

Review

Lactic Acid Fermentation in the Food Industry and Bio-Preservation of Food

Yulma Lizbeth Aguirre-Garcia, Sendar Daniel Nery-Flores , Lizeth Guadalupe Campos-Muzquiz ,
Adriana Carolina Flores-Gallegos , Lissethe Palomo-Ligas, Juan Alberto Ascacio-Valdés,
Leonardo Sepúlveda-Torres  and Raúl Rodríguez-Herrera * 

School of Chemistry, Universidad Autónoma de Coahuila, Saltillo 25280, Mexico;
y.aguirre@uadec.edu.mx (Y.L.A.-G.); sendar.nery@uadec.edu.mx (S.D.N.-F.); l.campos@uadec.edu.mx (L.G.C.-M.);
carolinaflores@uadec.edu.mx (A.C.F.-G.); lissethe_palomo@uadec.edu.mx (L.P.-L.);
alberto_ascaciovaldes@uadec.edu.mx (J.A.A.-V.); leonardo_sepulveda@uadec.edu.mx (L.S.-T.)

* Correspondence: raul.rodriguez@uadec.uadec.mx

Abstract: Studies on fermentation by acid lactic bacteria (LAB) have confirmed the presence of strains with attributes of considerable relevance for food processing. These strains, in addition to their ability to modify the texture and flavor of foods, possess beneficial properties for human health. They enhance food quality by making it more nutrient-rich and contribute to food preservation. The production of lactic acid, vitamins, exopolysaccharides, and bacteriocins, among other compounds, confers these properties to LAB. In the realm of preservation, bacteriocins play a crucial role. This is because bacteriocins act by inhibiting the growth and reproduction of unwanted microorganisms by interacting with the cell membrane, causing its rupture. This preservative effect has led LAB to have widespread use during food processing. This preservative effect has led to widespread use of LAB during food processing. This review highlights the importance of fermentation carried out by LAB in the food industry and in the bio-preservation of foods. These findings emphasize the relevance of continuing investigations and harness the properties of LAB in food production.

Keywords: lactic acid bacteria; fermentation; bio-preservation; bacteriocins



Citation: Aguirre-Garcia, Y.L.; Nery-Flores, S.D.; Campos-Muzquiz, L.G.; Flores-Gallegos, A.C.; Palomo-Ligas, L.; Ascacio-Valdés, J.A.; Sepúlveda-Torres, L.; Rodríguez-Herrera, R. Lactic Acid Fermentation in the Food Industry and Bio-Preservation of Food. *Fermentation* **2024**, *10*, 168. <https://doi.org/10.3390/fermentation10030168>

Academic Editor: Alessandra Pino

Received: 31 January 2024

Revised: 9 March 2024

Accepted: 12 March 2024

Published: 15 March 2024



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1. Introduction

Fermentation is a process that involve the utilization of substrate compounds by microorganisms to carry out their metabolism, generating final byproducts that can be exploited in various fields such as the pharmaceutical, chemical, and food industries [1]. An example of these microorganisms is lactic acid bacteria (LAB), which can be found in the form of cocci or non-sporulating rods. They can produce lactic acid through carbohydrates fermentation, which serves as their sole or primary source of carbon [2]. During this fermentation process, LAB induce a rapid acidification of the substrate by producing organic compounds such as lactic acid, acetic acid, ethanol, enzymes, and bacteriocins. Among their byproducts, lactic acid stands out as the main product of this biochemical process [3].

Lactic acid bacteria represent a type of microorganism of considerable importance, given their utility in the food industry, ranging from nutritional contribution to the optimization of food preservation processes. The most used genera are *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Enterococcus*, and *Streptococcus*. However, their scope is not limited to these genera, as there is a wide variety of bacteria, and research and exploration continue [1]. This group of bacteria can induce alterations in food taste and texture and has been utilized for the production of a wide variety of commercially interesting products, such as fermented meats, cereals, and vegetables. Fermented dairy products are among the most common [4]. Additionally, LAB fermentations can contribute to the digestibility of foods, provide health benefits, and are widely recognized for food preservation by reducing the

risk of deterioration associated with unwanted microorganism activity [5]. Moreover, these fermentations can also aid in valorizing food waste through the release of bioactives, presenting significant advantages from a sustainability and green perspective as an ecological approach [6].

This essential role in preservation intertwines with the current trend where consumers are redirecting their preferences towards nutritional and health benefits, in addition to the pursuit of more natural and sustainable food products. Typically, the use of preservatives involves the application of chemical compounds or physical methods such as refrigeration or freezing. Nevertheless, this trend has spurred the exploration of more sustainable alternatives for preservatives [5].

Bio-preservation has evolved as an antimicrobial strategy aimed at enhancing food safety and extending the shelf life of products through the implementation of biological systems. This approach involves the use of LAB and their metabolites, which, as mentioned earlier, possess antagonistic activities capable of inhibiting or eradicating microorganisms that could compromise the integrity of the food, thereby improving both safety and shelf life [7]. During the fermentative process, LAB release, in addition to lactic acid, low molecular weight proteins called bacteriocins. These compounds are primary metabolites composed of polypeptides, proteins, or protein complexes synthesized by bacteria through ribosomal machinery. Bacteriocins play a fundamental role by exhibiting antimicrobial activity, as they have the ability to inhibit the growth and reproduction of various bacteria. Their mechanism of action primarily involves interaction with the cell surface, increasing permeability and causing detrimental effects to the affected bacteria. Furthermore, bacteriocins can interfere with cell wall production, as well as nucleic acid and protein synthesis, consolidating their significant contribution to food bio-preservation [8].

The main purpose of this review is to investigate the fermentation processes of LAB and their significance in the food industry, assessing their various applications and benefits in food production and preservation.

2. Acid Lactic Bacteria

LAB constitute a group of Gram-positive bacteria, forming a diverse array of microorganisms present in the form of cocci or non-sporulating rods. They exhibit characteristics such as resistance to acidic conditions, aerotolerance, and immobility. Their main distinctive feature is the production of lactic acid as the primary product of carbohydrate fermentation [4]. In terms of taxonomy, most LAB are found in the phylum *Firmicutes*, class *Bacillus*, and order *Lactobacillales*, which comprises six families, *Lactobacillaceae*, *Leuconostocaceae*, *Enterococcaceae*, *Aerococcaceae*, *Streptococcaceae* and *Carnobacteriaceae*, with over thirty genera and 300 species described [4]. Additionally, the *Bifidobacterium* genus, a part of lactic acid bacteria, belongs to the *Actinobacteria* phylum. This taxonomic panorama demonstrates the diversity and complexity of LAB, emphasizing the importance of their study.

It is interesting to note that, despite their association with nutrient-rich habitats such as vegetables, milk, and meats, LAB have also been identified in environments such as the intestine, vagina, feces, and oral cavity, highlighting their versatility and presence in various zones. Over time, LAB have undergone a shift in their ecology; while they were once found in places like petroleum-contaminated soils or plant tissues, some bacterial strains have found a new ecological niche in the mammalian intestine, where a reservoir of up to 100 trillion microorganisms can be found [5]. This phenomenon has given rise to what we know as the intestinal microbiota, a complex and diverse community of microorganisms that play an essential role in host health and well-being. Their influence extends from improving the digestion and metabolism of foods and their nutrients to stimulating the immune system. The intestinal microbiota also plays a fundamental role in maintaining an intestinal barrier, acting as a protective shield against potential infections caused by pathogenic microorganisms, in addition to crucially contributing to the organism's homeostasis [9].

3. Lactic Acid Bacteria Fermentations

LAB derive their energy through the phosphorylation of sugar molecules and can be divided into two main categories: homofermentative and heterofermentative. Additionally, there are bacteria that exhibit both fermentation pathways, known as heterogeneous bacteria [10].

Bacteria classified as homofermentative have the ability to exclusively degrade hexoses, relying mainly on glycolysis. These bacteria are characterized by the production of two molecules of lactate from one molecule of glucose. Some examples of LAB strains that utilize the homofermentative pathway are *Lactobacillus plantarum* and *Pediococcus pentosaceus* [11]. On the other hand, bacteria classified as heterofermentative are notable for their ability to degrade both hexoses and pentoses. In the case of pentoses, they employ the pentose phosphate pathway, involving the action of specific enzymes such as ribose-5P epimerase and phosphoketolase. This process leads to the generation of lactate, carbon dioxide, and either ethanol or acetate as fermentation products [5,12]. *Limosilactobacillus fermentum*, *Lentilactobacillus buchneri*, *Lentilactobacillus parabuchneri*, and *Levilactobacillus brevis* are the most representative bacteria among obligate heterofermentative LAB [10].

In addition to this classification based on their fermentation mode (Figure 1), LAB can be identified according to their morphology, temperature growth ranges, and through phylogenetic analysis, using criteria established by Orla-Jensen in 1919 [5].

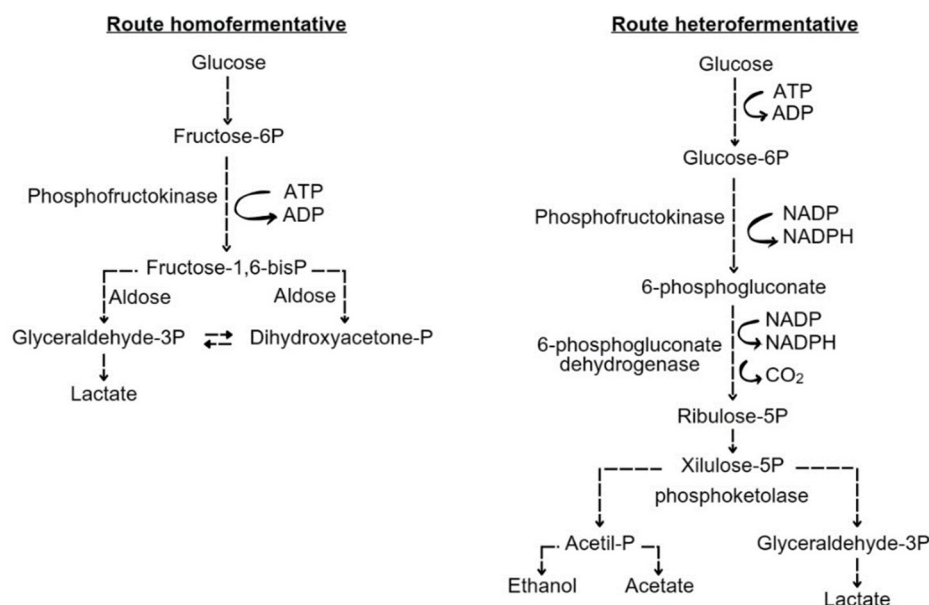


Figure 1. Metabolic pathways of homofermentative and heterofermentative LAB [12].

4. Products Synthesized by LAB of Importance in the Food Industry

Lactic acid bacteria (LAB) generate, during their fermentation processes, a variety of compounds of interest in addition to bacteriocins (Figure 2). Some of the compounds generated during fermentation by LAB (lactic acid bacteria), such as organic acids, bacteriocins, vitamins, and free amino acids, have the ability to penetrate microbial and fungal membranes, accumulating intracellularly in the cytoplasm of these microorganisms, conferring antimicrobial properties to LAB. Furthermore, specific metabolites also play a role in limiting the formation of free radicals, providing LAB with antioxidant activities in food [6]. Their significance lies in understanding and harnessing these natural products, not only for their roles in fermentation processes but also for their potential application in various industries, including the food industry [4].

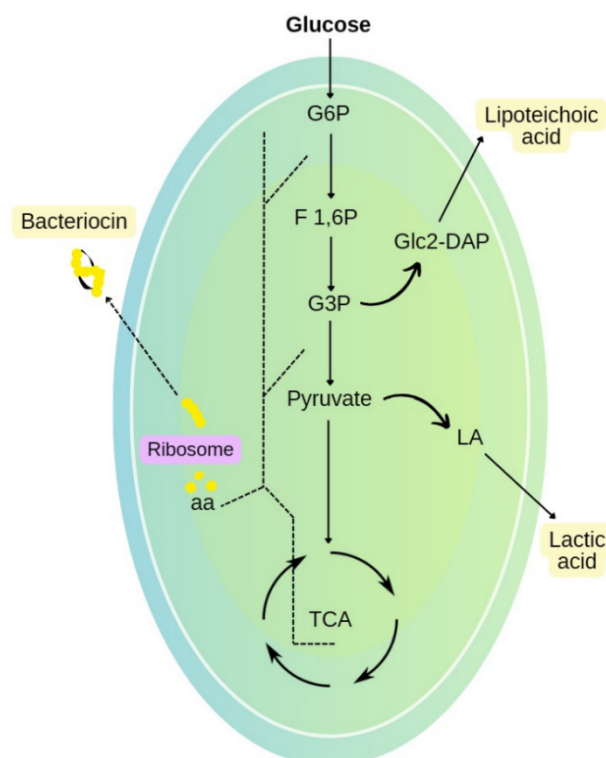


Figure 2. Some extracellular products of acid lactic bacteria fermentation [4].

4.1. Lactic Acid

Lactic acid is one of the principal and most important compounds produced by LAB fermentations. This compound can be obtained through both heterofermentative and homofermentative pathways, presenting two enantiomers: D-lactic and L-lactic [2]. Its applications span various fields, including medicine, the chemical industry, agriculture, environmental protection, and, notably, the food industry. It is worth noting that D-lactic is not metabolizable by animal cells, so most biotechnological production focuses on L-lactic [13].

The decrease in pH resulting from the production of organic acids such as lactic acid exerts antagonistic effects on the growth of bacteria, yeasts, and filamentous fungi. The effectiveness of pH reduction through LAB fermentation against *Salmonella* spp. has been confirmed by Daliri et al., 2020 [14]. In an assessment of lactic acid's impact on pathogen development, its greater efficiency against Gram-positive bacteria compared to Gram-negative ones was observed. Furthermore, an increase in lactic acid concentration was found to correlate with enhanced efficacy in inhibiting the growth of pathogenic microflora in foods [15]. Among the microorganisms most commonly used for the production of this organic acid are *Streptococcus*, *Lactobacillus*, *Bifidobacterium*, *Tetragenococcus*, and *Lactobacillus delbrueckii*, with the latter being the most widely utilized in the food industry [16].

In the food sector, there is a marked interest in enhancing both the yield and purity of L-lactic during food processing. Various strategies have been implemented for this purpose, such as the addition of nutrients like glucose or vitamin B during the fermentation process [17] and pH control in the medium [18]. In the case of batch production of L-lactic, optical purity of 100% and a yield of 99.22 g/L has been achieved through fermentation with the genetically modified strain of *L. paracasei* and glucose addition [19]. Fermentation by *Enterococcus mundii*, with the addition of glucose, xylose, and cellobiose, has also been carried out, achieving a yield of 0.870 g g⁻¹ and an optical purity of L-lactic exceeding 99% [4].

The strategies can vary, ranging from nutrient addition to pH control of the medium (Table 1). When comparing the mentioned strategies, the effectiveness of fermentation with the genetically modified strain of *L. paracasei* in obtaining highly pure L-lactic acid is highlighted. On the other hand, fermentation carried out employing *E. mundii*, with the

addition of glucose, xylose, and cellobiose, also demonstrates high optical purity and good yield, showcasing their effectiveness in L-lactic acid production. This analysis suggests that the choice of strains and their specific fermentation conditions can have a significant impact on the yield and purity of lactic acid.

Table 1. Comparison of modifications to lactic acid fermentation conditions conducted for the enhancement of lactic acid yield.

Microorganism	Variation in Fermentation Conditions	Yield	Reference
<i>Pediococcus acidilactici</i> ZY271.	Glucose, Vitamin B, pH control	61.6%	[17]
<i>Lactobacillus pentosus</i> MAX2.	pH 7	70%	[18]
<i>L. paracasei</i>	Glucose	99.22%	[19]
<i>E. mundii</i>	Glucose, xylose, cellobiose	87%	[4]

4.2. Gamma-Aminobutyric Acid

Gamma-aminobutyric acid (GABA) is a non-protein amino acid of crucial importance in the human central nervous system. The main intracellular synthesis pathway is the L-glutamate decarboxylation reaction, where glutamate decarboxylase (GAD) acts as a key rate-limiting enzyme. GAD and pyridoxal-5'-phosphate actively participate in the decarboxylation of L-glutamate, thereby generating GABA [2]. Several fundamental genes have been identified in the regulation of GABA, such as the *gadR* gene present in *L. brevis* D17, which not only controls GABA synthesis but also the bacterium acid resistance; its complete removal results in the total disruption of GABA synthesis [20].

In the field of the food industry, strains of lactic acid bacteria (LAB) capable of safely producing GABA can play a significant role as functional ingredients. These strains have been used in various products, including fermented cereals, cheeses, fermented milks, and yogurts. A notable example is the use of *L. brevis* and *Bacillus subtilis* during fermentation of traditional Chinese soy, resulting in a remarkable increase in GABA concentration in the final product [21].

4.3. Vitamins

LAB have the capability to synthesize a variety of vitamins, including riboflavin, folic acid, vitamin C, cobalamin, and others [2]. This attribute holds potential applications in the food industry, enabling nutritional fortification of foods and adding value to LAB. Riboflavin, also known as vitamin B2, is a compound that can be produced by LAB through the riboflavin synthase-encoding genes organized in a *rib* operon. The products of this operon, RibC, RibB, RibA, and RibH, play crucial roles in catalyzing the conversion of GTP and 5-phosphate ribose into riboflavin. While LAB can naturally produce this vitamin, genetic modification, as seen in specific cases such as *Lactobacillus plantarum* and *Lactococcus lactis* JC017, more effectively enhances riboflavin secretion [2,22].

Folic acid, a water-soluble B vitamin, is synthesized by the *Streptococcus*, *Lactobacillus*, and *Lactococcus* genera [23]. Due to its significance in nucleotide and protein biosynthesis, and considering that humans cannot synthesize this vitamin, its consumption is important, with potential implementation for food products [2]. LAB have the ability to synthesize folic acid through various pathways, including the Pterin and p-aminobenzoic acid (pABA) branches. It is noteworthy that, in some cases, such as with *Lactobacillus*, the addition of pABA to the fermentation medium becomes necessary for the successful synthesis of this vitamin [2].

4.4. Exopolysaccharides

Exopolysaccharides, as macromolecules derived from the polymerization of monosaccharides, play a crucial role in the food industry and are receiving increasing interest

due to their probiotic functions and their ability to impart new properties to fermented foods. These substances significantly influence the physiological function of lactic acid bacteria (LAB) [24]. Bacteria such as *Streptococcus thermophilus*, *Limosilactobacillus reuteri*, *Lactocaseibacillus casei*, and *L. plantarum* are examples of LAB capable of synthesizing these exopolysaccharides [2].

In the realm of the food industry, exopolysaccharides offer diverse applications, especially in fermented dairy products. A notable example is glucan, an exopolysaccharide that not only acts as a gelling and stabilizing agent but also enhances yogurt properties when strains such as *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* are used. Additionally, other exopolysaccharides exhibit antioxidant properties and have a notable capacity to retain water and oil, making them promising for the food industry and opening possibilities for cosmetic applications [2]. This highlights the versatility and benefits of exopolysaccharides across various industries.

4.5. Antioxidants

Antioxidants are compounds with a remarkable ability to neutralize free radicals, and their association with human health has sparked growing interest. The focus on the antioxidant properties of LAB has significantly increased, as extensive reports have highlighted their capacity to synthesize antioxidant metabolites. In the antioxidant domain of LAB, attention is directed toward the metabolism of phenolic substances and the limited ability of some bacteria to synthesize glutathione. Certain LAB, such as *L. brevis*, *L. fermentum*, and *L. plantarum*, have demonstrated the ability to metabolize phenolic acids through the decarboxylase and reductase enzymes [2].

The ability for strains of *Bifidobacterium*, *Lactobacillus*, and *Enterococcus durans* to reduce the concentration of free radicals, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (Cizeikeiene et al., 2021), has been mentioned. This finding was confirmed in a study involving 16 LAB strains (*Leuconostoc mesenteroides* MG860; *Leu. citreum* MG210; *P. acidilactici* MG5001; *Pediococcus pentosaceus* MG5078; *Weissella cibaria* MG5223, MG5090, MG5215, and MG5285; *L. brevis* MG5250, MG5280, MG5306, and MG5311; *Latilactobacillus curvatus* MG5020; and *Latilactobacillus sakei* MG5048 and MG5031) isolated from fermented foods, demonstrating their ability to alleviate oxidative stress by eliminating DPPH and ABTS radicals [25]. Additionally, another study revealed that strains like *L. salivarius* MG242, *L. plantarum* MG989, *B. bifidum* MG731, and *Bifidobacterium lactis* MG741, when exposed to heat, reduced oxidative stress by inhibiting NO production through the decreased gene expression of iNOS/COX-2 in LPS-activated RAW264.7 cells [26].

In the food domain, LAB's ability to increase the sulforaphane content, a molecule with antioxidant properties, in broccoli puree is noteworthy [27]. Furthermore, *L. plantarum*, in blueberry fermentation, demonstrates its ability to convert fruit polyphenols into phenolic metabolites with strong antioxidant activities [28]. Moreover, LAB has been employed to enhance camel milk, exhibiting higher antioxidant capacity during storage compared to commercially fermented milks [29].

Taken together, these findings suggest that these lactic acid bacteria strains can play a beneficial role by providing antioxidant properties, thereby supporting their potential application in the food industry to enhance the quality and shelf life of food products.

4.6. Bacteriocins

Bacteriocins are antimicrobial peptides released by ribosomes, with a length ranging from 20 to 60 amino acids. These peptides, which are cationic and hydrophobic, are produced by bacteria to protect themselves from other bacteria or pathogens [30]. Due to this, they are excellent candidates for preventing food deterioration caused by unwanted bacteria, as they encompass both Gram-negative and Gram-positive groups [8]. Generally, bacteriocins are low molecular weight molecules; during their synthesis process, they are accompanied by a leader sequence. During maturation, this leader sequence is cleaved or

separated; subsequently, the mature bacteriocin is released from the cell, ready to carry out its antimicrobial functions [4]. They can be classified into three classes based on their structure and properties (Table 2):

Class I: Known as antibiotics, these are thermo-stable peptides consisting of 19 to 50 amino acids (Figure 3). They incorporate polycyclic amino acids characteristic of thioethers, such as lanthionine, methyl-lanthionine, dehydroalanine, and 2-amino isobutyric acid [31]. Based on their charge, this class is further subdivided into two types. Type A sub-group molecules are helical in shape, flexible, and have a positive charge, with a molecular weight ranging from 2 to 4 kDa. These peptides induce the creation of pores in the cell membrane of unwanted microorganisms, leading to depolarization of the cytoplasmic membrane. Type B antibiotics are peptides with a molecular weight of 2–3 kDa and exhibit either a lack of net charge or a negative net charge. These are globular molecules that directly interfere with cellular enzymatic reactions, affecting, for example, cell wall synthesis. Their action targets crucial processes in cellular functioning, influencing the cell's biochemical machinery specifically [31].

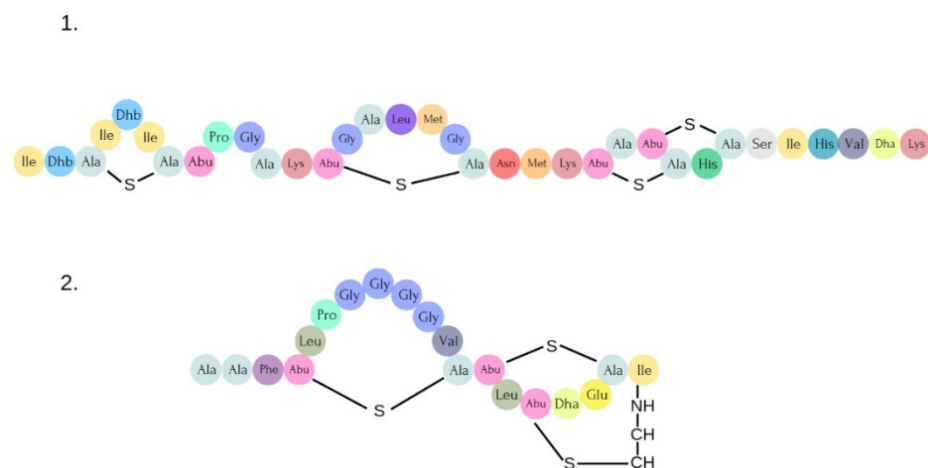


Figure 3. Structure of class I bacteriocins; Nisin of type A (1) and mersacidin of type B (2) [32].

Class II: Bacteriocins of class II are smaller thermo-stable peptides with a molecular weight less than 10 kDa. They have an amphiphilic helical structure, allowing them to insert into the cell membrane of the pathogenic microorganism, inducing depolarization and, consequently, cell death. This class is further subdivided into three types: Type A bacteriocins are characterized as monomers and present a consensus sequence at the N-terminal end comprising Tyr-Gly-Asn-Gly-Val-Xaa-Cys. Type B includes two-component bacteriocins, as their antimicrobial activity is generated by two different peptides present in their structure, which collaborate synergistically. Type C bacteriocins encompass circular bacteriocins (Figure 4), which have two transmembrane segments facilitating pore formation in the target cell [31].

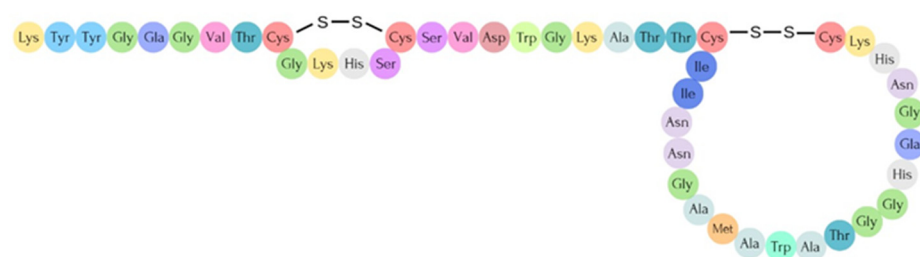


Figure 4. Structure of pediocin PA-1; class II bacteriocin [33].

Class III: Bacteriocins of this class are large peptides with a molecular weight exceeding 30 kDa. These are generally classified as lytic proteins, often categorized as murein hydrolases. They are also thermo-stable but are not well-characterized [31].

Table 2. Classification of bacteriocins [34].

Classification	Features	Subcategories	Examples
Class I bacteriocins (lantibiotics)	Lanthionine or peptides containing β -lanthionine	Type A (linear molecules) Type B (globular molecules)	Nisin, subtilin, epidermine Mersacidin
Class II bacteriocins	Heterogeneous class of small thermostable peptides	Subclass IIa (antilisterial pediocine bacteriocins type) Subclass IIb (composed of two peptides) Subclass IIc (other bacteriocins)	Pediocin, enterocin, sakacin Plantaricin, lactacin F Lactococcin
Class III bacteriocins	Large thermolabile peptides		Helveticin J, millericin B

4.6.1. Bacteriocins in Food Bio-Preservation

Food deterioration is a constant concern in the food industry. Improper handling can not only lead to significant economic losses and the accumulation of waste but can also result in food poisoning due to the production of toxins by the pathogenic microorganisms present in the food [35].

Bio-preservation involves extending the freshness and elevating the safety levels of food by using microorganisms or the compounds that they generate [36]. Fermentation has been employed as a method for the bio-preservation of food, thanks to its ability to produce antimicrobial compounds and alter various factors that inhibit the growth of harmful microorganisms in food. When it comes to fermentation carried out by LAB, their involvement in food fermentation processes goes beyond merely enhancing food taste and texture. These bacteria possess antimicrobial capabilities attributed to the production of a wide range of metabolites, including hydrogen peroxide, ethanol, and diacetyl, as well as acids such as propionic, ascorbic, benzoic, acetic, and lactic acids. Additionally, their ability to compete for nutrients adds to their defense arsenal against pathogenic microorganisms [37].

Despite implementation of these elements, ensuring a microbiologically safe product is not guaranteed, as adjustments to food may be necessary in certain circumstances to meet the demands and sensory preferences of consumers. This adjustment process can promote the survival of certain unwanted microorganisms in the final product, posing risks to safety, as undesirable microorganisms may persist in the final product. In this scenario, bacteriocins, whether isolated naturally or produced by LAB, emerge as an optimal strategy to address this challenge. The inclusion of bacteriocins in the fermentation process not only contributes to extending the shelf life of foods but also enhances microbiological safety.

Bacteriocins are of great interest due to their potential application in the food industry. However, it is important to note that not all LAB strains have the ability to synthesize these metabolites, emphasizing the inherent functional diversity within this group of microorganisms [38]. These strains, referred to as bacteriocinogenic lactic acid bacteria, are highly promising options for use as starter cultures, giving them significant relevance in the processes of applying fermented foods [5].

The use of bacteriocins in the food industry has been extensively studied, primarily during the production of dairy products such as cheese and yogurt, as well as in foods like vegetables, eggs, and meat products (Table 3). They can be incorporated into foods by using purified or semi-purified bacteriocins as an additional component, by including an ingredient previously fermented with bacteriocin-producing LAB or by using a starter culture that generates the desired bacteriocin [31]. Some of the most-used bacteria for food preservation include nisin, enterocin, pediocin, leucocin, and sukacin, among others [39].

Table 3. Some bacteriocins from LAB used for food preservation (Modified from [30]).

Producing Strain	Bacteriocin	Food Application
<i>Lactobacillus lactis</i> spp.	Nisin	Cheddar cheese Milk and milk products Meat and sausages
<i>L. lactis</i> MG1614	Enterocin A	Cottage cheese
<i>Enterococcus faecium</i> WHE 81	Enterocins A and B	Munster cheese
<i>E. faecium</i> F58	Enterocins L50A and B	Goat's milk and goat milk's cheese
<i>P. acidilactici</i> MCH14	Pediocin PA-1	Dried sausages and fermented meat products Salad dressings Fish fillets and chicken meat
<i>Leuconostoc gelidum</i> UAL187	Leucocin A	Meat Milk product, fresh meat, and sausage
<i>Carnobacterium maltaromaticum</i> UAL307	Carnocyclin A, carnobacteriocin BM1, and piscicolin 126	Milk products and meat
<i>Lactobacillus sakei</i>	Sukacin	Meat product

4.6.2. Mechanism of Action of Bacteriocins

Bacteriocins possess diverse inhibition mechanisms (Figure 5), which can be broadly categorized into two main groups: those exhibiting bactericidal effects by primarily targeting the cell envelope and those with bacteriostatic effects that impact gene expression and protein production. Some, especially those affecting Gram-positive bacteria, act by targeting the cell envelope. Certain class I bacteriocins, like nisin, exemplify this mechanism; they inhibit peptidoglycan synthesis by attacking lipid-II in the cell membrane or utilize this lipid-II as a coupling agent to facilitate pore formation, thereby inducing cell death [31].

Additionally, certain bacteriocins can inhibit or eliminate pathogenic bacteria by binding to the mannosyl-phosphotransferase system associated with the cell envelope, similarly inducing pores in the cell membrane [40]. In the case of bacteriocins affecting Gram-negative bacteria, they interfere with the metabolism of DNA, RNA, and proteins through enzymatic activities involving DNase, RNase, or phospholipase [31].

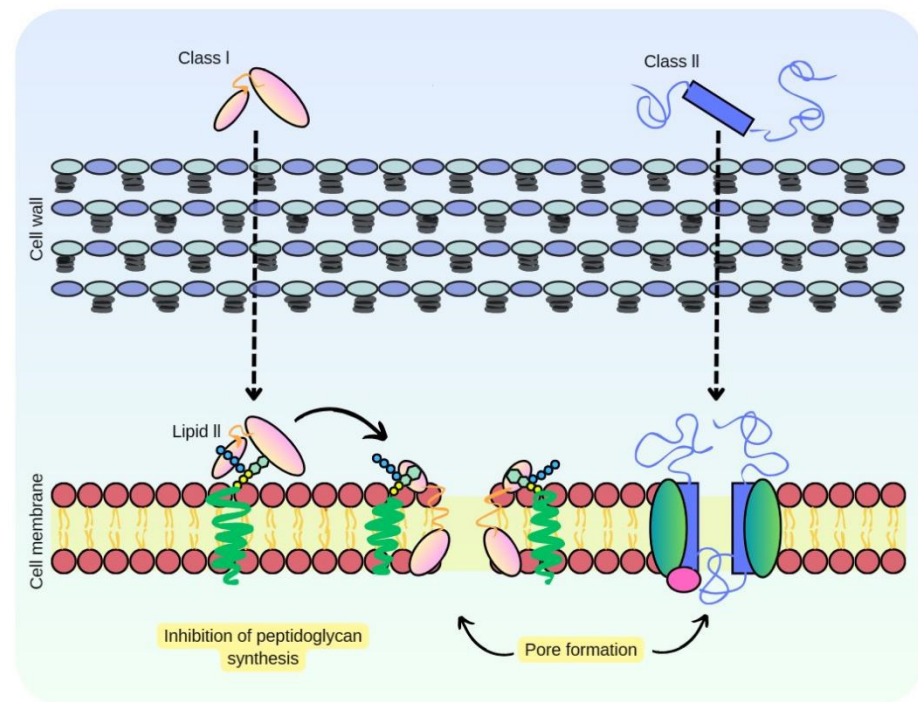


Figure 5. Examples of mechanism of action of bacteriocins on Gram-positive bacteria [41].

4.6.3. Nisin

Nisin is one of the bacteriocins approved by both the United States Food and Drug Administration (FDA) and the World Health Organization (WHO) for use as a preservative during food processing. This approval is attributed to its degradability and non-toxic reactions [30,42]. Its antimicrobial activity is due to its interaction with lipid-II receptors on the bacterial cell wall, which promotes the inhibition of cell wall synthesis and pore formation [43].

Its applications in food products encompass dairy products, desserts, ice creams, sauces, soups, fruit juices, bakery items, and more [42].

Nisin can be naturally obtained through extraction from lactic acid bacteria (LAB) strains like *Lactococcus* or *Streptococcus* without requiring any genetic modification [30]. Alternatively, it can be obtained from genetically modified strains aimed at enhancing its antibacterial effects. An example is Nisin Z, obtained naturally from *L. lactis* or through genetically modified *L. lactis* [44]. This bacteriocin exhibits antimicrobial activity against various Gram-positive bacteria such as *Listeria*, *Staphylococcus*, and spore-forming bacteria like *Bacillus* and *Clostridium*. It is commonly used to prevent the spoilage of various dairy products by inhibiting the growth of *Bacillus cereus*, *Clostridium botulinum*, and *Clostridium perfringens*. Nisin has been widely employed in cheeses to prevent *Clostridia* growth and for the inhibition or elimination of *Listeria monocytogenes* and *Staphylococcus aureus* to extend a product's shelf life [40]. However, it is crucial to note that Nisin tends to lose a significant portion of its activity at pH levels higher than 7, limiting its use in dairy products [40].

In addition to its presence in dairy products, *L. monocytogenes*, *B. cereus*, and *C. botulinum* can be found in bakery products and beverages, causing their deterioration and posing safety risks. Therefore, Nisin is also valuable for extending the shelf life of these foods (Table 4) [30].

Table 4. Use of nisin as a food preservative and its antimicrobial activity [30].

Food Products	Targeted Pathogens	Advantages	Disadvantages
Cheddar cheese	<i>L. monocytogenes</i> and <i>S. aureus</i>	The acidic and bitter taste of cheese can be improved.	Costly and low in stability.
Milk products	<i>B. cereus</i> , <i>C. botulinum</i> , and <i>C. perfringens</i>	The taste is not affected.	Loss of activity at pH above 7
Meat and sausages	<i>L. monocytogenes</i> , <i>B. cereus</i> , and <i>C. botulinum</i>	The taste is not affected.	Loss of activity at pH above 7
Bakery and beverage products	<i>L. monocytogenes</i> , <i>B. cereus</i> , and <i>C. botulinum</i>	The taste is not affected.	Loss of activity at pH above 7

4.6.4. Enterocin

Enterocin, a bacteriocin originating from the lactic acid bacterium *Enterococcus*, has obtained significant attention for its potential in inhibiting a diverse array of pathogenic microorganisms responsible for food deterioration. Notably, the four identified classes of enterocins include class I and II, which stand out due to their ability to inhibit various pathogens. What adds to their appeal is their remarkable stability across a wide pH range and under heat conditions. Despite these promising characteristics, enterocins of classes I and II face hurdles in gaining approval from regulatory bodies such as the FDA and the WHO. The reluctance to endorse these bacteriocins is rooted in concerns associated with enterococci's potential connection to human infections. Some strains of *Enterococcus* may harbor virulence factors and genes related to antibiotic resistance, contributing to the cautious approach. While most lactic acid bacteria categorized as food-grade are generally deemed safe for use in food products, the use of purified enterocins is deemed more suitable [40].

Enterocins produced by *Enterococcus faecalis* are effective in inhibiting *B. cereus*, *Bacillus macroides*, *Paenibacillus* spp., and *S. aureus* in fresh vegetables, soybean sprouts, and canned fruits and vegetables. It has also been demonstrated that enterocins are useful for preserving dairy and dairy products such as cottage cheese for the inhibition of *L. monocytogenes* [30]. This bacteriocin has also proven its utility as a preservative in juices such as orange, pineapple, apple, and peach [45].

4.6.5. Lactacin

Lactacins are a type of bacteriocins produced by *L. lactis*, including lactacin 3147 and lactacin 481. Lactacin 3147 exhibits antimicrobial activity directed against pathogenic bacteria significant in food deterioration and another LAB. This bacteriocin has proven effective in inhibiting *Listeria* and *Bacillus* in formulations such as infant milk, cottage cheese, and other cheeses. On the other hand, lactacin 481 shows a medium spectrum of activity, primarily targeting another LAB, *L. monocytogenes*, and *Clostridium tyrobutyricum*. In products like refrigerated fresh cheeses, a decrease in the count of *L. monocytogenes* has been observed, although complete elimination of this microorganism has not been achieved [40].

4.6.6. Pediocin

Pediocin is a class II bacteriocin produced by strains of *Pediococcus* such as *P. acidilactici*, *P. claussenii*, *P. cellicola*, *P. damnosus*, *P. ethanolidurans*, *P. inopinatus*, *P. parvulus*, *P. pentosaceus*, and *P. stilesii* [46]. It exhibits high stability at high temperatures and freezing, across a broad pH range, and even in the presence of enzymes such as RNase, DNase, lipase, and lysozyme. It has shown greater efficacy than nisin against various pathogens found in foods, including *L. monocytogenes*, *S. aureus*, *E. coli*, and *Pseudomonas* [40]. Its use proves to be a useful strategy for the preservation of meat products, inhibiting *L. monocytogenes*,

C. perfringens, and *C. botulinum*. In sausages, it has been employed as a preservative and shown to inhibit *L. monocytogenes* for up to 60 days under refrigeration at 4 °C [46]. This bacteriocin exhibits good stability in aqueous solutions, making it useful for milk and dairy products, where it has demonstrated a reduction in the count of *L. monocytogenes* in products such as cream, cottage cheese, and cheese sauces [40].

Some considerations when using pediocin as a food preservative include its proteolytic degradation, solubility variation, and the potential for adsorption by other food components. Additionally, the type of food must be considered to obtain an appropriate amount of the bacteriocin, especially when adding the producing strain directly to the food matrix [46].

4.6.7. Aureocin

Aureocin A70, a bacteriocin classified within class II, is produced by strains of *S. aureus* isolated from commercial pasteurized milk. This remarkable bacteriocin has revealed its potent bactericidal effect, especially against Gram-positive bacteria, highlighting its ability to effectively attack the presence of *L. monocytogenes*. In specific trials, Aureocin A70 demonstrated its efficacy by significantly reducing the count of *L. monocytogenes* in contaminated skimmed milk, achieving complete elimination of the bacteria within 7 days of the process [40].

Furthermore, a variant named Aureocin 4181 has been developed, derived from Aureocin A70, which exhibits an even more potent bacteriolytic action, especially against *S. aureus*. Studies reveal that Aureocin 4181 shows an activity two to four times higher than Aureocin A70 when facing *S. aureus* [47]. These findings underscore the distinctive ability of Aureocin A70 and its derivative Aureocin 4181 to effectively address the presence of various pathogenic bacteria, offering promising insights into the quest for advanced antimicrobial solutions.

4.6.8. Lactococo

The Lactococo BZ bacteriocin, synthesized by specific strains of *L. lactis*, showed broad antibacterial activity targeting both Gram-positive and Gram-negative bacteria. Its effectiveness has been notably demonstrated in suppressing the proliferation of *L. monocytogenes*, showing satisfactory results in both skimmed milk and whole milk over an extended storage period spanning 25 days [40].

5. Industrial Applications of LAB in Food Products

LAB play a prominent role in the food industry, actively participating in the production of fermented foods and beverages, such as cereals-based beers, dairy products, and cheeses, among others (Figure 6). For millennia, LAB fermentation occurred spontaneously, harnessing microorganisms naturally present in the food microbiota. While these traditional techniques persist, technological advancements and evolving dietary demands have driven the adoption of processes that enhance the control, standardization, and organoleptic characteristics of foods. The introduction of fermentation starter cultures has facilitated the industrialization of these production methods [48].

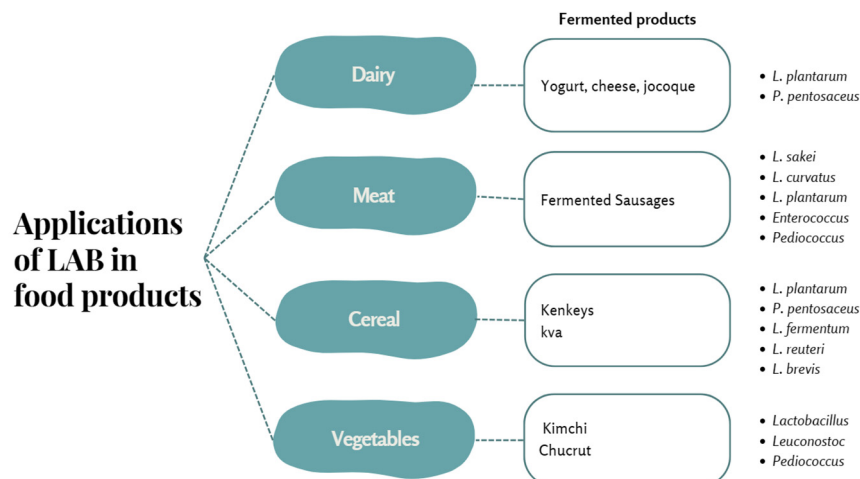


Figure 6. Examples of LAB-fermented foods.

In the dairy industry, LAB take center stage in various stages, from the production of dairy beverages through milk acidification to the crafting of cheeses and their maturation process [29]. The production of fermented milks, whether with whole, partially, or completely skimmed milk, involves pasteurization or sterilization before undergoing LAB fermentation. This process yields a variety of products, including yogurt and “jocoque,” with yogurt being the most widely distributed LAB-fermented dairy product globally that is produced industrially. The key functions of LAB in dairy product production include the generation of organic acids, milk coagulation, whey synthesis, sugar reduction, and aroma and flavor formation, as well as the inhibition of undesirable microorganisms and the contribution of probiotic capacities to foods. Additionally, these bacteria help prevent rancidity by reducing lipolysis and play a role in gas production, resulting in characteristic holes in certain types of cheeses [49].

LAB also contribute to the production of fermented sausages, with strains like *L. sakei*, *L. curvatus*, *L. plantarum*, *Enterococcus*, and *Pediococcus* widely used in this category. Their involvement not only imparts unique sensory characteristics to sausages but also enhances their safety and preservation. Furthermore, fermentation, where LAB play a significant role in inhibiting undesirable microorganisms, extends the shelf life of meat products.

They also play a role in the production of fermented cereal-based products, especially in Asia and Africa. Notable examples include Kenkey, a traditional food benefiting from fermentation by these bacteria, offering distinctive flavors and improving the digestibility and nutritional quality of the product. Similarly, Kvas, a beverage made from malt flour, undergoes fermentation processes driven by LAB. Fermented vegetables are another category derived from LAB fermentation. Kimchi, a fermented vegetable gaining considerable popularity worldwide, is obtained through spontaneous fermentation by strains of *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. This fermentation process enhances the flavor and texture of kimchi and provides health benefits by increasing the presence of probiotics in the food [50]. The diversity of fermented cereal and meat products underscores the versatility and significance of LAB in the food industry, contributing to the sensory complexity, safety, and durability of foods.

6. Advances in Patents on Applications of LAB and Their Fermentations in Food

Heller et al., in 2014, mentioned the impact of *Lactobacillus curvatus* on the bioconservation of food products under refrigerated conditions. Production of bacteriocins by this strain, even at refrigeration temperatures, makes it particularly useful for the bioprotection of ready-to-eat foods under refrigerated conditions. The need to identify strains with this capability is crucial, as the removal of oxygen from food does not guarantee the effective elimination of unwanted bacteria [51]. In the field of preservation, the obtention of a mutated strain of LAB, *Lactobacillus brevis*, stands out. It is specifically designed to

act as a preservative by inhibiting the growth of fungi in foods, pharmaceuticals, and cosmetic formulations [52]. Erkes et al., in 2016, patented a sophisticated method for reducing Gram-negative bacteria in fermented food products. This method combines a red wine extract, obtained through dealcoholization, concentration, and drying, with a culture that includes at least one LAB strain producing class IIa bacteriocins. Notable strains include *Carnobacterium maltaromaticum*, *Carnobacterium piscicola*, *Carnobacterium divergens*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactococcus lactis*, *Leuconostoc carnosum*, *Leuconostoc gelidium*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* [53].

Bhargava et al. present a patent that stands out for its focus on probiotic fermented foods and beverages derived from plants with the active participation of lactic acid bacteria [54]. This method involves the cultivation and incubation of vegetable substrates using activated LAB in a probiotic starter culture composition, aiming to acidify the vegetable substrate within a specific pH range, cool it, and refrigerate it to delay fermentation. Finally, Gilleladden et al. describe a method for producing food products that can be stored at room temperature. This process involves subjecting the food product with a pH between 3.4 and 4.4 to a heat treatment, followed by aseptically adding LAB strains for room temperature storage. The long-term viability of specific strains, such as *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum*, and *Lactobacillus Delbrueckii* subsp. *bulgaricus*, is a critical criterion in this application [55].

7. Future Challenges

Despite LAB playing an indispensable role in the food industry by generating byproducts during their fermentations, the evolving landscape continues to present significant challenges in their application. The emergence of mutant strains resistant to bacteriocins poses a threat to food preservation and safety, especially in products fermented by LAB. This phenomenon underscores the need for research and understanding of the resistance mechanisms employed by pathogens against these antimicrobial molecules, along with the identification of innovative strategies to counteract this issue and develop efficient food preservation strategies using LAB.

However, an additional obstacle is genetically modified bacteria, which exhibit significant improvements in the production of byproducts with preserving properties. This enhancement is linked to the inherent capacity of these modified strains to play a fundamental role in food preservation. Although genetically modified foods have gained acceptance over the years, transforming this field into a new frontier of study and opportunities, substantial challenges persist.

Controversies and debates regarding the long-term safety of genetically modified foods continue to emerge, complicating consumer acceptance. This scenario highlights the need for a careful evaluation of risks and benefits associated with the adoption of genetically modified LAB strains as a food preservation strategy. The enhanced capability of these strains to generate preserving byproducts not only redefines the paradigm of food preservation but also raises ethical and practical questions that require careful consideration.

Addressing concerns about their safety, transparency, and effective communication is of great importance to foster broader acceptance of these strains and fully capitalize on their numerous benefits. In this context, continuous research and scientific outreach play crucial roles in providing accurate and well-founded information that contributes to an informed decision-making process for both consumers and the food industry.

Furthermore, it is essential to consider that both the application of bacteriocins and reduction in lactic acid do not constitute broad-spectrum strategies. Not all LAB bacteria possess these capabilities, emphasizing the importance of identifying strains with such capacities and assessing their antimicrobial potential against specific pathogens. This knowledge is crucial for optimizing the utility of LAB as preservative agents.

8. Futures Trends

Fermentation by LAB shows significant potential in the food industry. As mentioned, it not only contributes to the sensory transformation of foods, enhancing flavor and texture, but also improves food safety by helping in food preservation. Additionally, fermentation has the capability to enhance the nutritional profile of foods, benefiting the consumer by promoting intestinal health and bolstering the immune system. With such numerous benefits and properties, the expansion of BAL in vegetables, grains, legumes, and other non-dairy foods, which is their most common utility, is a strategic route to maximize their potential. However, if there is a desire to further expand their utility and upscale, it is necessary to drive the optimization of processes for these products. Additionally, there is a need to continue to look for new microorganisms that are safe and can be utilized in the food industry. In the case of their use as a bio-preservative, exploring strains with the ability to produce bacteriocins emerges as another key strategy for fermentation, along with identifying the optimal conditions for the activity of these bacteriocins to ensure their effectiveness in different environments. These improvements can be crucial to gain better benefits, ultimately translating into competitive advantages in the market and an elevation in the value of lactic acid fermentation. Fermentation by LAB not only promises a bright future in the food industry but also provides opportunities for continuous improvement and innovation in terms of food quality and safety. The responsibility lies in strategically recognizing and harnessing this potential, thereby developing products that go beyond the conventional ones.

9. Conclusions

LAB play a fundamental and multifaceted role in the food industry, actively contributing to the creation of diverse products through fermentation, with a particular focus on bacteriocins. These microorganisms, encompassing species from the *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Enterococcus*, and *Streptococcus* genera, are not only prevalent in fermented dairy products but also wield substantial influence during fermentation processes of fruits, vegetables, and meats. Their impact extends beyond the enhancement of flavor and texture, as LAB significantly augment the digestibility of food and fulfill a critical function in food preservation. This preservation role is pivotal, reducing the risk of deterioration attributed to the presence of undesirable microorganisms.

The continual advancement of research and development in the realm of LAB and their bacteriocins unveils promising potential, particularly in the domain of food bio-preservation. The inherent capacity of these bacteria to impede or eradicate harmful microorganisms through bacteriocin production emerges as a sustainable alternative to conventional chemical preservatives. This paradigm, recognized as bio-preservation, seamlessly aligns with the burgeoning consumer demand for healthier and more environmentally sustainable options compared to other industrial processes. Bacteriocins, as pivotal byproducts arising from LAB fermentations, stand out as invaluable instruments in manifesting antimicrobial activity. Their prowess in disrupting the structure and functionality of pathogenic bacteria positions them as potent agents, enhancing both the safety and shelf life of preserved foods. A comprehensive understanding of these intricate processes and the exploration of their applications in the food industry are imperative to fully exploit the advantages offered by LAB and their metabolites.

However, amid these promising prospects, substantial challenges loom. The escalating resistance of microorganisms to antibiotics constitutes a pressing concern, necessitating continual attention to sustain the efficacy of preservation strategies rooted in LAB and bacteriocins. Furthermore, the acceptance of genetically modified LAB strains, exhibiting enhancements in bacteriocin production, introduces considerations of risks and benefits that demand thorough scrutiny to ensure their efficacious and secure integration into the ever-evolving landscape of the food industry.

Author Contributions: Conceptualization, Y.L.A.-G. and R.R.-H.; methodology, S.D.N.-F.; software, J.A.A.-V. and S.D.N.-F.; validation, A.C.F.-G., L.G.C.-M. and L.P.-L.; formal analysis, R.R.-H.; investigation, S.D.N.-F. and A.C.F.-G.; resources, R.R.-H.; data curation, J.A.A.-V.; writing—original draft preparation, Y.L.A.-G. and R.R.-H.; writing—review and editing, L.S.-T.; visualization, L.P.-L.; supervision, L.G.C.-M. and L.S.-T.; project administration, S.D.N.-F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors have no conflicts of interest to declare.

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