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# The Role of Bacteria in Food Safety and Preservation

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بسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيم

قُلْ بِفَضْلِ اللَّهِ وَبِرَحْمَتِهِ فَبِذَٰلِكَ فَلْيَفْرَحُوا هُوَ خَيْرٌ مِّمَّا يَجْمَعُون

صَدَقَ اللَّهُ الْعَظِيمَ

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# The Role of Bacteria in Food Safety and Preservation

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# List of Abbreviation

| **Abbreviation** | **Full Term** |
|---|---|
| **CFU** | Colony Forming Unit |
| ***E. Coli*** | *Escherichia Coli* |
| **EFSA** | European Food Safety Authority |
| **EPA** | Environmental Protection Agency |
| **EPS** | Exopolysaccharides |
| **FAO** | Food and Agriculture Organization |
| **FDA** | Food and Drug Administration |
| **FRS** | Fermented Rice Slurry |
| **GC-MS** | Gas Chromatography–Mass Spectrometry |
| **GG** | Lactobacillus rhamnosus GG |
| **GMP** | Good Manufacturing Practice |
| **GRAS** | Generally Recognized As Safe |
| **HACCP** | Hazard Analysis and Critical Control Points |
| ***KP*** | *Klebsiella pneumoniae* |
| ***L. mono*** | *Listeria monocytogenes* |
| ***LA*** | *Lactobacillus Acidophilus* |
| ***LA-5*** | *Lactobacillus acidophilus LA-5* |
| **LAB** | Lactic Acid Bacteria |
| ***LGG*** | *Lactobacillus rhamnosus GG* |
| **MHA** | Mueller-Hinton Agar |
| **MRS** | De man, Rogosa and Sharpe (Culture medium) |
| **MRS agar** | De man, Rogosa and Sharpe Agar |
| ***MRSA*** | *Methicillin-Resistant Staphylococcus aureus* |
| **NA** | Nutrient Agar |
| **PVDC** | Poly Vinylidene Chloride |
| **SEM** | Scanning Electron Microscope |
| **SPSS** | Statistical Package for the Social Science |
| **TSB** | Tryptic Soy Broth |
| ***VRE*** | *Vancomycin-resistant Enterococcus* |
| ***VRSA*** | *Vancomycin-resistant Staphylococcus aureus* |
| **WHO** | World Health Organization |

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## Abstract

This research focuses on the use of probiotics, particularly *Lactobacillus plantarum L4*, for food preservation. Due to the increasing demand for natural and safe food preservation methods, this study aims to explore the antimicrobial properties of this probiotic strain against harmful bacteria such as *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus*, as well as fungi like *Aspergillus niger* and *Aspergillus flavus*. The method used was the agar-diffusion assay, where the strain showed strong antagonistic activity against all the tested microorganisms. This activity is due to the production of organic acids like lactic acid, as well as other bioactive substances that were found to be protein-based, suggesting that they could be bacteriocins. These bioactive substances were stable even at temperatures above 100°C for 16 minutes and remained effective in a pH range from 4 to 7. Moreover, the antimicrobial activity of *Lactobacillus plantarum L4* did not diminish even after heat treatment, which indicates its potential for long-term use in food preservation. The findings suggest that *Lactobacillus plantarum L4* can be used as a potential natural preservative in food products, offering an alternative to synthetic preservatives. This research highlights the potential of probiotics in improving food safety, shelf life, and quality, with a focus on their antimicrobial properties, stability, and environmental safety.

**Keywords:**

Probiotics, Antimicrobial, *Lactobacillus Plantarum L4,* Food preservation, Bacteriocin

# Introduction

Food is the basic need of every human being, and efficient techniques are essential to minimize food spoilage. When a food item's nutritional content, texture, and flavor degrade to the point where it is no longer safe to eat, it is said to have spoiled. Since there was usually little to no food left over after daily meals, food preservation was not an issue in the past. However, when there was an excess of food available, the need for food preservation and storage increased at an unknown point. Food storage became essential for survival during times of scarcity, allowing people to survive when fresh food was in short supply [1].

To reduce spoilage and extend shelf life, chemical preservatives have commonly been used. The use of synthetic chemical preservatives in food has significantly increased. However, numerous scientific studies have found a connection between a number of health issues and the use of certain dietary additives. Ingesting chemical preservatives has been linked to hyperactivity and other neurophysiological problems, depending on age and a number of other circumstances. Additionally, some preservatives have been related to heart damage, lung problems, and other negative effects, while others have been found to be carcinogenic [2].

At the same time, advances in food processing and manufacturing techniques, including enhanced thermal [3]and non-thermal processing has increased [4] and has a positively impact on both the global food market's expansion and the food's shelf life [5]. A longer and more intricate food chain may be necessary to meet the increased demand for minimally processed, fresh cut, and ready-to-eat items, as well as the introduction of new foods and industrial techniques, which may increase the risk of microbial contamination [6]. In order to satisfy the changing needs of the whole food supply chain, from "farm to fork," there is a constant search for novel and complementing food preservation technology [1].

Because of that, scientists are trying to find natural ways to keep food safe. One of these ways is using helpful bacteria, like probiotics. These are bacteria that not only help with digestion, but they can also stop harmful bacteria from growing in food. Some of these bacteria also produce substances called bacteriocins, which kill harmful pathogens in food [1].

Probiotics are live microorganisms that provide health advantages to the host when consumed in sufficient quantities [7]. They have been linked to better digestive health and stronger immune responses. Probiotics help to restore the gut microbiome's composition and enhance the beneficial functions of gut microbial communities, such as reducing gut inflammation and preventing various intestinal diseases. They stimulate the production of defenses and immunoglobulin A (IgA) within the host's body, which helps inhibit the growth of harmful pathogens [8]. The possible antimicrobial properties of probiotics, along with their health benefits, have led to the exploration of different probiotic sources, formulations, and delivery methods in food products. Appropriate strains are chosen for the development of food products that incorporate probiotic bacteria in large-scale production [9].

For probiotic formulations to provide the claimed health benefits, there must be sufficient amounts of live bacteria (>106 cfu/mL or g of food) present at the time of ingestion [10]. Numerous strategies, such as encapsulation, guarantee that the necessary quantity of probiotics is supplied. One of the most often used components in functional foods is probiotics, and much funding has been drawn to create innovative food formulations and technologies [10]. The creation of capsules, powders, liquids, and traditional food forms that are added to baked goods, dairy products, candies, and beverages are examples of novel probiotic delivery methods. With a new preference for non-dairy matrices, these are spreading quickly [10].

Probiotics and their byproducts are examples of bioactive compounds that can be used to suppress harmful microorganism's growth and thus lengthen the lifespan of food products [11]. Probiotics and postbiotics have been used to stop the growth of harmful microbes and the corruption they cause because of their strong antibacterial properties. According to recent studies, postbiotics can be employed as novel antibacterial agents and may be a good substitute for probiotic cells [12].

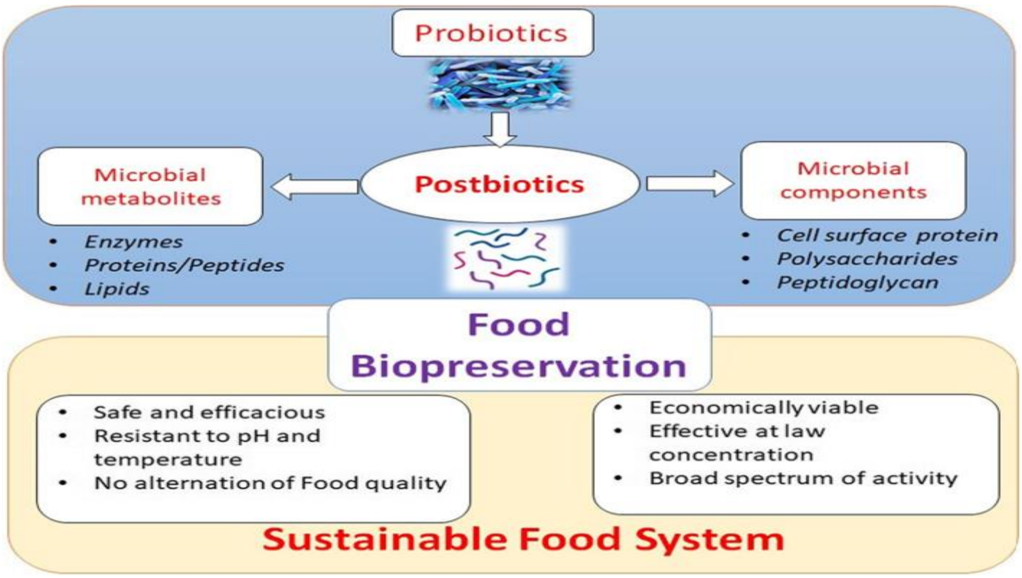


**Fig. 1.** Postbiotics are linked to probiotic in food preservation. (Source **[13]**)

Postbiotics are any compounds that a microbe produces or releases during its metabolic process and that have a positive effect on the host, either directly or indirectly. Postbiotics carry fewer dangers because they don't include live microorganisms [14].

Postbiotics are suitable for commercial use since they are non-toxic, easily transportable less costly to store, and have shelf lives of up to five years. Anti-proliferative and cholesterol lowering properties their antibacterial properties come from postbiotics like peptides, organic acids, fatty acids, bacteriocins, and H2O2 molecules. Postbiotics can be used to maintain and eradicate bacterial biofilm formation in foods as well as for food bio-preservation [13].

A variety of compounds with antimicrobial activity are produced by bacteria and yeasts used in food production, including organic acids (lactic, acetic, and propionic acids), diacetyl, H2O2, reuterin, bacteriocins, and enzymes that have bacteriostatic, bactericidal, fungistatic, and fungicidal action against pathogens and spoilage microorganisms. Their antibacterial activity mechanisms are different. They can be used as natural bio-preservatives to extend the shelf life of food and beverages because they are produced by GRAS microorganisms [15].

Lactic acid and acetic acid synthesis are the primary mechanisms by which lactic acid bacteria exhibit their antibacterial activity. Hydrogen peroxide, diacetyl, ethanol, phenolic acid, propionic acid, sorbic acid, benzoic acid, and protein compounds are all produced. Lactic acid bacteria produce organic acids that change the medium's pH and prevent the formation of putrefactive pathogens and toxic microorganisms. They can also directly affect microbial cells by acting as bacteriocins, which are peptide-based antibacterial compounds [15]. In addition to being safe food bio-preservatives, bacteriocins of food lactic acid bacteria can be used to effectively kill food pathogens and sensitive Gram-positive spoilage food bacteria. They can also be used to kill harmful Gram-negative bacteria in foods that may contain them [16].

Numerous benefits of lactic acid bacteria bacteriocins make them a good option for use in effective food bio-preservation techniques: Since lactic acid bacteria have been involved in food fermentations for centuries and have a history of safety, their metabolites are widely accepted to be safe. None of the lactic acid bacteria's bacteriocins has demonstrated any harmful effects on eukaryotic cells, and their proteinaceous nature guarantees their safety because digestive proteases keep them inactive [17].

Antimicrobial compounds can be imported as pure chemicals or as viable cells of microbial strains that grow in food and produce these metabolites in the food to guarantee food safety. In addition to the direct addition of living lactobacilli and lactic acid cocci cells to food products, bacteriocins of lactic acid bacteria are being used as bio-preservatives [18].

Modern technologies in food production focus on preserving the vitality of probiotics during the storage or post-harvest stages of food products. There have also been efforts to incorporate non-viable microorganisms to derive health benefits [19].

Depending on the intended application and the type of food, bacteriocins can be employed either directly or in combination with other therapeutic approaches [20]. Since lactic acid bacteria bacteriocins are proven to meet consumer and industrial needs for fresh, minimally processed foods, they are used as an addition [21, 22]. These bacteriocins have become more about the use of synthetic preservatives. They are now being studied more as safe and effective compounds that can help improve food safety without affecting food quality [16].

Bacteriocins are natural substances made by some bacteria to kill or stop the growth of other harmful bacteria. They are like small proteins that help good bacteria protect themselves and their environment. Bacteriocins are safe and can be used in food to keep it fresh and prevent it from spoiling. Many scientists are interested in them because they can be a natural and healthy way to preserve food instead of using chemicals [16].

Low molecular weight antimicrobial peptides are known to be produced by almost all living organisms as a component of their defensive mechanisms [23]. Almost all bacterial species, especially Gram-positive bacteria, ribosomally produce bacteriocins [24]. Because of its antimicrobial activity against harmful bacteria and food spoilage, bacteriocin has drawn interest from food scientists as a natural food bio-preservative [9] [25]. According to [26], bacteriocins are peptides or complex proteins that are produced by bacteria and have antibacterial properties against other bacteria as well as food-borne pathogens and food spoilage bacteria, primarily closely related species. This bio-preservative technique uses the antagonistic actions of microorganisms and their byproducts to eliminate undesirable germs in order to preserve food and extend its shelf life [25].

Numerous studies carried out in recent years have shown that different bacteriocins also have antimicrobial properties against some Gram-negative bacteria [27] and even some viruses [28], despite the fact that they are mainly efficient against bacterial species that are closely related to the producer species and particularly against Gram-positive bacteria [29].

Moreover, it has been understood that various bacteriocins are effective against biofilms, which can cause significant problems in the food industry [30]. bacteriocins are not considered to be real antibiotics [31]. Bacteriocins are proteinaceous by nature and are quickly broken down by the body's digestive enzyme protease, which sets them apart from therapeutic antibiotics. These principles make it easy to distinguish between bacteriocins and antibiotics [31].

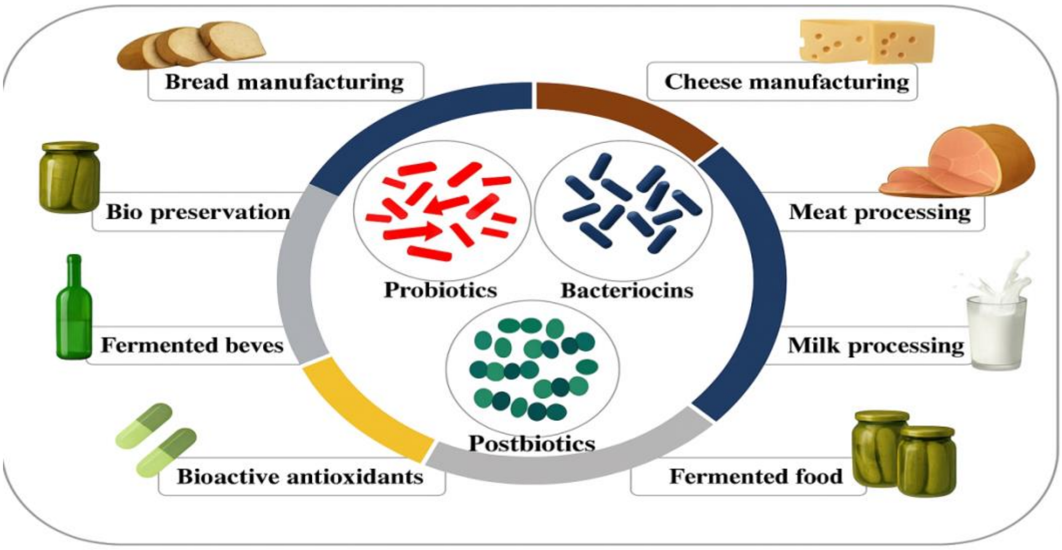


**Fig. 2.** Overview of the components used in bio-preservation. (Source **[32]**)

However, there are a few significant barriers that restrict the usage of bacteriocins in food. In fermented foods, lactic acid bacteria (LAB) are commonly utilized as starter or adjunct cultures. Using bacteriocins that are effective against LAB cultures in fermented foods can have a number of negative effects on food production and quality. In addition, proteolytic enzymes can alter bacteriocins with peptide structures, and bacteriocin stability in various foods may be limited [33]. Recent research [34, 35] has shown that adding bacteriocins or bacteriocin-producing bacteria with GRAS (Generally Recognized as Safe) status to film and coating materials used in foods can successfully overcome all of these restrictions [35].

So in this research, we will look at how bacteria can be used to keep food safe and fresh without using chemicals. We will also see how these bacteria work, and why they are a good option for food preservation. This way, we can make food healthier and safer for everyone [1].

## Bio-preservation for Food

Bio-preservation means using natural and safe ways to protect food from spoilage. Instead of using chemicals, we use good bacteria or the substances they produce to stop the growth of harmful microbes. This helps keep the food fresh for a longer time. Bio-preservation is better for health and the environment, and people prefer it because it's more natural. Bio-preservation techniques may require less energy than other preservation techniques, which would save energy and lower greenhouse gas emissions. These techniques also stand out as being essential to the production of sustainable food. In this research, we will focus on three main things used in bio-preservation: probiotics, postbiotics, and bacteriocins [1].

Using protective cultures of microbes to preserve food is known as bio-preservation, or bio-conservation [36]. By stopping the development of harmful microbes it involves using harmless microorganisms or their byproducts to extend food's shelf life and safety [37]. As shown in Fig.3, bio-preservation uses beneficial microbes to inhibit spoilage organisms. Fermentation is a common bio-preservation method that produces alcohols, organic acids, and other compounds by breaking down complex food molecules with the help of naturally occurring bacteria or by adding them. This procedure improves the food's overall quality by enhancing its flavor and aroma. It can be challenging to include probiotics into food products, though, because some strains can generate off tastes that could reduce customer approval [1]. Consequently, it's essential to choose strains that don't substantially change the original flavor, especially in products containing high amount of fat. To reduce flavor changes, characteristics of the product or raw material should be taken into account while choosing strains for bio-preservation [9].

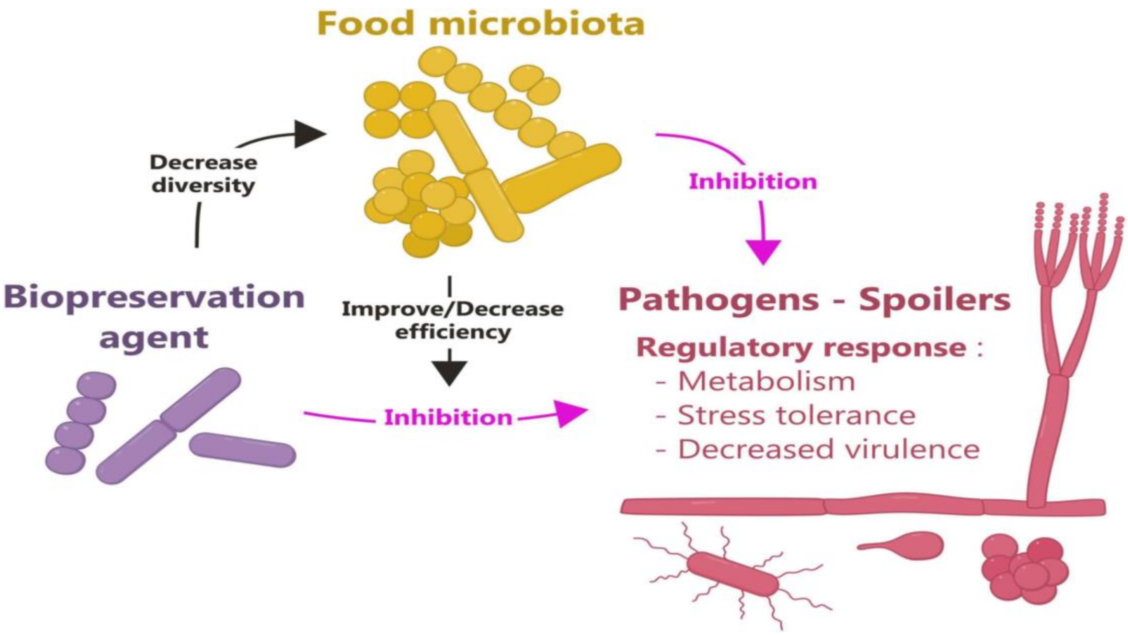


**Fig. 3.** Bio-preservation processes in food. (Source **[38]** )

In industrial settings, lysozymes, bacteriophages, LAB, and their bacteriocins are common bio-preservatives. Lysozymes are naturally occurring enzymes that are present in body secretions are valued for their capacity to break down biofilms. Effective bio-preservatives, bacteriophages are viruses that infect bacteria and have strong antibacterial properties. Complex proteins or peptides known as bacteriocins have antibacterial properties against closely related bacterial species [1].

Because of their dynamic properties and ability to create bacteriocins, LABs are especially noteworthy. Some strains of probiotics and bacteriocins that fight harmful and food-rotting microbes can be produced in LAB [39]. Probiotics are positioned as potential natural food bio-preservatives due to their capacity to produce bacteriocin and other antagonistic and antibacterial qualities. The advantages of using bacteriophages and endolysins as food bio-preservatives have been brought to light by recent developments in food safety. Numerous uses of probiotic microbes and bacteriocins with possible antibacterial action to improve food preservation are being researched [1].

## The Need for Bio-preservation

Heating, drying, freezing, pickling, edible coating, and high-pressure processing are a few examples of traditional food preservation techniques [40]. These techniques use various treatments or additions to inactivate and/or suppress harmful germs. However, knowledge of microbiology is not required for these techniques. Nutrients and sensory qualities are lost during pasteurization and other high-heat procedures. In addition, non-thermal processing methods such as food irradiation are linked to ethical and social issues addition to safety concerns [9]. Alternative food preservation techniques are required in order to effectively and sustainably solve modern food safety and quality concerns, as a result of the shortcomings in the current systems. Even though traditional methods are still used, they are no longer enough because of changes in consumer habits, technology, and health concerns [41].

The abundant supply of food in developed nations, along with shifts in socioeconomic conditions, demographic trends, and consumer perceptions of A number of distinct issues in the field of food preservation have been brought about by nutrition, dietary preferences, food selection trends, technological advancements, and competition among food processors [42]. Customers voice worries over the healthfulness and safety of foods that contain a range of nonfood additives, including preservatives [1]. The quantity and diversity of chemicals utilized have grown exponentially in comparison to prior years [1]. Reports about potential health risks from consuming certain preservatives and other compounds currently in use, such nitrite and saccharine, as well as additives that were once used but are now prohibited, have weakened consumers' trust in the safety and wholesomeness of the foods they eat [43]. Regulatory organizations are concerned about the usage of suitable food preservatives to ensure the safety of these products. As concerns over synthetic additives and preservatives in food continue to rise, the importance of bio-preservation methods grows, as they offer a more natural and safer alternative to prevent foodborne diseases caused by harmful microorganisms [1].

Foodborne disease outbreaks are brought on by biological agents known as foodborne pathogens. When a toxigenic pathogen establishes itself in a food product and creates a toxin that is afterwards consumed by the human host, or when a pathogen is consumed and becomes established in the host, foodborne diseases result [44]. There are about 200 known foodborne illnesses, and the most serious ones affect individuals with weakened immune systems, such as the elderly and the very young [45, 46]. *Yersinia enterocolitica, Salmonella* spp*., Shigella* spp*., Staphylococcus aureus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Cronobacter sakazaki, Escherichia coli, Listeria monocytogenes,* and other common foodborne pathogens are frequently associated with foodborne illnesses [44].

Since spore-foaming bacteria typically exhibit resistance to physical treatments, including thermal processing, they pose a serious risk to food that has been heated. Under the right circumstances, these spores might grow and spread through the product. While *Staphylococcus aureus and Clostridium botulinum* can produce heat-tolerant toxins, foodborne pathogens like *Clostridium botulinum, Clostridium perfringens, Bacillus subtilis,* and *Bacillus cereus are excellent* examples of spore-forming, extremely heat-resistant bacteria [9]. The majority of foodborne pathogens are mesophilic, meaning their ideal growth temperatures fall between 20 and 45°C however, some, like *Listeria monocytogenes* and *Yersinia enterocolitica*, may grow in a cold environment or at temperatures lower than 10°C (psychrotrophs) [47].

In response to these growing concerns over synthetic preservatives and foodborne illnesses, bio-preservation has emerged as a safer, more natural solution, utilizing food-grade bacteria that produce antimicrobial metabolites. This puts food-grade starting culture bacteria that produce antimicrobial metabolites or bio-preservatives in a special position, especially when it comes to their immediate application. Over time, their safety has been proven, and customers have employed them in one way or another, most notably health-conscious consumers [42]. Consumers and regulatory bodies grow more aware of the problem of food safety, and the food industry's responsibility to guarantee the safety of its products is better understood. Stricter hygienic regulations are also required due to an increase in the population's vulnerable members, especially in developed countries. Demand for bio-preservation rises as a result of customer demands for more convenience, such as less frequent shopping, and improved food quality stability to prevent food loss from rotting. At the same time, consumers want "natural" and "fresh" food free of chemical additives [48]. Given the increasing use of food-grade bacteria for bio-preservation, the application of probiotics becomes even more important in controlling pathogenic microorganisms in food [48].

Probiotics have been shown in the literature to suppress pathogenic bacteria in food products. Probiotics have also been shown to reduce pathogens in vivo in the human body [49]. The precise way that probiotics reduce pathogens in food products during processing and storage seems to be different from how they work in the human body. For example, probiotics' synthesis of short-chain fatty acids lowers the pH of fermented dairy products and makes the environment unfavorable to the growth of some harmful bacteria. Furthermore, when added to food products, some probiotics have the ability to create bacteriocin. These are advantageous because they stop more germs from entering the body through food products. The mechanisms by which probiotic microbes remain in the human body after ingesting may include a competitive advantage for nutrients, the release of bacteriocins or other antimicrobial agents that are effective against pathogenic microorganisms, and the inhibition of pathogenic microorganisms from adhering to the intestinal epithelium [49, 50]. However, future studies should look more closely at the synergistic nature of probiotics' ability to suppress pathogens in food products and in the human body after ingestion [50].

## I. Probiotics as Bio-preservatives

Probiotics involve the replacement of harmful bacteria and the introduction of beneficial bacteria into the gastrointestinal tract. Non-dairy probiotic foods are in high demand due to dietary allergies, lactose intolerance, vegetarianism, and veganism [51]. Fermented dairy products like yoghurt, cheese, and different kinds of fermented beverages like sour milk are traditionally linked to the delivery of probiotics into the human body. Non-dairy probiotic meals, primarily drinks, can also be used to administer probiotics. Probiotic distribution involves both fermented and non-fermented non-dairy foods. The majority of fruit and vegetable-based probiotic drinks are made without fermentation [52]. Commonly used probiotics and beneficial microorganisms in dairy and non-dairy plant-based food products are listed in Table 1. Research indicates that adding probiotics to food products may have positive health effects. Probiotics have become more popular in a number of countries' functional food markets as a result of increased consumer awareness. In order to determine and confirm the customer acceptance for probiotic food items, sensory properties are essential. Probiotics will mostly be used in nutrition and medicine in the future. Under the guidance of medical professionals, probiotic uses in the prevention and treatment of various diseases must be taken into consideration. The food industry can later create and promote this. In the interest of consumers, the whole concept behind the inclusion of probiotics in food product development should be carried out and spread in a scientific manner [53, 54].

The potential application of bacteriocins, produced by antagonistic microorganisms including probiotics, as natural preservatives has attracted the attention of the food industry in recent years [55]. There are numerous uses for probiotics in the dairy and food industries. There are several probiotic foods and beverages available on the market today. Customers think probiotic beverages are a more reliable source of active ingredients, which is why they are so popular [56].

In the food industry, probiotics are essential, especially in the dairy sector. In order to produce functional foods like yoghurt, cheese, and ice cream, as well as non-dairy items like cereals, chocolates, and other confections, as well as processed meat products, probiotic bacteria are usually encapsulated [57, 58]. Numerous businesses have created probiotics in the form of pills, tablets, or capsules. However, the food industry has very few encapsulated probiotic products. Furthermore, the study of cell biology and encapsulation technology will surely help to produce many different and marketable probiotic products [59]. The most often utilized probiotics in the dairy sector are strains of the genera *Lactobacillus* and *Bifidobacterium*. Other genera such *as Propionibacterium, Peptostreptococcus, Pediococcus, Leuconostoc, Enterococcus, Saccharomyces* and *Streptococcus* are slowly gaining popularity in the field of probiotic foods and beverages [60, 61]. In a recent study, a probiotic drink enhanced with mango juice was created using *Lactobacillus acidophilus LA-5*. Mango juice improved the viability of the probiotics. When exposed to in vitro gastrointestinal digestion, this formulation enhanced the probiotic's tolerance. The same study's sensory analysis of mango juice showed that when the concentration of mango juice rose, so did the beverages' sensory scores [62].

The stability of probiotic microorganisms can be affected by the properties of the food matrix, storage conditions, and the passage through the gastrointestinal tract. The properties of the food matrix play a major role in supporting the activity and survival of microorganisms. The primary factors influencing a food product's safety, consistency, and efficacy are its production process. Traditionally, both fermented and non-fermented foods are used to give probiotics. Probiotic bacteria in microencapsulated form are what the current food business seeks to include into unfermented meals. There are three main ways to introduce microbial strains into the human body. Additionally, probiotics are delivered through traditional fermented meals as pharmaceutical products and/or functional foods [63].

**Table.1.** Commonly used probiotics and beneficial microorganisms in dairy and non-dairy plant based food products [9].

| Genus | Probiotic/Potential Probiotic Strain | Food Matrix |
|---|---|---|
| *Lactobacillus* | *Lactobacillus rhamnosus* GG | Kefir |
| | *Lactobacillus casei* ATCC 393 | Fermented milk |
| | *Lactobacillus casei* Q14 | Yoghurt |
| | *Lactobacillus casei* 01 | Sheep milk ice cream |
| | *Lactobacillus paracasei* LBC-81 | Maize-based beverage |
| | *Lactobacillus plantarum* L7 | Rice-based fermented beverage |
| | *Lactobacillus acidophilus* NCIMB 8821 | Oat flour and barley malt beverage |

| Genus | Probiotic/Potential Probiotic Strain | Food Matrix |
|---|---|---|
| | *Lactobacillus reuteri* NCIMB 11951 | Oat flour and barley malt beverage |
| | *Lactobacillus fermentum* ATCC 9338 | Prickly pear juice |
| | *Lactobacillus fermentum* KKL1 | Rice-based fermented beverage |
| *Bifidobacterium* | *B. longum subsp. longum* YS108R | Fermented milk |
| | *B. animalis* | Milk supplemented with seaweed extract |
| | *B. breve* | Probiotic-fermented blended juices |
| *Saccharomyces* | *Saccharomyces cerevisiae* KU200284 | Kefir |
| *Pediococcus* | *Pediococcus pentosaceus* Lbf2 | Soursop juice |
| | *Pediococcus acidilactici* CE51 | Probiotic orange juice |
| | *Pediococcus pentosaceus* | Fermented soybean milk |
| *Propionibacterium* | *Propionibacterium freudenreichii subsp. Shermanii* | Probiotic feta cheese / chocolate |
| *Streptococcus* | *Streptococcus thermophiles* | Probiotic fermented oat flour |

## 1. Bio-preservative Properties of Probiotics

The major mechanisms of probiotic activity in the human body are depicted in Fig 4. One of the main advantages of probiotics is their antimicrobial properties. A number of commercially used probiotics and beneficial microorganisms showed various inhibitory actions against a variety of pathogenic and spoilage microorganisms in foods. The study of probiotic action mechanisms uncovers new roles for probiotic microbes. The selection of appropriate probiotic strains for particular applications can be easier by a complete understanding of the mode of action. Numerous mechanisms, such as modified gut microbiota, improved gut epithelial barrier, increased adherence to intestinal mucosa and epithelium, and immune system modulation to benefit the host, could explain the probiotics' antagonistic effects on other microorganisms. However, not all of these mechanisms are important for their bio-preservative properties [64].

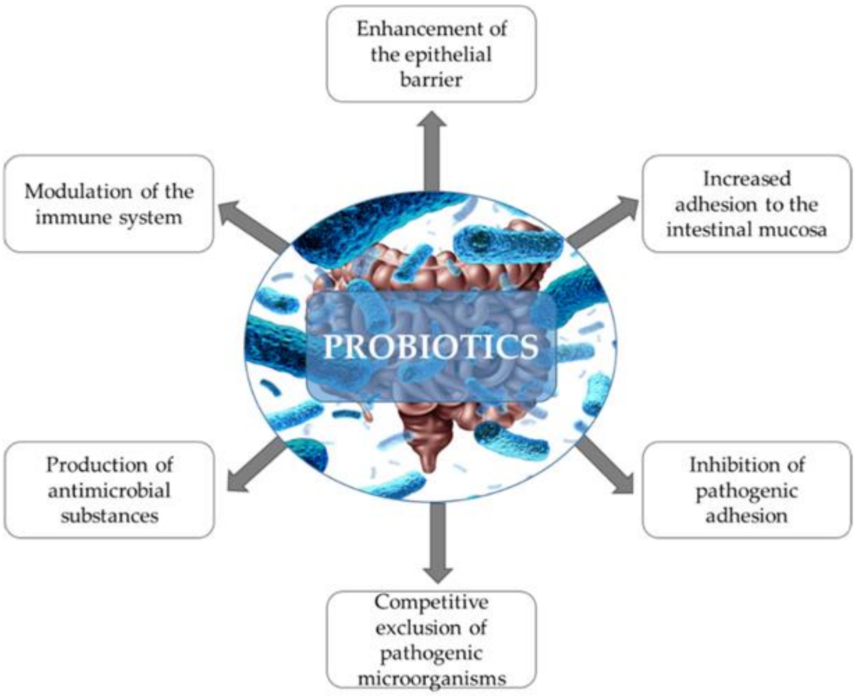


**Fig. 4.** Major mechanisms of probiotic activity in the human body. (Source **[9]**)

The primary source of probiotics' bio-preservative properties appears to be the production of antibacterial compounds. The production of ethanol, bacteriocins, and organic acids is mainly associated to the mechanism of probiotics' antibacterial activity, which in turn inhibits the growth of foodborne and spoilage microorganisms in food material [65]. Therefore, it is possible to see the production of bacteriocin by probiotic microbes as an essential part of their antagonistic capacity in bio-preservation. However, more research is needed to fully understand this occurrence. Numerous potential candidates for application as bio-preservative agents in plant-based foods are suggested by research targeted to examining the antibacterial properties of probiotics and possible probiotic strains. For example, *Lactobacillus kefiri*, a strong probiotic strain that was isolated from kefir grains, has antibacterial properties, according to Likotrafiti et al. [66]. The growth of pathogenic *Clostridium difficile* is considerably inhibited when *Bifidobacterium longum* IPLA20022 and *Bifidobacterium breve* IPLA20006 are co-cultured using short-chain fructooligosaccharides as a carbon source. Potential probiotic strains that have been identified from food sources provide the significant benefit of being able to be used as bio-preservative agents in different food matrixes [67].

Through the process of creating a protective culture, probiotics are used in the business with the goal of biological preservation. It causes the membranes of harmful and spoiling bacteria to become permeable, which ultimately results in their death. Many more metabolites, in addition to those listed above, have been thoroughly reported in a large number of researches. These include carbon dioxide, ethanol, reutericyclin, reuterin, hydrogen peroxide, diacetyl compounds, acetone, and acetaldehyde, etc [9].

## 2. Bio-preservation: Mode of Action

One important way that bio-preservation is used in food processing is through fermentation. By generating organic acids, the indigenous microbiota starts the fermentation process and lowers the pH of the food matrix. This higher acidity prevents harmful and spoiling microbes from growing and spreading throughout the food. In the fermentative processes of many different food products, LAB is essential. Remarkably, lactic and acetic acid are the main metabolic byproducts produced by LAB. By creating an acidic environment when released into the food matrix, these acids prolong the food product's shelf life without the need for additional chemical preservatives [68].

Using bacteriocins or other metabolites made by particular strains of microorganisms is another method of bio-preservation. Nisin and pediocin are two examples that come from the metabolism of bacteria. These substances are cationic peptides with remarkable thermal stability and hydrophobic properties that are mostly found in gram-positive organisms. They work by rupturing the target microflora's membranes, which stops harmful and spoiling bacteria from growing and prolongs the shelf life of food items [69]. Foods can be bio-preserved by including certain enzymes with natural antibacterial qualities either during or after processing. Examples of these industrially used enzymes are lactoperoxidase and lysozyme. These inhibitory enzymes might be added externally from reliable sources or they can be found naturally in the meal. Food can also be biologically preserved by adding natural antimicrobial substances, such as certain peptides and chemical compounds. These techniques are also based on bio-preservation principles [70]. The bio-preservative method of microorganisms for meat-based products is depicted in Fig. 5. However, the way that bio-preservatives work will differ depending on the food product; many ways of working have been documented. For example, Kavesh et al. for meat [71] and Agriopoulou s. et al. for fruits and vegetables [72].

Bio-preservation is a powerful technique that uses natural resources, such as protective microorganisms or their byproducts, to prolong food's shelf life without sacrificing its nutritional value or sensory appeal. The pathogenic microbial burden is greatly decreased, guaranteeing safer intake, while the sensory qualities do not decrease. Moreover, it has been noted that certain bio-preservation methods improve the nutritional makeup of foods that have been preserved, highlighting their effectiveness in food preservation [1].

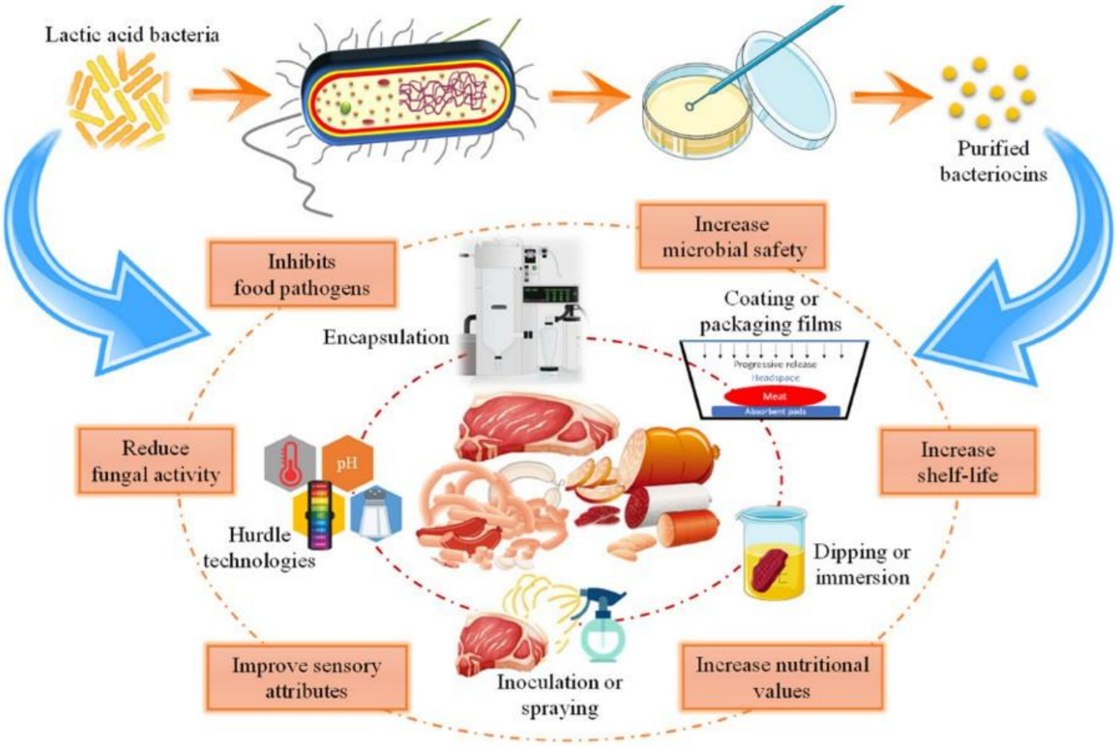


**Fig. 5.** Bio-preservative effect on meat and meat-based product (Source **[73]**)

## 3. Application in Foods

Probiotics, which are commonly used as supplements due to their many advantages, are increasingly preferred by consumers when added to functional foods rather than taken as pharmaceutical supplements. Probiotic strains such as *Bifidobacteria* help preserve fermented foods through fermentation, which improves their nutritional value and cosmetic appeal [63].

### 3.1. Poultry, Meat and Fish

It has been discovered that some meat-born LAB strains that are psychrotrophic, homo fermentative, and salt tolerant work well as bio-preservatives in cooked meat products [74]. LAB or their bacteriocins have been found to be efficient bio-preservative agents against *L. monocytogenes* in seafood [*75*]. Probiotic bio-preservative techniques have been the subject of extensive research in recent years to extend the shelf life of marine items. Green tea extracts, agar, and probiotic strains of *B. lactis* B94 and *L. paracasei* L26 have been used to create a bioactive coating on hake fillets, which has been examined for shelf life. It has been discovered that this multiple affects the fillets in a number of ways, including extending their shelf life by nearly a week and lowering their volatile spoiling indices. Additionally, the final product's beneficial LAB load increased noticeably [*76*]. Biochemical tests and 16S rRNA gene phylogenetic analyses identified 22 LAB isolates from a total of 132 LAB isolated from mussels whose cell-free supernatant showed action against *L. innocua* and *L. plantarum* as *E. mundtii*. The strain *E. mundtii* STw38 was used as a protective culture on fish paste that had been stored at 4 °C because none of the selected strains possessed any virulence characteristics. Air-packed and later vacuum-packed fish paste systems were used in the first step [77].

Bio-preservatives have also been used extensively in the meat industry to improve the quality and shelf life of meat during storage. It has been shown that *L. plantarum* and garlic extract work well together to inhibit the growth of *L. monocytogenes* and prolong the shelf life of ground beef. After applying this mixture to ground beef, the lipid oxidation was significantly reduced and the sensory rating increased as well [78]. For example, the differences between treated and untreated pork meat are shown in Fig. 6. At concentrations of 1600–2500 AU/mL, *lactococcin* BZ showed exceptional efficacy in reducing the numbers of psychrotrophic and mesophilic aerobic bacteria, LAB, total coliform, and fecal coliform bacteria. *Lactococcin* BZ showed strong antibacterial activity and inhibited the growth of *L. innocua* in meat samples. This indicates that *Lactococcin* BZ has a lot of potential for use as a biopreservative to increase the fresh beef's shelf life [79]. For the preservation of meat, *plantaricin* BM-1-activated polyvinylidene chloride (PVDC) film holds great potential. It has the ability to extend the shelf life of pork meat kept at 4 °C and reduce the growth of *L. monocytogenes* [80]. *Murraya koenigii berries* and *P. pentosaceus pediocin* were applied after *L. innocua* was intentionally inoculated in raw goat emulsion. During storage, there was noticeable reduction in *L. innocua*, demonstrating the combination's bio-preservative properties [81]. *E. coli* and *Salmonella* cell counts are significantly reduced when chitosan is added to beef, either by itself or in conjunction with *C. Maltaromaticum* UAL 307 as a protective culture [82].

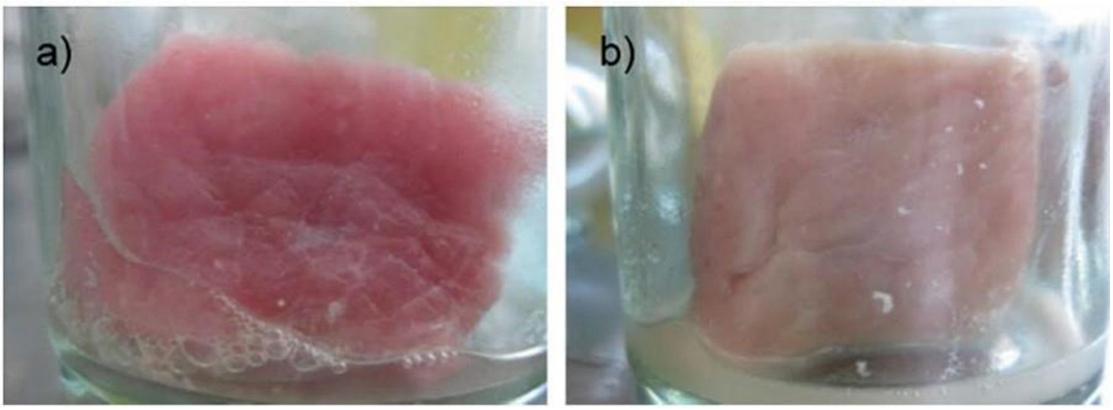


**Fig. 6.** Bacteriocin on pork meat preservation (**a**) Control (without bio-preservative); (**b**) Sample after 24 h (with bio-preservative) (Source **[83]**)

Studies also show the bio-preservative properties of probiotics in the poultry industry. The semi-purified bacteriocin BacFL31 was used to test the shelf life of frozen raw ground turkey meat at 200 and 400 AU/g. BacFL31 treatments successfully inhibited *Salmonella typhimurium* and other spoilage bacteria while also preventing the growth of *L. monocytogenes*. When BacFL31 was added, the turkey meat samples' shelf life was extended and their sensory qualities were improved while they were in the refrigerator [84]. But Kaveh et al.'s recent study from 2023 examined probiotics that act as bio-preservatives for meat and meat-based products [71].

## 3.2. Milk and Dairy Products

LAB has been used to aid fermentation in milk, increasing its acidity and extending its shelf life. Additionally, LAB-treated milk used to make cheese produced more EPS, which improved the cheese's rheological qualities and the generation of carbonyl compounds and acids. It also improved the cheese's sensory properties. Additionally, LAB-produced bacteriocins have a preservation effect [85]. Dairy products' hygienic safety is extended by the use of bio-preservative agents. Through their bactericidal and antibacterial activities, *lactococcin* BZ, *enterocin* KP, and their combination in full fat, half fat, and skim milk inhibited *L. monocytogenes* [86]. Because of its bacteriostatic activity, *L. plantarum* LpU4 and its purified *plantaricin* LpU4 could be utilized as bio-preservatives in cheese. It was especially active in acidic conditions [87]. Yogurt was bio-preserved with *L. pentosus* 22B to stop fungal degradation. The fungal response to concentrated extract following enzymatic treatments, GC-MS, and headspace were used to identify the antifungal compounds produced by *L. pentosus* 22B, which included organic acids, peptidic compounds, fatty acids, volatile compounds, and hydrogen peroxide. GC-MS [88]. The cross-section of the probiotic-fortified white cheese is shown in Fig. 7.

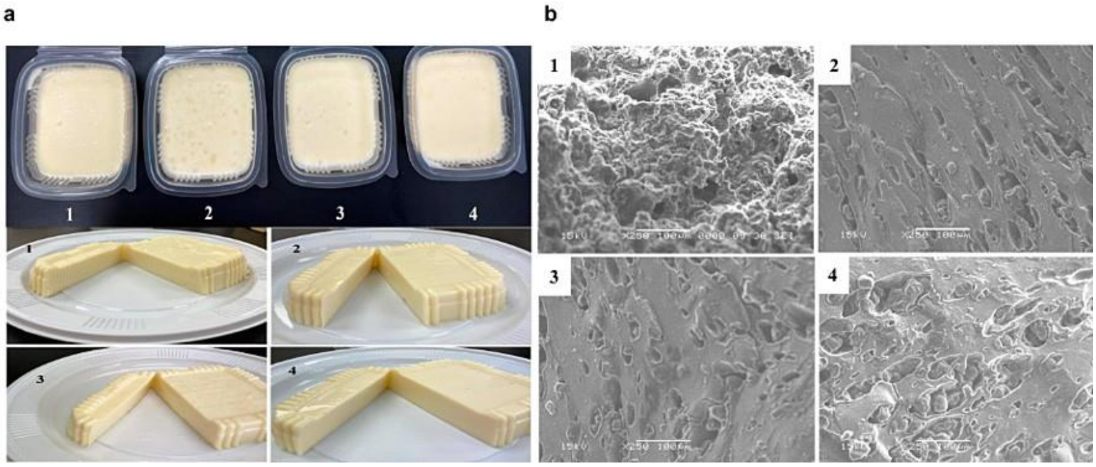


**Fig. 7.** Probiotics preserved white cheese **a)** Morphology of manufactured cheese using several strategies; **b)** Cross-section of cheese samples (SEM images) (1. Control cheese – High porous: less hardness, 2. Probiotic MG847589 cheese-less porous than 1, 3. Cheese with bacteriocin – smooth surface influencing high adhesiveness, 4. Cheese with probiotics and their bacteriocin – high hardness and high consumer acceptance) (Source [**89**])

## 3.3. Fruit

The safety and quality of fresh fruits or minimally processed fruit products, such fresh-cut fruits, have been improved by the use of probiotics and the natural microbiomes of fruits [9].

### 3.3.1. Melons

Melon and other fresh-cut fruits may become infected during processing and preparation (peeling, chopping, etc.). In particular, the fruit might spoil as a result of the pathogenic and spoiling bacteria getting access to its nutrients and growing [90]. Because melons are more likely to become contaminated during growing, postharvest handling, packaging, transit, distribution, or final preparation, they are particularly susceptible to multistate foodborne outbreaks. Consumption of tainted fresh-cut fruits has been the cause of multiple multistate outbreaks of listeriosis and salmonellosis during the past three decades [90, 91].

According to Ukuku et al. [92], the native microbial communities of the entire cantaloupe prevented *L. monocytogenes* from adhering to the rind surfaces, as well as from surviving and proliferating on the surfaces of the cantaloupe and homogenized fresh-cut surfaces. Furthermore, melons treated with either ethanol or chlorine had a considerably greater population of *L. monocytogenes* than melons that were either not disinfected or washed with water, indicating that disinfection treatment was harmful to the native microbial communities [92].

*Lactiplantibacillus plantarum* B2 and *Limosilactobacillus fermentum* (previously *Lactobacillus fermentum*) PBCC11.5, two possible probiotic strains that produce riboflavin, were found to have adequate inhibitory effects against *L. monocytogenes* on artifically contaminated melons. After 11 days of refrigeration, the vitality of *Lb. plantarum* B2 and *Lb. fermentum* PBCC11.5 was 3 × 108 CFU/g and 7.8 × 107 CFU/g, respectively (the probiotic strains' initial inoculation levels were 1 × 1010 CFU/mL). Probiotic enrichment had little effect on the fresh cut cantaloupes' primary nutritional and technical properties. However, after 11 days of storage, the addition of *Lb. plantarum* B2 produced certain unwanted sensory characteristics. Probiotic cantaloupe had a roughly two-fold increase in riboflavin concentration. These results imply that because of their antagonistic effects on fruit-originating *L. monocytogenes*, these two probiotic strains can improve the safety of minimally processed melons [93].

### 3.3.2. Apples

In sliced apples, the potential probiotic strain *Lactiplantibacillus plantarum* CIT3, which was isolated from apples, showed a strong inhibitory effect against *E. coli* and *L. monocytogenes*. Within 7 days of storage at 6 °C, products inoculated with *Lb. plantarum* CIT3 showed a significantly accelerated death of *E. coli*, reducing the pathogen numbers to undetectable levels. Furthermore, until the end of the storage period, *Lb. plantarum* CIT3 inhibited *L. monocytogenes* from growing. Additionally though to a lesser extent, *Lacticaseibacillus paracasei* (*Lb. paracasei*) M3B6 shown encouraging inhibitory activities against *E. coli* and *L. monocytogenes*. When present at concentrations more than 1.5 log CFU/g, these two possible biocontrol agents are proven to be effective. The results suggest these biocontrol agents improve safety against *E. coli* while providing an effective barrier against *L. monocytogenes* for at least 16 days of refrigeration storage. Additionally, these probiotic biocontrol agents were able to considerably reduce the growth of yeast, but they also caused the products to brown too soon, which had a detrimental impact on the products' sensory properties. However, the samples' color held up well for up to 7 days when stored at 6 °C [94].

*Lacticaseibacillus rhamnosus* (*Lb. rhamnosus*) GG (LGG) was applied to fresh-cut apple wedges, and its effect on instrumental eating quality measures and sensory acceptability was examined by Rößle et al. [95]. Sliced into skin-on wedges, the apple samples were then dipped in an edible buffer solution that contained about 1010 CFU/mL of *LGG*. After 10 days of storage at 2–4 °C, the viable LGG counts were higher than 108 CFU/g. Instrumental color values, shear values, soluble solid contents, titratable acidity, pH, and overall acceptability did not significantly differ between the probiotic apple wedges and the control, which was devoid of probiotics [95]. Another study showed that LGG did not degrade in quality after being stored at 5 and 10 °C for more than 28 days at quantities greater than 106 CFU/g on minimally processed apple wedges. *L. monocytogenes* numbers decreased by 1-log unit when LGG was present. Co-inoculation with LGG had no effect on *Salmonella*, despite a 1 log unit decrease in *L. monocytogenes* levels. This illustrates the strain-specificity of probiotics' capacity to suppress pathogens. Additionally, only during the first 14 days of storage did the viable counts of LGG following the simulated gastrointestinal digestion reach the acceptable levels (106 CFU/g). This suggests that the product offers extra probiotic advantages beyond its bio-preservation effect in the initial days of its shelf life [96].

In addition to LAB, some additional bacterial strains that have been shown to have probiotic qualities have also been shown to have antagonistic effects on food-borne pathogens. When *Gluconobacter asaii* was isolated from apple surfaces, it showed antagonistic effects on cut Golden Delicious apples during storage against *Salmonella enterica* Serovar Poona and *Listeria monocytogenes*. After two days of storage at 25 °C, *G. asaii* was able to grow on chopped apples and significantly reduced the populations of *L. monocytogenes*. After 5 days of storage, *G. asaii* decreased the *L. monocytogenes* population by around 2.1 to 2.8 log units at 10 °C, although the decrease in pathogenic populations was not considered significant. Within 7 days of storage at both temperatures, *G. asaii* can still lower the populations even at high inoculation levels of the pathogen. Furthermore, during 5 days of storage at 25 °C, *G. asaii* significantly reduced the populations of *S. enterica* Serova Poona. Since *S. enterica* Serova Poona is not a psychrotroph, it did not grow correctly at 10 °C. Most notably, despite reports that gluconobacter species produce browning on cut apple surfaces, *G. asaii* did not cause any browning in Golden Delicious cut apples [97].

In summary, these studies indicate that probiotic strains of *Gluconobacter asaii, Lacticaseibacillus paracasei M3B6, Lacticaseibacillus rhamnosus GG,* and *Lactiplantibacillus plantarum* CIT3 have significant antagonistic effects against common foodborne pathogens, extending the shelf life of fresh-cut apples [9].

### 3.3.3. Pears

The effectiveness of *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG (LGG) as probiotics against *Salmonella* and *L. monocytogenes* on fresh-cut pears at varying storage temperatures (5, 10, and 20 °C) was examined by Iglesias, Abadias et al. [98]. At 10 and 20 °C, LGG decreased the populations of *Salmonella* and *L. monocytogenes* by 2 and 3 log units, respectively. On the other hand, the pathogenic strains are not adversely affected by *Lb. acidophilus*. Regardless of the storage temperature, probiotic populations were kept between 107 and 108 CFU/g for the duration of the 10-day storage period. These findings imply that *L. monocytogenes* and *Salmonella* growth in fresh-cut pear wedges can be managed using LGG [98]. In a different study, Iglesias, Echeverría et al. [99]assessed the probiotic strain *Lb. rhamnosus* GG (LGG)'s antagonistic ability against a cocktail of 5 serovars of *Salmonella* and 5 *L. monocytogenes* serovars. During 9 days of refrigeration (5 °C), these were tested on fresh-cut pears under conditions similar to those used in commercial application. LGG maintained its survival in a changed atmosphere while controlling the growth of *L. monocytogenes*, resulting in a reduction of approximately 1.8 log-units in the pathogen population. *Salmonella*, however, showed no change. The quality parameters (soluble solids concentration and titratable acidity) were not substantially impacted by the application of LGG. However, the flavor perception of the product appears to be improved by the volatile compounds present in fresh-cut pear treated with LGG. According to a different study, LGG decreased *L. monocytogenes's* ability to adhere to and invade Caco-2 cells as well as its survival in the gastrointestinal tract [100]. These findings demonstrate that LGG alters *L. monocytogenes'* capacity for pathogenicity.

### 3.3.4. Oranges

Bacteriocin from the putative probiotic *Lactococcus lactis* AP2 was found to be stable at low temperatures for up to 72 hours and at acidic pH levels between 2 and 6. Because of these characteristics, it can be used to acidic foods as a bio-preservative. Purified bacteriocin outperformed sodium benzoate, a chemical preservative, in the study's ability to preserve orange and mixed fruit juice for more than 12 days at 4 °C. Additionally, it has been observed that the cell-free extract of *Lactobacillus lactis* AP2 inhibits the growth of *Shigella dysenteries, Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, and Bacillus cereus* [101].

### 3.3.5. Other fruits

Due to the higher content of ellagic acid, pomegranate juices fermented in monoculture with *Lactobacillus plantarum* POM1, *Lactobacillus plantarum* C2, *and Lactobacillus plantarum* LP09 were found to have improved antibacterial qualities. *Lactobacillus plantarum* LS5-fermented sweet lemon juice at 37 °C for 48 hours shown antibacterial activity against *S. Typhimurium* and *E. coli* O157:H7. Throughout the 28-day storage period at 4 °C, the probiotic juice's viability counts exceeded 107, and it also exhibited elevated pH, lactic acid, and antioxidant capacity [1].

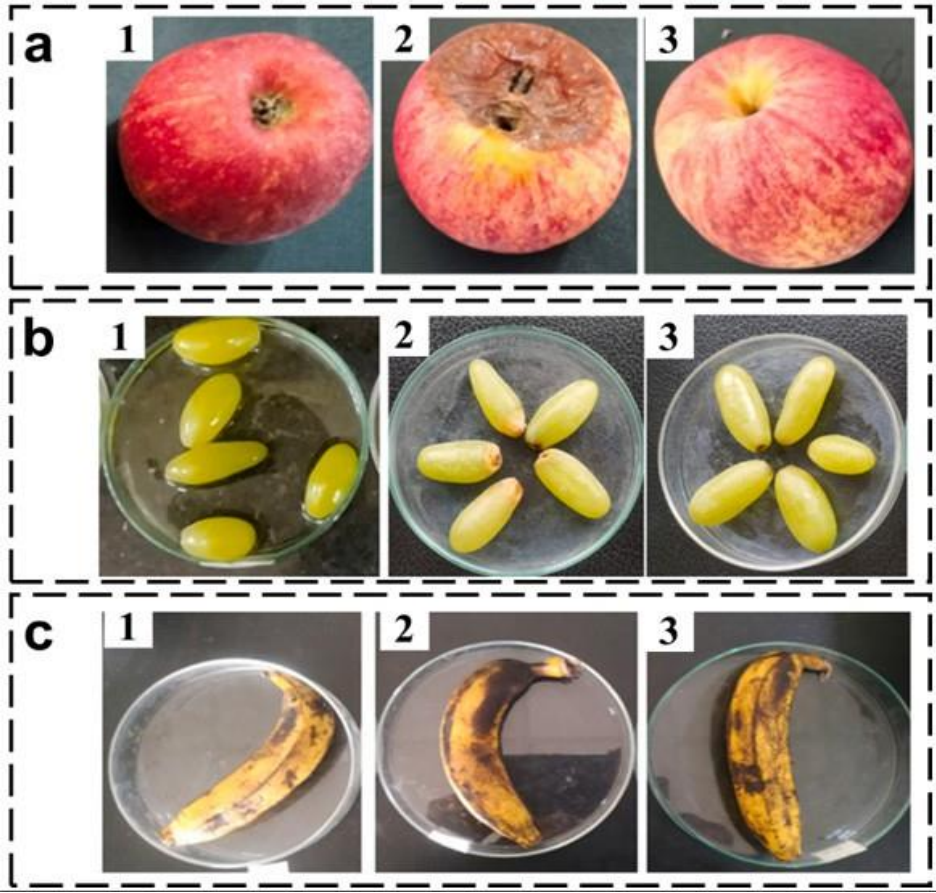


**Fig. 8.** Extending the shelf life of fruits with probiotics (*L. plantarum* DMR14). **a)** Apple (1) - 0 h, (2 & 3) - after 7 days without and with treatment**;** **b)** Grape (1) - 0 h, (2 & 3) - after 5 days without treatment and with; **c)** Banana (1) - 0 h, (2 & 3) - after 3 days without treatment and with. (Source **[102]**)

## 3.4. Vegetables

Post-harvest spoilage caused by fungi in tomatoes results in a greater economic loss for the food industry. In order to employ them as bio-preservatives for tomatoes inoculated with *Aspergillus flavus* and *Penicillium expansum*, Luz et al. [103] tested nine LAB strains isolated from tomatoes for antifungal activity against 33 fungal strains. The cell-free extracts of *Lactiplantibacillus plantarum* TR7 and *Lb. plantarum*

TR71 exhibited the strongest antifungal activity. The chemicals responsible for the antifungal activity were found to be volatile organic molecules, organic acids, and phenolic acids. When tomatoes were bio-preserved using the cell-free extracts of the two LAB strains mentioned above, the microbial counts dropped by 1.98–3.89 logs and 10 spores/g in comparison to the unfermented tomatoes [103].

Excellent adaptation to the minimally processed lamb's lettuce was demonstrated by *Lacticaseibacillus casei* V4B4 and *Lb. plantarum* V7B3, which inhibited pathogenic bacteria and controlled spoiling microorganisms. More significantly, the inclusion of these possible biocontrol agents had no effect on the products' color or appearance [94].

The application of *Pediococcus* spp. cell-free supernatant (15 mL/g) demonstrated improved button mushroom, corn, tomato, and strawberry preservation. The treated tomato and maize samples, for instance, stayed fresh for 13 and 20 days, respectively, while the untreated samples only lasted 6 days. Additionally, this study indicated that 100 g/L of *Pediococcus* spp. cell-free supernatant exhibits antibacterial activity against *Shigella* spp. and *E. coli.* More interestingly, as compared to the chemical preservatives sodium sulphate and sodium benzoate, the treatment with *Pediococcus* spp. showed improved preservation in these items. Food samples treated with *Pediococcus* species and their microbiological quality. Showed significantly less bacteria overall than those treated with chemical preservatives. These findings imply that *Pediococcus* species that produce bacteriocin extend the shelf life of some food types and can be employed as bio-preservatives [104].

## 3.5. Allied Applications

Cereal-based goods have been bio-preserved to improve their shelf life and sensory qualities. Customers' demands for high-quality, preservative-free foods can be met by using quinoa flour fermented with the antifungal *L. amylovorus* DSM19280 as a bio-preservative ingredient to produce gluten-free bread with enhanced nutritional value, bread quality, and safety due to its longer shelf life [105]. By using *Lactobacillus* species' antifungal properties, the shelf life of wheat bread was extended by six more days without mold when compared to the non-acidified bread [106]. Cereal-based fermented vegan goods were made using *L. plantarum* O21, which was collected from plants and successfully kept in a refrigerator for 21 days [107]. Recently, cereal-based probiotic biopreservatives were documented by Adesulu-Dahunsi et al. [108]. Bio-preservatives have an antifungal effect on wheat grains, as seen in Fig. 9.

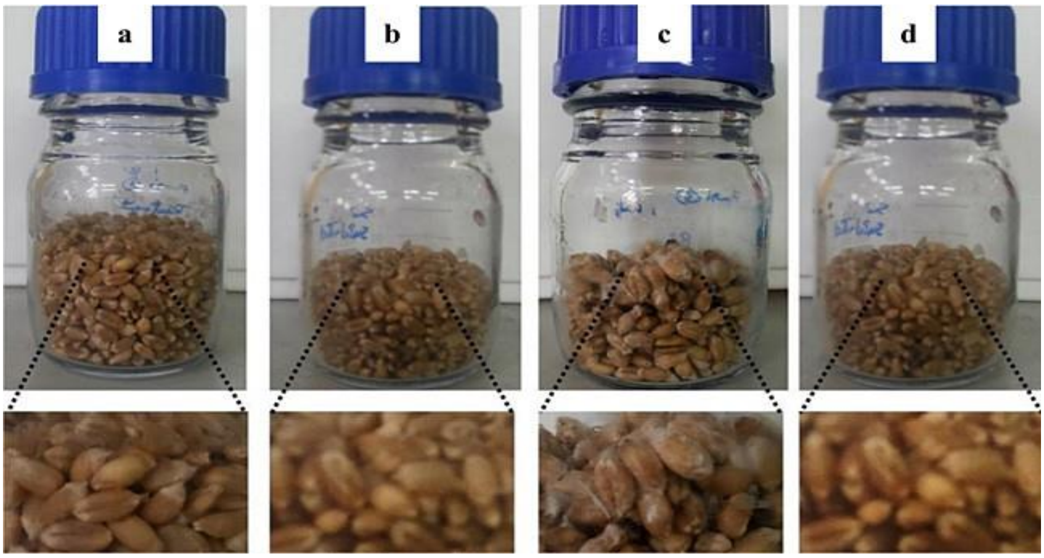


**Fig. 9.** Biopreservative on wheat grains (antifungal activity - *Lactobacillus* spp. RM1 supernatant against *A. parasiticus* after 15 days): **a)** Untreated control; **b)** Positive control treated with Lactobacillus spp. RM1 supernatant; **c)** Treated with *A. parasiticus* spores; **d)** Treated with *A. parasiticus* spores and *Lactobacillus* spp. RM1 supernatant. (Source [**109**])

Research has shown that bio-preservative probiotics have a wide range of uses in plant-based beverages, meats, and other goods, in addition to their traditional use in traditional foods. Udayakumar et al. (2022) extensively reviewed bio-preservative probiotics and their respective strains, shedding light on their diverse applications across different food categories [9]. Similarly, Verma et al. documented bacteriocins and their specific antibacterial application in several food industries [69].

## II. Postbiotics as Bio-preservatives

postbiotics have been used to prevent the proliferation of pathogenic microorganisms and their mediated corruption. Recent research suggests that postbiotics may be suitable replacement ingredients for the probiotic cells and also can be used as innovative antibacterial [110].

### 1. Production of postbiotics

The food that is most frequently used to provide probiotics is yoghurt. Probiotics are added to many items, both fermented (like cheese and yoghurt) and non-fermented (such cereal and chocolate bars). Probiotics may have difficulties surviving throughout manufacture and storage due to certain dietary properties, including acidity, water activity, particular chemical components, moisture, temperature, packaging permeability to oxygen, and duration. The majority of fermented milk's claimed health benefits have been confirmed. Probiotics, prebiotics, symbiotics, and postbiotics can also be found in infant formula [111, 112]. *Bifidobacterium breve* and *Streptococcus thermophilus* have been particularly used to make fermented infant formula. After the fermentation process, the bacteria are killed by spray drying. The newborn formula contains inanimate bacteria and fermentation byproducts. Several pediatric clinical studies have demonstrated its safety and postbiotic qualities, such as preventing thymus enlargement and alkaline stools in healthy-term infants, reducing the severity of severe diarrhea, improving immune markers and inflammatory responses, which may be linked to a few characteristics of gastrointestinal tolerance, and reducing digestive symptoms [113, 114, 115]. Considering that food supplements may have longer shelf lives than probiotic food supplements due to their lack of viability, this is a prospective market for the development of novel postbiotic products. The concept of postbiotics is likely to lead to an expansion in the variety of microorganisms utilized for functional purposes. Potential postbiotics have been investigated for species other than those from the family *Lactobacillaceae* or the generally benign genus *Bifidobacterium* that were not able to be managed live because of safety and health concerns [116, 117, 118]. Because postbiotics are stable across a broad temperature range, they can be added to foods and ingredients before heat processing without affecting their functionality. This could be advantageous to producers financially and technologically. When survival is not the primary determining factor, postbiotics can be used in drug carriers like food supplements and/or pharmaceutical goods since their proper dosage can be controlled during manufacture and storage conditions [119]. Several methods, such as sonication, enzymatic treatments, and chemical processes, are used in the creation of postbiotics. These techniques are essential for removing bioactive ingredients, altering microbial structures, and guaranteeing the durability and efficacy of postbiotics for a range of health-promoting uses. The desired postbiotic molecule, the microbial strain employed, the intended usage, and scalability considerations are some of the variables that influence the technique of postbiotic production. The best technique is carefully chosen by researchers and producers to optimize postbiotic product yield, quality, and safety [13]. Production of postbiotics through various techniques has been illustrated in Fig. 10.

Fermentation is the most prevalent postbiotic source in the food industry. Postbiotics are found naturally in a variety of milk-based products, as well as other foods like kefir, kombucha, yoghurt, and pickled vegetables. In addition to *Streptococcus, Akkermansiamuciniphila, Eubacateriumhallii, Faecalibacterium*, and *Saccharomyces boulardii*, the producer strains that can be used to extract the postbiotics in situ are mainly *Lactobacillus* and *Bifidobacterium* strains [120, 121, 122]. Numerous bacteriocins have also been identified, characterized, and may find use in industry in the future. Their extraction and characterisation will be influenced by the microbiological strains and growth conditions [13].

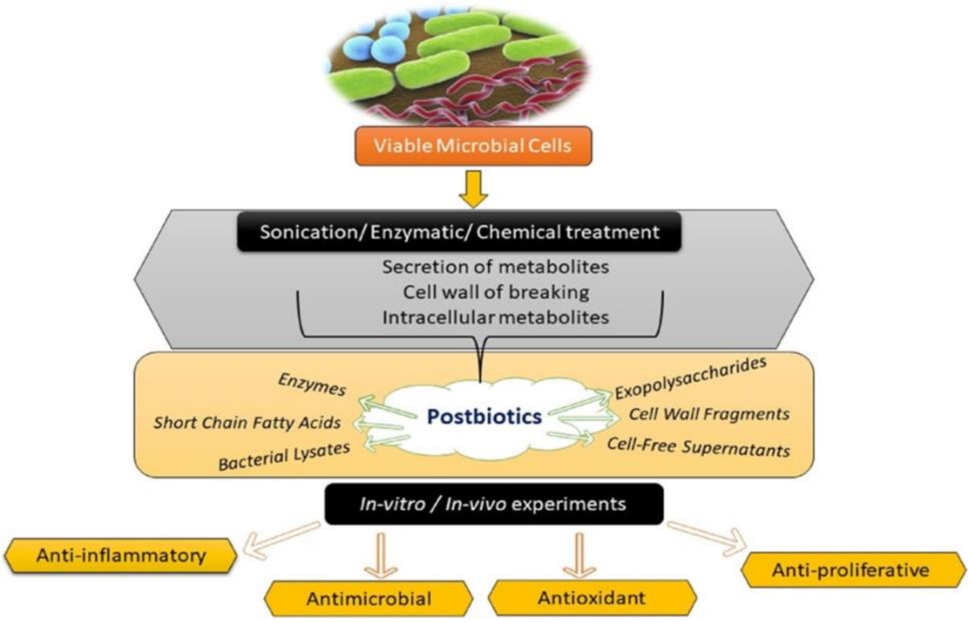


**Fig. 10.** Schematic representation for production of postbiotics through various techniques and its bioactivities. (Source [13])

## 2. Different classes of postbiotics and its bioactivities

The postbiotics can be classified based upon its chemical nature and its bioactivities. Various types of postbiotics and its role in food preservation have been discussed in this section [13].

### 2.1. Organic acids

One of the most important benefits of postbiotics in the food industry is their inhibition of food deterioration microorganisms. Organic acids are among the substances that can be used as antibacterial agents. Organic acids are known to be one of the key postbiotics. Bacterial fermentation produces two isomers of *lactic acid*, *L* and *D*, which effectively reduce pathogenicity. Acids such as citric and acetic acids also inhibit the development of infections by creating an acidic environment. By reducing pH levels in in vivo or in vitro conditions, organic acids such as acetic acids (pka =4.76) and *lactic acids* (pka = 3.86) inhibit the growth of infections. The inhibitory action of organic acids is linked to their effects on bacterial cell membranes. The main mechanisms at work here include preserving membrane integrity and lowering the pH inside the cell [123]. Energy generation and the antibacterial qualities of organic acids are related in two ways: they prevent or control the acidification of cellular cytoplasm. Three strains of *Lactobacillus plantarum* (P1, S11, and M7) produced organic acids (tartaric acid, acetic acid, lactic acid, citric acid, and malic acid), and Hu et al. (2019) [124] investigated the efficacy of these acids in killing *Salmonella* and *Escherichia coli*. They found that strains of *L. plantarum* secrete organic acids that inhibit the growth of pathogenic bacteria. By lowering their pH and acidifying their cell membranes, organic acids destroy microorganisms. Two organic acids with especially strong antibacterial properties are lactic and acetic acids. These results imply that new antibacterial agents for broad use in the food industry could be created by combining different organic acids [125, 126].

## 2.2. Fatty acids

Fatty acids and their derivatives can be used in place of antibiotics. Fatty acids have been known to have antibacterial qualities for more than a century. A hydrophilic carboxylic group is added to a saturated or unsaturated carbon chain to create fatty acids. Fatty acids are also recognized as potential postbiotics with strong antibacterial properties [127]. A long-chain fatty acid called Eicosapentaenoic acid (EPA) prevents Gram-positive bacteria from growing. Among other fatty acids, lauric and meristic acids have a potent inhibitory effect on the genesis and proliferation of microorganisms. By triggering cell lysis, increased membrane permeability, disruption of the electron transport chain, disruption of enzyme activity and structure, and production of functional/morphological changes in perceptive components such proteins, fatty acids have antimicrobial effects on bacteria. In 2020, Higashi and colleagues looked on the effect that fatty acids produced by *Lactobacillus acidophilus, Lactobacillus fermentum,* and *Lactobacillus paracasei* had on *Klebsiella oxytoca*. They found that the fatty acids in the probiotic bacteria lyse the cell membrane of *Klebsiella oxytoca*, stopping the bacterium from proliferating [128].

## 2.3. Hydroxyl radicals

Hydroxyl radicals, which have strong oxidative capabilities, can be produced from H2O2. The main metabolite of lactic acid bacteria, hydrogen peroxide, is produced by all bacteria but is usually seen in catalase-negative bacteria grown in aerobic conditions. Hydrogen peroxide (H2O2) concentration is the main determinant of the inhibitory and antibacterial effects, which can vary based on several factors. Numerous factors, such as specific bacterial strains and environmental factors (pH and temperature), can also affect bacterial concentration. H2O2's antibacterial characteristics are a result of its potent oxidizing capabilities, which harm bacterial cytoplasmic protein structures [129]. *Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus,* and *Lactobacillus rhamnosus breve* were tested for their ability to combat methicillin-resistant *Staphylococcus aureus* (MRSA) in vitro. According to the study, probiotic bacteria can produce hydrogen peroxide, which inhibits the growth of *Staphylococcus aureus*. These results suggest that postbiotics, such as hydrogen peroxide, can be used as a viable alternative to antibiotics in the fight against food deterioration and infections [122].

# 3. Applications of postbiotics in food biotechnology

It is not feasible to inexpensively embed a sizable amount of the preservative in the food's matrix since this would cause the food to become contaminated because microbiological deterioration, particularly mold growth, begins on the food's surface. One possible remedy for these problems has been proposed: using food packaging to extend the shelf life of food [130].

## 3.1. Removal of biofilm

There are numerous microorganisms that can be one or more different types and have varied growth rates. A biofilm is a complex community of microorganisms in a protein or carbohydrate matrix. Biofilms can be produced by microorganisms such as fungi and bacteria [131]. Gram-positive and gram-negative bacteria both possess these skills. Bacterial resistance to antibiotics during the biofilm phase is one of the main issues the world is currently dealing with. These include surface adhesion that is both reversible and irreversible, microclone formation with exopolysaccharide synthesis, and other production processes. Irreversible biofilms and colony components must be controlled for the food product business to guarantee food safety. When cleaning and disinfecting surfaces, biofilms produced by the food industry are harder to remove [132, 133]. *Yersinia enterocolitica, Listeria monocytogenes, Staphylococcus aureus,* and *Campylobacter jejuni* are some of the main bacteria that form biofilms. Numerous methods have been used to control and get rid of bacterial biofilms. Using postbiotics is an inventive way to get rid of biofilms. Recent research has shown that postbiotics are effective in breaking down bacterial biofilms [**132**, **133**]. In one investigation, a biofilm formed on polystyrene surfaces by *Lactobacillus monocytogenes* was cured by probiotic bacteria *Lactobacillus casei* 431, *Lactobacillus acidophilus* LA5, and *Lactobacillus salivarius*. Postbiotics have been found to prevent biofilm formation. The authors demonstrated that the main cause of the decrease in *L. monocytogenes* biofilm was the absence of postbiotics that contained bacteriocin and organic acids. In the food product business, postbiotics are a practical way to stop bacterial biofilm from growing [134, 135].

## 3.2. Development of active food packaging

One of the most creative approaches to food packaging is "active packaging," which adapts to shifting consumer tastes and market trends [136]. Flavors, odors, antimicrobials, antioxidants, moisture, ethylene, carbon dioxide, and substances that emit CO2 are the main targets of active packaging methods. A number of factors, such as the food product itself, the type of packaging, and other environmental factors, influence how long food lasts on the shelf. By including antimicrobial agents (AAs) of plant, animal, and microbiological origin, or their metabolites, antimicrobial nanoparticles, etc., in the packaging, an active packaging system called "antimicrobial active packaging" shields food from microbial decomposition during storage and transportation [86]. The antimicrobial efficacy cannot be precisely predicted because of a number of environmental conditions, including temperature, relative humidity, light intensity, and the quantity of moisture in food, that can impact the survival of probiotics in bioactive packaging and the generation of antimicrobial compounds. Furthermore, the thermal, barrier, and mechanical properties of the packaging material can be changed by the consumption of bacterial cells. These factors allow postbiotics to be used in antimicrobial packaging techniques that employ live bacteria [13].

## III. Bacteriocin as Bio- preservatives

*Archaebacteria* and *Eubacteria* are among the many bacteria that create bacteriocins, which are antimicrobial peptides or proteins. For thousands of years, people have used bacteriocins in fermented foods due to their strong antibacterial qualities. Bacteriocins are categorized based on their size, inhibitory activity spectrum, and mode of action. Among the many beneficial qualities that bacteriocins provide are their resistance to heat and pH fluctuations as well as their capacity to stop the onset and spread of gastrointestinal infections. Important elements of bacteriocins' antibacterial mechanism include their effects on the morphological and functional characteristics of bacterial peptides, their prevention of spore formation, and their pore generation on pathogenic cell membranes [137, 138]. In a study, Wang et al. (2018a, b) [139, 140] used bacteriocins from fish-isolated *Lactobacillus plantarum* LPL-1 against *Listeria monocytogenes*. Consequently, it was found that the bacteriocins may inhibit the growth of *L. monocytogenes* by acidifying its cell membrane and creating holes in the bacterial membrane. In a separate study [138] and his associates investigated the efficacy of bacteriocins generated by *Lactobacillus taiwanensis* against *Salmonella gallinarum* and *E. coli*. Consequently, it was found that the bacteriocin of *L. taiwanensis* may lyse the membranes of pathogenic bacteria, destroying their protein structures and impeding their growth. According to the results of the aforementioned investigations, bacteriocins can be used as a method to lessen the germs that can cause food to spoil [138].

### 1. Classification of Bacteriocins

Numerous characteristics, including molecular weight, antibacterial spectrum, mode of action, thermal stability, amino acid composition, and posttranslational changes, are used to categorize bacteriocins [141]. Furthermore, based on the structure of their cell walls, bacteriocins can be divided into two primary groups: those produced by Gram-positive bacteria and those produced by Gram-negative bacteria [142].

## 1.1. Gram-Positive Bacteriocin Producers and Their Bacteriocins

Based on factors like size, biosynthetic techniques, biological activities, and structural characteristics, bacteriocins generated by Gram-positive bacteria are categorized into four major types Fig. 11. [142]

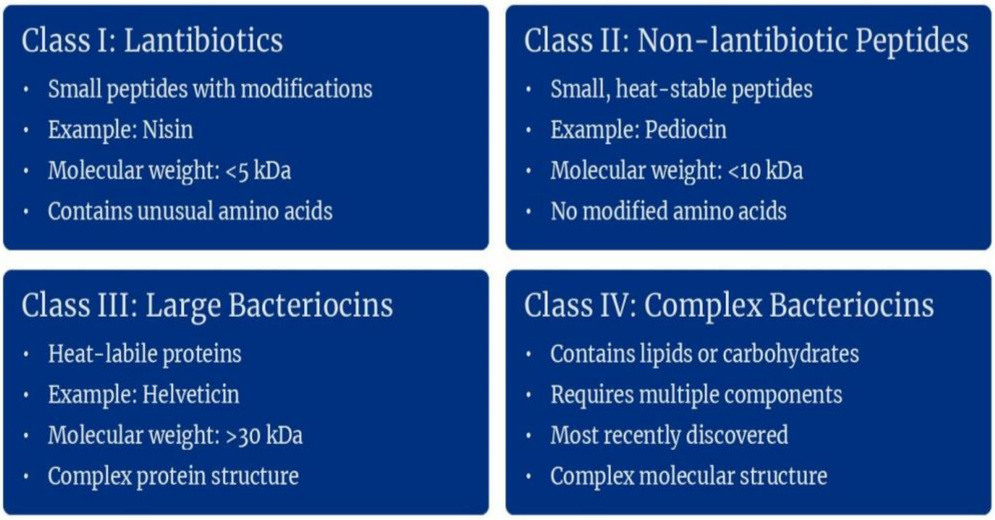


**Fig. 11.** Classification and prominent features of bacteriocins produced by Gram-positive bacteria. (Source **[142]**)

## 1.2. Gram-Negative Bacteriocin Producers and Their Bacteriocins

Bacteriocins are mostly produced by Gram-positive bacteria, mainly LAB, but some Gram-negative bacteria can also produce bacteriocins. *E. coli* produces colicins and microcins, which are gram-negative bacteriocins. Moreover, colicin and bacteriocins that resemble microcins are also seen (Fig.12) [143].

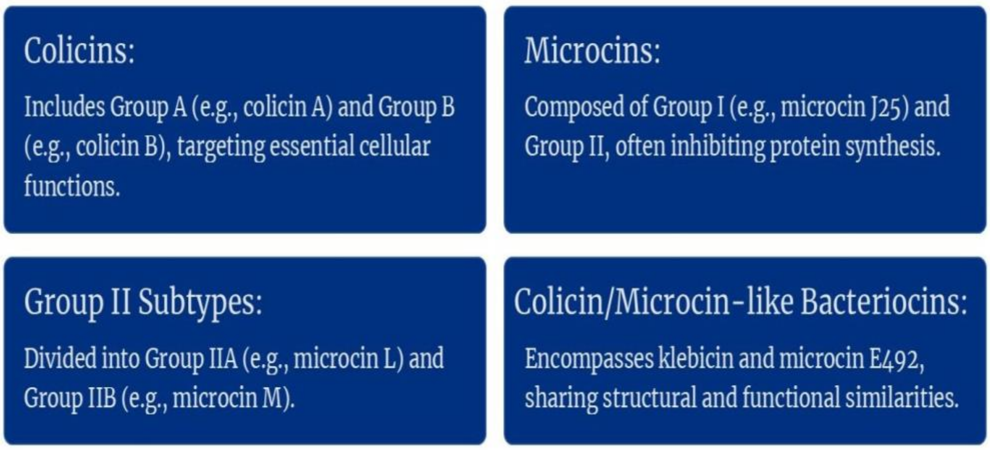


**Fig. 12.** Classification and prominent features of bacteriocins produced by Gram-negative bacteria. (Source **[143]**)

## 2. Nature of bacteriocin

The majority of bacterial lineages produce these proteins, which are essential for identification and have a corresponding immune system for both host and self-defense against infections. Depending on their type and environmental adaptability, the majority of bacteriocin-producing bacteria come from a variety of sources. Because of their ability to adapt, one microbe outperformed the other in the product they produced. Gram negative bacteria, gram positive bacteria, and archaea are the three basic categories based on these [25].

During the stationary phase, the Archaea produce a unique family of antimicrobial peptides called archaeocins that resemble bacteriocins [144]. By secreting archaeocins, the producer strain lyses the target cells and lessens competition in the surrounding environment. Bacteriocins, on the other hand, are first isolated from Gram-negative bacteria. The bacteriocin family was first discovered by a colicin from *E. coli* that was found to be an antibacterial protein. This colicin dominated many relevant investigations until recently [145]. The fact that many species of Gram-negative bacteria are capable of producing colicin-like proteins limits the number of bacteriocin-producing strains in addition to *E. coli.* Important sample examples of bacteriocins of other Gram-negative bacteria are the pyocins of *Pseudomonads*, the cloacins of *Enterobacter cloacae*, the alveicins of *Hafniaalvei*, the marcescins of *Serratiamarcescens*, and the klebicins of *Klebsiella pneumonia* [146].

Due to their relative size, the majority of bacteriocins in this group are heat-labile peptides. Microcins like *E. coli's* microcin V are an exception to this rule. It has the characteristic of being heat stable and having very few peptides. The primary drawback of Gram-negative bacteria's bacteriocins that restricts their industrial-scale applications is their limited range of antibacterial action. This characteristic highlights the more appropriate bacteriocin types that are generated by Gram-positive bacteria [146].

Numerous bacteriocins are also produced by gram-positive bacteria. Gram-positive bacteriocins are a special helpful tool for many industrial and therapeutic uses because of their much wider inhibitory spectrum and non-toxic properties on eukaryotic cells. Because of its GRAS (generally regarded as safe) potential for human ingestion, lactic acid bacteria (LAB), a collection of phylogenetically diverse Gram positive bacteria with some similar morphological, metabolic, and physiological features, have garnered a lot of attention in this regard [147]. LAB is known as "lactic acid bacteria" because of the lactic acid that is produced during their fermentation process. At least 50% of the carbon in sugars is transformed into two isomers of lactic acid by a LAB member throughout this process. Based on numerous physiological and morphological characteristics, this group of bacteria exhibits a wide range of diversity. Cocci, bacilli, or coccobacilli are Gram-positive bacterial strains having a variety of physiological traits that can be members of LAB. These are very important in food and medicine because of their safe nature and beneficial metabolic products (such bacteriocins, diacetyl, acetoin, hydrogen peroxide, reuterin, reutericyclin, organic acids, and antifungal peptides). The majority of bacteriocins can be extracted from meat, dairy, and vegetable products; some can also be extracted from fish products [25].

## 3. Application of Bacteriocin In food industry

Food pathogens, which lead to food degradation and ultimately illness from food borne diseases, are among the most worrying problems in the food sector [148]. Bacteriocins' ability to be used in a variety of technological applications is largely dependent on their antibacterial properties. In this sense, the rapid rise and dissemination of germs that are resistant to several drugs highlights the significance of research investigations aimed at identifying infections, alternate strategies for preventing This makes bacteriocins with broad-scale antibacterial action potentially useful natural antimicrobials for a variety of industrial uses [149].

Technology is still in its infancy when it comes to protecting food from microbial contamination, which has led to significant financial losses and unfavorable health outcomes for people. Nevertheless, more recent research indicates that bacteriocins are mostly employed in the food business and are a biological defense against food rotting [150]. Lactic acid bacteria create these bacteriocins, which have a lot of promise to satisfy this need in the food industry. Lactic acid bacteria create bacteriocin, which is widely acknowledged to be a harmless chemical that is inert and non-toxic to eukaryotic cells when used in food preservation. They have a broad antibacterial spectrum against numerous food-borne pathogenic and spoilage bacteria, are inactivated by digestive proteases, have minimal effect on gut microbiota, and are resistant to heat and pH [151]. They also exhibit a bactericidal mode of action, which typically targets the bacterial cytoplasm membrane. They are also capable of genetic manipulation and do not exhibit antibiotic cross-resistance. Lactic acid bacteria have a preservation effect because their metabolic activity produces lactic acid, acetic acid, hydrogen peroxide, and bacteriocin [152]. These characteristics of bacteriocins can be used in the food industry to extend food shelf life, provide extra protection against temperature abuse, lower the risk of food-borne pathogens propagating through the food chain, decrease the need for chemical preservatives, and lessen the financial losses brought on by food spoilage [153]. They also allow for the application of less harsh heat treatments without sacrificing food safety: improved preservation of food nutrients and vitamins, as well as Foods' organoleptic qualities allows for the promotion of new food varieties [154]. Bacteriocins serve a variety of purposes in the food sector. These have dominated food safety and preservation and are found in dairy products, meat and poultry items, fish and marine foods, vegetables, and beverages [155, 156]. Commercially accessible bacteriocin preparations for food applications include nisin and pedocin, and their applications are steadily expanding [157]. Many experts believe that the most well-known Class I bacteriocin, Nisin (marketed as Nisapline), is recognized globally as a biopreservative in specific industrial applications [158].

## 4. Application of bacteriocin in canned food products Alcoholic beverages

Due to the fact that yeasts cannot use nisin, it can be used to keep wine or beer from spoiling. It can keep working during fermentation without affecting the brewing yeast strains' flavor, growth, or fermentative efficiency. Therefore, it could be utilized to decrease the time-temperature combination of pasteurization and increase the beer's shelf life [158]. Nisin may also be used to lower the quantity of sulfur dioxide needed for wine production in order to prevent bacterial deterioration [159].

## 5. Application of bacteriocin in seafood products

Bacteriocin use in seafood products: Because Gram-positive and Gram-negative bacteria are typically thought to be safe, producers of LAB in food processing are particularly interested in this application. The use of several bacteriocins made by lactic acid bacteria in food has been authorized. The only bacteriocins now in use in commerce are Pediocin PA-1 and the nisin group produced by *Pediococcus acidilactici* [160]. Three approaches have typically been used when using bacteriocins for food bio-preservation [161]. LAB is injected into food, resulting in the production of bacteriocin. For the products to be used properly, the LAB must be able to produce bacteria and produce bacteriocin. Using bacteriocins, either naturally occurring or artificially generated, as a food preservative. Incorporating bacteriocin as a food additive into a product that has undergone fermentation using a strain that can produce it [31].

## 6. Application of Bacteriocin in fish products

Degradation of fresh fish, even if vacuum-packed fresh fish and shellfish might occasionally have problems due to pathogenic species like *Clostridium botulinum* and *Listeria monocytogenes*. Furthermore, *L. monocytogenes* implanted into frozen thawed salmon was inhibited in its growth, the quantity of all aerobic bacteria was reduced, and the shelf life of fresh chilled salmon was increased by Nisin and Microgard [162]. Later, the study demonstrated the noteworthy effects of lactic acid, sodium chloride, and/or nisin in rainbow trout, while demonstrated the influence of LAB cultures on the control of fish pathogenic bacteria [163]. Salmon that has been cold-smoked with *L. sake* or *C. divergens* is protected against listeria [164].

## Conclusion

The growing consumer demand for minimally processed, natural, and safe food products has highlighted the importance of biopreservation techniques as a sustainable alternative to synthetic chemical preservatives. This shift is particularly relevant in the plant-based and seafood industries, where probiotics and bacteriocins are emerging as promising solutions for extending shelf life and improving food safety. Probiotics have been widely studied for their applications in the dairy and non-dairy food sectors, with increasing research focusing on their role in food safety and preservation. They not only inhibit the growth of pathogens but also provide functional health benefits to consumers. In seafood, LAB-derived antimicrobial compounds, including bacteriocins and extracellular polymeric substances (EPS), have demonstrated effectiveness in controlling spoilage and pathogenic bacteria. Furthermore, LAB strains produce antioxidants that neutralize free radicals, adding an extra layer of health benefits. Despite their advantages, bacteriocins, such as nisin, face certain challenges, including limited activity spectrum, instability at high temperatures, and susceptibility to enzymatic degradation. Overcoming these limitations through innovative approaches, such as combining probiotics with non-thermal preservation technologies, could revolutionize food preservation. Future research should focus on developing probiotic-based bio-preservation systems that are more stable, efficient, and environmentally friendly, offering a sustainable alternative to traditional preservatives while ensuring food quality and safety.

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