate from fruits and vegetables [6]. Hence, the objective of the current review was to summarize biopreservative properties of the probiotics and potential probiotics/beneficial microorganisms used in plant-based food matrices.

2. The Need for Biopreservation

Foodborne pathogens are biological agents that cause foodborne illnesses, leading to foodborne disease outbreaks. Foodborne illnesses occur when a pathogen is ingested and establishes itself in the host or when a toxigenic pathogen establishes itself in a food product and produces a toxin, which is then ingested by the human host [7]. More than 200 foodborne diseases have been identified, and most severe cases occur in those patients who have compromised immune system function for example, very old and very young [8,9]. *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Cronobacter sakazaki*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Vibrio* spp., and *Yersinia enterocolitica* are some of the most common foodborne pathogens which more commonly linked with foodborne diseases [7].

Suppression of foodborne pathogens in food products by probiotics has been reported in the literature. Similarly, pathogen suppression by probiotics in vivo in the human body has also been described [10]. The exact probiotic mechanism in pathogen suppression in food products during processing and storage seems to differ from that within the human body. For example, the production of short-chain fatty acids by probiotics lowers the product pH in fermented dairy foods and creates an unfavorable environment for certain pathogenic microorganisms. In addition, certain probiotics can produce bacteriocin when incorporated into food products. These are beneficial as they prevent the ingestion of higher numbers of pathogens with food products. When probiotic microbes are in the human body after ingestion, the mechanisms may be associated with the prevention of pathogenic microorganisms from attaching the intestinal epithelium, with a competitive advantage for nutrients and with the release of bacteriocins or other antimicrobial agents active against pathogenic microorganisms [10–13]. Nonetheless, the synergistic nature of pathogen suppression by probiotics in food products and after ingestion within the human body should be further investigated in future research.

Biopreservation is defined as the utilization of non-pathogenic microorganisms or their metabolites to increase the shelf life and enhance food safety by the destruction or inhibition of undesirable microorganisms present in food [14]. Fermentation is one of the most common forms of food biopreservation. Naturally present or externally added microorganisms are involved in the breakdown of complex food compounds along with producing alcohols, organic acids, and various industrial preparations. The fermentation results in the production of aroma and taste components, a process which improves the organoleptic properties of the food [15]. However, one of the main challenges when fortifying food products with probiotics is obtaining consumers' acceptance due to the development of off-flavors by certain probiotic strains. Due to this reason, alterations in organoleptic properties in some groups of raw materials or products are not recommended, for example, in products with higher fat content. Thus, for this sort of raw materials/product, strains that cause no or negligible alterations to the original flavor should be recommended for biopreservative purposes. This suggests that the selection of a strain as a biopreservation agent should be based on the nature of the raw material or product.

The most widely used biopreservatives in industrial applications are lysozymes, bacteriophages, lactic acid bacteria and their bacteriocins. Lysozymes are natural enzymes present in bodily secretions, known for their antibiofilm characteristics. Bacteriophages are viruses that infect bacteria, and their antibacterial properties make them an efficient biopreservative. Bacteriocins are biologically active complex proteins or peptides that show antimicrobial action against closely-related bacterial species [16]. Lactic acid bacteria (LAB) have gained special interest due to their dynamic characteristics and bacteriocin-producing ability. Among different probiotic strains, some strains are capable of producing bacteriocins that exert antimicrobial properties against certain pathogenic and food-spoilage microorganisms. The bacteriocin-producing ability of the probiotics, along with their other antagonistic/antimicrobial properties, have made them promising candidates as natural biopreservatives of food. The advancement in food safety has revealed the benefits of the use of bacteriophages and endolysins as food biopreservatives. It is aimed to explore the different applications of probiotic microorganisms and bacteriocins with potential antimicrobial activity, to preserve food [17].

Consumers are not only aware of the risk derived from foodborne pathogens, but are also aware of the consequences of artificial chemical preservatives used to inhibit them. Food producers face conflicting challenges due to strict food safety requirements set by the regulatory bodies and policymakers. Examples of conventional methods of food preservation are heating, drying, freezing, pickling, edible coating, and high-pressure processing [18]. These methods involve the inactivation and/or inhibition of pathogenic microorganisms through different treatments or additives. However, these methods do not involve the understanding of microbiology. Pasteurization and other high-heat treatments result in the loss of nutrients and sensory properties. Further, non-thermal processing techniques like food irradiation are associated with safety concerns, along with social and ethical issues. These disadvantages in the existing food preservation systems create the need for alternative food preservation methodologies that can address modern food safety and quality concerns efficiently and sustainably [19]. Spore-forming bacteria are a major threat in heat-treated food as these spores show typical resistance to physical treatments including thermal processing. These spores can germinate and grow out in the product under suitable conditions. Foodborne pathogens such as *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus subtilis* and *Bacillus cereus* are some good examples of spore-forming and highly heat-resistant bacteria, whereas *Staphylococcus aureus* and *Clostridium botulinum* are capable of producing heat-tolerant toxins. Most of the foodborne pathogens are mesophilic (optimum growth temperatures ranges 20–45 °C) and some are capable of growing under refrigerated conditions or temperatures less than 10 °C (psychrotrophs), such as *Listeria monocytogenes* and *Yersinia enterocolitica* [20]. In this context, the antagonistic effects and antimicrobial properties of the probiotic microorganisms may be beneficial to the inhibition

and suppression of the growth of foodborne pathogens during food processing as well as refrigerated storage.

Biopreservation is of high interest due to its ecological sustainability and consumer-friendly nature. Unlike artificially synthesized chemical preservatives that have toxic effects over long-term usage, biopreservatives offer little or no harmful health effects [21,22]. This ecofriendly preservation technique is used as an alternative to chemical additives to extend the shelf life of various plant-based food and beverages [23]. Public awareness about the importance of maintaining a balance in the gut microbiota and enhancing mucosal defenses against pathogens is rapidly increasing.

The lifestyle changes have resulted in the globalization of the food market. In recent years, the consumers willing to choose minimally processed foods and functional products. Complex and lengthier food chains are involved in the processing of these food products, which increases the risk of microbial contamination and thus raises safety concerns. New preservation techniques are being researched to meet consumer expectations for natural and superior overall quality in food. Biopreservation being an alternative food preservation methodology, maintains hygienic quality, increases product shelf life, and minimizes the impact of nutritional and organoleptic properties of various perishable food products. Variation in consumer dietary patterns and the shift towards the consumption of vegan and organic foods have created a demand for biopreservation techniques and their implementation in the plant-based food and beverage industry. The need for biopreservation is a reflection of increased consumer awareness [16].

3. Role of Probiotics in the Food Industry

Probiotics involve the replacement of harmful bacteria and the introduction of beneficial bacteria into the gastrointestinal tract. Dietary allergies, lactose intolerance, vegetarianism and veganism have created a demand for non-dairy probiotic foods [24]. The delivery of probiotics into the human body is traditionally associated with fermented dairy foods like yoghurt, cheese, and various types of fermented beverages such as sour milk. Probiotic delivery can also be achieved through non-dairy probiotic foods, mainly beverages. Both fermented and non-fermented non-dairy foods play an important role in probiotic delivery. The probiotic beverages of fruit and vegetable origin are mostly formulated using non-fermentation methods [25]. Commonly used probiotics and beneficial microorganisms in dairy and non-dairy plant-based food products are listed in Table 1. Scientific experimentation has proved the possible health benefits of incorporating probiotics in food commodities. The expansion of consumer awareness has resulted in popularity for the probiotics in functional food markets in some countries. Sensory properties are given high importance to determine and validate consumer acceptance for probiotic food products. In the future, use of probiotics will mostly be common in medicine and nutrition. It is necessary to consider probiotic applications in preventing and treating various diseases under the guidance of medical professionals. This can later be developed and promoted by the food industry. The whole ideology of probiotic inclusion in food product development should be conducted and spread scientifically and in the interest of consumers [26,27].

The potential application of bacteriocins, produced by antagonistic microorganisms including probiotics, as natural preservatives is gaining the attention of the food industry in recent years [28]. Probiotics have a wide range of applications in the food and dairy industry. Probiotic beverages and foods that are dairy-based are widespread in the current market. The reason for the popularity of probiotic beverages is that the consumers believe it to be a more reliable source of active ingredients [29].

Probiotics play an important role in the food industry, predominantly in the dairy sector. Probiotic bacteria are commonly encapsulated for producing functional foods, e.g., yoghurt, cheese, ice cream, as well as non-dairy products like cereals, chocolates, various confectioneries and processed meat products [30,31]. Several companies have formulated probiotics as pills, tablets or capsules forms. Yet, very few encapsulated probiotic products are found in the food industry. Furthermore, the understanding of cell biology and

encapsulation technology will undoubtedly help to develop various novel and commercial probiotic products [32]. In the dairy industry, strains of the genera *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics. Other genera such as *Propionibacterium*, *Peptostreptococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Saccharomyces* and *Streptococcus* are slowly gaining popularity in the field of probiotic foods and beverages [33,34]. In a recent study, *Lactobacillus acidophilus* LA-5 has been used to develop a probiotic drink enriched with mango juice. The addition of mango juice enhanced probiotic viability. As a result of this formulation, the probiotic tolerance was improved when exposed to in vitro gastrointestinal digestion. Sensory analysis of mango juice from the same study revealed that the sensory score of beverages increased with the increase in mango juice concentration [35].

The stability of probiotic microorganisms can be affected by the characteristics of the food matrix, storage conditions, and the passage through the gastrointestinal tract. The longevity and activity of microorganisms are largely supported by the characteristics of the food matrix. The method of production of a certain food product contributes mainly to the safety, consistency, and effectiveness of the food product. Traditionally, probiotic delivery is carried out through the use of fermented and non-fermented foods. The modern food industry aims to include probiotic microorganisms in unfermented foods in a microencapsulated form. Probiotic microbial strains can be introduced into the human body in three major forms. Further, probiotics are administered through traditional fermented foods either as functional foods and/or pharmaceutical products [5].

Table 1. Commonly used probiotics and beneficial microorganisms in dairy and non-dairy plant-based food products.

Genus	Probiotic/Potential Probiotic Strain	Food Matrix	Reference
<i>Lactobacillus</i>	<i>Lactobacillus rhamnosus</i> GG	Kefir	[36]
	<i>Lactobacillus casei</i> ATCC 393	Fermented milk	[37]
	<i>Lactobacillus casei</i> Q14	Yoghurt	[38]
	<i>Lactobacillus casei</i> 01	Sheep milk ice cream	[39]
	<i>Lactobacillus paracasei</i> LBC-81	Maize-based beverage	[40]
	<i>Lactobacillus plantarum</i> L7	Rice-based fermented beverage	[41]
	<i>Lactobacillus acidophilus</i> NCIMB 8821	Oat flour and barley malt beverage	[42]
	<i>Lactobacillus reuteri</i> NCIMB 11951	Oat flour and barley malt beverage	[42]
	<i>Lactobacillus fermentum</i> ATCC 9338	Prickly pear juice	[43]
<i>Bifidobacterium</i>	<i>Lactobacillus fermentum</i> KKL1	Rice-based fermented beverage	[44]
	<i>B. longum</i> subsp. <i>longum</i> YS108R	Fermented milk	[45]
	<i>B. animalis</i> <i>B. breve</i>	Milk supplemented with seaweed extract Probiotic-fermented blended juices	[46] [47]
<i>Saccharomyces</i>	<i>Saccharomyces cerevisiae</i> KU200284	Kefir	[48]
	<i>Pediococcus</i>	Soursop juice	[49]
<i>Pediococcus</i>	<i>Pediococcus pentosaceus</i> Lbf2	Probiotic orange juice	[50]
	<i>Pediococcus acidilactici</i> CE51	Fermented soybean milk	[51]
	<i>Pediococcus pentosaceus</i>		
<i>Propionibacterium</i>	<i>Propionibacterium freudenreichii</i> subsp. <i>shermanii</i>	Probiotic feta cheese	[52]
<i>Streptococcus</i>	<i>Streptococcus thermophilus</i>	Probiotic chocolate, novel probiotic fermented oat flour	[53,54]

4. Biopreservative Properties of Probiotics

The major mechanisms of probiotic activity in the human body are depicted in Figure 1. Antimicrobial properties are one of the core benefits associated with probiotics. A number of commercially used probiotics and beneficial microorganisms have demonstrated various inhibitory actions against a variety of pathogenic and spoilage microorganisms in foods. The study of mechanisms of probiotic action reveals novel functions of probiotic microor-

ganisms. A clear understanding of the mode of action enables the selection of suitable probiotic strains for specific applications. The antagonistic effects of the probiotics on other microorganisms may be due to a number of mechanisms including gut microbiota modification, enhanced gut epithelial barrier, increased adherence to the epithelium and intestinal mucosa, and immune system modulation to offer benefits to the host [55]. However, not all of these mechanisms are important for their biopreservative properties.

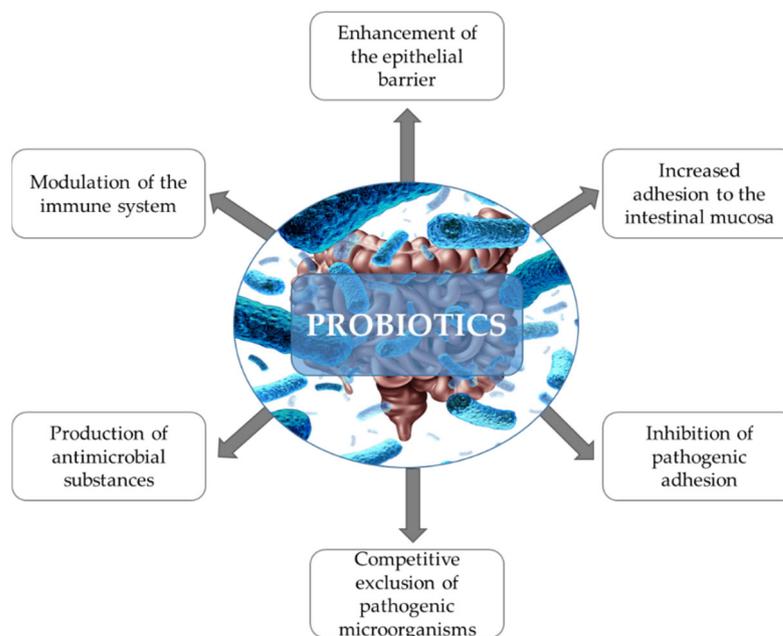


Figure 1. Major mechanisms of probiotic activity in the human body.

The production of antimicrobial substances seems to be the main contributor to the biopreservative properties of probiotics. The mechanism of probiotics' antimicrobial activity is largely associated with the formation of organic acids, ethanol, and bacteriocins and thereby the inhibition of foodborne and spoilage microorganisms present in food material [56]. Hence, bacteriocin production by probiotic microorganisms can be considered as an integral part of their antagonistic ability in biopreservation. However, this phenomenon warrants further research. Research dedicated to investigating the antimicrobial properties of the probiotics, as well as potential probiotic strains, provides suggestions for a long list of potential candidates that can be used as biopreservative agents in plant-based foods. For instance, Likotrafiti et al. [57] reported the antimicrobial properties of *Lactobacillus kefir* which is a potent probiotic strain isolated from kefir grains. Co-culturing of *Bifidobacterium longum* IPLA20022 and *Bifidobacterium breve* IPLA20006 in the presence of short-chain fructooligosaccharides as carbon source significantly reduces the growth of pathogenic *Clostridium difficile* [58]. Among these, potential probiotic strains isolated from food sources have the great advantage of using as biopreservative agents in another food matrix.

5. Probiotic Applications for Improved Shelf Life in Vegetables, Fruits and Other Miscellaneous Plant-Based Foods

In terms of probiotic deliver, probiotic-enriched vegetables and fruits are considered to be an ideal alternative to probiotic dairy products. This is because these food formulations can better meet the needs of vegans/vegetarians, the lactose-intolerant population, and individuals prefers low-cholesterol intake or those who are allergic to animal proteins [59]. There is a continuous increase in demand for ready-to-eat fresh vegetables and fruit products, largely due to the consumers' interest in fresh and healthy foods with an easy means of preparation [60]. Being low-acid foods, exhibiting higher moisture content and having a high number of cut surfaces, cut fruits and vegetables provide ideal conditions for microbial

growth, including growth by pathogenic and spoilage microorganisms [60]. These products are therefore highly susceptible to causing outbreaks of multistate foodborne diseases caused by common pathogens such as *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [60,61]. To ensure the safety of ready-to-eat fresh-cut fruits and vegetables, safe production methods and proper disinfecting procedures are essential [60].

The native microbial communities established on the food are ideal sources to isolate potential probiotic strains exhibiting inhibitory effects against contaminating food-borne pathogens. The organisms have the advantage of being part of the natural microbial community already established on the target product, which may facilitate their colonization of and survival on the produce when applied in appropriate numbers [61]. Trias and others [60] reported that indigenous LAB occurred at densities ranging from 10^2 to 10^6 cfu g⁻¹ on fresh vegetables and fruits in which the genera *Lactobacillus* and *Leuconostoc*, and to a lesser extent *Weissella*, *Enterococcus* and *Lactococcus* species, were found to be the most abundant. Although there is a large diversity of indigenous LAB present in fresh fruit and vegetables, only a few bacteria have been identified as having antagonistic capabilities against foodborne pathogens. Research evidence suggests that natural microbiota present on fresh fruits and vegetables compete with pathogens for physical space and nutrients and/or by producing antagonistic compounds that negatively affect the viability of the pathogenic microorganisms [62].

5.1. Fruits

Probiotics and natural microbiomes of fruits have been used in improving safety and quality aspects of fresh fruits or minimally processed fruit products such as fresh-cut fruits.

5.1.1. Melons

Fresh-cut fruits such as melon can be contaminated during preparation (peeling, cutting, etc.) and processing. Specifically, the spoilage and pathogenic microorganisms may gain access to the nutrients inside the fruit and then multiply, causing deterioration [63]. Melons are highly susceptible to multistate foodborne outbreaks since they have a higher chance of contamination during growth, postharvest handling, packing, transportation, distribution, or final preparation. During the last three decades, several multistate outbreaks of salmonellosis and listeriosis have occurred due to the consumption of contaminated fresh-cut fruits [63,64].

Ukuku et al. [62] reported that native microbial communities of the whole cantaloupe inhibited attachment to rind surfaces, as well as the survival and growth of *L. monocytogenes* on cantaloupe surfaces and homogenized fresh-cut surfaces. Moreover, disinfection treatment was found to be detrimental to the native microbial communities, as the population of *L. monocytogenes* was significantly higher in melons treated with either chlorine or ethanol than that given no disinfection treatment or washed with water [62].

Riboflavin over-producing potential probiotic strains *Lactiplantibacillus plantarum* B2 and *Limosilactobacillus fermentum* (previously *Lactobacillus fermentum*) PBCC11.5 were reported as exhibiting satisfactory inhibitory effects against *L. monocytogenes* on artificially contaminated melons. The viability of *Lb. plantarum* B2 and *Lb. fermentum* PBCC11.5 were 3×10^8 CFU/g and 7.8×10^7 CFU/g at the end of the refrigerated storage for 11 days (initial inoculation levels of the probiotic strains were 1×10^{10} CFU/mL). The major technological and nutritional parameters of the fresh-cut cantaloupes were unaffected by probiotic enrichment. However, the addition of *Lb. plantarum* B2 resulted in some undesirable sensory attributes after 11 days of storage. The riboflavin content increased approximately two-fold in probiotic cantaloupe. These results suggest that these two probiotic strains can enhance the safety of minimally processed melons due to their antagonistic effects on *L. monocytogenes* of fruit origin [59].

5.1.2. Apple

The potential probiotic strain *Lactiplantibacillus plantarum* CIT3, isolated from apple, showed a significant inhibitory effect against *E. coli* and *L. monocytogenes* in sliced apples. Products inoculated with *Lb. plantarum* CIT3 showed significantly accelerated the death of *E. coli*, lowering the pathogen numbers to undetectable levels within 7 days of storage at 6 °C. Moreover, *Lb. plantarum* CIT3 inhibited the growth of *L. monocytogenes* until the end of the storage period. *Lacticaseibacillus paracasei* (*Lb. paracasei*) M3B6 also showed promising inhibitory effects against *E. coli* and *L. monocytogenes*, although to a lesser extent. These two potential biocontrol agents are found to be effective when they are present at levels higher than 1.5 log CFU/g. Results suggest that these biocontrol agents create an effective hurdle against *L. monocytogenes* for at least 16 days of refrigerated storage, while increasing the safety against *E. coli*. Furthermore, these probiotic biocontrol agents were able to significantly inhibit the growth of yeast, although the sensory properties of these products were negatively affected as these biocontrol agents resulted in the premature browning of the products. However, the color of the samples remained acceptable for up to 7 days of storage at 6 °C [65].

Rößle et al. [66] studied the applicability of *Lacticaseibacillus rhamnosus* (*Lb. rhamnosus*) GG (LGG) to fresh-cut apple wedges and its effect on instrumental eating quality parameters and sensory acceptability. Apple samples were cut into skin-on wedges and were dipped in an edible buffer solution containing approximately 10¹⁰ CFU/mL of LGG. The viable LGG counts at the end of the 10-day long storage (at 2–4 °C) period was above 10⁸ CFU/g. No significant difference was observed in instrumental color values, shear values, soluble solid contents, titratable acidity, pH and overall acceptability between the probiotic apple wedges and control which did not contain probiotics [66]. Another study demonstrated that LGG survived at concentrations higher than 10⁶ CFU/g on minimally processed apple wedges for over 28 days of storage at 5 and 10 °C without any quality deterioration. In the presence of LGG, *L. monocytogenes* counts were reduced by 1-log units. Although *L. monocytogenes* counts were reduced by 1 log unit, *Salmonella* was not affected by co-inoculation with LGG. This demonstrates the strain specificity of the pathogen inhibition ability of probiotics. Further, the viable counts of LGG after the simulated gastrointestinal digestion were satisfactory and within the recommended levels (10⁶ CFU/g) only during the first 14 days of storage. This indicates the additional probiotic benefits of the product in addition to the biopreservation effect during the early days of product shelf life [67].

Not only LAB, but certain other bacterial strains reported to possess probiotic properties have also been reported to have antagonistic effects against food-borne pathogens. *Gluconobacter asaii*, isolated from apple surfaces, exhibited antagonistic effects against *Listeria monocytogenes* and *Salmonella enterica* Serovar Poona on cut Golden Delicious apples during storage. *G. asaii* was able to grow on cut apples and significantly reduced the *L. monocytogenes* populations after 2 days of storage at 25 °C. At 10 °C, although the reduction in pathogenic populations was not significant, *G. asaii* reduced the *L. monocytogenes* population by ~2.1–2.8 log units after 5 days of storage. At high inoculation levels of the pathogen, *G. asaii* is still able to reduce the populations within 7 days of storage at both temperatures. In addition, *G. asaii* significantly reduced *S. enterica* Serova Poona populations within 5 days of storage at 25 °C. *S. enterica* Serova Poona did not grow properly at 10 °C as it is not a psychrotroph. Most importantly, *G. asaii* did not cause any browning in Golden Delicious cut apple although it has been reported that gluconobacter species cause browning on cut apple surfaces [61].

In summary, these studies suggest that probiotic strains of *Lactiplantibacillus plantarum* CIT3, *Lacticaseibacillus paracasei* M3B6, *Lacticaseibacillus rhamnosus* GG and *Gluconobacter asaii* possess considerable antagonistic effects against common foodborne pathogens and thereby extend the shelf life of fresh-cut apples.

5.1.3. Pears

Iglesias, Abadias et al. [68] studied the effectiveness of the probiotics *Lactobacillus acidophilus* LA-5 and *Lb. rhamnosus* GG (LGG) against *Salmonella* and *L. monocytogenes* on fresh-cut pear at different storage temperatures (5, 10 and 20 °C). LGG reduced *Salmonella* and *L. monocytogenes* populations by 2- and 3-log units, respectively, at 10 and 20 °C. In contrast, *Lb. acidophilus* has no antagonistic effect on the pathogenic strains. During the 10 days of storage, probiotic populations were maintained around 10^7 – 10^8 CFU/g irrespective of the storage temperature. These results suggest that LGG can be used to control the growth of *Salmonella* and *L. monocytogenes* in fresh-cut pear wedges [68]. In another study, Iglesias, Echeverría et al. [69] evaluated the antagonistic capacity of the probiotic strain *Lb. rhamnosus* GG (LGG) against a cocktail of 5 serovars of *Salmonella* and 5 serovars of *L. monocytogenes*. These were assayed on fresh-cut pear at conditions simulating commercial application over 9 days of refrigerated storage (5 °C). During the storage, LGG controlled the growth of *L. monocytogenes* (the pathogen population reduced by ~1.8 log-units) and survived during storage in a modified atmosphere. However, no effect was observed on *Salmonella*. Application of LGG did not significantly affect the quality attributes (titratable acidity and soluble solids content). However, the volatile compounds found in fresh-cut pear treated with LGG seems to positively affect the flavor perception of the product (47). Another study demonstrated that LGG caused a reduction in the survival of *L. monocytogenes* in the gastrointestinal tract, as well as adhesion and invasion into Caco-2 cells [70]. These results show that LGG modifies the pathogenic potential of *L. monocytogenes*.

5.1.4. Oranges

In another study, it was found that bacteriocin from the potential probiotic *Lactococcus lactis* AP2 was stable at low temperatures for up to 72 h and acidic pH from 2 to 6. These qualities mean it can be used as a bio-preservative in acidic foods. The study showed that purified bacteriocin expressed better results compared to the chemical preservative (sodium benzoate) in preserving orange and mixed fruit juice during cold storage (4 °C) for over 12 days. Moreover, the cell-free extract of *Lc. lactis* AP2 was reported to inhibit the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteries*, *Staphylococcus aureus* and *Bacillus cereus* [71].

5.1.5. Other Fruits

Pomegranate juices, fermented with *Lactobacillus plantarum* POM1, *Lactobacillus plantarum* C2 and *Lactobacillus plantarum* LP09 in monoculture, were reported to have enhanced antimicrobial properties due to increased concentration of ellagic acid (Filannino et al., 2013). A probiotic sweet lemon juice fermented with *Lactobacillus plantarum* LS5 (37 °C for 48 h) showed antibacterial activity against *S. Typhimurium* and *E. coli* O157:H7. The probiotic juice is characterized by increased pH, lactic acid and antioxidant capacity and recorded viability counts over 10^7 throughout the storage period (4 °C for 28 d) (Hashemi et al., 2017).

5.2. Vegetables

Post-harvest spoilage caused by fungi in tomatoes results in a greater economic loss for the food industry. Luz et al. [72] screened 9 LAB strains isolated from tomato for antifungal activity against 33 fungal strains and then used as biopreservatives of tomato inoculated with *Penicillium expansum* and *Aspergillus flavus*. The highest antifungal activity was observed in the cell-free extracts of *Lactiplantibacillus plantarum* TR7 and *Lb. plantarum* TR71. Organic acids, phenolic acids and volatile organic compounds were identified as responsible compounds for the antifungal activity. Biopreservation of tomatoes with the cell-free extracts of the above two LAB strains decreased the microbial counts by 1.98–3.89 logs and 10 spores/g compared to those which were not fermented [72].

Lactocaseibacillus casei V4B4 and *Lb. plantarum* V7B3 showed excellent adaptability to the minimally processed lamb's lettuce to control spoilage microorganisms and inhibit pathogenic microorganisms. More importantly, the appearance and color of the products were not affected by the addition of these potential biocontrol agents [65].

Application of the cell-free supernatant of *Pediococcus* spp. (15 mL/g) showed enhanced preservation of strawberries, corn, tomatoes, and button mushrooms. For example, although untreated tomato and corn samples remained fresh only for 6 days, the treated samples of tomato and corn remained fresh for 13 days and 20 days, respectively. Moreover, this study suggested that 100 g/L of cell-free supernatant of *Pediococcus* spp. demonstrate antimicrobial potential against *E. coli* and *Shigella* spp. More interestingly, the treatment with the *Pediococcus* spp. demonstrated enhanced preservation in these products compared to the chemical preservatives sodium sulphate and sodium benzoate. The microbial quality of food samples treated with *Pediococcus* spp. showed significantly lower total bacterial counts compared to those treated with chemical preservatives. These results suggest that bacteriocin-producing *Pediococcus* species provide enhanced shelf life to certain food varieties and can be used as biopreservatives [73].

5.3. Plant-Based Milk Analogues and Other Miscellaneous Products

Plant-based milk substitutes and analogous products are becoming highly attractive among consumers at present. However, one of the major hygienic concerns associated with the production of beverages from plant origin is high microbial contamination of the raw materials. For instance, one of the major safety concerns associated with boiled and fermented quinoa products is the spontaneous growth of opportunistic pathogens expressing thermal resistance (e.g., *Enterobacteriaceae*). However, after fermentation with *Lactiplantibacillus plantarum* DSM9843 (at 30 °C for 2 days) viable counts of *Enterobacteriaceae* in boiled quinoa milk (5.3 log CFU/mL) reduced below the detection limits (<1 CFU/mL). During the storage of 28 days at 4 °C, *Lb. plantarum* was the predominant microorganism found in the medium which maintained around 10⁶–10⁷ CFU/mL [74].

Bifidobacterium animalis has successfully been employed to maintain the microbiological quality of cashew nut milk without affecting whiteness or the beverage's sensory acceptance. The probiotic bacteria were able to survive in the food matrix with counts above 10⁷ CFU/mL over a 30-day storage period at 4 °C [75].

Lee et al. [76] demonstrated that probiotics *Lactococcus lactis* subsp. *lactis*, *Lactobacillus reuteri*, and *Pediococcus acidilactici* were better utilized with β-glucooligosaccharides (β-GOS), derived from barley β-glucan, leading to an approximately 25% increase in nisin-Z production which would elevate the antimicrobial activity of the probiotics [76].

Nissen et al. [77] showed that certain compounds produced during the fermentation of hemp milk selectively enhance the growth of beneficial microbes such as probiotics (e.g., *Lactobacillus fermentum*, *Lb. plantarum* and *Bifidobacterium bifidum*), whereas certain compounds such as terpenes inhibit the growth of harmful bacteria.

Trias et al. [60] have identified 18 isolates composed of *Lactobacillus plantarum*, *Weissella cibaria*, *Leuconostoc mesenteroides*, *Leuconostoc citreum* and *Lactococcus lactis* strains from 700 samples of 36 types of fruits, vegetables, ready-to-eat salad and fresh-cut individual products. More importantly, none of the bacteria promoted spoilage reactions such as browning, pectinolytic activity or production of off-odors. Among the different strains isolated, *Leuconostoc* species showed the best antagonistic capacities over a wide range of microorganisms [60]. However, the use of *Leuconostoc* species as a food additive is limited to the predominance of heterofermentative species, which may negatively influence the organoleptic properties of the final product [78].

5.4. Miscellaneous Food Items

Modern-day consumers are interested in healthier diets. The cholesterol content in dairy products and allergic reactions to milk proteins have led to the development of non-dairy probiotic products. A plethora of possibilities for probiotic product development

is offered by plant materials like legumes, cereals, vegetables, fruits, and their combinations [24]. There is an increasing demand for the use of natural antimicrobial substances to safeguard foods and to replace synthetic additives in foods. Different probiotic microorganisms, specifically lactic acid bacteria, can be a promising fulfilment of this demand or consumer request. The metabolites produced by probiotics are hydrogen peroxide, diacetyl compounds, acetone, acetaldehyde, ethanol, reutericyclin, reuterin, carbon dioxide and most importantly natural acids and bacteriocins. The ability to use the metabolite bacteriocin produced by probiotics shows its effectiveness as a biopreservative. This antibacterial methodology could be a potential solution for raising concerns about pathogenic bacteria and antibiotic-resistant strains in plant-based foods. Recombinant probiotics with good antimicrobial characteristics can be developed using probiotic microorganisms [79].

Lactiplantibacillus plantarum is one of the species of LAB that is used as an important element of the fermentation process employed in probiotic fermented food products. *Lb. plantarum* produces exopolysaccharides, lactic acid and bioactive compounds that are antibacterial in nature. These show a potential activity against the foodborne pathogens present inside a human intestine [80]. The antifungal strains of LAB act as high-potential biological substance that produces 'bread' of improved quality, safety, and nutrition. The shelf-life extension can be achieved either by the involvement of probiotics in bread formulations or by their incorporation into active packaging as antimicrobial films [81]. Plant-based yoghurt alternatives are predominantly produced using soy or coconut (low in protein and high in saturated fat).

Lactobacillus xylosus exhibits probiotic, as well as bacteriocinogenic, activities. These species produced bacteriocins that inhibited *S. aureus* and *E. coli*. The antibacterial activity was found to be retained at a wide range of pH and temperature treatments. Reduction in bacterial load was observed due to the presence of bacteriocins. This study reveals the usage of *L. xylosus* strain as potential probiotics and usage of their bacteriocins as bio-preservatives [82]. Hence, *L. xylosus* can be potentially used in plant-based food and beverages as well.

The lactic acid bacteria isolates, derived from a coconut palm nectar which is fermented naturally, were subjected to assessments to determine antibiotic sensitivity, antimicrobial activity, and antioxidant properties. The isolates exhibited good probiotic properties, along with promising antifungal and antibacterial activities. This shows that lactic acid bacteria isolated from coconut palm nectar is favorable to be used as preservatives in functional fermented foods [83]. The synergistic action of lactic acid bacteria which is a probiotic can be used to bio preserve emulsified foods and cosmetic products. Besides adding the biomolecules that possess antimicrobial properties directly, adding living bacteria with a bioprotective nature is a promising approach to produce safer food products [84].

Lactobacillus brevis SG1, a potent bacteriocin-producing probiotic strain, isolated from a traditional Nigerian fermented cereal showed promising antifungal effects against *Candida albicans* and *Penicillium citrinum*. Brevicin SG1 is the responsible bacteriocin isolated from this strain and reported to exert its antagonistic effects through fungiolytic activity. Moreover, the bacteriocin is stable at a broad pH range, an attribute which makes it a suitable candidate both for biopreservation and probiotic usage [85].

Various probiotics used in biopreservation of plant-based food products are given in Table 2.

Table 2. Examples of probiotics used in biopreservation of vegetables, fruits, and other miscellaneous plant-based food items.

Type	Food Product	Probiotic/s Used in Biopreservation	Mode of Biopreservation	Reference
Cereal-based	Fermented Oat flour	<i>Lb. plantarum</i>	Fermentation—low pH	[86]
	Probiotic roasted chickpeas	<i>Lb. plantarum</i> 299v	Exclusion of pathogenic microorganisms	[87]
	Probiotic soymilk	<i>B. breve</i> strain Yakult	Fermentation—low pH	[88]
Vegetable-based	Probiotic blanched cabbage	<i>Lb. paracasei</i> LMG P22043	Exclusion of pathogenic microorganisms	[89]
	Probiotic cabbage juice	<i>Lb. plantarum</i> <i>Lb. rhamnosus</i> <i>Lb. brevis</i>	Fermentation—low pH	[90]
	Probiotic beetroot juice	<i>Lb. casei</i> 431 <i>Lb. acidophilus</i>	Fermentation—low pH	[91]
	Probiotic tomato juice	<i>Lb. casei</i> <i>Lb. plantarum</i> <i>Lb. delbrueckii</i>	Fermentation—low pH	[92]
Fruit based	Probiotic cut apple	<i>Lb. plantarum</i> 299v	Increased antioxidant activity—delayed oxidation	[93]
	Probiotic enriched apple snacks	<i>Lb. plantarum</i> SICC	Production of anti-microbial bioactive compounds	[94]
	Probiotic apple juice	<i>Lb. paracasei</i> ssp. <i>paracasei</i>	Fermentation—low pH	[95]
	Probiotic pineapple juice	<i>Lb. casei</i> NRRL B-442	Fermentation—low pH	[96]
	probiotic orange juice powder	<i>Lb. plantarum</i> 299v <i>P. acidilactici</i> HA-6111-2	Production of anti-microbial bioactive compounds	[97]
	Probiotic orange juice	<i>P. acidilactici</i> CE51	Fermentation—low pH	[50]
	Probiotic cantaloupe juice	<i>Lb. casei</i> NRRL B-442	Fermentation—low pH	[98]
	Probiotic pomegranate juice	<i>Lb. plantarum</i> <i>Lb. delbrueckii</i>	Fermentation—low pH	[99]
	Cut Honeydew melon	<i>Lb. casei</i> NCIMB 4114	Increased antioxidant activity-delayed oxidation	[100]
	Table olives	<i>Lb. rhamnosus</i> <i>Lb. paracasei</i> <i>B. bifidum</i> <i>B. longum</i>	Bacterial cell adhesion to the fruit surface	[101]

6. Future Insights

Probiotics have a promising role in reducing human susceptibility to pathogens. The interpretation of the data available becomes difficult due to the use of different probiotic strains, the treatment duration, the dosage, and a small trial size. All probiotics do not employ the same mechanism and they show different responses to different strains of microorganisms. Hence, probiotics are ‘strain specific’. This makes it necessary to determine the optimal species, doses and formulations while using probiotics in plant-based food systems [102].

The combination of more than one approach, also known as hurdle technology, can be used to obtain desired results with better efficiency than other methods [103,104]. The limitations or disadvantages associated with one technique can be removed or rectified by combining it with other methods to achieve safety and efficacy. Biopreservation can be combined with other treatment methods to suit the challenges of food safety. To exemplify this, a study on fresh-cut conference pear has been considered. The combination of calcium treatment in the post-harvest phase, immersion in an antioxidant solution and

biopreservation with probiotics were used to enhance the safety and overall quality of fresh-cut pears. The probiotic microorganism *Lacticaseibacillus rhamnosus* GG was used to evaluate the antioxidant activity and total phenolic content in fresh-cut fruits [105].

The plant-based food products supplemented with probiotic microorganisms are carefully formulated to deliver the desired benefits to consumer health while effectively utilizing its biopreservative properties to enhance the shelf life of food products. Novel trends, such as replacing animal-based milk with probiotic carrier plant-based milk, exemplify the effective utilization of probiotics in non-dairy foods [106]. The modern food industry focuses on making the preservation techniques safer and more effective. The environmental concerns, toxicity and side effects caused by long-term usage of chemical preservatives makes it necessary to employ probiotic microorganisms in the field of food preservation. Biopreservation is known to be the future of food preservation. As a technique, it shows better preservative effects than other methods, along with delivering health benefits to humans, fostering sustainability, and positively impacting the environment [107]. However, minimizing microbial contamination during processing, storage and distribution is the key factor in ensuring safety of both plant-based and animal-derived food and beverages [108].

7. Conclusions

Today's consumers are looking for food products that are minimally processed, more natural and safer for consumption. Probiotics have a wide range of applications in the dairy industry. As such, exploring their applications in the non-dairy plant-based food sector is necessary due to the increasing demand for plant based-food products. Further, novel probiotic applications in plant-based food systems are becoming highly popular. In the era of pathogens showing antibiotic resistance, the method of 'biopreservation' is of high importance. The application of probiotics seems to be a promising biopreservative method to increase the shelf life and final quality of plant-based foods and beverages. The study of probiotic characteristics and probiotic delivery helps to explore possible applications of probiotics in the field of food safety—an area that has not been explored much. Further studies should be focused on replacing chemical preservation methodologies with biopreservation for safety and environmental concerns. Further, probiotic treatment in this regard is not just an alternative to chemical treatment, but also a health-promoting technique.

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