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Review

Bio-Preservation of Meat and Fermented Meat Products by Lactic Acid Bacteria Strains and Their Antibacterial Metabolites

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Abstract: Meat and some meat products are highly perishable due to their high-water content, pH, and high content of nutrients. Therefore, spoilage control in these products is one of the critical challenges in the food industry. On the other hand, the increasing widespread awareness about the undesirable effects of synthetic preservatives has promoted the breakthrough of the use of natural compounds or bio-preservation technology. Bio-preservation implies the application of microorganisms or their metabolites to extend the shelf life of food products. In this regard, according to the ancient and safe use of fermentation by lactic acid bacteria (LAB), their application in the bio-preservation of meat and meat products is gaining more attention. Thus, more understanding of the potential of LAB and their metabolites in the control of pathogens in meat and meat products can create new horizons in the production of safe and functional products with long shelf life. So, this article aims to review the recent knowledge about the bio-preservation of meat and meat products by LAB and their metabolites. Also, their antibacterial mechanism and potential for use in hurdle technology are discussed. The outcome of this review literature shows the high potential of various LAB strains and their metabolites especially bacteriocins as bio-preservatives in meat and meat products for extending their shelf life. In this regard, their combined use with other novel technologies or natural antibacterial compounds as hurdle technology is a more effective method that can compete with synthetic preservatives.

Keywords: bio-preservation; fermentation; lactic acid bacteria; meat products; natural anti-microbial

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1. Introduction

Meat and meat products are rich sources of nutrients for humans due to their high content of vitamin B groups, protein, essential amino acids, and minerals. Also, they provide a favorable environment for the growth of several microorganisms due to their ideal pH, nutrient factors, and high water activity [1]. The main bacteria involved in meat spoilage include the genera *Brochothrix*, *Enterobacter*, *Acinetobacter*, *Moraxella*, *Pseudomonas*, *Leuconostoc*, and *Proteus*, meanwhile some of them (i.e., Enterobacter, Pseudomonas) secrete biogenic amines, which might cause food safety issues [2]. Furthermore, meat and meat products may be contaminated by pathogenic microorganisms such as *Campylobacter jejuni*, *Clostridium botulinum*, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella* spp., *Yersinia enterocolitica*, and *Staphylococcus aureus* [3,4].

One of the main concern for the meat industry is the spoilage of fresh meat and meat products caused by microbial contamination [5]. The meat industry applies different techniques to inhibit microbial growth and the production safe products with the suitable and

desired shelf life [6]. Accordingly, the most common applied techniques include physical (e.g., drying, freezing, heat treatment, packaging, and curing) and especially chemical (e.g., use of synthetic preservative compounds) methods [7]. Nevertheless, chemical additives have many disadvantages such as the alteration of the nutritional and organoleptic properties of foods [8,9]. Also, the carcinogenicity and toxicity of many chemicals such as nitrates have been proven. Nitrates are the most common chemicals used in the meat industry for the inhibition of microbial growth, retardation of lipid oxidation, development of better flavor, taste, and aroma, and preserve the color of the meat. In fermented sausages, their conversion into nitrites by microbial nitrate reductases can inhibit the growth of spoilage bacteria such as *Clostridium* spp. [10]. It has been reported that their excess consumption can have dangerous effects on consumer health due to the formation of carcinogenic nitrosamines [11]. For example, nitrates can generate nitric oxide by nitrosation reactions, which can undergo a reaction with secondary amines and form N-nitrosamines [12,13]. So, the increasingly negative perceptions of synthetic preservative chemicals, the greater attention of consumers towards food quality, and increasing demand for high nutritional and synthetic chemical-free products has promoted the food industry to replace traditional preservation methods with green techniques, such as active packaging, modified atmosphere packaging, high hydrostatic pressure, pulsed electric fields, and bio-preservation [14–17]. In this field, bio-preservation is the most reliable and potent technique closely related to "from farm to fork" strategy. Bio-preservation is considered a method to extend the shelf life of food products using compounds derived from plants, animals, bacteria, or fungi [18]. However, most researchers focus on bio-preservation by using beneficial microorganisms and/or their antimicrobial compounds [19].

In this context, lactic acid bacteria (LAB) have attracted more attention than other bio-preservative microorganisms due to different reasons such as their encapsulation capability by extrusion during the production of the antimicrobial film [20] and their GRAS status approved by the U.S. Food and Drug Administration (FDA) as a preservative in some food [3,21]. On the other hand, increasing the demand for natural preservative methods in line with environmental protection led to an increase in researchers' interest in finding efficient and sustainable preservative methods. In this regard, bio-preservation with LAB bacteria has no negative effects on consumers' health and environment and have recently gained more attention as a useful and sustainable approach for the production of functional foods that lead to the sustainability of the consumers' health [22,23]. So, the use of LAB and/or their metabolites, either alone or in combination with a low amount of natural or synthetic preservatives and moderate physicochemical treatments, may be an efficient solution to extend the shelf life and enhanced food safety (e.g., dairy products, fermented meat, and meat products) without negative effects on their nutritional quality [24]. Accordingly in the last two decades, intensive investigations have been focused on LAB and their antimicrobial metabolites to discover new LAB strains with food preservation potential to be used in sustainable preservative methods [25].

Hence, this review summarizes the current research on the bio-preservation of meat and meat products by LAB and their metabolites. Moreover, their mechanism of action and their application in hurdle technology and active packaging will be discussed.

2. Fermented Meat Products and Their Health-Beneficial Properties

In the past, different techniques were used and developed for the preservation of meat and meat products, starting with adding some ingredients, such as salt and sugar to reduce microorganisms without an exact understanding of their preservative mechanisms. But today, the use of microorganisms in terms of fermentation of meat and meat products is known as an effective preservation method [5]. Microbial enzymatic activities during fermentation leads to various physicochemical and microbial changes based on the meat components (natural or added components) [26]. Fermentation can occur by two pathways: (1) the use of natural microflora of meat or (2) the use of starter cultures such as lactic acid bacteria and micrococci. Lactic acid bacteria breakdown the carbohydrates and micrococci

reduce nitrates and nitrites to nitric oxide that leads to production of volatile and non-volatile compounds and flavor and odor changes of the product [27]. Also, fermentation causes different health-beneficial properties in fermented meat products in comparison to non-fermented ones such as antioxidant, antimicrobial, anti-hypertensive, and antithrombotic [28,29]. Furthermore, some nutritional components are produced during fermentation that have the potential to prevent diabetes, cancers, and allergic sensitization [30]. More studies are needed to discover other health-beneficial potentials of these products and their exact mechanisms. Also, these products should be evaluated in terms of food safety.

3. A Brief Overview on LAB

LAB are part of the natural microbial flora of fermented meats and the intestinal microbiota of humans. These aerotolerant bacteria are mainly non-sporing, Gram-positive, Catalase-negative, and have either a spherical-shaped or rod-shaped cell (Figure 1) [31]. LAB are microaerophilic organisms and preferably require anaerobic conditions for growth. They play an important role in food fermentations; in fact, LAB can ferment carbohydrates to high amounts of lactic acid as the final product (homofermentative bacteria); in addition to lactic acid, heterofermentative bacteria produce acetic acid, carbon dioxide, and ethanol, as by-products [32]. These organisms are acidophilus with the optimum acidic pH values of 5.5–6.2, but few can tolerate pH as low as 3.0 [33]. LAB are Generally Regarded As Safe (GRAS) according to the FDA and the European Food Safety Authority (EFSA) that have granted many LAB species Qualified Presumption of Safety status (QPS) [25,34,35]. Lactic acid bacteria possess considerable bioactive properties such as cholesterol reduction and antimicrobial properties, which has led to an increased interest in their effective role as preservatives in innovative food preservation technology, much more than their application in traditional fermentation [36–38]. The antibacterial activity of LAB strains has been proven in different studies [39]. It has been reported that different LAB strains secrete various compounds that inhibit bacterial growth such as diacetyl, phenyl-lactate, organic acids, hydroxy fatty acid, hydroxy phenyl-lactate, hydrogen peroxide, propionate, and cyclic dipeptides. These bacteria also secrete biosurfactants, bacteriocins (i.e., acidophilin, lactacin, bifidocin, helveticin, plantarim, pediocin, bulgaricin, diplococcin, and nisin), and bacteriocin-like inhibitory substances [40,41].

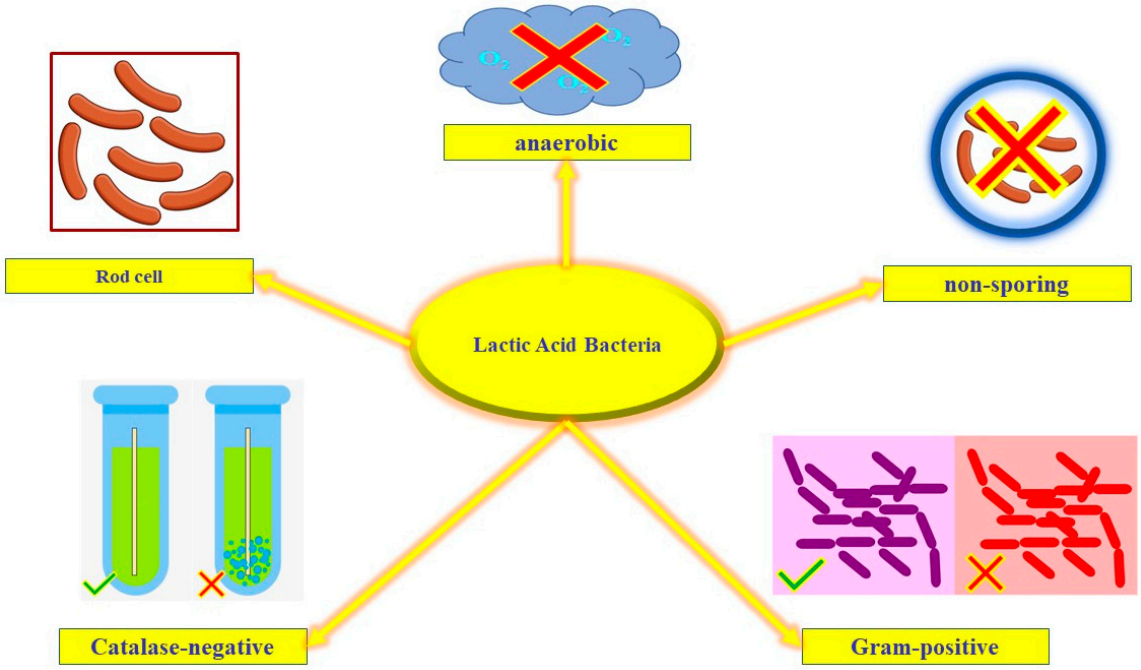

Figure 1. Schematic of properties of lactic acid bacteria.

3.1. LAB Strains Involved in Fermented Meat Products

The technological characterization of LAB strains involved in the fermentation process of meat is essential to select the best strain to be utilized as starter cultures [42]. The genera *Lactobacillus*, *Carnobacterium*, *Weissella*, *Pediococcus*, *Enterococcus*, and *Leuconostoc* are the main LAB that play crucial role in the fermentation [42,43]. The list of some of GRAS LAB that are most commonly used in bio-preservation of meat and meat products are mentioned in Table 1. Members of the genus *Lactobacillus* are usually the dominant species in most fermented meat products, but in some slightly acidified sausages, both *Enterococcus* and *Lactobacillus* are present in similar amounts [43]; nevertheless, *Lactobacillus plantarum* and *Lactobacillus curvatus* are the most common LAB species in fermented sausages [26]. It has been reported that in many fermented sausages, *Lactobacillus sakei* has the most adaptability due to a higher maximum growth rate, higher final cell density, and a shorter lag phase [44,45]. It should be noted that in Southern European sausages, the most and least common species are *Lactobacillus sakei* and *Pediococci* spp., respectively [46]. Also, molds, such as *Penicillium chrysogenum* and *Penicillium nalgiovense*, are commonly used for sausage ripening in Southern Europe [47]. In artisan sausages from Southern Europe, a strain of *Enterococcus faecium* grows increasingly during the early stages of fermentation, producing a bacteriocin [48]. It has been reported that yeast genera, especially *Debaryomyces hansenii*, can be found in fermented meat products with appropriate organoleptic characteristics [49,50]. In the next section, some data from the literatures on the bio-preservation of fermented meat products by application of starter LAB will be reviewed.

Table 1. Some of LAB species that are most commonly used in meat preservation.

Genus	Species	Genus	Species
Lactobacillus	*Lactobacillus delbrueckii* *Lactobacillus bulgaricus* *Lactobacillus gallinarum*	*Lacticaseibacillus*	*Lacticaseibacillus paracasei* *Lacticaseibacillus rhamnosus* *Lacticaseibacillus casei*
	Lactobacillus gasseri *Lactobacillus lactis* *Lactobacillus helveticus*	*Pediococcus*	*Pediococcus acidilactici* *Pediococcus pentosaceus* *Pediococcus parvulus*
	Lactobacillus reuteri *Lactobacillus acidophilus* *Lactobacillus curvatus* *Lactobacillus sakei* *Lactobacillusalivarius*	*Leuconostoc*	*Leuconostoc mesenteroides* *Leuconostoc citreum* *Leuconostoc pseudomesenteroides* *Leuconostoc carnosum*
Lactiplantibacillus	*Lactiplantibacillus pentosus* *Lactiplantibacillus plantarum*	*Latilactobacillus*	*Latilactobacillus sakei* *Latilactobacillus curvatus*
	Lactiplantibacillus brevis *Lactiplantibacillus casei*	*Limosilactobacillus*	*Limosilactobacillus fermentum* *Limosilactobacillus reuteri*

3.2. Bio-Preservation of Meat and Meat Products by LAB and Their Metabolites

As mentioned above, bio-preservation strategies are based on the application of natural compounds derived from microorganisms, plants, or animals for extending the shelf life of food products [18]. But most studies circumscribe the bio-preservation concept to the application of microorganisms such as LAB or their metabolites to enhance food safety and extend the shelf life of food products. Generally, the most important approach is to use of microorganisms or their metabolites with antimicrobial activity against food spoilage bacteria and especially foodborne pathogens [25]. A desirable bio-preservation compound should only show antimicrobial activity against the targeted spoilage or pathogenic microorganisms and should not adversely affect the intestinal microbial flora of consumers [18].

LAB can be used directly as a functional ingredient in meat and meat products or as starters in fermentation processes. When directly applied, LAB can be added in freeze-dried

or fresh cultures in different ways such as addition to fresh meat, meat batter formulation, or spraying on the surface of ready-to-eat meat products or fresh meat [25].

Traditionally, LAB have been widely used in fermentation processes, converting carbohydrates to lactic acid and producing biologically active compounds such as antibacterial and antifungal peptides, diacetyl, organic acids, and flavor precursors [51].

3.2.1. Bio-Preservation of Fermented Meat Products by Starter LAB

LAB play a key role in the production of fermented meat products resulting in texture and flavor improvement along with product preservation and finally the extension of shelf life. The strong buffering capacity and the low carbohydrate content of fresh meat lead to mild fermentation without variations in the organoleptic properties of food products. In naturally fermented meats, the addition of sugar leads to lactic acid production, pH decrease, and consequently protein denaturation by LAB activity [3]. The bio-preservation of meat and meat products by starter LAB has been reported in different studies as summarized in Table 2. For example, Nikodinoska et al. [52] evaluated the effect of *L. plantarum* PSC20 on microbial quality (*L. monocytogenes* and *Salmonella)* of Chorizo sausage. They reported that *L. plantarum* PSC20 caused a significant reduction in *L. monocytogenes* but did not have a significant effect on *Salmonella*. In another study, Lucumi-banguero et al. [53] revealed that *Lb. plantarum* and *Lb. sakei* have the maximum inhibition effect on *P. aureus* and the minimum effect on *S. typhimurium* and *S. marcescens*.

Table 2. The bio-preservative effect of LAB in meat and fermented meat products.

Product	Bio-Preservative LAB	Application Method	Targeted Microorganisms	Results	References
Vacuum-packaged fresh beef	*Lb. curvatus*	live culture and its bacteriocins, lactocin 705, and lactocin AL705	*L. monocytogenes* and *Br. thermosphacta*	Reduction in targeted microorganisms	[54]
Dry fermented sausage	*P. acidilactici*	as a starter culture	*L. monocytogenes*	Reduction in *L. monocytogenes* counts by 3.3 log CFU/g	[55]
Sliced fresh beef	*P. acidilactici* and *P. pentosaceus*	as a starter culture	*S. typhimurium* and *L. monocytogenes*	Reduction in *Enterobacteriaceae*, *Staphylococcus*, coliforms, *L. monocytogenes* and *S. typhimurium*	[56]
Vacuum packed raw beef	*L. curvatus*	as a starter culture	*B. thermosphacta* *Pseudomonas* sp.	No considerable bio-protective effect on *Pseudomonas* sp. Significant inhibition effect on *B. thermosphata*. Controlling the growth of natural spoilage lactic acid bacteria in meat	[57]
Beef meatballs	Bacteriocin produced by *L. plantarum*	0.3% bacteriocin	*E. coli* and *S. typhimurium*	Reduction the total plate counts. Inhibition effect on *E. coli*	[58]
Fresh beef meat	Lactococcin BZ	various amounts of lactococcin BZ (200–2500 AU/mL)	Total aerobic, psychrotrophic and mesophilic bacteria, lactic acid bacteria, faecal coliforms, total coliforms, and *L. innocua*	Reduction in mesophilic, coliforms, psycotrophic. 3.9 log reduction in LAB. 6 log reduction in *L. innocua.*	[59]

Table 2. *Cont.*

Product	Bio-Preservative LAB	Application Method	Targeted Microorganisms	Results	References
Sucuk sausages	*L. plantarum* strains (S50, S51, S72, S74)	as starter cultures	*L. monocytogenes*	Reduction in the *L. monocytogenes* counts by 2.74 log CFU/g in the presence of *L. plantarum* S50	[60]
Chorizo sausage	*L. plantarum* PSC20	as protective cultures	*L. monocytogenes* and *Salmonella*	Reduction in *L. monocytogenes* No significant effect on *Salmonella*	[52]
Minced beef	*Lb. acidophilus*	as protective cultures	*Enterobacteriaceae*, *coliform* and *S. aureus*	Inhibition effect on *S. aureus*	[61]
Chorizo	*Lb. plantarum* and *Lb. sakei*	as protective cultures	*L. monocytogenes*, *E. coli*, *S. typhimurium*, *S. marcescens*, *S. aureus*, *P. aeruginosa*, and *P. vulgaris*	The most inhibition activity on *P. aureus*, and the least on *S. typhimurium* and *S. marcescens*.	[53]
Fresh red beefminced meat	*Lb. acidophilus* and *B. animalis*	400 AU/g Bac FL 31 bacteriocin	*S. aureus*	Reduction in the *S. aureus* by 0.89 and 0.55 CFU/g by *Lb. acidophilus* and *B. animalis*, respectively	[62]
Fermented sausage	*L. curvatus* 54M16	as starter cultures	*Enterobacteriaceae* and staphylococci	Reduction in Staphylococci and Enterobacteriaceae	[63]
Raw and roasted pork	*Lb. paracasei F2I2*	spraying with bacterial suspension	Staphylococci and coliforms	Reduction in Staphylococci and coliforms by more than 3.83 and 3.48 log CFU/g	[64]
Fermented pork meat sausage	*L. curvatus* 54M16	as cultures	*L. monocytogenes*	Reduction in *L. monocytogenes* by 0.51 log CFU/g	[65]

3.2.2. Bio-Preservation of Meat and Meat Products by Bacteriocins from LAB

As mentioned above, LAB produce antimicrobial compounds such as bacteriocins that have the potential to limit the growth of pathogen or spoilage microorganisms. It has been reported that more than half of almost all bacterial species can produce bacteriocins [66]. In this regard, Ren et al. [67] reported the noticeable antibacterial activity of *L. rhamnosus* sp. A5 against *E. coli*, *B. subtilis*, *Salmonella*, and *S. aureus*. They revealed that its purified bacteriocins have a pronounced inhibitory effect against *E. coli* and *B. subtilis*.

Bacteriocins are small bioactive peptides extracellularly released by Gram-negative and Gram-positive bacteria such as LAB [68]. In addition to extending the shelf life, bacteriocins have other benefits such as reducing the risk of transmission of pathogenic microorganisms and using a smaller amount in the application of synthetic preservatives [69]. However, bacteriocins are somewhat like antibiotics, but they have significant differences for many properties [70]. Bacteriocins are more active at wider pH ranges and are inherently more resistant to higher temperatures than antibiotics. Moreover, they have fast-acting antimicrobial mechanisms even at very low concentrations, so the development of resistant microbial strains in target bacteria is unlikely to be observed. In addition, the sensibility to proteases represents a limit as many Gram-negative bacteria produce proteases that will hydrolyze bacteriocins and thus inhibit the activity of bacteriocins [71]. Generally,

bacteriocins are classified based on their molecular size, chemical structure, bacterial source, mechanism of action, and heat stability. Bacteriocins produced by Gram-positive LAB are divided into four groups (class I, class II, class III, and class IV).

Class I bacteriocins are thermo-stable, small (<5 kDa), ribosomally synthesized peptides with non-proteogenic thioether amino acids, lanthionine (Lan), and/or methyllanthionine (MeLan), so they are called lantibiotics [72].

Class II bacteriocins have molecular weight < 10 kDa. They are thermo-stable, hydrophobic, with 30–60 amino acids, contain a helical amphiphilic structure, which leads to the depolarization of the membrane of bacterial cell and consequently death of pathogens. These bacteriocins are called non-lantibiotics, as Lan or MeLan is absent [73].

Class III bacteriocins are heat-sensitive, large with molecular weight > 30 kDa [74]. This group have two subclasses, IIIa (bacteriolysin) and IIIb. The first subclass IIIa includes different bacteriocins such as lysostaphin and enterolysin that show their pathogenic effect by bacterial cell wall lysis. On the other hand, subclass IIIb such as helveticin M that act by dissipating the membrane and reducing the intracellular ATP concentration [75].

Class IV bacteriocins have complex structural moieties, so they are not considered as true bacteriocins and do not show antimicrobial potential. Some of colicins and microcins produced by *E. coli* belong to this group because of their antigenicity, microbial targets, and protein size [75]. The antibacterial effect of some bacteriocins from LAB are summarized in Table 3. For instance, de Azevedo et al. [76] evaluated the effect of bacteriocin-like inhibitory substances from *Pediococcus pentosaceus* in ready-to-eat pork ham. The results showed that in artificially contaminated ready-to-eat pork ham, bacteriocin-like inhibitors had considerable inhibition effect on the growth of *L. seeligeri* for 6 days. Chakchouk-Mtibaa et al. [77] investigated the effect of Bacteriocin BacFL31 from *Enterococcus faecium* on Turkey meat. They stated its considerable inhibition effect on *L. monocytogenes* and *Salmonella typhimuriu*.

Table 3. Antibacterial effect of bacteriocins from LAB in meat and meat products.

Product	Bactericins	Results	References
Ready-to-eat pork ham	bacteriocin-like inhibitory substances from *P. pentosaceus*	Inhibition of *L. seeligeri* by 1.74 log CFU/g—Lower weigh loss	[76]
Chicken meat pieces inoculated	Sonorensin from *B. sonorensis*	Inhibition of *L. monocytogenes* and *S. aureus*	[78]
Raw ground Turkey meat	Bacteriocin BacFL31 by *E. faecium* FL31	Inhibition of *L. monocytogenes* and *S. typhimurium* by 3.2 log CFU/g and *S. aureus* by 2.2 log CFU/g	[77]
Vacuum-packaged beef frankfurters	Semi-purified bacteriocins from *L. curvatus* or *L. sakei*	Reducing pathogens to below detection level	[79]
Vacuum-sealed hot dogs	Bacteriocins from *L. curvatus* L442 and *L. lactis* subsp. *cremoris* ATCC 14365	Reduction in *L. monocytogenes* by greater than 2 log CFU/hot dog	[80]
Fresh beef	Lactococcin BZ bacteriocin from *L. lactis*	Reduction in mesophilic, psychrotrophic, and lactic acid bacteria by 4.87, 3.50, and 3.94 log, respectively, and 1.90×10^4 and 1.04×10^2 CFU/g reduction in coliform and fecal coliform bacteria, respectively	[59]
Beef meat	Bacteriocins from *L. crustorum* MN047	Significant reduction in *E.coli* and *S. aureus* by 4.3 and 4.5 log CFU/mL, respectively	[81]
Raw chicken breast	Bacteriocin *XJS01* from *Ligilactobacillus salivarius*	47% reduction in *S. aureus*	[82]
Beef meat	Bacteriocin BM1300 from *L. crustorum* MN047	More antimicrobial effect against *E. coli* than *S. aureus*, 3.44 and 5.40 log CFU/mL, respectively	[83]

Table 3. *Cont.*

Product	Bactericins	Results	References
Fresh raw beef meat	Bacteriocin BM1122 from *L. crustorum* MN047	Inhibition effect on *S. aureus* and *E. coli* by 4.75 and 5.89 log CFU/mL	[84]
Fresh pork sausage	Bacteriocin from *L. curvatus* UFV-NPAC1	Reduction effect on *L. monocytogenes* as its counts ranged from 2 to 3.5 log CFU/g in control sample and ranged from 1.0 to 2.0 log CFU/g in treated sample through 10 days of storage	[85]
Chicken meat	Pediocin AcH/PA-1 from *P. pentosaceus* OZF	Reduction in *L. monocytogenes* counts by 3.8 log through 14 days of storage	[86]
Portuguese traditional fermented meat sausages	Pediocin PA-1 from *P. acidilactici* HA-6111-2	Reduction in *L. monocytogenes* to undetectable levels	[87]
Vacuum-packaged ready-to-eat meats	Bacteriocins from *L. curvatus*, *L. lactis*, *P. acidilactici*, *E. faecium*, and *E. thailandicus*	Reduction in *L. monocytogenes* by greater than 2 log	[88]

Synthesis and Mode of Action of Bacteriocins

The synthesis mechanism of bacteriocins can often be induced by population increase, stress conditions, nutrient shortage, cation surfactants, inhibitors, and the type of nitrogen, carbon, and phosphate sources in the media [89].

Bacteriocins are positively charged molecules with hydrophobic parts, and they interact electrostatically with phosphate groups (with negative charges) of cell membranes [14,90]. In other words, bacteriocins bind to receptors located on the cell wall of pathogen or spoilage bacteria, and then, bacteriocins kill the bacteria with various support mechanisms (Figure 2). Different mechanisms are involved in microorganisms' destruction by bacteriocin such as inhibition of nucleic acid synthesis, change in the cell translator mechanism, interference in the protein synthesis, and unbalanced functioning of the cytoplasmic membrane affecting cell permeability and energy use [91]. Moreover, lantibiotics inhibit target cells by creating pores on the membrane, causing disruption in the pH gradient or the transmembrane potential, leading to the leakage of cellular materials [14].

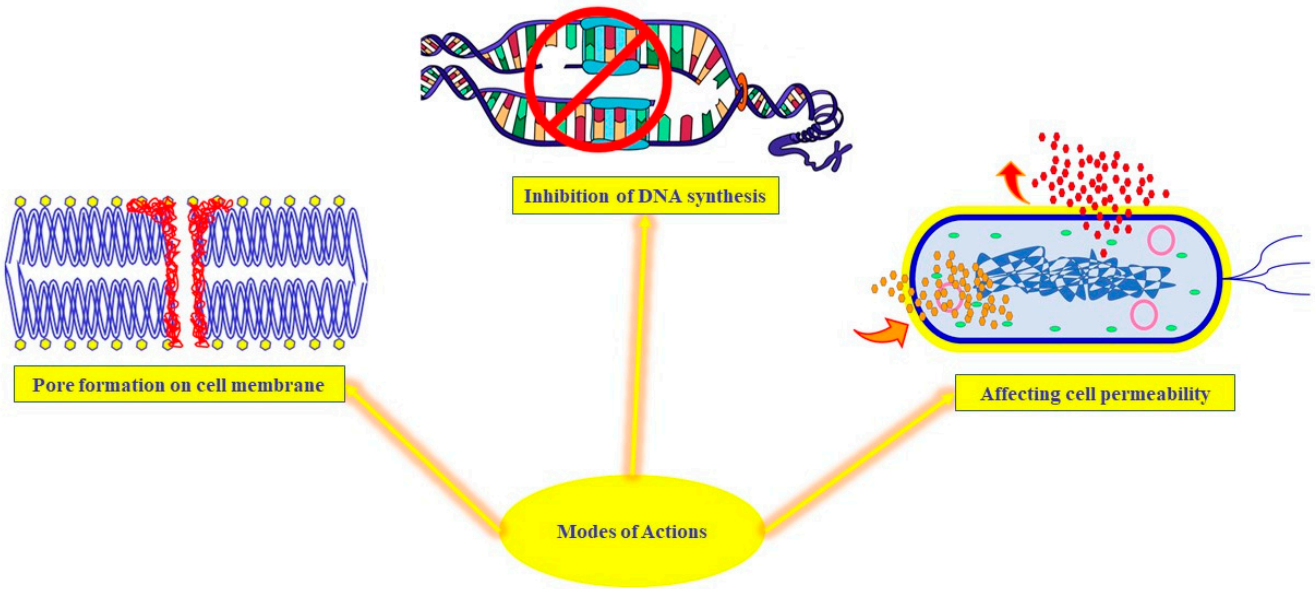

Figure 2. Modes of action of bacteriocins.

3.2.3. Bio-Preservation of Meat and Meat Products by Biosurfactants from LAB

As mentioned above, LAB produce different bioactive compounds including bacteriocins and bio-surfactants. In recent years, biosurfactants have reattracted much attention because of their considerable bioactive potential such as antibacterial, antiproliferative, and antioxidant activity [92,93]. Bio-surfactants are non-toxic, biodegradable, and amphiphilic compounds that are produced by various microorganisms such as LAB as secondary metabolites [94,95]. Biosurfactant are usually produce in two modes: cell-bound or excreted [96]. They have a diverse and complex chemical structure such as lipopolysaccharides, lipoproteins, and polysaccharide-protein complexes [97]. These compounds show distinct surface activity and can form micellar aggregates that lead to the reduction of the interfacial and surface tension [94,98,99]. Biosurfactants show appropriate activity at a wide range of pH and even under undesirable conditions such as salinities and high temperatures; so, they are suitable for food and pharmaceutical applications [100,101]. Their antimicrobial properties can play an effective role in the preservation of food products, including meat products. They exert their antimicrobial potential through various mechanisms such as: (1) The prevention of biofilm formation by the reduction of the bacterial interaction with the surface through alteration of the charge and wettability of the surface [94,102]; (2) Interference in the normal function of the microorganisms by interaction with their intracellular constituents [103]; (3) Destruction of the cell walls and membranes of microorganisms [104,105]. Different studies evaluated the antibacterial potential of biosurfactants from LAB on food pathogens; a summary of these studies is listed in Table 4.

Table 4. The antibacterial effect of biosurfactants from LAB on food pathogens with emphasis on meat and meat products.

Pathogens Sources	Biosurfactant	Results	References
Raw ground goat meat	Biosurfactants from *L. paracasei* and *L. casei*	Reduction in the total aerobic counts, *E. coli* MTCC 118, and *P. aeruginosa* MTCC 1934	[106]
Fresh beef	Biosurfactants from *L. paracasei*	100% inhibition of *Bacillus* sp. BC1, *S. aureus* STP1, and *S. xylosus* STP2	[107]
--------	Biosurfactants from *L. paracasei*, *L. delbrueckii,* and *L. acidophilus*	18, 47.36, and 30.92% inhibition of *S. aureus*, respectively	[108]
--------	Biosurfactants from *L. paracasei*	100% inhibition of *E. coli*, *S. agalactiae* and *S. pyogenes*	[109]
--------	Biosurfactants from *L. paracasei* ssp. *Paracasei* A20	Reduction in *C. albicans*, *S.aureus, and S. Epidermidis*	[110]
--------	Biosurfactants from *S. thermophilus* A	Antimicrobial activity against *S. epidermidis*, *S. aureus*, *S. salivarius*, *R. dentocariosa*, *C. albicans*, and *C. tropicalis* at concentration of 20 and 40 g/L	[111]
--------	Biosurfactants from *L. lactis* 53	Antimicrobial activity against *S. epidermidis*, *S. aureus*, *S. salivarius*, *R. dentocariosa*, *C. albicans*, and *C. tropicalis* at concentration of 20 and 40 g/L	[112]

3.3. LAB or Their Metabolites as a Part of Hurdle Technology

Hurdle technology refers to the combination of different preservative factors such as water activity (a_w), temperature, redox potential (Eh), and novel preservative techniques, such as gas packaging, natural extracts, essential oils, and bacteriocins, to create more selective and efficient defensive systems to overcome pathogenic and spoilage microorganisms [69]. So, LAB and their metabolites can be used as a part of hurdles technology that act synergistically and inhibit food spoilage in combination with other preservative agents. In this way, less intensities of technological treatment and/or doses of preservative agents are required [113]. In the application of different antimicrobial agents, it is very important to select the best combination, so that desirable preservative effects are achieved. In this regard, it has been reported that the addition of chelating agents makes the outer membranes of Gram-negative bacteria more permeable and sensitive to the hydrophobic peptides such as bacteriocins [114]. Also, freezing temperatures and modified atmosphere

packaging (MAP) in combination with LAB and their metabolites can be used in hurdles technology approaches [115]. Different studies have evaluated the effect of different LAB in combination with various bio-preservatives on the microbial quality of meat and fermented meat products (summarized in Table 5).

Table 5. The antibacterial effect of LAB or their metabolites in combination with other preservative agents in meat and meat products.

Product	Bio-Preservative Agent	Results	References
Ground beef	*L. reuteri* or *L. plantarum* in combination with garlic extract	1.4 and 1.5 log reduction of *L. monocytogenes* by using *L. reuteri* or *L. plantarum* in combination with 1% of garlic extract	[116]
Beef sausage	Bacteriocinogenic *Enterococcus mundtii* and 0.0075% ascorbic acid, 3% NaCl, 0.02% $NaNO_2$, 0.75% glucose and 0.75% sucrose	>2 log cfu/g reduction of *L. monocytogenes*	[117]
Sliced beef	Bacteriocin from *C. maltaromaticum* combined with steam and chitosan	No synergistic effect 2 log reduction of *S. typhimurium*, *E. coli* and *S. typhimurium*	[118]
Minced beef meat	*Mentha piperita* essential oil with semipurified bacteriocin	Reduction in *Enterobacteriaceae*	[119]
Frozen ground beef patties	Bacteriocin-producing *L. curvatus* and *L. lactis* in combination with Na2EDTA	1 log reduction of *E. coli*	[120]
Fresh chicken meat burger	*L. pseudomesenteroides* combined with MAP (50% CO_2 and 50% O_2)	Reduction in *L. monocytogenes* and *C. jejuni*	[115]
Fresh pork sausage	Combination of essential oils, nisin, nitrite, and organic acid salts, encapsulated	Reduction in *L. monocytogenes*	[121]
Alheira paste	*L. sakei* and *L. plantarum*, vacuum packed or packed under MAP (20% CO_2, 80% N_2)	2 log reduction in *L. monocytogenes* by *L. sakei*. No significant differences between vacuum or MAP	[122]
Sliced lombo	Combination of Bacteriocin from *P. acidilactici* with HPP	Reduction in *L. innocua*	[123]
Goat meat emulsion	Combination of Pediocin from *P. pentosaceus* and *Murraya koenigii* berries extract	Reduction in *L. innocua*	[124]
Ready-to-eat porkham	Bacteriocin-like inhibitory substances (BLIS) from *P. pentosaceus* and nisin	Inhibition of growth of *L. seeligeri*	[76]

LAB Application in Active Packaging

In addition to increasing demand for natural preservative agents, the demand for biodegradable and ecologically friendly packaging materials is increasing. On the other hand, the direct use of preservative agents in meat and meat products may reduce their bio-active properties such as their antimicrobial activity. So, in order to avoid this negative effect, the application of preservative agents as one of the components of active packaging is a suitable solution. Active packaging is a promising technology that actively modifies the internal environment of the food product package by interacting with the food over the storage time. Also, it is defined as an intelligent packaging system that alter and modify the environment inside the package and consequently the state of the food system in order to improve the food quality and extend the shelf life of the product [125,126]. In this field, anti-microbial packaging is an efficient type of active packaging that have attracted much

attention. Anti-microbial packaging has the ability to inhibit or kill pathogenic or spoilage food contamination microorganisms [127]. Antimicrobial agents can initially incorporate into the packaging materials and migrate into the food through partitioning and diffusion. Different approaches involve the incorporation of antimicrobial agents into food packaging, such as adding antimicrobial agents into the film formulation and adding them in the extruder when producing the film. Generally, different factors affect the properties of antimicrobial film such as the condition of casting process, the antimicrobial potential of the antimicrobial agent, physicochemical properties of film, mass transfer coefficient, and storage temperature [127,128]. In this regard, the application of preservative agents such as LAB and their metabolites in active packaging has various advantages such as controlled release and the need to lower the amount of these agents [113,129]. Various studies have investigated the preservative effect of LAB and their bacteriocins on the quality of meat and meat products (summarized in Table 6).

Table 6. The application of LAB or their metabolite in active packaging of meat and meat products.

Product	Bio-Preservative Agent	Results	References
Natural and artificial casings of meat product	Sakacin G from *L. curvatus*	Reduction in *L. innocua*	[130]
A pullulan film in ready-to-eat turkey breasts	Sakacin A from Lactobacillus sakei *DSMZ 6333*	Reduction in *L. monocytogenes*	[131]
An active polyvinylidene chloride film on fresh pork	Plantaricin from Lactocacillus plantarumBM-1	Reduction in *L. monocytogenes*	[132]
A novel biocomposite film made of poly lactic acid and sawdust particles on raw sliced pork	Pediocin PA-1/AcH	Reduction in *L. monocytogenes*	[133]

3.4. Kinetics Models for Microbial Inactivation

Different kinetic models have been widely used for predicting the inactivation patterns of microorganisms. In this regard, the first-order kinetic mode is employed for log-linear survival curves, while the Weibull, biphasic, and log-logistic models are used for non-log-linear inactivation patterns. The first-order kinetic mode, the Weibull, biphasic, and log-logistic are expressed by the following equations, respectively [134]:

$$log\frac{N_t}{N_O} = -\frac{t}{D_T} \quad (1)$$

$$log\frac{N_t}{N_O} = -bt^n \quad (2)$$

$$logN_t = logN_o + \log\left(k \times e^{-\alpha t} + (1-k) \times e^{-\beta t}\right) \quad (3)$$

$$log\frac{N_t}{N_O} = \frac{A}{1+e^{\frac{4\sigma(\tau-logt)}{A}}} + \frac{A}{1+e^{\frac{4\sigma(\tau+6)}{A}}} \quad (4)$$

where N_O and N_t are the initial and surviving populations of bacteria at any time (CFU/g), t is time (min), D_T is defined as the time at which 90% of the bacterial population is inactivated, b is the inverse of the shape factor (1/min), n is the shape parameter (dimensionless), α and β are the inactivation kinetic rate constants (1/min), σ is the maximum inactivation rate (log (CFU/g)/log min), τ is the log time to attain the maximum inactivation rate (log min), A is the log increase in population. The statistical criteria are used to determine the goodness of fit of the kinetic models for describing the survival data.

4. Conclusions

The further negative perceptions related to the increase of synthetic preservative chemicals in meat products and the increased consumer focus on the relationship between the daily diet quality and health has led the food researchers to replace synthetic preservatives

with natural compounds. Generally, an effective bio-preservation compound should only show antimicrobial activity against the targeted spoilage or pathogenic microorganisms and should not adversely affect the intestinal human microbial flora. In this regard, LAB and their metabolites have been widely studied due to their high preservative activity, which can potentially be used in the bio-preservation of meat and fermented meat products. In this paper, a general overview of LAB was given with reference to the species involved in fermentation of meat products. The genera of *Lactobacillus*, *Carnobacterium*, *Weissella*, *Pediococcus*, *Enterococcus,* and *Leuconostoc* are the leading LAB that play an essential role in fermentation. LAB produce various biological compounds such as antibacterial and antifungal peptides, diacetyl, organic acids, etc. In this field, bacteriocins are small bioactive peptides that are released by Gram-negative and Gram-positive bacteria such as LAB and induce a shelf-life extension of meat products. On the other hand, the combined use of LAB or their metabolites with other preservatives (nisin, natural extracts, essential oils, etc.) and novel techniques (MAP, HPP, active packaging, etc.) as hurdle technology, significantly increase their preservative effect. Moreover, the incorporation of LAB and their metabolite in active packaging is a more efficient method in their application as bio-preservatives in meat and meat products in comparison to direct application. As a result, these conclusions in the application of LAB in bio-preservation will pave the way for commercial use of LAB and their metabolites, especially bacteriocins, in the meat industry as natural preservatives to replace synthetic compounds. In addition, the combination use of these microbial bio-preservatives with other antibacterial compounds has an effective result on the shelf life and security of meat products. However, it is necessary to evaluate and characterize the novel bacteriocins and enhance suitable preservation techniques to avoid resistance.

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