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Biopreservation of Food using Probiotics: Approaches and Challenges

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Abstract

Food preservation has received a paramount focus throughout history, prompting the use of various methods such as chemical additives, thermal treatments, and nonthermal approaches to prolong the shelf life of food. In this regard, biopreservation is emerging as a promising alternative owing to its eco-friendly nature and minimal toxicity effects. It involves harnessing natural microorganisms and their byproducts to enhance both the nutritional value and longevity of food products. This review delves into the role of probiotics and postbiotics in biopreservation, elucidating their beneficial impact on human health and their potential as 'safe' food preservatives. It covers a spectrum of pro/post-biotic organisms, including bacteria and yeast, alongside different types of biopreservatives, their mechanisms of action, and applications across diverse food categories. Furthermore, the review assesses the influence of biopreservation on food quality and sensory attributes. However, commercialization hurdles loom, particularly concerning safety and regulatory compliance, necessitating thorough scrutiny before widespread implementation.

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Introduction

Food is the basic need of every human being, and efficient techniques are essential to minimize food spoilage. Food spoilage refers to the deterioration of a food item's nutritional value, texture, and taste to the extent that it becomes unsafe for consumption. In ancient times, food preservation was not a concern as there typically was no or minimal leftover food after daily meals. However, the necessity for food storage and preservation ascended at an indeterminate point when there was a surplus of food available. During periods of scarcity, storing food became imperative for survival, enabling individuals to endure when fresh food was scarce. Food

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preservation has undergone significant evolution since ancient times, adapting to the needs and advancements of each era up to the present century. Fig. 1a summarizes the preservation techniques evolvement in food processing.

There has been a notable increase in the utilization of synthetic chemical preservatives in food. However, substantial scientific research has emerged linking the consumption of these food additives to various health concerns. Based on the age and a range of other factors, hyperactivity and other neurophysiological challenges may result due to ingesting chemical preservatives. Furthermore certain preservatives have been identified as carcinogenic, while others are associated with respiratory issues, heart damage, and other adverse.[1] In recent decades, increases in the usage of advanced food processing and manufacturing methods such as advanced thermal processing,[2] nonthermal processing,[3] and food manufacturing methods positively influence the shelf life of the food as well as food market growth globally.[4] Introducing novel foods, new manufacturing processes, and the growing demand for minimally processed, fresh-cut and ready-to-eat products may require a longer and more complex food chain, increasing the risk of microbiological contamination.[5] Hence, there is an ongoing quest for innovative and complementary food preservation technologies that meet the evolving demands of the entire food supply chain, from “farm to fork.”

According to the Food and Agriculture Organization-World Health Organization, probiotics are "living microbes" that benefit the host when supplied in suitable proportions. Several studies have demonstrated that various microorganisms such as lactic acid bacteria (LAB), *Bifidobacteria including Bifidobacterium infantis, B. longum, B. lactis, Escherichia coli, and yeast species such as Saccharomyces cerevisiae, S. boulardii, and S. lactis*, among others, possess probiotic properties and can be utilized for various beneficial purposes.[6] Postbiotics are non-viable bacterial products or metabolic byproducts that probiotic bacteria produce during fermentation (Fig. 1b). These include short-chain fatty acids, organic acids, enzymes, peptides, and vitamins, among others. Postbiotics can have a positive impact on health by affecting the gut microbiota, immune system, and overall host biology. They are increasingly being recognized for their potential therapeutic applications in gut health, immune response modulation, and disease prevention or treatment.[7] These postbiotic mechanisms (Fig. 1c) have the capability to enhance the effectiveness of their molecules in the human gut without necessitating the destruction of their own cellular structure. Thus, it can result in heightened affinity for immune receptors or prolonged retention of active compounds within the host. The cell wall serves as a protective barrier, shielding against swift degradation by digestive enzymes and immune reactions within the host. Conversely, in the case of vaccines, preserving cellular integrity is paramount, often involving the removal or inactivation of the most harmful or pathogenic elements.[7] Fig. 1d illustrates the publication trends from the year 2004 to 2024 in the Scopus database by giving the keywords “Biopreservation” AND “Probiotics” OR “Postbiotics” AND “Food” (excluding review articles, books, book chapters, and short surveys). This review comprehensively evaluates the current biopreservation methods employing probiotics for food biopreservation, elucidating their applications, safety regulations, and future prospects within this field.

Biopreservation for Food

Biopreservation, also referred to as bioconservation, involves preserving food through the use of protective cultures of microorganisms.[9] By preventing the growth of harmful bacteria it involves employing harmless microbes or their metabolites to increase the safety and shelf life of food.[10] A popular biopreservation technique is fermentation, in which complex food molecules are broken down by naturally occurring microorganisms or by their addition, resulting in the production of alcohols, organic acids, and other substances. This process enhances the flavor and aroma of food, improving its overall quality. However, incorporating probiotics into food products can be challenging due to the development of off-flavors by certain strains, which may affect consumer acceptance. Therefore, it's important to choose strains that do not significantly alter the original flavor, especially in products with higher fat content. The selection of strains for biopreservation should consider the characteristics of the raw material or product to minimize flavor alterations.[11]

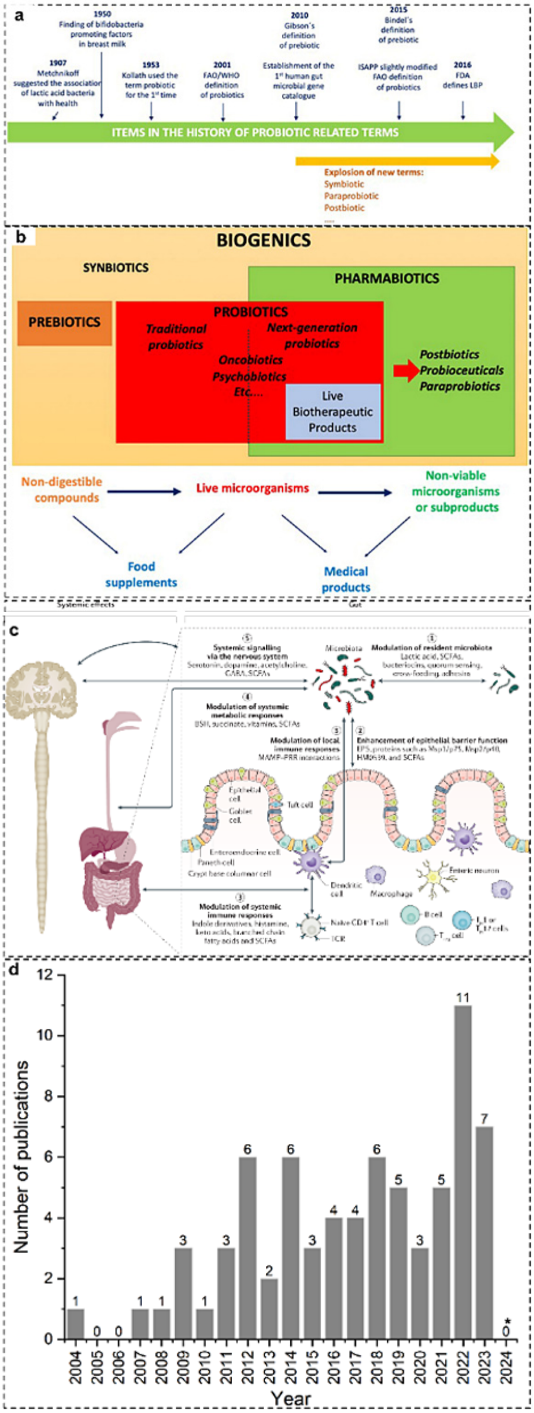

Fig. 1. a) Timeline of probiotic-related terms (Source[8]); b) Scheme of the main terms related to the probiotic field (Source[8]); c) Postbiotics mechanism (Source[7]) (SCFAs: Short Chain Fatty Acids, EPS: Exopolysaccharide, PRR: Pattern Recognition Receptor, TCR: T-Cell Receptor, BSH: Bile salt hydrolase, T_{reg} cell: Regulatory T cell, MAMP: Microbe Associated Molecular Pattern, TH: T helper cell); d) Publication trends from 2004-2024; scientific articles on biopreservation of foods (*accessed on 07.02.2024 through SCOPUS)

Popular biopreservatives widely utilized in industrial settings include lysozymes, bacteriophages, LAB, and their bacteriocins. Lysozymes, which are natural enzymes found in bodily secretions, are valued for their ability to disrupt biofilms. Bacteriophages, viruses that infect bacteria, possess strong antibacterial properties, making them effective biopreservatives. Bacteriocins are complex proteins or peptides with antimicrobial activity against closely related bacterial species. LABs are particularly noteworthy due to their dynamic characteristics and capacity to produce bacteriocins. Certain probiotic strains within LAB can generate bacteriocins that combat pathogenic and food spoilage microorganisms.[12] The bacteriocin-producing capability, combined with other antagonistic and antimicrobial properties of probiotics, positions them as promising natural biopreservatives for food. Recent advancements in food safety have highlighted the benefits of utilizing bacteriophages and endolysins as food biopreservatives. There is ongoing exploration into various applications of probiotic microorganisms and bacteriocins with potential antimicrobial activity to enhance food preservation.

Importance of Biopreservation

The abundant supply of food in developed countries, combined with changes in socioeconomic, demographic patterns, consumers' concepts of nutrition, preferences for various types of foods, food selection patterns and technological innovations, and competition among food processors, has created several unique problems in the area of food preservation.[13] Consumers express concerns about the safety and wholesomeness of foods containing a variety of nonfood additives, such as preservatives. In comparison to previous years, the number and variety of chemicals used has increased exponentially. Consumers' faith in the safety and wholesomeness of the foods they eat has been shaken by reports of possible health hazards from the consumption of some preservatives and other additives currently in use, such as nitrite and saccharine, as well as additives previously used but no longer permitted.[14] The use of appropriate food preservatives to assure the safety of these goods is a source of consternation for regulatory organizations.

In this sense, antimicrobial metabolites or biopreservatives produced by food-grade starting culture bacteria are in a unique position, particularly in terms of their ability to be used right away. Their safety has been established over time; they have been and continue to be used by customers in some manner, most notably by health-conscious consumers.[13] Regulatory agencies and consumers are more aware of the food safety issue, and there is a greater understanding of the food industry's obligation to ensure the safety of its goods. Also, a rise in the number of vulnerable persons in the population, particularly in industrialized nations, necessitates stricter hygiene standards. The need for greater food quality stability in order to avoid food loss due to spoiling and consumer need for more convenience, such as less frequent shopping, but yet demanding "fresh" and "natural" food free of chemical additives results in increased demand for biopreservation.[15]

Biopreservation Versus other Preservation Methods

Biopreservation methods offer several advantages over traditional preservation methods, such as thermal, chemical, and nonthermal techniques. This methods typically utilize natural microorganisms, bacteriocins, or enzymes that are considered safe for consumption. In contrast, chemical preservatives may raise concerns about potential health risks or side effects. This aspect is particularly important for food products, where consumers prioritize natural and minimally processed ingredients.[15] Unlike thermal preservation methods like pasteurization or sterilization, which can degrade the nutritional content of food by destroying heat-sensitive vitamins and enzymes, biopreservation techniques generally have minimal impact on the nutritional quality of preserved products and that keeps the food's flavor, texture, and nutritional value intact.[16]

Biopreservation methods are often more environmentally sustainable compared to chemical preservatives, which may have adverse effects on ecosystems and human health. These techniques utilize natural processes and renewable resources, reducing the environmental footprint associated with food preservation.[17] It can successfully prevent the growth of infections and spoiling bacteria, extending the shelf life of perishable goods. This helps to reduce food waste and distribution costs, contributing to economic and environmental sustainability. Biopreservation can be applied across various industries, including food, pharmaceuticals, and cosmetics, to preserve a wide range of products.[18] This versatility makes biopreservation an attractive option for manufacturers seeking sustainable preservation solutions that can meet diverse needs. Some biopreservation methods, particularly the use of specific probiotic cultures or bacteriocins, can target specific microorganisms while leaving beneficial microbes intact. This selective inhibition of spoilage organisms can help maintain microbial balance and enhance product safety. Compared to other preservation methods, biopreservation techniques may require less energy input, contributing to energy savings and reducing greenhouse gas emissions. These methods also stand out as indispensable for sustainable food production.[17]

Probiotics as Biopreservatives

Probiotics are an efficient class of microorganisms that can act as protective cultures in foods. Rather than just preserving and extending the shelf life of foods, they also add to the nutritional profile of the foods by maintaining good gut health. Broadly speaking about probiotic preservation, a natural technique of enhancing the shelf life of food, there are two methods in which probiotics are applied. Biopreservation can be through the probiotic strains of organisms in the food or via metabolic products such as bacteriocins, postbiotics released or externally added to the food. At the same time, this biological preservation can be either by externally added probiotic strains or by inherent microbiota in the food. Such foods can be broadly referred to as functional foods in the market due to their higher functionality in maintaining good human health.[19] This section will cover the postbiotics and their mode of action.

Postbiotic Metabolites

Postbiotic metabolites play a significant role in shaping the gut environment and influencing host health. They can interact with the gut epithelial cells, immune cells, and other microbes in the gut, contributing to various physiological processes. For example, SCFA, such as acetate, propionate, and butyrate, have been extensively studied for their roles in maintaining gut barrier integrity, modulating immune responses, and regulating energy metabolism. The composition and concentration of postbiotic metabolites can vary depending on factors such as the type of probiotic bacteria present in the gut, dietary habits, and overall gut health. Research on postbiotic metabolites is ongoing, with increasing interest in their potential therapeutic applications for promoting gut health, modulating immune function, and preventing or treating various diseases.[7]

Antimicrobial Proteins/ Peptides

The probiotics bacteria and yeast can synthesize antimicrobial proteins or peptides exhibiting antimicrobial activity in the foods. From the LAB, the maximum amount of bioactive peptides production, bacteriocins takes place. These bacteriocins are majorly attracted in microbiology, owing to the inhibitory action against gram-positive and gram-negative bacteria in foods. Numerous strains of LAB from various genera and species, such as *Lactococcus lactis, Streptococcus thermophilus, L. acidophilus, L. plantarum, L. sake, L. curvatus, Leuconostoc mesenteroides, L. carnosum, L. gelidum, Pediococcus acidilactici, P. pentosaceus, P. parvulus, Enterococcus faecalis, E. faecium, and B. bifidum*, have been identified as producers of bacteriocins.[20] Recently, in a study on the antimicrobial protein extracted from the *Pediococcus* spp in dairy samples treated with fresh strawberries, tomatoes, fish, and button mushrooms, researchers observed activity for 15 days against *E. Coli* and *Shigella spp.*. Results were compared with FDA-approved chemical preservatives such as sodium benzoate and sulfite. Interestingly, biopreservative exhibited better results than other preservatives.[1]

Dhanda *et al*. (2023) reported the *P. acidilactici* NCDC 252 peptides for dairy products, fruit beverages and wine.[21] Similarly, studies have reported that yeast produces antimicrobial proteins for this biopreservative application, known as killer toxins or zymocins. Researchers investigated phenolic metabolites' antibacterial and antioxidant capabilities *in vitro* against pure flavonoid naringenin and its prenylated derivatives (strain of *S. cerevisiae,* a Generally Recognized as Safe (GRAS) organism). The study aimed to assess their potential as organic food preservatives. *Pichia anomala* is a yeast found in various sources, such as dairy products, plants, fruits, and human digestive tracts. Its production of ethanol, carbon dioxide, and ethyl acetate is thought to be the cause of its antagonistic effects against many rotting organisms of plant products used for food or feed.[22] The bacterial and yeast-based antimicrobial proteins/peptides on foods are exclusively documented by Teneva & Denev.[20]

Enzymes

One of the emerging categories of food biopreservatives, called antibacterial enzymes (known as "phage lysins" or "enzybiotics = enzymes + antibitics"), can directly destroy the membrane of the bacteria.[23] The enzymes were used for the catalytic activity to enhance the antimicrobial activity with LAB or probiotic bacteria. For instance, catalytic enzymes (catalase, proteinase K, lipase) were used in a biopreservation study on probiotic bacteria to enhance antifungal activity.[24] However, the ongoing research for new enzymes and probiotics is a novel approach that holds promise for finding thermostable and easily producible forms in heterologous hosts, or it may possess synergistic effects. This approach involves exploring diverse environments for enzymes with desirable characteristics, including heat resistance and compatibility with industrial-scale production processes. By studying enzymes from extremophiles or employing directed evolution techniques, researchers can identify variants with enhanced stability under high temperatures. Additionally, utilizing heterologous hosts, *E. Coli* or *L. lactis* for enzyme production offers scalability and simplifies production.[23] Similarly, *E. lactis* probiotic assessment study produced a strain that exhibited potential enzymatic activities.[25] Overall, the quest for novel enzymes, coupled with probiotics, is an advancement of biotechnological techniques and holds significant potential for overcoming current limitations in producing and applying enzybiotics in the food industry.

Organic Aids

LAB is a group of gram-positive bacteria that can convert carbohydrate substrate into organic acids such as acetic, propionic, lactic, succinite and formic acid. Other than creating unfavorable conditions for the microorganisms, it enhances the food taste, appeal and consumer acceptance.[26] The diverse functional properties of organic acids make them valuable across a range of industries, including food, chemical, cosmetic, pharmaceutical, and beverage sectors. In the biopreservation of tomatoes, the LAB strains isolated from the tomato and sourdough execute the antifungal action against *P. expansum* and *Aspergillus flavus*. Regarding biopreservation techniques, lactic, phenyl lactic, pyrazin and acetic acid derivatives can be related to the antifungal action.[27] In recent documentations of biopreservation in foods from probiotics, the authors Abouloifa *et al*. and Nasrollahzadeh *et al*. documented the organic acids and their mechanism on antifungal action.[28,29]

Amino acids

Bacteriocins, typically produced by LAB, are antimicrobial peptides with 30-60 amino acids and an amphiphilic helical structure.[30] All the peptides or protein-based biopreservatives consist of amino acids, but very few studies reported this amino acids-based composition on antimicrobial activities. The probiotic bacteriocin (*Lacticaseibacillus rhamnosus*) extracted from the mixed fruit juices reported hydrophobic amino acid composition (I–K–K–V-T-I). This novel bacteriocin is used for antibacterial and antibiofilm activity.[31] Similarly, for *B. licheniformis* MCC 2514 strain, the genome sequencing study reported that essential amino acids synthesis acts as a biopreservative agent.[32] Due to amino oxidase activity, LAB can decarboxylate amino acids, which significantly reduces the biogenic amine accumulation during fermentation.[33] Nevertheless, there is a shortage of documentation concerning the amino acids component in probiotics for the assessment of biopreservation.

Short-Chain Fatty Acids (SCFAs)

The generation of SCFAs by probiotics results in a reduction in the pH of fermented dairy products, creating an inhospitable environment for specific pathogenic microorganisms.[11] The major four SCFAs, 3-hydroxy decanoic acid, 3-hydroxy-5-dodecenoic acid, 3-hydroxydodecanoic acid, and 3-hydroxytetradecanoic acid, from *Lactiplantibacillus plantarum* MiLAB 14 reported inhibitory activity against fungal or mold. These fatty acids' detergent-like properties alter the cellular membrane of target microbes.[34] Besides the SCFAs, hydroxy acids such as unsaturated monohydroxy fatty acids demonstrated antifungal activity, whereas saturated hydroxy fatty acids and unsaturated fatty acids derived from oleic and stearic acids showed no such effects. The authors reported that monohydroxy fatty acid acts as an antifungal agent due to the effect of double bond and a hydroxyl group along a C18 aliphatic chain in its structure. Nasrollahzadeh *et al*.[29] reviewed antifungal action through probiotics or probiotic strain-produced fatty acids.

Other Metabolites from Probiotics

The exopolysaccharides (EPS) consist of glucose and galactose units, linked by β 1-4/6 bonds. These EPS sources from LAB exhibit antimicrobial activity.[35] Additionally, this acts as a preservative material through coating on tomatoes.[36] Similarly, more studies on this EPS are related to food and biopreservative application.[37,38]

Probiotics are utilized in the industry with a view of biological preservation works by the mechanism of becoming a protective culture. It permeabilizes the membranes of spoilage and pathogenic bacteria, leading to their ultimate destruction. In addition to the metabolites mentioned above, numerous other metabolites have been extensively documented in numerous studies. These include hydrogen peroxide, diacetyl compounds, acetone, acetaldehyde, ethanol, reutericyclin, reuterin, and carbon dioxide etc.[11] In particular, Nasrollahzadeh *et al*.[29] exclusively reviewed reports on antifungal activity and Agripoulou *et al*.[39] documented antibacterial of from the probiotics/probiotic strains.

Biopreservation: Mode of Action

Fermentation serves as a significant application of biopreservation in food processing. During fermentation, the indigenous microflora initiates the process by producing organic acids, subsequently lowering the pH of the food matrix. This increased acidity impedes the growth and proliferation of pathogenic and spoilage microorganisms within the food. LAB plays a pivotal role in fermentative processes across various food products. Remarkably, LAB primarily generates lactic and acetic acid as metabolic byproducts. These acids, upon release into the food matrix, establish an acidic environment, thus extending the shelf life of the food product without requiring the addition of external chemical preservatives.[40]

Another approach to biopreservation involves the use of bacteriocins or other metabolites produced by specific microorganism strains. Examples include nisin and pediocin, which are derived from bacterial metabolism. These compounds, primarily originating from gram-positive organisms, are cationic peptides with hydrophobic characteristics and exceptional heat stability. They function by disrupting the membranes of target microflora, thereby preventing the growth of pathogenic and spoilage microorganisms and extending the shelf life of food products.[41] Certain enzymes with inherent antimicrobial properties can be incorporated into foods during or after preparation, constituting a form of biopreservation. Examples of such enzymes utilized in industry include lysozyme and lactoperoxidase. These inhibitory enzymes may naturally occur within the food or be externally supplemented from authentic sources. Additionally, natural antimicrobial compounds, including specific peptides and organic compounds, can be introduced to biologically preserve food. These methods also align with the principles of biopreservation.[24] Fig. 2 illustrates the biopreservative mechanism on microorganisms for meat-based products. However, the mode of action of biopreservatives will vary based on food commodities; different modes of action have been reported. For instance, S Agriopoulou *et al*. for vegetables and fruits,[39] and Kavesh *et al*. for meat.[30]

Biopreservation emerges as a potent method leveraging natural resources, including inherent protective cultures or their added metabolic products, to extend the shelf life of foods without compromising their sensory properties or nutritional value. Rather than diminishing, the sensory attributes remain unaltered, while the pathogenic microbial load is significantly reduced, ensuring safer consumption. Moreover, some biopreservation techniques have been observed to enhance the nutritional profile of preserved foods, further underscoring their efficacy in food preservation.

Application in Foods

Probiotics and postbiotics, widely used as supplements for their multitude of benefits, are increasingly preferred by consumers when incorporated into functional foods rather than taken as pharmacological supplements. As such, they have been added to various food matrices. Through fermentation, probiotic strains like Bifidobacteria contribute to the preservation of fermented foods, enhancing both their appeal and nutritional value.[43]

Poultry, Meat and Fish

Certain strains of meat-born LAB that are salt tolerant, homo fermentative and psychrotrophic are found effective as biopreservatives in cooked meat products.[44] The same is the case with seafood, where LAB or their bacteriocins were found effective as biopreservative agents against *L. monocytogenes*.[45] Extensive studies have been carried out in recent years to increase the shelf life of seafood products using probiotic biopreservative methods. A bioactive film has been reconstituted from green tea extracts, agar and probiotic strains of *L. paracasei* L26 and *B. lactis* B94 on hake fillets and tested for shelf life. This film has been found to have multiple effects on the fillets, pertaining to increasing shelf life by nearly a week as well as decreasing the volatile spoilage indices. Also, a notable increase was found in the beneficial LAB load in the end product.[46] From a total of 132 LAB isolated from mussels, biochemical assays and 16S rRNA gene phylogenetic studies revealed 22 LAB isolates whose cell-free supernatant showed activity against *L. innocua* and *L. plantarum* as *E. mundtii.* There were no virulence factors in any of the strains that were chosen, and the strain *E. mundtii* STw[38] was used as a protective culture on fish paste that had been kept at 4 °C. Air-packed and later vacuum-packed fish paste systems were used in the first step. *E. mundtii* STw38 survived both storage conditions and was successful in limiting the growth of local vegetation in fish paste. According to these study findings, STw38 is a promising strain for fish biopreservation.[47]

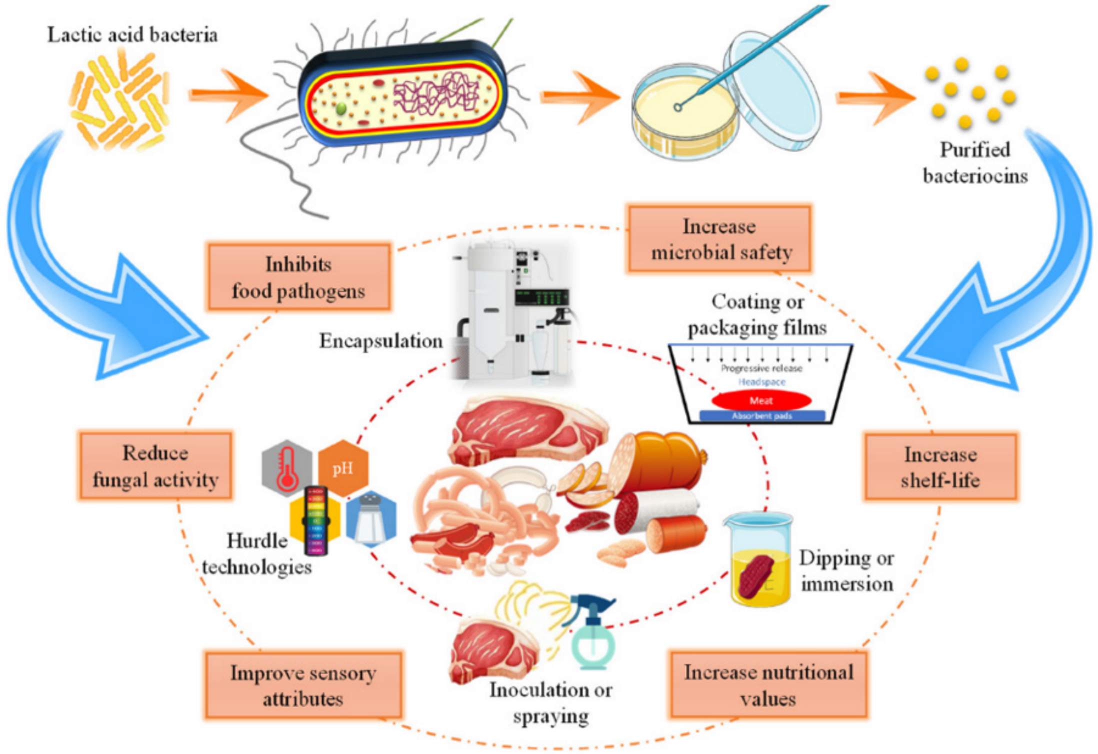

Fig. 2. Biopreservative effect on meat and meat-based product (Source[42])

The meat industry also had many applications using biopreservatives to increase the meat quality and shelf life during the storage period. The combination of *L. plantarum* and garlic extract was shown to be effective in reducing *L. monocytogenes* growth and extending the shelf life of ground beef. The lipid oxidation was considerably reduced, and the sensory rating was also higher after using this combination in ground beef.[48] For instance, Fig. 3 shows the treated and untreated pork meat differences. *Lactococcin* BZ has been particularly efficient in lowering the counts of psychrotrophic and mesophilic aerobic bacteria, LAB, total coliform and fecal coliform bacteria at concentrations of 1600 - 2500 AU/mL. *Lactococcin* BZ reduced the development of *L. innocua* in meat samples and demonstrated substantial antibacterial action. This indicates that there is great potential for using *Lactococcin* BZ as a biopreservative to extend the shelf life of fresh beef.[49] Plantaricin BM-1-activated polyvinylidene chloride (PVDC) film has a lot of promise for meat preservation. It has great potential to reduce *L. monocytogenes* development in pork meat and extend the shelf life stored at 4 °C.[50] *L. innocua* was artificially inoculated in raw goat emulsion, and a combination of pediocin from *P. pentosaceus and Murraya koenigii* berries was applied. A significant decrease was seen in the *L. innocua* during storage, which shows the biopreservative property of the combination used.[51] Adding chitosan alone or in combination with *C. Maltaromaticum* UAL 307 as a protective culture to beef decreases *E. Coli* and *Salmonella* cell counts considerably.[52]

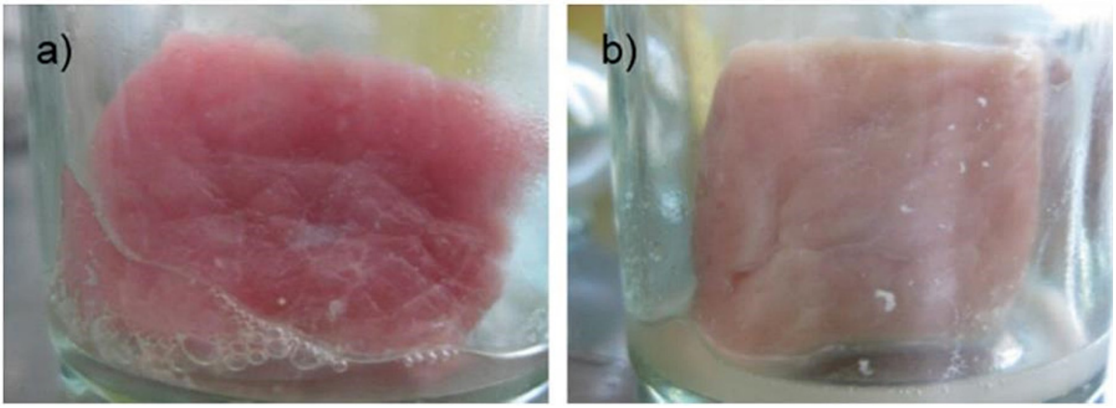

Fig. 3. Bacteriocin on pork meat preservation (a) Control (without biopreservative); (b) Sample after 24 h (with biopreservative) (Source[54])

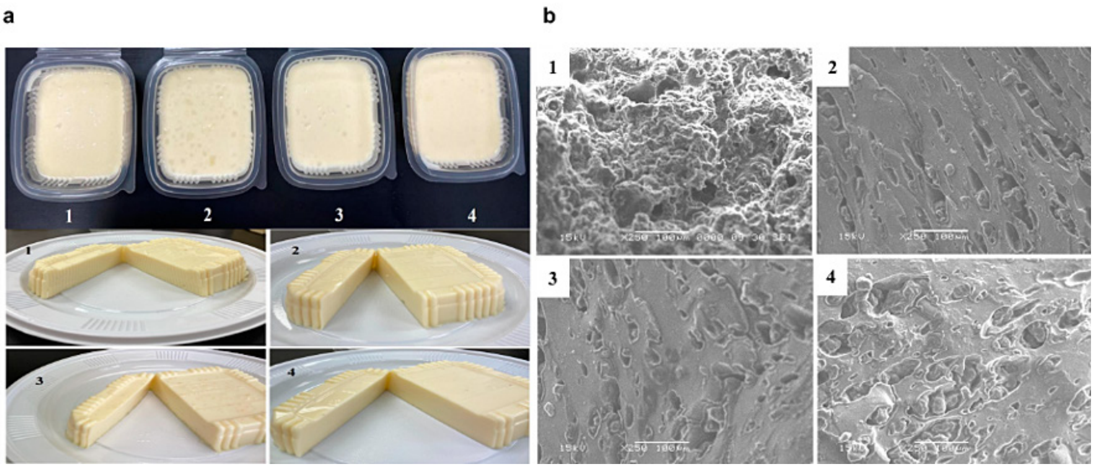

Fig. 4. Probiotics preserved white cheese a) Morphology of manufactured cheese using several strategies; b) Cross-section of cheese samples (SEM images) (1. Control cheese – High porous: less hardness, 2. Probiotic MG847589 cheese-less porous than 1, 3. Cheese with bacteriocin – smooth surface influencing high adhesiveness, 4. Cheese with probiotics and their bacteriocin – high hardness and high consumer acceptance)(Source[59])

There are studies that show the biopreservative nature of probiotics in the poultry industry, too. The shelf life of frozen raw ground turkey meat was tested using the semi-purified bacteriocin BacFL31 at 200 and 400 AU/g. Treatments with BacFL31 prevented the growth of *L. monocytogenes* and effectively stopped other spoilage bacteria, including *Salmonella typhimurium*. The addition of BacFL31 increased the turkey meat samples' shelf life and enhanced their sensory characteristics while they were refrigerated.[53] However The recent documentation from Kaveh *et al*. (2023) reviewed biopreservative probiotics for meat and meat-based products.[30]

Milk and Dairy Products

LAB has been utilized in milk to aid fermentation, which increases acidity, leading to extended shelf life. Moreover, milk utilized for cheese production treated with LAB increased EPS in cheese, leading to improved rheological properties and carbonyl compounds and acids produced, and also enhancingsensory qualities. Bacteriocins produced by LAB also provide a preservative effect.[55] Biopreservative agents help prolong the hygienic safety of dairy foods. *Lactococcin* BZ, enterocin KP, and their combination in full fat, half fat, and skim milk inhibited *L. monocytogenes*, through their bactericidal and antibacterial activity.[56] In cheese, *L. plantarum* LpU4, as well as its purified plantaricin LpU4 could be used as biopreservatives due to its bacteriostatic activity, and it was more active at acidic condition.[57] *L. pentosus* 22B was used as a biopreservative in yogurt to prevent fungal deterioration. The antifungal compounds produced by *L. pentosus* 22B, including organic acids, peptidic compounds, fatty acids, volatile compounds, and hydrogen peroxide, were identified using the fungal response to concentrated extract after enzymatic treatments, GC-MS, and headspace GC-MS.[58] Fig. 4 illustrates the cross-section of the fortified white cheese using probiotics.

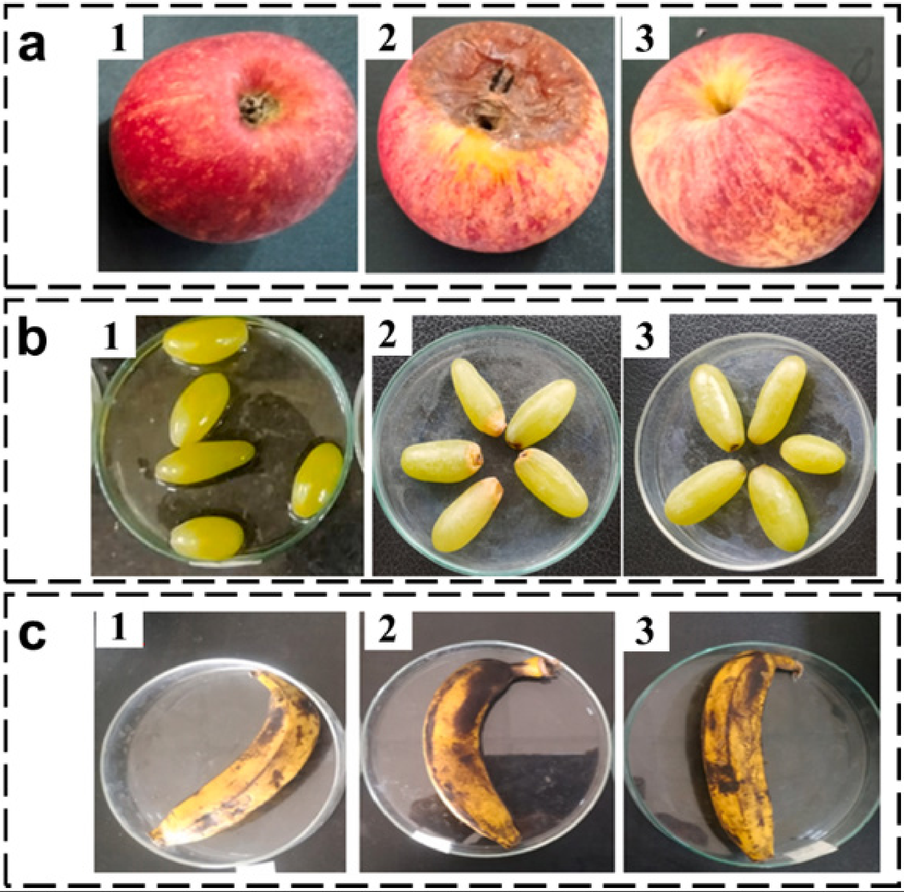

Fig. 5. Extending the shelf life of fruits with probiotics (*L. plantarum* DMR14). a) Apple (1) - 0 h, (2 & 3) - after 7 days without and with treatment; b) Grape (1) - 0 h, (2 & 3) - after 5 days without treatment and with; c) Banana (1) - 0 h, (2 & 3) - after 3 days without treatment and with (Source[67])

Fruits and Vegetables

This preservation method has been demonstrated to have increased the shelf life of vegetables by inhibiting pathogenic and spoilage microorganisms. A control of microbial flora that is undesirable is achieved and instead, beneficial microflora is found to have increased. The only drawback was that this novel technology was found to be efficient only in a narrow pH range. Also, *P. pentosaceus* produces pediocin, which is effective as a biopreservative against *L. monocytogenes* in fresh vegetables.[60] On fresh-cut pear, the antagonistic potential of the probiotic strain *L. rhamnosus* GG against a cocktail of 5 serovars of *Salmonella* and 5 serovars of *L. monocytogenes* was tested under commercially relevant circumstances. Probiotics suppressed the population of *L. monocytogenes* on fresh-cut pear, suggesting that this was a more suitable bacteria for this pear fruit since the quality of the fruit remained unaffected.[61] Fig. 5 illustrates the treated fruits with probiotics. *L. plantarum* B2 and *L. fermentum* PBCC11.5 were used to inoculate fresh-cut cantaloupe. Furthermore, it has been shown to have a high capacity to decrease *L. monocytogenes* levels.[62] Phage formulations and bacteriocin-producing strains have shown excellent effects against *L. monocytogenes* and seem to be a potential decontamination agent for leafy greens to reduce the development of spoilage-causing organisms.[63] Similarly, for the broccoli juice biopreservation study, *L. plantarum* ATCC 8014 strain was reported.[64] *Salmonella* and *L. monocytogenes* were intentionally injected at a concentration that would seldom occur on fresh-cut melon held at 5 and 10 °, and the biopreservative culture *P. graminis* CPA7 inhibited their growth.[65] Recent documentation from Sri Sneha and Dharumadurai reported the list of bio preservatives for fruits and vegetables.[66]

Allied Applications

Biopreservation methods have been used in cereal-based products to enhance their sensory qualities and shelf life. The combination of quinoa flour fermented with the antifungal *L. amylovorus* DSM19280 serves as a great potential biopreservative ingredient for producing gluten-free bread with improved nutritional value, bread quality, and safety due to extended shelf life, thus meeting consumer demands for high-quality, preservative-free foods.[68] Antifungal abilities of *Lactobacillus spp.* were used to prolong the shelf life of wheat bread, compared to the non-acidified bread, shelf life extension of six additional mold-free days was achieved.[69] *L. plantarum* O21 obtained from the plant origin was used to make cereal-based fermented vegan products and successfully stored in refrigerated condition for 21 days.[70] Recently, cereal-based probiotic biopreservatives were documented by Adesulu-Dahunsi *et al*.[71] Fig. 6 shows that biopreservatives act as an antifungal agent on wheat grains.

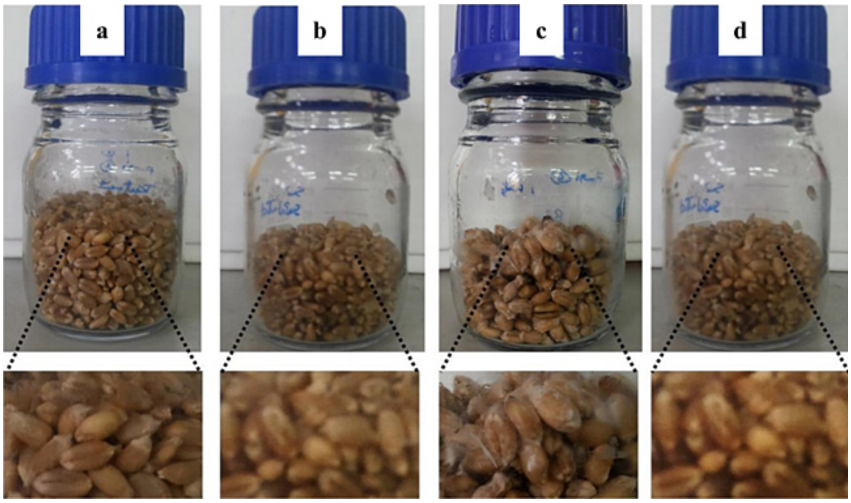

Fig. 6. Biopreservative on wheat grains (antifungal activity - Lactobacillus spp. RM1 supernatant against *A. parasiticus* after 15 days): a) Untreated control; b) Positive control treated with Lactobacillus spp. RM1 supernatant; c) Treated with *A. parasiticus* spores; d) Treated with *A. parasiticus* spores and Lactobacillus spp. RM1 supernatant (Source[72])

Apart from their conventional usage in traditional foods, research has demonstrated the broad range of applications for biopreservative probiotics in plant-based beverages, meats, and various other products. Udayakumar *et al.* (2022) extensively reviewed biopreservative probiotics and their respective strains, shedding light on their diverse applications across different food categories.[11] Similarly, bacteriocins and their particular antimicrobial application in different food industries were documented by Verma et al.[41] Overall, Table 1 provides the last few years' documents on probiotics in biopreservatives for different food industries applications.

Table 1. Probiotics - Biopreservation application in food industries

Probiotics/ Probiotics strains	Name of the source	Mode of activity	Field/domain/ Category of application	Target organism	Key findings	Refere -nces
L. plantarum MYS44	Fermented finger millet wine	Antifungal	Poultry	*A. parasiticus* MTCC 411	• Major antifungal compounds were oleic acid, octanoic acid, butanamide, and decanoic acid derivatives • Poultry feed sample reduced the aflatoxin B1 content by 34.2%	75
Leuconostoc mesenteroides 68 (Le.m.68), *C. divergens* 468 (C d.468)	Sushi, cold -smoked salmon, gravlax	Antimic-robial	Fish – Salmon juice	*L. innocua*	• Exhibits antimicro -bial activity and growth in low-temperature storage conditions (4 °C) • Selected strains were able to grow at 4 °C for 17 d	76
Penaeus monodon	Black tiger shrimp	Antibac-terial	Fish	*Vibrio alginolyticus, Pseudomonas stutzeri*, *Aeromonas hydrophila,*	• Bacteriocins displayed an amide bond at 1652 cm^{-1}, indicating that the substance was a protein • Preventing microbiological deterioration, improving the hygienic quality and prolonging the seafood products' shelf life	77
Bacillus	Fermented	Antimi-	Fish	*Lactobacillus*	• Isolate can able	78

coagulans BDU3	fish (Ngri)	crobial		*spp*. MTCC 10093	to tolerate pH as low as 2.0 and up to 0.2% bile salt concentration • Greater antimic-robial potency with lower cytotoxicity	
L. plantarum DY4-2	Cutlassfish	Antimi-crobial	Fresh turbot (*Scophthalmus maximus*) fillets	*Aeromonas sobria, P. fluorescens, V. parahae -molytic, L. monocytoge -nes, P. aeruginosa,*	• In 12 days storage study (4 °C), there were 2.7 log units of reduced *P. fluorescens* • It had the potential to act as a biopre-servative for aquatic products	79
L. innocua CECT 910, L. innocua CECT 4030 and L. innocua CECT 8848 from Colección Española de Cultivos Tipo (CECT), L. innocua TA-1.17	Addition with lysozymes	Antimi-crobial	Meat – Fuet, cooked ham, Dairy - fresh cheese	*L. innocua*	• 3.4 log cycle reduction after 6 days at 4 °C	80
L.plantarum TN8	Poultry gastroint-estinal tract	Antiba-cterial	Meat – beef	*L. monocyto -genes, Salmonella spp.*	• Better quality inclusive color, lipid oxidative stability, and texture parameters	81
Bubalus bubalis	Raw buffalo milk	Antimic -robial	Dairy - Milk	*E. coli, S. aureus, L. monocytog -enes, Salmo -nella enterica subsp. enterica*	• Complete Listeria monocytogenes inhibition was observed after 6-8 days	82
Enterococcus durans LM01C01, LM05C01, EP1	Kefir grains or milk	Antimic -robial	Dairy - Cream cheese	*E. coli, S. aureus*	• LM01C01 resists acid and grows in the presence of bile • It tolerates potassium sorbate and NaCl ingredients	83

L. paracasei MG847589	Traditional Egyptian Karish cheese	Anti-myco -toxigenic	Dairy – Soft white cheese	*S. aureus, A. parasiticus, A. parasiticus*	• Decreased antimicrobial count • Improved sensory scores	59
L. lactis 537	Fresh herb	Antimic -robial	Dairy - Milk	*Lb. plantarum* ATCC14917, *L. monocyto -genes* ATCC19111, *S. aureus* ATCC9144	• Inulin significantly enhanced the growth and nisin production • Prebiotic inclusion for the development of a milk-based symbiotic product	84
Lacticasei-bacillus rhamnosus CLK_01	Fermented juice	Antibac -terial, antibiofilm	Biopreser-vatives for food	*V. Parahemo-lyticu, E. coli, Shigella dysen -teriae, S. ser. Choleraesuis, S. ser. Enter-itidis, Salmo nella ser. Enteritidis, P. aeruginosa, Klebsiella pneu-monia, Aeromonas hydrophila, S. aureus, S. argenteus, Micrococcus luteus, S. agalactiae*	• Exhibited a broad antimicrobial spectrum against foodborne pathogens • It also caused permeability and deformation of bacterial pathogenic cells	31
L. plantarum S61	Fermented green olive	Antibact -erial, antifungal	Fruit – Apple, orange	*Rhodotorula glutinis,Cand -ida pelliculosa, Penicillium digitatum, Rhizopus ory zae A. niger, L.monocyto-genes (ATCC 19,117), Fusa-rium oxysporum, S. enterica (ATCC 14,028), P. aeruginosa (ATCC 49,189), S. aureus (ATCC 6538)*	• Ability to lower Apple Juice levels of *Salmonella enterica* (ATCC 14,028) • *L. plantarum* S61 and /or its bioactive substan -ces offer a viable approach to biocontrol against diseases and spoilage microbes	85

B. cereus NS02	Buffalo milk	Antimic -robial	Fruit – Apple juice	*L. monocy-togenes*	• Heat stable low molecular weight peptide biopreser vative with effective antilisterial activity in the food system	86

Recent studies highlighted the potential application of biopreservatives extended to edible food packaging applications. These packaging materials act as a functional carrier for pre/pro/post-biotics to enhance food safety, quality and shelf life.[73] Postbiotics of FreshQ combined with nanocellulose, the antimicrobial membrane was developed for food contact applications.[74]

Effect of Preservatives on Food Quality and Sensory Attributes

Biopreservation, which involves the use of a mixture of LAB bacteriocins (nisin) and bacteriocin-producing bacteria, is now regarded as an important aspect of the food industries hurdle technology. As a result, synergistic effect clearly plays a role in preventing pathogenic and spoilage microorganisms' growth of resistant bacteria (gram-negative) by affecting outer-membrane permeability. Further, it can improve the food's sensory, chemical, and microbial qualities, significantly impacting food safety, shelf life extension, and health requirements.[87] In a research investigation of fermented black chokeberry juice formulated in a yogurt-style product, it was found that when compared to regular yogurt, the juice enriched with probiotics (*L. plantarum*) exhibited higher antioxidant capacity and increased viability of beneficial microorganisms, particularly when in a freeze-dried form.[87] Similarly, another study on yogurt explained positive sensory results.[88] A Cheddar cheese study reported probiotics incorporated have a higher score than the other cheese.[89] A recent document on the fortification of cheese using probiotics attained a higher score than others.[59] Many studies reported that the addition of probiotics results in enhancements of the sensory and texture of the food products.[11,20] Homayouni *et al*. (2021) recently documented their perspective on the food safety aspect of postbiotics applications.[90]

Safety and regulatory aspects

Regulatory frameworks for food prioritize safety and guidelines for health benefit claims. However, the absence of a consensus on postbiotic definitions has stalled regulations for probiotics. The European Union focuses on live microbe safety, employing the Qualified Presumption of Safety list assessing historical safe use and human exposure. Evaluations consider strain-level antibiotic resistance to mitigate risks. Postbiotics face novel food and health claim regulations; novel foods demand rigorous safety evaluations, including toxicity data. Recent trends suggest assessing inanimate bacteria, potentially postbiotics, is simpler than live bacteria (probiotics). Safety assessments for inanimate bacteria as novel foods have occurred, with varying authorization processes. Presently, meeting regulatory requirements for postbiotics appears less complex than for probiotics in the EU. In the EU, using the term "postbiotics" on food or food supplements requires health claim approval by the European Food Safety Authority and systematic approval as a novel food. Additionally, recent EU regulations pertaining to medical devices explicitly exclude "living organisms" from their scope.[91] The acceptability and implementation of foods available in the market depend greatly on consumer awareness, less inhibition by consumers and utilization of the apt strains in foods. The effect of biopreservation by probiotics and such foods treated in human populations that are widely vulnerable is of great concern. Pregnant women, lactating mothers, infants and old age people constitute a considerable portion of such populations, and the effects of such preservative measures on them are yet to be holistically approached. The probability of such foods generating transmissible antibiotic resistance and the aftermath of probiotics in weight gain is also an area of great concern. The usability of this to severely ill patients and immune-compromised consumers is not yet completely formatted. Thus, become potential challenges in the implementation of such a novel natural preservative technique.

The main obstacle in the commercialization of this technique is the viability of the method at elevated temperatures, along with the heat stability and sensitivity of the microbiota involved. Also, the selection of microorganisms depends on a wide range of characteristics such as their viability and activity, safety as per norms, adherence to epithelial tissues of the gut in the human host, resistance to bile and acid in the human digestive tract, production of metabolites that can be antimicrobial components, capability to colonize in the gastrointestinal tracts of humans. Much research is further required to establish and utilize this novel technology in the food sector. If applied efficiently, this natural preservative technique can overcome many of the health concerns and issues that society faces today.

Conclusion

The use of probiotic bacteria in a variety of applications, including the food industry and the health sector, has already influenced this field of research. This article assesses the probiotic's safety, functionality, and technical aspects. In the seafood sector, antimicrobial compounds such as bacteriocins, polymeric chemicals such as extracellular polymeric substances (EPS – Biopolymer), and biosurfactants are often utilized. Additionally, LAB strains have been observed to produce antioxidants that may scavenge free radicals, such as superoxide anions and hydroxyl radicals. However, using biopreservatives comes with certain disadvantages; for instance, bacteriocins have a limited spectrum of activity, diffuse in solid matrices, get inactivated by proteolytic enzymes, interact with bacteriocin-resistant bacteria and other food ingredients, are unstable at higher temperatures and the application of protective cultures is challenging. In the future, it may combine with other nonthermal approaches, clearing the path and developing new hurdle technologies to extend food shelf life. Pro/Post-biotics may become more common biopreservatives of food in the future, giving consumers possible health benefits as well as providing natural, sustainable substitutes for conventional chemical preservatives.

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Conflict of Interest

The authors declare no conflict of interest.

Authors Contribution

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