Review Article

Bacteriocin and its Current Application as a Food Packaging Film Component against Spoilage: A Narrative Review

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ABSTRACT

Food spoilage still concerns the manufacturing of food products. Several factors contributed to this problem, including temperature during preservation or storage, moisture and water content, and the majority are due to bacteria that cause spoilage. Lactic Acid Bacteria (LAB) have several uses, including as either starter cultures or probiotics due to their antimicrobial compounds. LAB produce bacteriocins, which have a significant role in biopreservation. The incorporation of bacteriocins into different packaging films has proven to control the spoilage bacteria growth and at the same time improve food quality. However, the Food and Drug Administration (FDA) has only approved a few LAB bacteriocins as biopreservatives, wherein nisin is the most popular. Packaging immobilization of other bacteriocins that have a broader antimicrobial scope has become an interest. This narrative review discusses the current applications of bacteriocins as food packaging film components against spoilage. A total of 20 studies were retrieved from PubMed, GoogleScholar, ScienceDirect, and ResearchGate, restricting from 2013-2021. The gathered literature works reported novel bacteriocins that can be candidates as biopreservatives due to their broader antimicrobial scope and inhibitory effects. However, there is still a need for further investigation of the newly discovered LAB bacteriocins on their packaging application and inhibition of food pathogens.

Key words: Lactic acid bacteria, Bacteriocins, Biopreservation, Food packaging, Antimicrobial packaging, Spoilage.

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INTRODUCTION

Various ways to preserve foods have always been in demand due to transportation issues and environmental concerns. Studies about bacterial growth inhibition in foods that cause food spoilage are still a hot topic to cater using natural preservatives capable of extending the food products' shelf life. Bacteria such as Lactic Acid Bacteria (LAB) are known for its use in food preservation. Likewise, LAB is often used as a starter culture or as probiotics due to its antimicrobial compounds. Production of bacteriocins is one characteristic needed when used in the food industry, which are antimicrobial peptides that continuously act as a preservative candidate due to their antibacterial or inhibitor activity against foodborne pathogens. Over the years, foodborne pathogens are still the main problem related to food spoilage. Fortunately, bacteriocins are proven to inhibit various Gram-positive and Gram-negative bacteria. These bacteriocins are induced into food packaging and can inhibit other Gram-positive bacteria that develop in food surfaces.[1]

The discovery of the LAB producing bacteriocins that prevents spoilage to foods led to the birth of food

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packages incorporated with LAB bacteriocins. However, factors such as temperature and pH can hugely affect or influence the activity of bacteriocins in inhibiting food-borne pathogens.[2] There are four classes of bacteriocins known and used in different fields, namely Class I, II, III, and IV bacteriocins. Bacteriocins like Nisin and Pediocin are the most commonly used natural preservatives against pathogenic food microorganisms.[3] Bacteriocins not only prevent the spoilage of food but also extend the food's shelf life as well. One of the applications of bacteriocins in food preservation is through food packaging. Food packaging induced with bacteriocins prevents food from being contaminated by inhibiting bacterial growth in food surfaces. Furthermore, various researchers and innovators of food packages incorporated with LAB bacteriocins are beginning to step up their studies, improving these food packages by creating edible packages and films fused with active agents that can control and prevent the growth of microbial contamination.

Bacteriocins have proven their role as a natural preservative. However, spoilage is still a concern despite their application on food packaging. The possible reason for this is that Gram-positive or Gram-negative bacteria only can be inhibited by the Generally Recognised as Safe (GRAS) bacteriocins approved by Food and Drug Administration (FDA). There is an emergence of potential bacteriocins than nisin to be applied on food packaging. Several authors have also tried to develop edible packaging films with bacteriocins to prevent food spoilage. However, some of these still lack further application or experiment on food to prove their antimicrobial activity. The purpose of this review is to give readers knowledge about the existing current trends in food packaging with LAB bacteriocins. Also, this might raise other potentiality of LAB bacteriocins in food packaging. Hence, the content of this narrative review thoroughly focuses on the widely used LAB bacteriocin, their mode of action against foodborne pathogens, and their application as biopreservatives and food packaging components against spoilage bacteria. The wonders of LAB Bacteriocins incorporated in food packaging could be advantageous in many different sectors and aspects such as in the food industries, world trade, environment, and many more. This review aims to present the existing studies that focus on the capability of LAB bacteriocins incorporated in food packaging to prevent further spoilage in food. Also, explain the other potential of LAB bacteriocins in edible packaging in the form of a narrative review.

METHODOLOGY

Approach

The review speaks of the given available articles for biopreservation and food preservation and literature that contains reports on its application.

It pertains to the LAB that can produce bacteriocins, different spoilage bacteria saw, and its mode of action. Including bacteriocins' that work as food preservatives, the current development of incorporation of it in antimicrobial films led to the idea of active/edible food packaging.

The addition of newly discovered potential bacteriocins for food preservation includes Plantaricin, e.g., Plantaricin LR14, Plantaricin C; Thuricin, e.g., Thuricin CD; and Cerein, e.g., Cerein 7. Shown and discussion of other bacteriocins present in food products and active edible packaging such as chitosan, bacterial cellulose, cassava starch, polylactic acid (PLA).

Literature Search Strategy

The searches were retrieved from four online databases, Google Scholar, PubMed, ResearchGate, and ScienceDirect. The combination search terms used include "lactic acid bacteria" AND ("bacteriocin" OR "nisin") AND ("active packaging" OR "food packaging" OR "edible film") AND ("antimicrobial activity" OR "spoilage" OR "shelf life extension" OR "biopreservation" OR "biopreservative"), with publication date restriction, to retrieve recent trends on bacteriocin food packaging application.

Eligibility Criteria

This review focuses on the current application of bacteriocin as a food packaging film against spoilage, restricting the included studies and articles primarily from the period of 2013 – 2021. Other inclusion criteria include (a) bacteriocins used as packaging components and (b) packaging with bacteriocins against spoilage, and (c) potential bacteriocins as biopreservative against spoilage with recommendation for their packaging application.

On the other hand, studies without full-text available and are only presented as abstracts, non-English literature, letters, academic correspondence, editorials, study protocols, pilot studies, papers discussing LAB but not involving bacteriocins, scholarly studies or reports about food packaging without bacteriocins, and have no relevance to any application against food spoilage were all excluded and omitted from the entire master source of this review's research references.

RESULTS

The initial articles that the authors have searched are about 2,728 articles. A total of 705 removed duplicate studies. Then, 2,023 screened studies for the title and abstract. After this, 166 articles were assessed and reviewed for full-text availability and inclusion criteria. Lastly, including about 20 articles passed for the eligibility criteria in this review. Figure 1 illustrates the process of selecting the articles to be included in this narrative review.

Spoilage Bacteria

Food spoilage occurs in the change of food sensory based on its flavor, visual, tactile, and olfactory. Different microorganisms and chemicals contaminate the food that leads to food spoilage. However, spoilage of food depicts its appearance rather than the problem of the disease it causes due to the reason that the majority of microorganisms responsible for food spoilage do not cause pathogenic effects to humans.[24] The risks of food spoilage may happen at any time,

Table 1: Characteristics of the studies included.

Study	Bacteriocin, LAB species and strain	Food application, Packaging material, Other components combined, Strategy	Target pathogen, Antimicrobial activity against spoilage bacteria
[4]	Nisin. *Did not directly isolate from LAB*. Purchased from Sigma-Aldrich.	Ready-to-eat (RTE) deli turkey meat. PLA films. Chitosan, lauric arginate (LAE), and both chitosan and LAE. (1) Direct coating and (2) double-layer film treatment, with or without flash pasteurization treatment.	*Listeria innocua*. Plate count method (Colony forming units [CFU]/cm^2). Chitosan + nisin reduced more *L. innocua* than chitosan alone Chitosan + LAE is more effective in reducing *L. innocua* Flash pasteurization significantly reduced the *L. innocua*
[5]	Plantaricin BM-1 *Lactobacillus plantarum* BM-1.	Sliced cooked ham. Polyamide- polyethylene bags (vacuum-packaged). (1) Incorporation (incorporated- plantaricin batch) and (2) direct surface application on one side (applied- plantaricin batch).	*L. monocytogenes*. Plate count method (CFU/g). Applied-plantaricin batch markedly decreased viable counts of *L. monocytogenes* during the 35-day storage period Both incorporated- and applied-plantaricin batches delayed the *L. monocytogenes* growth Incorporated-plantaricin batch had better inhibitory effect than applied-plantaricin batch
[6]	Sakacin A. *Lb. sakei* DSMZ 6333.	RTE thin-cut veal meat (carpaccio). Polyethylene (PE)-coated paper. Gelatin from cold water fish skin, glycerol, and water. Coating.	*L. innocua*. Plate count method (CFU/g) and zone of inhibition test. Sakacin A-PE-coated paper sheets have decreased *Listeria* population after 48 hr Slightly reduced aerobic population was also observed in meat slices stored in contact with sakacin A-coated sheets after 48 hr
[7]	Nisin. *Did not directly isolate from LAB*. Purchased from Maxinis®.	Cooked ham. Biodegradable Polyhydroxybutyrate/ Polycaprolactone (PHB/PCL) film blended with (1) Cloisite® 30B, and (2) Cloisite® 10A nanocomposite films. Adsorption at different concentrations and temperatures.	*L. plantarum* CRL691. Zone of inhibition test. PHB/PCL + nisin films exhibited an effective inhibitory activity against *L. plantarum* CRL691 Extended shelf life and effective against the target pathogen No synergistic antimicrobial effects observed in the combination of organo-clays fillers and nisin
[8]	Plantaricin BM-1. *Lb. plantarum* BM-1.	*No food application*. (1) PE, (2) low-density PE (LDPE), and (3) high-density PE (HDPE) films. Soaking, absorption, and coating.	*L. monocytogenes* CMCC 54003. Zone of inhibition test. Clear and obvious zones of inhibition: PE (14.33 mm); LDPE (15.00 mm); HDPE (15.33 mm)

Continued...

Table 1: Cont'd.

Study	Bacteriocin, LAB species and strain	Food application, Packaging material, Other components combined, Strategy	Target pathogen, Antimicrobial activity against spoilage bacteria
[9]	Nisin. *Lactococcus lactis*.	Ultra-filter (UF) cheese. Bilayer cellulose-chitosan-zinc oxide (ZnO) nanocomposite film. Chitosan, zinc oxide nanoparticles, and Tetraethyl orthosilicate (TEOS). Sol-gel method.	*L. monocytogenes*. Disc covering test and zone of inhibition test. Nisin-incorporated chitosan-cellulose films exhibited antimicrobial activity during 4 ± 1°C and 22 ± 1°C storage, and significantly reduced *L. monocytogenes* viable counts Sol-gel method effectively retain nisin in the film
[10]	Nisin. *Did not directly isolate from LAB*. Purchased from Sigma-Aldrich.	*No food application*. Bacterial nanocellulose membrane (from *Gluconacetobacter xylinus*). EDTA solution (10 mM, 20 mM, or 40 mM). Immersion at different concentrations.	*E. coli*, *S. aureus*, and *Pseudomonas aeruginosa*. Zone of inhibition test. Nisin without EDTA solution inhibited *S. aureus*, but did not inhibit *E. coli* and *P. aeruginosa* Nisin with 10 mM EDTA solution inhibited all pathogens Both *E. coli* and *P. aeruginosa* were inhibited in nisin with 20 and 40 mM EDTA solution
[11]	Nisin. *Did not directly isolate from LAB*. Purchased from Shanghai Yuanye Bio-Technology Co., Ltd.	*No food application*. PLA film. Cold plasma treatment and coating.	*L. monocytogenes*. Zone of inhibition test. Cold plasma treatment affected the adsorption capacity of the PLA film Inhibited *L. monocytogenes* due to improved adsorption of nisin after cold plasma treatment
[12]	Bacteriocin DY4-2. *Lb. plantarum* DY4-2.	Turbot fillets. Polyvinyl chloride bags. Immersion.	*P. fluorescens*. Plate count method (CFU/mL) and zone of inhibition test. Reduced *P. fluorescens* during 12-day storage at 4°C Delayed the deterioration of turbot fillet quality
[13]	*Unidentified bacteriocin*. *Lactobacillus sakei* subsp. *sakei* 2a.	*No food application*. Bacterial cellulose membranes (from *G. xylinus*). Entrapment and immersion.	*L. monocytogenes*. Plate count method (CFU/mL) and zone of inhibition test. Exhibited zone of inhibition against *L. monocytogenes* Entrapped bacteriocins in bacterial cellulose membranes' nanofibers exhibited a significant antimicrobial activity
[14]	Bacteriocin 7293. *W. hellenica* BCC 7293.	Pangasius fish fillet. PLA/sawdust particle biocomposite film (PLA/SP). Diffusion coating.	*L. monocytogenes*, *S. aureus*, *P. aeruginosa*, *Aeromonas hydrophila*, *E. coli*, and *Salmonella* Typhimurium. Spot-on-lawn method and plate count method (CFU/cm^2). Exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria Sawdust particles may improve the embedding of bacteriocin 7293 into PLA film
[15]	Plantaricin BM-1. *Lb. plantarum* BM-1.	Fresh pork meat. Polyvinylidene chloride (PVDC) films. Soaking and adsorption.	*L. monocytogenes*. Zone of inhibition test. Exhibited inhibition zones against *L. monocytogenes* (16.28+0.37 mm) Inhibited *L. monocytogenes* growth during 24-hr culture
[16]	Enterocin BacFL31. *E. faecium* FL31.	Ground beef meat. Sterile plastic bags. Aqueous peel onion extract. Supplementation.	Enterobacteriaceae. Aerobic plate counts (APC), aerobic psychrotrophic counts (PTC), and Enterobacteriaceae counts. Reduced Enterobacteriaceae growth and *L. monocytogenes* ATCC 19117

Continued...

Table 1: Cont'd.

Study	Bacteriocin, LAB species and strain	Food application, Packaging material, Other components combined, Strategy	Target pathogen, Antimicrobial activity against spoilage bacteria
[17]	Raw bacteriocin. *Lb. plantarum* and *Lb. paracasei*.	Catfish and pork. Bacterial cellulose membrane. Loading.	*E. coli*, *S. aureus*, and *B. subtilis*. APC and Zone of inhibition test. Inhibited *E. coli*, *S. aureus*, and *B. subtilis*
[18]	Nisin (Z). *L. lactis* subsp. *lactis* I8-7-3 LC 113942.	Bigeye snapper and tiger prawn. Thermoplastic starch/polybutylene adipate terephthalate (TPS/PBAT) film (vacuum-packaged). Gelatin with LAE. Coating.	*S.* Typhimurium ATCC 14028 and *V. parahaemolyticus* ATCC 17802. Plate count method (CFU/g) and zone of inhibition test. Gelatin-TPS/PBAT films with combination of Nisin and LAE reduced and eliminated population of *Salmonella* on both refrigerated tiger prawn and bigeye snapper raw slices
[19]	Plantaricin BM-1. *Lb. plantarum* BM-1.	Fresh pork meat. PE terephthalate/polyvinylidene chloride/retort casting polypropylene (PPR) plastic multilayer film (vacuum-packaged). Chitosan. Coating.	*L. monocytogenes*. Plate count method (CFU/g). PPR alone and with chitosan only exhibited an increased counts of *L. monocytogenes* PPR with both chitosan and plantaricin BM-1 reduced *L. monocytogenes* viable counts and could markedly inhibit their growth at 4°C storage
[20]	Nisin. *L. lactis*.	Fresh, raw beef, fresh, raw chicken breast, and RTE turkey breast. Composite antimicrobial film (CAF), *made from biopolymer-poured PE film*. Pullulan, gelatin, xanthan gum, glycerol, thymol, LAE, and biopolymer solution. Mixing and combining.	Shiga toxin-producing *E. coli* (STEC), *Salmonella* spp., *L. monocytogenes* and *S. aureus*. Plate count method (CFU/cm^2) and zone of inhibition test. CAF with nisin alone has no significant inhibition against cocktails of four pathogens CAF with LAE is more effective than CAF containing nisin
[21]	Nisin (A). *Did not directly isolate from LAB*. Purchased from Cayman chemical.	Cheddar cheese. (1) Gelatinized and (2) non-gelatinized resistant starch polymers. Sodium alginate (0.5, 1 and 2 %). Microencapsulation.	*Clostridium tyrobutyricum*. Zone of inhibition test. Encapsulated nisin reduced *C. tyrobutyricum* viable count after incubation for the 1st week at 4°C and become undetected from 2nd week up to 4th week Nisin beads exhibited inhibition zone against *Pediococcus acidilactici* UL5
[22]	Nisin. *Not mentioned*.	Papaya fruit. LDPE. Chitosan. Layer-by-layer and coating.	*E. coli*, *B. subtilis*, *L. monocytogenes*, *Klebsiella pneumoniae*. Plate count method (CFU/g). Exhibited low number of colonies than unwrapped and LDPE-wrapped Extended shelf life
[23]	Bacteriocin BM1029. *Lb. crustorum* MN047.	Beef meat. Sterile bags. Soaking.	*E. coli* and *S. aureus*. Zone of inhibition test. Reduced *E. coli* viable counts Exhibited inhibition zone against Antibiotic-resistant *E. sakazakii* 6-16 (1), *L. monocytogenes* CMCC 54004, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923

Selection Strategy

Members of this review divided themselves into two groups: The first group was the reviewers that initially screened the potential studies to be included in this review. The author's adviser acted as the reviewer that assessed any discrepancies found in the initial selection of eligible studies to be used in this review. Then, the same group evaluated and verified titles and abstracts for their relevance. The second group conducted the full-text screening. One of the authors tabulated and summarized the eligible full-text references used for this review.

Data Extraction

The characteristics of eligible studies such as the first author's name, publication year, LAB species and

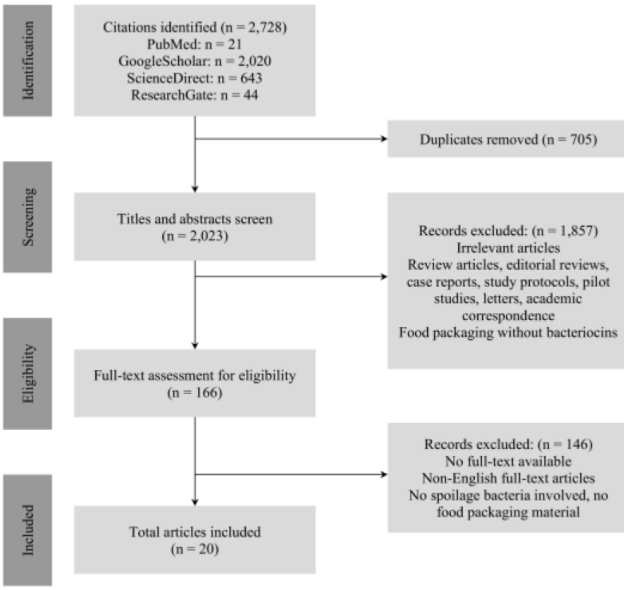

Figure 1: Flow chart for study selection process.

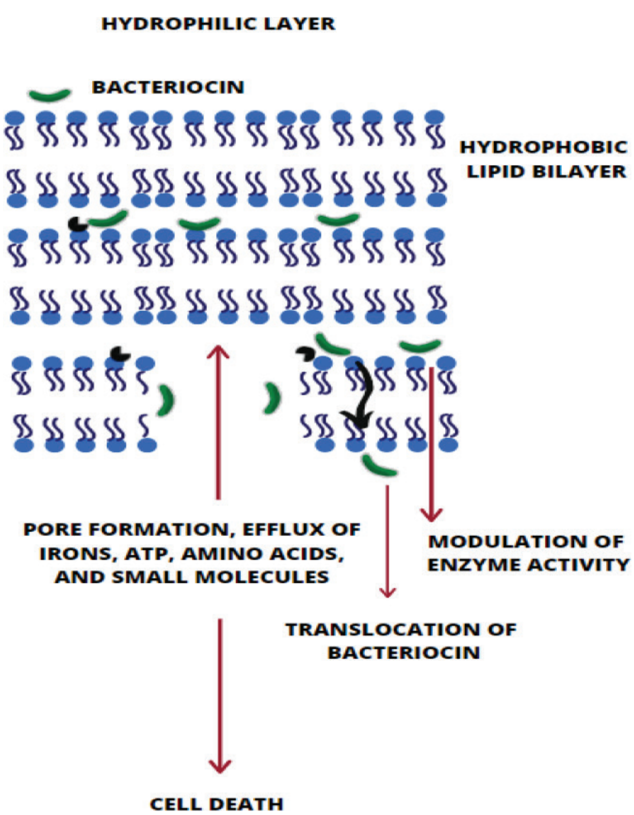

Figure 2: LAB bacteriocin Mode of Action.
The killing mechanism of bacteriocin involves binding with its receptors, membrane pore formation in the cytoplasm, enzyme activity modulation and translocation.

strain, bacteriocin, the components combined with the bacteriocin, packaging material and strategy, food application, target pathogen, and antimicrobial activity against spoilage bacteria were extracted and mentioned in detailed by three authors in Table 1.

especially when foods are in unsuitable conditions that cause spoil.

Various reasons why food spoilage occurred are caused by extrinsic and intrinsic factors.[25] Extrinsic factors cause oxygen, temperature, and humidity of food spoilage. Temperature is the primary cause of food spoilage's extrinsic factor because it influences the cell's thermal appearance. However, food spoilage's intrinsic factors are pH, moisture content (water activity), and nutrient content.

Most foods spoil easily because there is often a succession of different nutrients that microbes need to rise. The most common organisms in food are fungi and bacteria. Fungi such as yeasts and molds are unicellular/single-cell organisms and multicellular/organisms with several cells. Bacteria that dominate fungi and are also unicellular.

Microorganisms, including food fermentation bacteria can multiply up to where they can be capable of spoiling food. In reality however, only certain species of bacteria have been related to the spoilage of food, dictated by the characteristics of the bacteria, the food and its storage conditions. The predominant non-spore forming bacteria that are related to food spoilage are the following bacteria: *B. thermosphacta*, *Carnobacterium*, *K. zopfii*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Weisella*. These cause discolorations, production of gas, off-colors, off-flavors, production of slime, and pH decrease when spoiling food. There are also spore-forming bacteria that contribute to spoilage, namely Bacilli which are aerobic and Clostridia which are strictly anaerobic.[26]

Table 2 enumerates the various examples of bacteria that cause spoilage in different types of food, namely meat products, milk, fruit, and vegetables. Meat and seafood both have *Brochothrix*, Carnobacteriaceae, and Shewanellaceae as their spoilage bacteria. On the other hand, Enterobacteriaceae and *Lactococcus* are the similar spoilage bacteria of meat and milk, Staphylococcaceae and lactic acid bacteria for meat and fruit, and *Clostridium* spp. for meat and vegetables. *Bacillus* bacteria causes spoilage in three types of food products, namely milk, fruits, and vegetables. Lastly, *Pseudomonas* spp. can cause spoilage in meat, seafood, milk and fruit.

Lactic Acid Bacteria

LAB are Gram-positive bacteria that can be found in various fermented foods with a high tolerance for high thermal stress and low pH. Its primary role in the food industry is developing fresh, safe, pleasant, and stable foods for human consumption.[43] LAB were used with edible polymer matrices, resulting in the extended

Table 2: Examples of different food spoilage and its causative microorganisms.

Food	Spoilage Bacteria	References
Meat	*Aeromonadaceae*, *Carnobacteriaceae*, *Enterobacteriaceae*, *Leuconostocaceae*, *Listeriaceae*, *Moraxellaceae*, *Pseudomonadaceae*, *Shewanellaceae*, *Staphylococcaceae*	[27-35]
- Poultry	*Brochothrix thermosphacta*, *Serratia proteamaculans*, LAB, Mesophiles, *Pseudomonas*, Psychotropic	
- Lamb	bacteria, *Clostridium* spp., Enterobacteriaceae	
- Beef	*Brochothrix*, *Carnobacterium*, *Lactococcus*, *Leuconostoc*	
- Pork	*Brochothrix*, *Lactobacillus*, *Leuconostoc*, *Pseudomonas*, *Rahnella aquatilis*	
Seafood	*Brochothrix*, *Carnobacterium*, *Photobacterium*, *Pseudoalteromonas*, *Pseudomonas*, *Psychrobacter*, *Shewanella*	[36-39]
Milk	*Acinetobacter lwoffii*, *Aerococcus urinaeequi*, *Bacillus licheniformis*, *Enterobacter kobei*, *Lactococcus lactis*, *Pseudomonas* spp., *Serratia ureilytica*, *Yersinia enterocolitica*	[40]
Fruit	*Bacillus* sp., *Escherichia* sp., *Klebsiella* sp., *Staphylococcus* sp.	[41]
Vegetables	*Bacillus*, *Clostridium* spp., *Corynebacterium*, *Erwinia carotovora*, LAB, *Micrococcus*, *Pseudomonas* spp., *Rhizopus* spp., *Xanthomonas*	[42]

food product's shelf life. Various studies discussed the different types of biopolymer materials that contain active probiotics such as cassava starch with *L. casei*,[44] sodium alginate with *L. lactis*,[45] and isolate Pea Protein/MC/Sodium caseinate/HPMC with *Lb. plantarum*.[46] Likewise, it can also synthesize bacteriocins and exopolysaccharides.[47]

Bacteriocin produced by Lactic Acid Bacteria

A group of Gram-positive and Gram-negative bacteria ribosomally synthesized substances of protein structure possessing antimicrobial activities, called bacteriocins, are produced by various bacteria such as LAB and certain members of archaea.[48] Bacteriocins have been used mainly for food preservation due to their specific mode of action. If incorporated into packaging films, they presumably migrate into the food through diffusion and reduce the growth of spoilage and pathogenic microorganisms. They can selectively inhibit or kill common foodborne pathogens and food-spoiling bacteria, increasing shelf life and maintaining food quality and safety. Hence, numerous LAB that synthesize bacteriocin are classified according to their mechanism. Classification of bacteriocin is divided into four namely the Class I, II, III, and IV. These classifications are established to distinguish bacteriocin from antibiotics.[48]

Class I typically undergo the process of posttranslational modification. The first group is subdivided into two: Type A and Type B lantibiotics. The difference between the two subgroups is that Type A lantibiotics enclose the linear peptides, which act by permeabilization. On the other hand, Type B lantibiotics carry a chargeless or negative charge since it consists of globular molecules that act variably. Among all the bacteriocins that belong to the group, Nisin is the most prominent or most recognized.[49]

Class II are thermostable and are defined as the non-lantibiotics group.[49] The similarity between Class I and Class II bacteriocins is the transporter proteins of ATP-binding cassettes. Generally, Class II bacteriocins do not undergo substantial posttranslational modification processes. Targeting bacteria that are Gram-positive, LAB, and the genera *Listeria, Clostridium,* or *Enterococcus* are included in their spectrum. Furthermore, it is divided into four subclasses which makes it the largest group. First, Subclass IIa, or the pediocin-like bacteriocin, has a strong mechanism against the genus *Listeria*. Second, is the Subclass IIb which contains a disulfide bridge and is bound to dipeptide bacteriocins. This subclass evinces an interesting high homology of sequence. Third, Subcass IIc bacteriocins that also contain a disulfide bridge and enclosed cyclic peptides. Finally, the Subclass IId which encloses bacteriocins according to their structure, secretion action, and other mechanisms.

Contrary to the other classes, Class III are thermolabile as defined by Karpinski and Szkaradkiewicz.[51] Lysostaphin, Enterolysin A, and Helveticin are included in this class.

Moreover, Class IV have ample activity of lipids and carbohydrates in their molecules. It inhibits both Gram-positive and Gram-negative bacteria. These are somehow similar to bacteriocin substances and therefore do not conform to the bacteriocin concept. Substances similar to bacteriocin have broader potential use compared to bacteriocins due to their firmness over a broad range of pH and wide spectrum bactericidal components.[48] Therefore, the overwhelming majority of bacteriocins coming from the Gram-positive bacteria, specifically LAB are shown to eradicate a diverse group of Gram-positive bacteria and have also gained attention through recent years[50] which justifies their

Table 3: LAB-producing Bacteriocin. Retrieved from "Lactic Acid Bacteria and Bacteriocins" by Zhang.[50]

Bacteriocin	Bacteriocin Producing/Secreting Strain	Classification	References
Bacteriocins Produced by *Lactobacillus*			
Plantaricin Plantaricin LR14 Plantaricin C Plantaricin S Plantaricin NC8	*Lactobacillus plantarum*	Class I Class I Class II Class II	[48]
Gassericin Gassericin A Gassericin T Gassericin M	*Lactobacillus gasseri* LA 39	Class IIc	[48,51]
Sakacin Sakacin A	*Lactobacillus sake* 706	Class IIa	[48,52]
Lactacin Lactacin B Lactacin F	*Lactobacillus acidophilus* N2 *Lactobacillus acidophilus* 11088		[48]
Lactocin Lactocin S Lactocin 27	*Lactobacillus sakei* *Lactobacillus helveticus*	Class II	[48]
Helveticin Helveticin J	*Lactobacillus helveticus* 481	Class III	[48,53]
Bacteriocins Produced by *Bifidobacterium*			
Bifidin Bifidin I	*Bifidobacterium bifidum 1452*		[48]
Bifilong Bifilong Bb-46	*Bifidobacterium longum Bb-46*		[48]
Bifidocin B	*Bifidobacterium bifidum NCFB 1454*		[48]
Thermophilicin B67 Thermophilicin L23		Class III	[48,54]
Bacteriocins Produced by *Lactococcus*, *Enterococcus*, and Other Species			
Nisin	*Lactococcus lactis*	Class I	[48,53]
Lactococcin Lactococcin A	*Lactococcus lactis*	Class II	[48]
Enterocin Enterocin A Enterocin AS-48 Enterocin B Enterocin 1146	*Lactococcus lactis* *Enterococcus faecalis* *Enterococcus faecalis W3* *Enterococcus faecalis*	Class IIa	[48]
Pediocin Pediocin A Pediocin PA-1 Pediocin AcH Pediocin JD Pediocin Bac Pediocin SJ-1	*Pediococcus (P. acidilactici, P. cerevisiae, P. pentosaceus)* *Pediococcus lactis* *Pediococcus lactis* *Pediococcus lactis H* *Pediococcus lactis* *Pediococcus lactis* *Pediococcus lactis*	Class II	[48,53]
Enterolysin Enterolysin A	*Enterococcus* *Enterococcus faecalis* LMG 2333	Class III	[48]
Leucocin Mesenterocin 5 Leucocin A Leucocin S	*Leuconostoc* *Leuconostoc mesenteriodes* *Leuconostoc gelidum*	Class IIa	[47,48]

potential application in food industry as natural preservatives.

The Table 3 lists the LAB strains, their bacteriocin production, bacteria they inhibit, and classification. Class I are heat-stable, and the predominant Gram-positive bacteria that produce them include *Enterococcus* and *Lactobacillus* for instance. Enterocin is a bacteriocin of Class IIa, whereas Gassericin is a bacteriocin of Class IIc. Among all of them, plantaricin and nisin are considered to be the most essential bacteriocins.

Plantaricins and Nisin - The Two Most Important Bacteriocins

Plantaricins (*Lb. plantarum)* and Nisins *(L. lactis)* are both highly active against Gram-positive bacteria which is why they are considered as the limelight in the food industry. Plantaricins have all their peptides act synergistically to produce precursors containing a double glycine moiety which is essential in LAB cultures.[48] Nisin, on the other hand, exhibits a wide range of antimicrobial scope, hence bagging the GRAS status with its hydrophilic and hydrophobic properties[48] in 1988 by the FDA.[55]

Mode of Action

Generally considered as very potent microorganisms, most bacteriocins are capable of inhibiting their target organisms' growth by modulating the enzyme activity, as well as by forming pores. They bring their killing game mainly through membrane permeabilization, cell envelope attacks and restriction of both gene expression and protein production.

Two classes of bacteriocins are mainly involved in this mechanism, namedly Class I and Class II. Class I works by restricting the peptidoglycan synthesis through inhibition of lipid-II present on the organism, which primarily transports the peptidoglycan subunits of the cytoplasm to the cell wall. Once this happens, correct synthesis of the cell wall cannot proceed causing death to the cell. On the other hand, Class II bacteriocins work smart by manipulating and posing the lipid-II as a docking molecule to lead the permeabilization, allowing membrane insertion and pore formation.[56] The sentence must be written as follows: Once they have established themselves inside the cell, immediate cell death occurs as seen in Figure 2. Although potent to both closely related and diverse range of microorganisms, bacteriocins are safe for human consumption. Similarly, the wedge model is assumed to be most accurate based on the pattern and interconnections between the compound of the lantibiotic model, the outer layer of the target plasma membrane, and nisin. Bacteriocin first collides with a fragile cell through an electrostatic bond between a cationic or anionic charged phospholipid in the plasma membrane and through a bacteriocin molecule that resists water (hydrophobic). Within the lipid bilayer, peptide permeation depletes the ordered bilayer locally. As such, bacteriocins exert their activity by establishing pores and complexes of ion channels in the plasma membrane of a sensitive bacteria temporarily. Due to the breakdown of pH gradient and membrane potential, the activity of the proton pump is compromised. By contrast, through the system called barrel stave or carpet, bacteriocins that are classified as Class II remove excess lipids in the plasma membrane during the permeabilization process. As a result of a targeted modification of the lipid bilayer, a transitory and centralized perforation occurs.[49]

LAB Bacteriocins as Biopreservatives

The exploitation of antimicrobial properties of certain microorganisms on the prevention of food spoilage and development of pathogenic bacteria is called biopreservation. This approach aims to reduce chemical additives usage such as nitrites, sodium chloride, and organic acids. Many biopreservation studies have concentrated on LAB's antagonistic behavior towards spoilage and pathogenic bacteria, making them effective as biopreservatives. That said, the use of such bacterial viruses or bacteriophages to extract pathogenic bacteria from food has received significant attention in the past decade. As LAB is vying for nutrients, hydrogen peroxide, lactic and acetic acids, and peptide bacteriocins are examples of active antimicrobials that are also found in their metabolites.[57]

Since bacteriocins are perceived to have a bactericidal activity naturally, it is applied in preservation of food. They can inhibit the development of undesirable microorganisms typically found in food, thereby preventing spoilage.[56] One of the vital attributes regarding why bacteriocins are acquiring notoriety in the food industry today is due to its safety for humans to consume as compared to various chemical preservatives with the same aim. They are also steady and consistent, hence once incorporated in the food matrix can resist the pH, high heat temperature and enzymes.

At present, incorporating bacteriocins into food products can be in three unique ways: a) as a component for pure bacteriocin additives, b) as fermentates, or c) as starter cultures.[56] For instance, crude extracts are integrated mostly into vegetable and meat products.

From 1995 to 2015, the field of bacteriocin continued to develop. Accordingly, more bacteriocins originating from LAB have been characterized in detail. Though these LAB bacteriocins exhibit promise for various applications, their use for biopreservation have shown considerable potential.

Currently, the only bacteriocins licensed as food preservatives are pediocin PA-1 and nisin, made with *P. acidilactici* and *L. lactis*, respectively. Nisin is commercially available as Nisaplin by Danisco. However, pediocins are available commercially as Alta 2431 and Microgard™ from Quest International.[58] Other bacteriocins running for attaining a status to be commercially available to be utilized as biopreservatives are being isolated and characterized, such as cerein, plantaricin, subtilin, and thuricin.[59]

Table 4 lists down the LAB bacteriocins that are being utilized or studied for biopreservation, as well as the bacteria that they inhibit. Nisin, pediocin, reuterin, and reutericyclin are the only LAB bacteriocins currently being used as food biopreservatives. These are proven to inhibit Gram-positive microorganisms that contribute to food spoilage and possibly diseases as well, like *Staphylococcus*, which is the common bacteria they act upon. Nisin and reutericyclin show activity against *Clostridium* and *Streptococcus*. *Lactobacillus*, *Leuconostoc*, and *Listeria* are inhibited by pediocin and its various types. Reuterin also acts against *Listeria*, as well as Penicillium and a few other Gram-negative pathogens like *Escherichia coli* DH5α and *Salmonella enterica* spp.

Plantaricin, thuricin, and cerein also inhibit *Staphylococcus* (plantaricin), *Listeria* (plantaricin and verein), *Clostridium* (thuricin), and *Bacillus* (cerein). They all have great potential in biopreservation applications but they are still being studied and not yet utilized. Other examples of bacteriocins with promising results in inhibiting food spoilage and pathogenic bacteria are gassericin, sakacin, lactatin, lactotin, bifidocin B, thermophilicin B67. thermophilicin B23, lactococcin, and enterocin.

Trends and Applications of LAB bacteriocin Incorporation in Food Packaging/Antimicrobial Films

Over the years, the incorporation of LAB bacteriocins in food packaging reduced the growth of spoilage bacteria. Bacteriocins in packaging benefit the food products for they effectively act as natural food protectants or coating the products' surfaces. Researchers are continuously investigating and developing food packaging containing other potential bacteriocins. According to Mapelli,[65] class IIa bacteriocins (e.g., enterocin, leucocin, plantaricin BM-1, and sakacin A) are more effective in dealing with several food pathogens causing contamination and spoilage than nisin. Hence, other bacteriocin classes are still of interest if they could potentially inhibit food pathogens, such as mentioned beforehand.

Table 1 listed some examples of the food products packaged with bacteriocins and their antimicrobial activity. PE is a film commonly used for packaging and adsorption of the antimicrobial solution. Zhang[8] compared three films, namely PE, LDPE, and HDPE, coated with plantaricin BM-1. All these films showed inhibitory effects against the target pathogen, *L. monocytogenes*. However, this study did not apply the treated films to any food. Hassan[21] are the first to investigate and develop microcapsules with nisin from alginate and resistant starch and then applied to cheddar cheese.

There is an emerging interest and demand in using biodegradable and edible packaging. Active biodegradable packaging is any material type used for dressing (i.e., coating or wrapping) various kinds of food to prolong their quality and shelf life that is immediately eaten and digested together with food with or without further removal. PLA is an example of a biodegradable film that can be an alternative to plastics. Woraprayote[14] developed PLA/SP, a biodegradable packaging, and coated with bacteriocin 7293. Table 1 also listed a few examples of edible packaging with bacteriocin. Divsalar[9] used the sol-gel method to prepare the edible cellulose-chitosan-ZnO nan-ocomposite bilayer film, which retained nisin in the edible packaging. This result supported the theory that the sol-gel technique plays a role in maintaining biological compounds in polymer-based edible films.

Some authors tried to improve the adsorption and incorporation of bacteriocins into the packaging material to enhance their inhibitory effects. As previously discussed, Divsalar[9] used the sol-gel method to regulate the nisin release from nan-ocomposite film into UF cheese. On the other hand, Hu[11] also used cold plasma-treated PLA films and coated them with nisin. The result of this treatment was an improvement in the capacity of nisin to adsorb into the PLA films. Unfortunately, they did not apply these films to any food product. Moreover, the antimicrobial activity of bacteriocins is dependent on temperature and pH during food storage, interaction with additives and components of food; enzyme activity; interactions of microbial; and characteristics of spoilage bacteria.[53] Divsalar[9] found and supported that nisin is highly stable at low or refrigeration temperatures, and its anti-bacterial activity gradually declines as the temperature increases from 4°C to 22°C.

Table 4: Bacteriocins and their target pathogens.

	Target Pathogen	References
	Bacteriocins used for Biopreservation	
Nisin	*Staphylococcus aureus*, *Clostridium botulinum*, and *Streptococcus hemolyticus*	[48]
Pediocin - Pediocin A - Pediocin PA-1 - Pediocin AcH - Pediocin JD - Pediocin Bac - Pediocin SJ-1	*Lactobacillus*, *Leuconostoc*, *Staphylococcus aureus*, and *Listeria monocytogenes*	[48,53]
Reuterin	*Penicillium expansum*, *Staphylococcus aureus*, *Salmonella enterica* spp., *Listeria monocytogenes*, and *Escherichia coli DH5*	[60]
Reutericyclin	*Staphylococcus aureus*, *Streptococcus* spp., and *Clostridium difficile*	[61]
	Newly Discovered Bacteriocins with Strong Potential for Food Biopreservation	
Plantaricin - Plantaricin LR14 - Plantaricin C - Plantaricin S - Plantaricin NC8	*Staphylococcus aureus*, and *Listeria monocytogenes*	[48]
Thuricin - Thuricin CD - Thuricin H	*Clostridium difficile*	[59,62]
Cerein - Cerein 7 - Cerein 8A	*Bacillus cereus*, and *Listeria* spp.	[59,63]
	Other Examples of Bacteriocins	
Gassericin - Gassericin A - Gassericin T - Gassericin M	*Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*	[48,51]
Sakacin - Sakacin A	Many *Lactobacilli* and Gram-positive bacteria	[48,52]
Lactacin - Lactacin B - Lactacin F	*Enterococcus faecalis*, *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus*, and *Lactobacillus helveticus*	[48]
Lactocin - Lactocin S - Lactocin 27	*Listeria monocytogenes*	[64]
Bifidin	*Micrococcus*	[48]
Bifilong - Bifilong Bb-46	*Eschirichia coli*, *Salmonella typhimurium*, *Bacillus cereus,* and *Staphylococcus aureus*	[48]
Bifidocin B	*Listeria monocytogenes, Enterococcus, Bacillus, Leuconostoccus,* and *Phanerococcus*	[48]
Thermophilicin B67	*Salmonella enterica* subsp. *enterica serotype* N-15	[48]
Thermophilicin B23	*Listeria monocytogenes*	[54]
Lactococcin	*Listeria monocytogenes*	[48]
Enterocin - Enterocin A - Enterocin AS-48 - Enterocin B - Enterocin 1146	*Listeria monocytogenes*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Clostridium butyricum*, and *Clostridium perfringens*	[48]
Enterolysin - Enterolysin A	*Lactobacillus*, *Lactococcus*, *Pediococcus*, *Enterococcus*, *Bacillus*, *Listeria monocytogenes,* and *Staphylococcus*	[48]
Leucocin - Mesenterocin 5 - Leucocin A - Leucocin C - Leucocin S - Carnocin	*Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, and *Escherichia coli*	[47,48]

LAB bacteriocins are promising biopreservatives; however, most of them still lack the approval of the FDA to become GRAS. Nisin gained approval from Food and Agricultural Organization (FAO) and FDA for its safe use as a food preservative,[58] but it does not have broad inhibitory effects, especially for Gram-negative bacteria. Nonetheless, the exhibition of LAB bacteriocins to inhibit food pathogens is a step for the possibility for existing food packaging to change and thus prevent rapid food spoilage.

DISCUSSION

LAB bacteriocins' potential in preventing food spoilage can level up in the food packaging industries. Only a few LAB bacteriocins were noted as GRAS and incorporated with food packaging. Remarkably, Nisin and Pediocin are the only bacteriocins that are considered safe for human consumption. However, these two are some of the bacteriocins capable of inhibiting some Gram-positive bacteria. Several studies have apprised in the biopreservative application of novel bacteriocins. It shows that they can potentially inhibit both Gram-positive and Gram-negative bacteria that cause spoilage on various food products. One example of this is the Lactocin MM4 which can be an alternative to nisin due to its antimicrobial activity. However, its food packaging application is still not well-studied.

Recently, studies have focused on the incorporation of LAB bacteriocins other than nisin into food packaging. Plantaricin, sakacin A, and bacteriocin 7293 were incorporated into fish products, meat products, and ready-to-eat products, as summarized in Table 1. Other studies isolated potential bacteriocins that also showed promising inhibitory effects against spoilage bacteria. For example, bacteriocin DY4-2, isolated from *Lb. plantarum* DY4-2, effectively reduced the population of *P. fluorescens* in turbot fillets, while enterocin BacFL31, isolated from *E. faecium* FL31, also reduced *L. monocytogenes* in ground beef. However, these two bacteriocins lack application on different packaging films. Hence, further conduction of studies is necessary to evaluate the antimicrobial activities of these bacteriocins in various food packaging materials. Some studies have also tried and employed other strategies to incorporate bacteriocins into packaging films. These strategies enhanced the immobilization of bacteriocins into packaging films. However, the studies gathered still have not proven to be safe when applied to food products.

CONCLUSION AND RECOMMENDATIONS

Owing to its nontoxic, safe, non-immunogenic, thermostability properties and broad bactericidal activity, LAB bacteriocins are considered great biopreservative agents. Its potent killing ability against food-borne pathogens when induced in both anti-microbial packaging and edible films is now climbing the charts for food industry innovation and development. Likewise, it helps share a unique flavor and texture to the food products. Aside from extending the foods' shelf life, bacteriocin-induced packaging helps the environment and consumers to have a more natural alternative option. It can be a substitute to the food preservatives that have a chemical antimicrobial mixture that could be potentially toxic for long-term use, and as well as for the plastic food packaging causing pollution. Novel bacteriocins with potential application as biopreservative still need further improvement in their safety in different conditions and antimicrobial activity. Hence, other unnoticed LAB bacteriocins infused into food packaging to decrease food spoilage may be possible. The authors recommended further research about the efficient value of temperature and moisture content to bring out the maximum effectiveness of bacteriocin-induced packaging. Also, they recommend further study about the bacteriocins that could be potentially used in packaging to enhance its use. Considering these discoveries, LAB bacteriocin as a biopreservative could change the current food packaging and save our remaining natural resources.

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Authors' Contributions

F.L.A., S.M.D., K.J., J.A.M., R.S.O., M.J.P., C.R.P., and F.H.S. participated in the conceptualization and design, data acquisition, and writing of the manuscript. J.A.M. and C.R.P. composed the abstract. M.J.P. and F.H.S. wrote the introduction. All authors contributed to the screening and assessment of each article included in the study, as well as the analysis and interpretation of the data gathered. F.H.S., M.J.P., and R.S.O. wrote the discussion and provided the conclusion and recommendations. B.H. provided guidance and assistance during the writing of the manuscript. All authors read, reviewed, and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LAB: Lactic Acid Bacteria; **FDA:** Food and Drug Administration; **GRAS:** Generally Recognised as Safe; **PLA:** Polylactic Acid; **RTE:** Ready-to-eat; **LAE:** Lauric Arginate; **PE:** Polyethylene; **PHB/PCL:** Polyhydroxybutyrate/Polycaprolactone; **LDPE:** Low-density Polyethylene; **HDPE:** High-density Polyethylene; **UF:** Ultra-filter; **TEOS:** Tetraethyl orthosilicate; **PLA/SP:** Polylactic Acid/sawdust particle biocomposite film; **PVDC:** Polyvinylidene chloride; **TPS/PBAT:** Thermoplastic starch/polybutylene adipate terephthalate; **PPR:** Polyethylene terephthalate/polyvinylidene chloride/retort casting polypropylene; **CAF:** Composite antimicrobial film; **FAO:** Food and Agricultural Organization.

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