

Research Article

Lactic Acid Bacteria as Biological Food Preservatives: Genomic Insights and Applications

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Abstract

Lactic acid bacteria (LAB), a group of Gram-positive microorganisms, are recognized as biological agents for food preservation and pathogen management due to their ability to suppress spoilage and pathogenic microbes. These bacteria produce a variety of antimicrobial chemicals, including antifungal and antibacterial agents, which effectively suppress the growth of harmful pathogens and enhance food safety. This review aims to explore the functional capabilities of LAB as bio-control agents and biological food preservatives, with a focus on their taxonomy and diverse applications in the food and health industries. The utilization of LAB as biological preservation agents is especially advantageous due to their Generally Recognized as Safe (GRAS) classification, which makes them a safer option to standard chemical preservatives. LAB provides a biological control strategy that uses living organisms to manage infections and prevent food spoilage. Their methods of action include hyper-parasitism, predation, antibiotic synthesis, lytic enzyme activity, and generation of host resistance. The potential and applications of LAB go beyond food preservation; they also play an important role in the prevention of infectious diseases due to their antibacterial properties. LAB provides an environmentally friendly and effective alternative to chemical preservatives. Further research on the optimization of LAB strains for specific applications and improved genetic engineering could expand their industrial and therapeutic roles. By emphasizing the benefits of LAB in enhancing food safety and quality, this paper advocates for their integration into contemporary food preservation practices as a sustainable alternative to chemical preservatives.

Keywords

Biological Food Preservation, Biocontrol Agents, Lactic Acid Bacteria, Food-borne Pathogens, Food Spoilage Bacteria

1. Introduction

Lactic acid bacteria (LAB) are a group of Gram-positive microorganisms characterized by their cocci or rod shapes, catalase-negative properties, and non-spore-forming nature. These fastidious organisms exhibit a high tolerance for low

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pH levels [1, 2]. LABs are widely recognized for their fermentation capabilities, which not only enhance food safety but also improve the nutritional value of various foods, providing numerous health benefits for humans [3]. Historically, LAB have played a vital role in food preservation by inhibiting the growth of pathogenic and spoilage microorganisms. Commonly used in food fermentation processes they produce a range of metabolites that contribute to their beneficial health effects [4]. Additionally, LAB are classified as GRAS, making them a favorable choice for food applications. These microorganisms are also known for their potential as sources of antimicrobial agents and are frequently employed as biological control agents [5]. LAB produces both antibacterial [6] and antifungal compounds [7]. Their biological control strategies include inhibiting the growth of pathogenic microorganisms, colonizing the gastrointestinal tract, and employing mechanisms such as bacteriocins, organic acids, and hydrogen peroxide.

Moreover, LAB have potential applications as antidiabetic agents, cholesterol-reducing agents, immune system modulators, drug-delivery vehicles, and in promoting mental and emotional well-being [8]. Genomic analysis of LAB has progressed significantly with the availability of sequenced genomes, offering more profound insights into their evolutionary divergence. Studies reveal an ongoing trend of genome size reduction among LAB [9]. Diversity LAB are ubiquitous, found in various natural environments such as dairy (fermented), meat, and vegetable sources, as well as in the gastrointestinal and urogenital tracts of humans and animals, soil, and water [10, 11]. The potential of LAB as biological catalysts for the production of various organic compounds in both food and non-food sectors lies in their diversity, metabolic and stress tolerance features, and the use of genetic and metabolic engineering tools to enhance their capabilities. LAB's growth characteristics, nutritional requirements, metabolism, and production of antimicrobial compounds are crucial for inhibiting the growth of pathogens and spoilage organisms, thus contributing to the production of fermented foods [12].

Some LAB strains go beyond acid production, synthesizing specific antimicrobial proteins like bacteriocins, which act against harmful bacteria while preserving a favorable microbiome within food systems [13]. Despite LAB's efficacy, challenges remain in optimizing their application as bio-preservatives. A significant issue is the variability in preservation capacity among LAB strains, which often display differences in antimicrobial production and effectiveness under varying environmental conditions. For instance, the bacteriocins produced by LAB may need specific conditions for stability and efficacy, limiting their use in certain food types [14]. Additionally, while many LAB strains have GRAS status, their genetic variability and adaptability to environmental pressures present challenges for standardized applications across different food systems [15]. Therefore, the objectives of this study are to investigate the functional capa-

bilities of LAB as biological food preservatives and bio-control agents by examining their antimicrobial properties against foodborne pathogens in various food matrices, assessing the stability and efficacy of bacteriocin and organic acid production under diverse storage conditions.

2. Diversity of LAB: Their Classification, Distribution, and Sources

2.1. Classification and Physiology of LAB

LABs are classified within the phyla *Firmicutes* and *Actinobacteria*, class *Bacilli*, and orders including *Atopobium*, *Bifidobacterium*, and *Lactobacillales*. Their classification can be further refined based on cellular morphology, glucose fermentation patterns, growth temperature ranges, and sugar utilization [90]. Techniques such as 16S rRNA gene sequencing also enhance LAB classification [16]. Key genera in this group include *Aerococcus*, *Alloiococcus*, *Bifidobacterium*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* [17].

Among these, *Lactobacillus* is the most prevalent genus, comprising over 100 species primarily found in carbohydrate-rich environments [18]. Most *Lactobacillus* species are isolated from the gastrointestinal tracts of humans and animals, with significant numbers also originating from vegetables and their fermented products [19]. In contrast, *Leuconostoc* species are commonly found in chilled meats and clinical samples, as well as in plant material and fermented dairy products [20]. *Pediococcus* species are typically associated with spoilage in fermented beverages like beer, while *Lactococcus* species are predominantly found in dairy products, with fewer reports in fermented plant materials [21]. *Lactobacillus* species are facultative anaerobes, Gram-positive, non-motile, and non-sporulating, exhibiting tolerance to acidic conditions. They can be classified as either homofermentative or heterofermentative [20]. Due to their health benefits, certain LABs are used as probiotics, biological food preservatives, and biocontrol agents against foodborne pathogens. Probiotics are live microorganisms, such as bacteria or yeast, that support human or animal health and are found in dietary supplements and fermented foods like yogurt [22]. For an organism to qualify as a probiotic, it must be non-pathogenic, classified as GRAS, tolerant of low pH and high bile salt concentrations, and accepted by the immune system without triggering antibody formation [23]. Additionally, probiotics should not transfer antibiotic resistance genes to pathogens through horizontal gene transfer.

LABs also serve as biocontrol agents due to their preservative capabilities, which stem from their production of various broad-spectrum antimicrobial compounds. Most LABs have GRAS status, playing a vital role in food fermentations that

contribute to both desirable sensory properties and microbiological safety [22]. The antimicrobial effects of LAB are primarily due to their production of lactic acid, acetic acid, and other organic acids, as well as compounds like hydrogen peroxide, diacetyl, ethanol, phenolic substances, and proteinaceous materials. Some LAB strains can also produce bacteriocins, which have garnered interest as a novel method for controlling foodborne pathogens [24]. Utilizing natural and microbiological compounds to prevent food spoilage presents a promising solution to economic losses from microbial contamination and helps reduce foodborne illnesses [25].

2.2. LAB: Distribution and Sources

LABs are prevalent and constitute a significant portion of the microbial community in milk and dairy products. Various LAB species play essential roles in the daily production of these items [26]. In subtropical regions, traditional fermented products are mainly associated with thermophilic LAB, whereas Western and Northern European countries tend to favor products that utilize mesophilic bacteria [27]. LAB can be categorized based on the fermentation products generated from glucose. Homofermentative LABs, such as *Lactococcus*, *Pediococcus*, and *Streptococcus*, exclusively produce lactic acid from glucose fermentation. Conversely, heterofermentative LAB, including *Leuconostoc* and *Weissella*, generate equal amounts of lactate, carbon dioxide, and ethanol during glucose fermentation [28]. LAB has played a crucial role in food fermentation for millennia, marking one of the oldest techniques for food preservation. The use of LAB for fermenting milk products dates back to around 6,000 BC, with applications in meat fermentation beginning around 1,500 BC, and vegetable fermentation emerging around 300 BC [29].

These bacteria are commonly found in various environments, including decomposing plant matter, fruits, dairy items, fermented meats and fish, cereals, beets, pickled vegetables, potatoes, sourdough, silages, and beverages. They can also be present in juices, sewage, and within the cavities of humans and animals [30]. In humans, LAB primarily colonizes the oral cavity, ileum, and colon, and they are the predominant microbial residents of the vagina. Specific LAB species are linked to the mouth, intestines, and vagina in mammals, while others are associated with fermented seafood, such as Plaasom, a traditional Thai fermented fish [31].

2.3. Biological Food Preservation and Biocontrol Agents

2.3.1. LAB as a Biological Food Preservative

Bio-preservation extends food safety and shelf life using living microorganisms, notably LAB, and their metabolites. LAB's preservation capability stems from their production of hydrogen peroxide, diacetyl, organic acids (lactic, acetic, and propionic), lactis, nisin, enterocins, carbon dioxide, ethanol,

diacetyl, acetaldehyde, and acetoin, antifungal compounds, pediocin, bacteriocins, and antibiotics like reuterin [32] (Table 1). Their antimicrobial mechanisms involve multiple metabolites produced during fermentation, with organic acids such as lactic, acetic, and propionic acids inhibiting microbiota by affecting transport processes and membrane potential. The effectiveness of LAB in preservation is largely due to their production of organic acids that lower the pH of food, enhancing shelf life and safety by quickly inhibiting pathogens [33].

1) Lactic Acid

Lactic acid, produced by LAB, has a long-standing role in the food industry. Found naturally in many fermented foods like yogurt and sauerkraut, it serves as an acidulant, flavoring agent, and spoilage inhibitor in various products [34]. Its inhibitory mechanism involves the non-dissociated form penetrating the cytoplasmic membrane, leading to cytoplasmic acidification and energy depletion for bacterial growth [35]. LAB comprises around 20 genera, including *Lactobacillus* and *Streptococcus* [36].

2) Acetic Acid

Acetic acid, present in vinegar, inhibits the growth of bacteria, yeasts, and fungi, demonstrating bacteriostatic effects at 0.2% and bactericidal activity above 0.3%, particularly at low pH levels [37]. Acetic acid produced by heterofermentative LAB enhances aroma and prevents spoilage in sourdough. Studies have shown that acetate can improve intestinal defense against pathogens like enterohemorrhagic *E. coli* O157 [38].

3) Hydrogen Peroxide (H₂O₂)

LAB production of hydrogen peroxide inhibits foodborne pathogens and supports preservation, especially at refrigeration temperatures. However, it can negatively affect the organoleptic properties of meat products. LAB strains like *Lactobacillus sakei* and *Pediococcus acidilactici* exhibit catalase activity, helping mitigate rancidity [39]. The bactericidal effect of H₂O₂ is influenced by concentration, pH, and temperature.

4) Bacteriocins

Bacteriocins are biologically active peptides or proteins with antimicrobial properties, primarily effective against closely related bacterial species. Often cationic and amphiphilic, they can permeabilize membranes. In the food industry, bacteriocins reduce the need for chemical preservatives and lower the intensity of heat treatments. They are typically combined with traditional preservation methods, enhancing the sensory and nutritional qualities of food [39]. The production of bacteriocins is a dynamic process that contributes to food preservation through ongoing interactions [25]. To maximize their use, it's vital to study bacteriocin-producing LAB strains suited for specific food environments. These strains can serve as starter, adjunct, or protective cultures, generating enough bacteriocins during processing to inhibit pathogens [40].

5) Enterocins

Bacteriocin-producing *Enterococcus* strains are commonly isolated from various food sources, including sausage, fish, vegetables, and dairy products like cheese. Besides enhancing the unique flavors of these foods, *Enterococcus* species serve as protective agents against pathogens such as *L. monocytogenes*, frequently found in meat and dairy [41]. Enterocins exhibit antimicrobial properties that make them valuable for food preservation, effectively controlling pathogens like *L. monocytogenes* [42]. These bacteriocins are effective against several foodborne pathogens, including Gram-negative bacteria [43].

6) Reutericyclin

Reutericyclin is the first known low-molecular-weight antibiotic produced by LAB. Research indicates that both Gram-positive and Gram-negative bacteria, as well as various fungi and yeasts, are sensitive to reutericyclin, which targets cellular structures such as the cytoplasmic membrane [44]. Its mechanism of action is similar to weak organic acids, including acetic and sorbic acid. Reutericyclin can be introduced into food or pharmaceutical products through either the purified compound or metabolically inactive cells [44].

7) Pediocin

Pediocin is a group of cationic, membrane-permeabilizing peptides with 37-48 residues produced by LAB, specifically classified as class IIa bacteriocins with low molecular weight. Produced by *Pediococcus acidilactici*, pediocin is commercially available as a fermentative powder and is effective against numerous foodborne pathogens, making it a potential food preservative [45]. Initially used in meat products, pediocin is now applied in dairy due to its stability in aqueous solutions, positioning it as an attractive option for dairy applications [25].

8) Lactis

Research indicates that lactis has broad antimicrobial activity suitable for various food applications [46]. Specifically, *Lactococcus lactis* sub-species lactis INIA 639 produces lactis in 481, which plays a role in releasing intracellular esterases

from *L. helveticus* strains during cheese ripening. This lysis is essential for esterases to access their substrates. Certain adjunct cultures can enhance lipolysis, resulting in the release of volatile free fatty acids (FFA) depending on cheese aging [47]. Unlike many LAB bacteriocins, lactis Z maintains its antimicrobial activity under alkaline conditions and at temperatures up to 100 °C.

9) Diacetyl, Acetaldehyde, and Acetoin

Heterofermentative LAB generate acetaldehyde through pyruvate decarboxylation, which then condenses to form α -acetolactate, later converted into diacetyl and then reduced to acetoin [45, 48]. Diacetyl imparts a buttery flavor to fermented dairy products, but its high concentrations for preservation limit its use. Acetaldehyde, usually present in lower concentrations, also helps control microbial contaminants alongside other LAB metabolites [49].

10) Carbon Dioxide

Carbon dioxide aids in preservation by exerting antimicrobial effects and creating anaerobic environments that replace molecular oxygen. Its antifungal activity arises from inhibiting enzymatic decarboxylation and accumulating within the lipid bilayer of membranes, disrupting permeability [50].

11) Nisin

Nisin is a versatile bacteriocin utilized in various food products, including liquids, solids, and canned goods. It can be added during processing as a solution or powder. For example, it is often incorporated into heated cheese during processing [51]. The concentration of nisin varies based on the food type, heat treatment severity, storage conditions, and desired shelf life. Nisin is effective in acidic foods (pH 3.5-8.0) and can be used in liquid egg products, pasteurization of milk, aged cheeses, and canned vegetables [44]. Its effectiveness is enhanced when combined with other preservation methods, such as lysozyme, lactates, or even bacteriophages, particularly against pathogens like *L. monocytogenes* [51].

Table 1. Groups of LAB Species Employed for Production and Their Targeted Pathogens.

S. N	LAB Groups/Species	Products	Targeted pathogens due to specific Bacteriocin	Reference
1	<i>Enterococcus faecium</i>	Enterocin X	<i>L. monocytogenes</i>	[53]
2	<i>Lactobacillus bulgaricus</i>	Carbon Dioxide	<i>Bacillus subtilis</i>	[54]
3	<i>Lactobacillus sakei</i>	Nisin	<i>Staphylococcus aureus</i>	[55]
4	<i>Lactococcus lactis</i> sub spp. <i>Cremoris</i>	Nisin	<i>L. monocytogenes</i> and <i>S. aureus</i>	[55, 56]
5	<i>Staphylococcus aureus</i>	Aureocin	<i>Bovine mastitis</i>	[56]
6	<i>Staphylococcus simulans</i>	Lysostaphin	<i>S. aureus</i>	[58]
7	<i>Carnobacterium maltaromaticum</i> C2	Carnobacteriocin X	<i>L. monocytogenes</i>	[59]
8	<i>Propionibacterium freudenreichii</i>	Propionic Acid	<i>L. monocytogenes</i> , <i>Clostridium</i> spp.	[53]
9	<i>Lactobacillus acidophilus</i>	Hydrogen Peroxide	<i>Bacillus subtilis</i> , <i>Salmonella</i> spp.	[60]

S. N	LAB Groups/Species	Products	Targeted pathogens due to specific Bacteriocin	Reference
10	<i>Lactobacillus sakei</i>	Sakacin P	<i>Listeria monocytogenes</i>	[61]
11	<i>Leuconostoc mesenteroides</i>	Diacetyl	<i>Listeria monocytogenes</i>	[59]
12	<i>Lactobacillus casei</i>	Lactic Acid	<i>Salmonella typhimurium</i>	[54]
13	<i>Lactobacillus brevis</i>	Acetic Acid	<i>E. coli</i> , <i>Bacillus cereus</i>	[55]
14	<i>Streptococcus thermophilus</i>	Hydrogen Peroxide	<i>Pseudomonas aeruginosa</i>	[54]
15	<i>Enterococcus hirae</i>	Enterocin P	<i>S. aureus</i> , <i>Salmonella</i> spp.	[53]
16	<i>Lactococcus lactis</i>	Carbon Dioxide	<i>S. aureus</i> , <i>Listeria monocytogenes</i>	[57]
17	<i>Lactobacillus delbrueckii</i>	Hydrogen Peroxide	<i>Staphylococcus aureus</i> , <i>E. coli</i>	[62]
18	<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	Nisin	<i>Bacillus cereus</i>	[63]
19	<i>Saccharomyces cerevisiae</i>	Ethanol	Not pathogen-specific; fermentation applications	[64]
20	<i>Bifidobacterium bifidum</i> NCFB 1454	Bifidocin B	<i>Bacillus</i> , <i>Enterococcus</i> , <i>Lactobacillus</i> , <i>Leuconostoc</i> , <i>Listeria</i> , and <i>Pediococcus</i>	[63]
21	<i>Lactobacillus reuteri</i> TMW 1.656	Reutericyclin	<i>Escherichia coli</i> , <i>Salmonella enterica</i>	[65]
22	<i>Enterococcus faecium</i>	Enterocin A	<i>Listeria monocytogenes</i>	[66]
23	<i>Pediococcus pentosaceus</i> ATCC 43200	Pediocin AcH	<i>Listeria innocua</i> , <i>Bacillus cereus</i>	[73]
24	<i>Streptococcus thermophilus</i>	Carbon Dioxide	<i>E. coli</i> , <i>Salmonella</i> spp.	[60]
25	<i>Lactobacillus plantarum</i>	Diacetyl	<i>S. aureus</i> , <i>Enterococcus faecalis</i>	[66])
26	<i>Lactococcus lactis</i>	Nisin	<i>Listeria monocytogenes</i>	[56]
27	<i>Lactobacillus casei</i>	Ethanol	Fermentation by-product; pathogen inhibition	[67]
28	<i>Lactobacillus reuteri</i> DSM 20016	Reutericyclin	<i>Bacillus subtilis</i> , <i>Clostridium difficile</i>	[68]
29	<i>Pediococcus acidilactici</i> UL5	Pediocin UL5	<i>Clostridium botulinum</i> , <i>Staphylococcus aureus</i>	[69]
30	<i>Lactobacillus plantarum</i> ML03	Ethanol	Fermentation by-product in pathogen control	[70]
31	<i>Lactobacillus reuteri</i> DSM 17938	Reutericyclin	<i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i>	[71]
32	<i>Pediococcus acidilactici</i> PA-1	Pediocin PA-1	<i>Listeria monocytogenes</i> , <i>Enterococcus faecalis</i>	[72]
33	<i>Enterococcus durans</i>	Enterocin B	<i>Clostridium perfringens</i>	[54]
34	<i>Lactococcus lactis</i>	Diacetyl	<i>Clostridium botulinum</i>	[57]

2.3.2. LAB as Bio-control Agents

"Biological control" or "bio-control" refers to using living organisms to manage pests and diseases in various biological fields. In entomology, this involves predatory insects and microbial pathogens to control pest populations. In plant pathology, it pertains to microbial antagonists that suppress diseases and host-specific pathogens that manage weeds. The organisms that achieve this suppression are known as biological control agents (BCAs) [74]. LAB play a role in modifying gut pH and demonstrating antagonistic effects against pathogenic bacteria by producing antimicrobial substances. They reduce pathogenic bacteria through competition for binding sites and nutrients [38]. Research indicates that LAB can combat diarrhea-causing pathogens by preventing their

adhesion to epithelial cells or by producing bacteriocins like nisin [75]. LAB also stimulates the immune system by secreting enzymes, such as lactase. Their significant advantages include being GRAS, cost-effective, and effective against microbial infections. Studies have shown that *Lactobacillus salivarius* can produce lactic acid, inhibiting the growth of *Helicobacter pylori*, which is linked to gastritis, peptic ulcers, and gastric cancer. Preliminary evidence suggests that probiotic bacteria may inhibit *H. pylori* colonization and activity, as demonstrated in mouse models [40].

1) Hydrogen Peroxide Production

The production of hydrogen peroxide (H_2O_2) is known to inhibit LAB [76]. According to [77] investigation, *Lactobacillus lactis* strains such as *L. casei* Shirota and *L. acidophilus* YIT 0070 can inhibit *Escherichia coli* O157. LAB, particu-

larly *Lactobacilli*, produce H_2O_2 , which antagonizes *Staphylococcus aureus*, a significant medical pathogen. Specific strains can inhibit *S. aureus* growth at concentrations of 0.18 mmol/l [78]. These *Lactobacilli* lack catalase, preventing them from converting H_2O_2 to water, leading to its accumulation and potential bacterial cell death.

2) Competition with Pathogens

LAB enhances intestinal health through various mechanisms, such as producing antimicrobial substances and stimulating mucosal immunity [44]. They also competitively inhibit pathogens from adhering to intestinal epithelial cells. *Lactobacillus* strains from the human gut have been shown to inhibit several anaerobic bacteria linked to gastroenteric infections. Mechanisms affecting *H. pylori* include antimicrobial production, improved gut barrier function, and competition for adhesion sites, although the significance of these mechanisms is not fully understood [78].

3) pH-Lowering Capacity of LAB

LAB, especially *Lactobacillus* strains, produce acetic, lactic, and propionic acids, lowering their environment's pH and inhibiting Gram-negative pathogens. Certain *Lactobacillus* strains generate lactic acid that suppresses *Salmonella enterica* growth. Additionally, they produce antibacterial agents with bactericidal effects [75].

2.4. Major Applications of LAB

2.4.1. Application of LAB in the Food Industry

For centuries, bacterial fermentation has preserved the nutritional value of perishable raw materials and extended the shelf life of food and beverages. Bacteriocins produced by LAB are particularly valuable to the food industry [79]. Research indicates that these antimicrobial compounds allow LAB to thrive in competitive environments, making them suitable as natural food-grade preservatives that consumers find more acceptable [44]. Many LAB strains produce various bacteriocins, some of which are patented for food applications. Strategies for using bacteriocins in food preservation include inoculating food with LAB as starter cultures, adding purified bacteriocins directly, and utilizing products fermented with bacteriocin-producing LAB in food formulations [80]. While *in-situ* bacteriocin production by starter cultures shows promise for fermented foods, non-fermented products like minimally processed meats or packaged salads require LAB strains that produce adequate bacteriocins without compromising sensory quality [81]. Nisin, one of the most researched bacteriocins, is widely employed in the food industry to inhibit spoilage and pathogenic bacteria such as *Listeria monocytogenes* and *Clostridium botulinum*. It is effective in a variety of foods, including canned, chilled, and ambient-stored products [36]. Nisin functions effectively at pH levels from 3.5 to 8.0 and is commonly used in pasteurized milk, aged cheeses, and canned vegetables [44].

2.4.2. Applications of LAB in Medicine

Lactic acid bacteria (LAB) are valuable in the medical field for their ability to inhibit pathogenic microorganisms. As non-pathogenic organisms and part of the normal human gastrointestinal tract (GIT) flora, LAB helps prevent colonization by opportunistic pathogens [36]. Some LABs produce natural compounds like bacteriocins and microcins, which can be used as clinical treatments or food preservatives. These bioactive substances, derived from enterobacteria, can effectively target specific Gram-negative bacteria [82]. For instance, hybrid bacteriocins from *E. coli* (Ent35eMccV) have been shown to inhibit *Enterohemorrhagic E. coli* and *Listeria monocytogenes* [36].

2.4.3. Applications of LAB in Vaccine Development

LABs are promising candidates for oral vaccine development due to their natural adjuvant properties. They can produce various heterologous antigens, such as those for *Helicobacter pylori* urease, *Yersinia pseudotuberculosis* LcrV antigen, and human papillomavirus type 16 EP7 antigen [83]. LAB's resistance to stomach acid allows them to survive transit through the digestive tract, which is crucial for effective oral vaccine delivery. To enhance LAB viability during gastrointestinal passage, techniques such as encapsulation in microparticles or liposomes have been developed, improving their adhesion to mucosal surfaces and facilitating better antigen delivery and uptake by gut-associated lymphoid tissue [11].

2.4.4. Application of LAB to Stimulate the Immune System

Research from recent years has supported the idea that LAB, including *Lactobacillus casei*, can stimulate immune responses in humans. A study found that *Lactobacillus casei* can enhance the production of circulating antibodies against pathogens, such as *Pseudomonas aeruginosa*, by promoting immune activation [84]. Another study reported that *Lactobacillus rhamnosus* HN001 supplementation significantly increased natural killer (NK) cell numbers in human subjects, contributing to enhanced immune defense [85]. Additionally, the use of milk fermented with *Lactobacillus casei* Shirota has been shown to increase NK cell cytotoxic activity, providing further evidence of LAB's role in immune modulation [40, 11].

2.5. Safety Assessment of LAB

Many LAB strains, such as *Lactobacillus* and *Bifidobacterium*, have established a solid safety profile through widespread use. These strains are vital components of the gut microbiota in healthy individuals, contributing significantly to various metabolic processes [36]. Given their importance in functional foods and overall human health, the International Dairy Foods Association (IDFA) has set guidelines for assessing the safety of microorganisms in food. Similarly, the

European Food Safety Agency (EFSA) outlines the safety of commonly used LAB, including *Lactobacillus* and *Bifidobacterium*, in its Qualified Presumption of Safety (QPS) document. Most LAB strains demonstrate a strong safety record and LAB-based products continue to be monitored post-market introduction [86].

2.6. Genomic Association with LAB

The metabolic properties of LAB have been utilized for food preservation for generations, with these practices being passed down through various food traditions that remain popular today. Fermented foods produced with LAB are widely consumed, contributing to global sales in the tens of billions of dollars annually. Recently, there has been increasing interest in commensal LAB due to evidence suggesting their role in promoting health and preventing infections [87]. The availability of sequenced genomes has facilitated a deeper understanding of the evolutionary changes in LAB, revealing a trend toward a significant reduction in genome size over time [88]. It appears that the last common ancestor of the *Lactobacillales* order lost approximately 600 to 1,200 genes while gaining fewer than 100 during its divergence from the Bacilli ancestor [89].

3. Conclusion

The application of LAB as natural food preservatives has emerged as a significant strategy to enhance food safety and increase shelf life. Their ability to generate different antimicrobial compounds, such as hydrogen peroxide, organic acids, and bacteriocins, makes them most effective in controlling spoilage microorganisms and food-borne pathogens. These important characteristics contribute to preserving nutritional value, reducing food spoilage, and minimizing the reliance on chemical preservatives, which aligns with consumers' growing demand for clean-label foods. Additionally, LAB holds substantial value in functional food applications due to their probiotic potential and health benefits, such as enhancing immune function and promoting gut health. LAB also demonstrates potential beyond food preservation, with applications in probiotics, vaccine development, and even mental health modulation. The genomic diversity and adaptability of LAB allow for targeted applications and potential genetic improvements for enhanced efficacy. Despite this versatility, more comprehensive studies are essential to optimize their use and expand their safety assessments across various food matrices.

Abbreviations

BCAs	Biological Control Agents
EFSA	European Food Safety Agency
FFA	Free Fatty Acids

GIT	Gastrointestinal Tract Flora
GRAS	Generally Recognized as Safe
H ₂ O ₂	Hydrogen Peroxide
IDFA	International Dairy Foods Association
LAB	Lactic Acid Bacteria
NK	Natural Killer Cell Numbers
QPS	Qualified Presumption of Safety

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Author Contributions

Samuel Adugna Gedefa: Conceptualization, Investigation, Methodology, Writing – original draft

Debebe Landina Lata: Validation, Writing – review & editing

Seid Mohammed Ebu: Funding acquisition, Resources, Supervision, Validation

Genet Lakew: Data curation, Funding acquisition, Supervision

Melaku Deju Ankye: Funding acquisition, Resources

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Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors declare no conflicts of interest.

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