Review article

Bacteriocın, Plantaricin and Pediocin Bıosynthesis in Lactic Acid Bacteria, Antimicrobial Mechanism and Applications as Food Preservatives: Review

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Abstract

Bacteriocins are antimicrobial peptide compounds derived from gram-positive and gram-negative bacteria. Bacteriocins can be cationic, anionic, and neutral. These compounds are synthesized in bacterial ribosomes and have a broad spectrum of activity against pathogenic bacteria. Some bacteriocins from lactic acid bacteria used in the food industry are nisin, diplococcin, acidochilin, bulgarican, helventicin, lactacin, and plantaricin. Bacteriocins produced by *Pediococcus* spp., which are known as pediocins, can be used as alternative preservatives in the food industry. Another type of bacteriocin is plantaricin, which is produced by a group of gram-positive bacteria, *Lactobacillus plantarum*, and can inhibit growth and kill a group of gram-negative bacteria that are pathogenic. Bacteriocins derived from lactic acid bacteria and used as biopreservatives have several advantages, namely a) they are not toxic and biodegrade easily because they are protein compounds; b) they do not harm the intestinal microflora because they are easily digested by enzymes in the digestive tract; c) they can reduce the use of chemical food preservatives; and d) they are highly versatile and can be utilized in various forms such as the form of bacteriocin-producing bacterial culture strains or purified or semi-purified bacteriocin compounds.

Keywords

antimicrobial;
bacteriocin;
food preservation;
pediocin;
plantaricin

1. Introduction

Bacteriocins are defined specifically related to colicin, a type of antibiotic protein synthesizing a component that can kill or inhibit bacterial cell growth [1] and its absorption ability depends on

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specific receptors on the sensitive part of the bacteria [2]. Other colicin-type inhibitory effects include relatively high molecular weight, sensitive inhibitory activity (generally restricted to Enterobacteriaceae strains), and genetically determined plasmid combinations [3]. It has now been found that most bacteriocins produced are from gram-positive bacteria. This does not follow the characteristics of colicin. Bacteriocins tend to be more active against gram-positive strains of bacteria [4]. Some bacteriocins, such as nisin, contain lanthionine, a modified amino acid formed after the translation of the molecule. Lactic acid bacteria (LAB) can produce lactic acid that is helpful for inhibiting the growth of unwanted microorganisms in food [5]. In addition to lactic acid, lactic acid bacteria can produce various antimicrobial substances. Some examples of these antimicrobial substances include hydrogen peroxide, diacetyl, organic acids, and bacteriocins. Bacteriocins are antimicrobial peptide compounds derived from gram-positive and gram-negative bacteria. Bacteriocins can be cationic, anionic, and neutral [6]. These compounds are synthesized in bacterial ribosomes and have varied activities and a broad antimicrobial spectrum. Bacteriocins are extracellular peptides or bioactive complexes produced by lactic acid bacteria. They are bactericidal or bacteriostatic against other types of bacteria, especially those derived from strains of bacteria closely related to the bacteria produced. However, in some cases, bacteriocins can also be broad-spectrum by inhibiting the growth of or killing bacterial strains unrelated to the bacteria that produce them [7].

Lactic acid bacteria are the group of bacteria that produce the most bacteriocins. Generally, the bacteriocins secreted by LAB are small cationic peptides with 30 to 60 amino acid residues and are heat resistant [8, 9]. Based on research that has been done, more than 50 different types of bacteriocins are produced by LAB [10]. Some bacteriocins from LAB that have been characterized are nisin produced from several *Lactococcus lactis*, Lactococcus A and B from *Lactococcus lactis* subsp. *cremoris*, pediocin from *Pediococcus acidilactici*, lactacin from *Lactobacillus johnsonii*, lactostrepsin from *Streptococcus cremoris*, and curvacin from *Lactobacillus curvatus* [11]. Bacteriocins are protein substances that generally have small molecular weights and have bactericidal activity through protein synthesis regulated by plasmids [12]. Bacteriocins play many roles and provide many benefits. They can act as antagonists, and it is because of this property that they are used as food biopreservatives. They are also able to inhibit the growth of gram-positive and gram-negative bacteria and thus are used as therapeutic agents [13]. Several species of lactic acid bacteria that have been known to produce bacteriocins include *Streptococcus lactis, Lactobacillus plantarum, Lactobacillus acidophilus, Pediococcus acidilactici, Enterococcus faecum, Enterococcus lactis, Leuconostoc mesenteroid*es, and *Listeria monocytogenes* [14, 15].

Bacteriocins are defined by several additional criteria: (1) they have a relatively narrow spectrum of activity targeting mostly bacterial strains associated with the bacteriocin-producing species, (2) the active compounds consist mainly of a protein fraction, (3) they have bactericidal properties, (4) they have specific receptors that can recognize and inhibit or kill target bacterial cells, and (5) their determinant genes are found in plasmids that have a role in production and immunity [16]. Bacteriocins produced by lactic acid bacteria are active components of food biopreservatives [17]. In addition, some bacteriocins are resistant and stable in heat treatment, making them applicable to the heating process. Bacteriocins also have irreversible properties, are easy to digest, positively affect health, and can be active at low concentrations [18]. In this review, the biosynthesis of plantaricin and pediocin bacteriocins in lactic acid bacteria, antimicrobial mechanisms, and their application as food preservatives are discussed.

2. Bacteriocin Classification

Bacteriocins produced by lactic acid bacteria are classified into three main classes based on their biochemical and genetic characteristics [19-21], namely:

1. Class I: Bacteriocins of this class are referred to as lantibiotics. The peptides in this bacteriocin are small molecular weight (<5 kDa), modified in the post-transcriptional phase, and contain one or more amino acids such as lanthionine, E-methyllanthionine, and dehydrated dehydroalanine and dehydrobutyrine residues. Based on the functional and structural groups, lantibiotics can be divided into types A and B.

a. Type A lantibiotics are cationic peptides extended by a lanthionine bridge. These peptides work by interfering with the cell membrane of the target organism (e.g., nisin, subtilin, and epidermin).

b. Type B lantibiotics are round and smaller peptides (up to 19 amino acid residues). These peptides work by interfering with the enzyme function of the target organism. For example, they inhibit cell wall biosynthesis (e.g., mersacidine, duramycin, and actagardin).

2. Class II: These are small molecular weight (<10 kDa) peptides that are unmodified and heat resistant. The members of this class of bacteriocins form a helical (amphipathic) structure with variable hydrophobicity and an E-Sheet structure. These peptides are stable when heated to 100°C-121°C. So far, more than 50 class II bacteriocins from lactic acid bacteria have been isolated and characterized. Class II bacteriocins include:

a. Class IIa: Pediocins, the largest and most studied subclass. These peptides have vigorous anti-listerial activity and show the most significant identity in sequencing (40-70%).

b. Class IIb: Two-peptide bacteriocins. These bacteriocins require a combination of two peptides for potent antimicrobial activity. Most of these bacteriocins are active when the peptides are present separately but are optimally active with both present. Exceptions include the peptides Lactocin G and Lactocin 705, which do not have antimicrobial activity when used separately.

c. Class IIc: sec-dependent bacteriocins. These bacteriocins will cross the cytoplasmic membrane via sec-dependent secretory pathways (e.g., acidosin B, divergicin A, bacteriocin 31, enterosine P, and listeriosin 743A).

d. Class IId: Bacteriocins without direct sequencing. Unlike other bacteriocins, these bacteriocins are synthesized without a primary N-terminal or sequencing signal. This subclass consists of the two-component bacteriocin enterosine L50 and the single peptide enterosine Q, produced by *Enterococcus faecium* L50, and Aurosin A70, produced by *Staphylococcus aureus* A70.

e. Class IIe: Bacteriocins with cyclic peptides. In contrast to linear bacteriocins, these bacteriocins become cyclic by forming a head-tail peptide binder. (Example: AS-48, gassericin A, and circularin A)

f. Class IIf: Other unmodified bacteriocins. This group contains other class II bacteriocins that do not resemble the structure and motifs of any subclasses.

3. Class III: Peptides with a significant molecular weight (>30 kDa) that are not heat-resistant. Only a few bacteriocins of this class have been identified (e.g., helveticin J, helveticin V, acidophilusin A, lactacin A, and B).

2.1 Bacteriocin biosynthesis and secretion

The production of bacteriocins requires at least four genes. One or two operons generally regulate these genes. One gene encodes a peptide precursor (pre-peptide), and then the second gene determines the immunity of the target organism (Figure 1). The third gene codes for the Human ATP-binding cassette (ABC) transporter (a membrane transporter), so the propeptides can cross the

membrane simultaneously [22]. Finally, the fourth gene encodes an accessory protein required when secreting bacteriocins (Figure 1). Histidine kinases are activated by mature-inducing peptides and regulate the phosphorylation response. The regulator of this response binds to DNA and activates genes. Prebacteriocin and preinduced peptides cross the membrane sequentially [23].

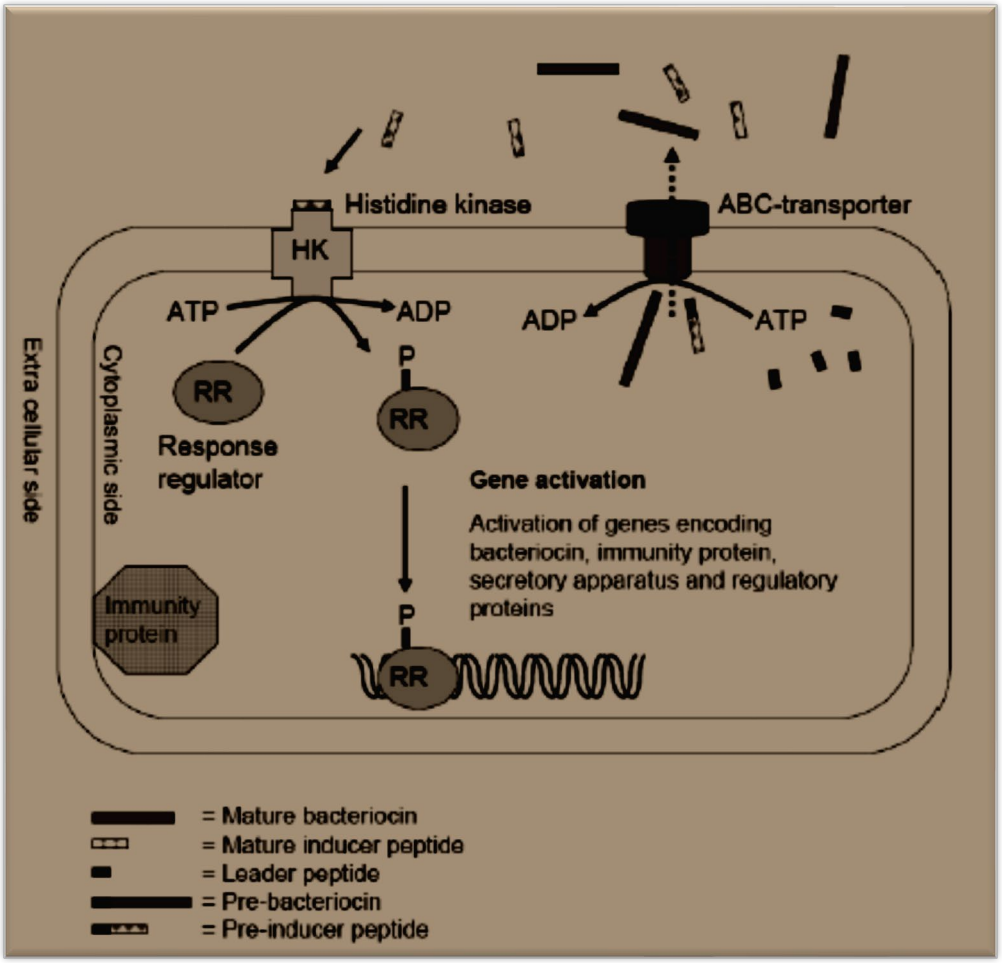

Figure 1. Mechanism of bacteriocin biosynthesis [13]

2.2 Mechanism of action of bacteriocin

Generally, bacteriocins from gram-positive bacteria are active against other types of gram-positive bacteria [1, 4]. However, some species (such as nisin) also have activity against gram-negative bacteria when the outer membrane of the bacteria is permeable [20]. Type A lantibiotics and some class II bacteriocins are membrane-activating peptides that destroy the cytoplasmic membrane integrity of target cells through pore formation [1, 2]. These bacteriocins cause leakage of low molecular weight metabolites or expulsion of proton pressure (Proton Motif Force, PMF) [2, 3]. PMF is an electrochemical gradient across the cytoplasmic membrane consisting of a membrane potential (ψ) and a pH gradient, which guides ATP synthesis and accumulates other ions and

metabolites. The decrease in target cell PMF, induced by bacteriocin activity, leads to cell death by stopping the energy-generating reaction [2, 3].

3. Plantaricin

One type of bacteriocin is plantaricin that is synthesized explicitly by a group of gram-positive bacteria. For example, *Lactobacillus plantarum* producing plantaricin can inhibit growth and kill a group of gram-negative pathogenic bacteria [24]. Some of the characteristics possessed by plantaricins are generally small, thermostable, and cationic or hydrophobic. These antimicrobial compounds are non-toxic to humans and easily degraded by proteolytic enzymes. They do not harm the intestinal microflora because digestive enzymes easily digest them, and moreover they are stable to changes in pH and temperature [25]. Plantaricins can inhibit the growth of pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Bacillus cereus*, and *Salmonella typhimurium* [26]. Plantaricins display promising antimicrobial properties and have the potential to be developed at the application stage. Bacteriocins such as plantaricins can be used as natural biopreservatives. They contain antimicrobial compounds that can damage or inhibit the growth of pathogenic bacteria and thus extend a food product's shelf life [27].

The mechanism of plantaricin IIA-1A5 in destroying bacterial cell membranes is by inhibiting the synthesis of cell membranes where plantaricin adheres to cell membranes and interferes with the permeability properties of bacterial cells and then form pores. When the membrane pores are enlarged, a severance of crosslinks between N-acetylglucosamine and N-acetylmuramic polypeptides, that are made up for peptidoglycan, occurs [28]. When crosslinking occurs, the bacterial cell wall will become easily damaged. The target of bacteriocins produced by lactic acid bacteria is the cytoplasmic membrane of sensitive bacterial cells, which can have fatal consequences for the survival of these cells. All living cells are limited by a cytoplasmic membrane that is selectively permeable and carries out active transport, thus playing a role in controlling components in the cell. When the integrity of the cytoplasmic cell membrane is disturbed, the substances in the cell escape from the cell, causing damage or cell death [29].

The initial step of the action of plantaricin is the attachment of plantaricin molecules to specific membranes (receptors) or non-specific membranes. When the bacterial cell membrane has been damaged, it will cause the release of protein and genetic material. Damage to bacterial cells due to antibacterial compounds can also be caused by damage to the hydrophobic bonds of components that make up cell membranes, such as proteins, phospholipids, and the dissolution of hydrophilic and hydrophobic components. When this occurs, the permeability of the cell membrane is increased, facilitating the entry of antibacterial components into the cell and the release of cell substances such as proteins and nucleic acids, and causes cell damage. Bacteriocins have various effects on the bacteria. Leukonosine S and plantaricin C19 have bacteriostatic effects [30]. Bacteriocins have bacteriostatic properties based on their ability to inhibit the viability of the growth rate of pathogenic bacteria. Several bacteriocins have bactericidal activity, such as bacteriocin PsVP-10, plantaricin C from *Lactobacillus plantarum* LL441, and plantaricin L-1. Bacteriocins have bactericidal properties by forming non-selective pores in the cell membrane, thus releasing cell contents and resulting in cell death [31].

In general, bacteriocins from LAB can fight other bacteria with bactericidal effects. The mechanism of bactericidal activity of some bacteriocins, in general, is as follows: (1) bacteriocin molecules come into direct contact with the cell membrane, and (2) this contact disrupts the membrane potential producing cytoplasmic membrane instability, and the cell becomes weak, (3) membrane instability can have an impact on the cell membrane [1, 7]. Formation of holes or pores in the cell membrane occurs through the process of interference with PMF (Proton Motive Force)

[31]. The administration of plantaricin-IIA1A5 showed a bactericidal effect, as indicated by observations of UV-absorbance, where turbidity occurs due to the presence of cell material (ions, proteins, and genetic material) from the inside of the membrane. The ability of plantaricin to damage cell membranes can be explained using two models, the barrel-stave model and the carpet model. The mechanism by which plantaricins destroy bacterial cell membranes according to the barrel-stave model begins with the absorption of the helical structure of the plantaricin to the cell membrane through the hydrophobic region [25].

In contrast, the hydrophilic region of the plantaricin molecule freely interacts with the solvent. After the plantaricin reaches the concentration limit, the plantaricin will form pores in the membrane, with the hydrophobic surface facing outward and the hydrophilic surface facing inward, resulting in cell leakage [25]. At the beginning of the attachment of the bacteriocins onto the membrane surface, the bacteriocins form tiny pores, which then enlarge as more bacteriocin molecules enter the cell, a process which can result in the release of cell material from the membrane and cell death. Leaks that occur due to the formation of holes in the cytoplasmic membrane are indicated by the activity of molecules that enter and leave the cell. The leakage that occurs impacts the decrease in the cellular pH gradient. In general, the formation of cytoplasmic holes due to the presence of bacteriocins causes changes in membrane potential gradients, the release of intracellular molecules, and the entry of extracellular substances [32]. This effect causes cell growth inhibition and results in cell death in cells sensitive to bacteriocins. The mechanism of plantaricin damage to cell membranes as described in the carpet model is indicated by the absence of pore formation on the cell membrane. Plantaricin will stick to the surface of the cell wall and will stretch like a carpet. Furthermore, plantaricin can cause lysis of all cell wall surfaces that have been covered. Cell wall lysis can have an impact on cell membrane damage. The cell membrane will be easily damaged because there is no cell wall as a wrapper and due to strong pressure from within the membrane. Without a cell wall, the cell membrane cannot withstand the osmotic pressure inside the bacterial cell, so the cell will burst [25].

4. Pediocin

Bacteriocins produced by *Pediococcus* spp. are known as pediocins, and they can be alternative preservatives for the food industry. Pediocin has been commercialized under the name AltaTM 2341 and has been used in American and European countries as a preservative, especially in meat products, to prevent the growth of *Listeria monocytogenes*. Pediocin belongs to class IIa bacteriocins, which are small (<10 kDa) peptides, cationic, hydrophobic, heat stable, strong anti-listeria, and not the result of post-translational modifications. Pediocin generally has a molecular weight (BM) ranging from 2.7-17 kDa. According to Ennahar *et al.* [33], pediocin can be used as a substitute for nisin because it has a wide pH range and is stable to heat. Pediocin's mode of action is bactericidal, involving binding, insertion, and pore formation processes that result in the wasting of proton motive force (PMF) and leakage of intracellular substances leading to the death of the target bacteria. Pediocin containing the motif of Tyr-Gly-Asn-Gly-Val amino acid residues can form pores with a barrel stave mechanism, where pediocin binds to specific receptors of bacterial cell membranes, making the hydrophobic surface of pediocin interact with membrane phospholipids. The target bacteria are hydrophobic, then the hydrophilic surface of pediocin creates a pore in the interior of the hydrophilic cell and creates a channel that causes leakage and cell death [34].

Pediocin, a bacteriocin from lactic acid bacteria, is not toxic and safe to use as a food preservative to prevent food damage caused by pathogenic bacteria. This is because proteolytic enzymes quickly degrade bacteriocins, so bacteriocin fragments do not last long in the human body. In addition, pediocin has peptides that are bactericidal activity against gram-positive bacteria,

including pathogenic bacteria but has no effect on eukaryotic cells and has high stability against high temperatures and low pH. This supports the application of pediocin as an antimicrobial in various foodstuffs. Some strains of *P. acidilactici* and *P. pentosaceus* can produce pediocin [33].

4.1 Production of pediocin

Pediocin is produced by *Pediococcus* sp., which are included in the group of lactic acid bacteria, namely gram-positive bacteria, cannot form spores, are catalase-negative, resistant to acid, with optimum growth pH between 4.0-4.5, and grow under microaerophilic to anaerobic conditions. *Pediococcus* is a bacterium with non-motile (not moving) characteristics and a spherical shape. Bacterial cells of *Pediococcus* are present in pairs or tetrads (composed of four cells) and are facultative anaerobes; requires an environment rich in nutrients containing growth factors and fermentable sugars. *Pediococcus* sp. are homofermentative (produce only lactic acid) and cannot use pentoses (C5-atom carbohydrates). The optimum growth temperature is 25-30°C, while the optimum pH is around 6.0. *Pediococcus* has species and strains that differ in their tolerance and resistance to oxygen, pH, temperature, antibiotic resistance, and NaCl. Some *Pediococcus* strains have one or more plasmids of various sizes, some of which can encode genes for carbohydrate fermentation and bacteriocin production [35].

Pediococcus acidilactici and *P. pentosaceus* are commonly used to ferment vegetables and meat. Both are the main species used for pediocin production, as starter cultures in fermentation processes and as probiotic supplements for humans and animals. *Pediococcus pentosaceus,* in general, has similar characteristics to *P. acidilactici*, where the two are only distinguished by growth temperature and resistance to salt. *Pediococcus pentosaceus* does not grow at a temperature of 50°C and is resistant to salt concentrations up to 10%, while *P. acidilactici* is the opposite. The optimum temperature for its growth is 28-35°C. Most strains are known to ferment glucose, ribose, galactose, arabinose, and fructose, to DL-lactate, while only a few strains can ferment lactose and xylose [36]. *Pediococcus pentosaceus* 2A2, used in this study, is a bacterium isolated from fresh beef sold in the Bogor regional market. Based on the 16S-23S ribosomal RNA sequence analysis, *P. pentosaceus* 2A2 was included in the *P. pentosaceus* ATCC 25745 group with a 97% NCBI BLAST match. The genome of *P. pentosaceus* ATCC 25745 contains a group of genes that regulate the bacteriocin system, which results in solid bacteriocin activity. Therefore, the producer has immunity to bacteriocins resulting from having specific immune proteins [37].

Pediocin production occurs in the exponential growth phase until it enters the stationary phase. Biologically, pediocin is synthesized as an inactive pre-peptide with one N-terminal group on the main peptide to be translated into a C-terminal propeptide. After the translation process, the principal peptide that carries the propeptide molecule will be transported from the cytoplasm to the outside through the membrane with ABC carrier bonds that act as an endopeptidase. This ABC carrier bond will cleave the main peptide so the propeptide portion can be released into the environment. In contrast, the principal peptide remains in the cytoplasm to be able to return to play a role in further pediocin synthesis. The propeptides released are pediocin molecules formed [19].

According to Reis *et al.* [38], the composition of the media will affect the production of bacteriocins and their antimicrobial properties. Most of the pH values for bacteriocin production are likely species or strain dependent. The synthetic medium commonly used for pediocin production is MRS (deMan Rogosa Sharpe). MRSB is a liquid medium commonly used to grow LAB. This synthetic medium is relatively expensive. Several studies have been conducted to produce bacteriocins using inexpensive media, such as molasses. Molasses is a by-product of cane sugar production, which contains sugar and organic acids, has a sucrose content of 44-50% with a pH of 5.5-6.5 obtained during the cane sugar bleaching process. It is the cheapest source of organic carbon and energy source for the growth of microorganisms. Molasses is generally exported abroad at low

prices, even in many places. This waste is the cause of environmental pollution problems due to the content of calcium oxide, which can reduce oxygen levels in the soil.

Bacteriocin production using molasses media has been widely carried out [2, 19] by using 4% molasses in MRSB media inoculated with LAB strain SCG 1223. The results showed that the highest bacteriocin activity against *E. coli* was obtained. In addition, according to De Vuyst and Leroy [13], the growth of *Lactobacillus plantarum* AMA-K in 10% molasses medium was equivalent to growth in MRSB, where the bacteriocin produced by *Lactobacillus plantarum* AMA-K had similar homology to pediocin PA-1. Cleveland *et al.* [7] also produced pediocin using *P. pentosaceus* 2A2 isolate in growth media containing 30% molasses at 37ºC for 24 h. Analysis with SDS-PAGE provides information that the molecular weight of pediocin crude extract (EKP) is 8.1 kDa. The EKP produced is heat resistant (80ºC for 30 min, 100ºC for 15 min, 121ºC for 15 m) and stable to pH 2.0, 4.0, and 6.0. The antimicrobial activity of the EKP obtained against the indicator bacteria *L. monocytogenes* ATCC 7644 resulted in a zone of inhibition that was not significantly different from that of MRSB. EKP's comparative test results against commercial synthetic preservatives, such as sodium benzoate (1000 ppm) and sodium nitrite (30 ppm and 300 ppm), resulted in a larger pediocin inhibition zone compared to commercial synthetic preservatives. This shows that pediocin can be effectively used as a food preservative, especially as a substitute for synthetic preservatives.

4.2 Characteristics of pediocin

Pediocin is one type of bacteriocin, an antimicrobial composed of peptides or short-chain proteins synthesized in the ribosomes. It can inhibit the growth of other bacteria, even at low concentrations. Although this bacteriocin can be categorized as an antibiotic, it is not an antibiotic. The main difference between bacteriocins and antibiotics is that these bacteriocins limit their antibacterial activity to strains that are closely related to the strains of the bacteria that produce them, generally of the same species, while antibiotics have a broader spectrum of activity and although their activity is narrower, their effects are not related to bacterial strains that have proximity to the producing bacteria. In addition, bacteriocins are synthesized in the ribosomes and produced in the primary growth phase, whereas antibiotics are generally secondary metabolites. Pediocin is a class IIa bacteriocin, one of the most studied bacteriocins, and has been widely characterized and used as a natural food preservative. According to Jack *et al*. [39], class I and IIa bacteriocins are the most abundantly available and have the potential for industrial applications.

Pediocin is a safe antimicrobial. Lavermicocca *et al*. [40] explained that this safe property was due to pediocin being a bacteriocin produced by Lactic Acid Bacteria (LAB), which is not toxic. The safe nature of LAB causes these microorganisms to be classified as food-grade microorganisms or known as generally recognized as safe (GRAS) microorganisms, which are microorganisms that are not at health risk. Protease enzymes can inactivate pediocin in the digestive system with little effect on the natural microorganisms present in the human digestive system. This bacteriocin does not affect the sensory quality of the food added [41].

Pediocins generally have varying molecular weights (BM). Pediocin SA-1 from *P. acidilactici* NRRL B5627 has a BM of 3.66 kDa, pediocin K23-2 produced by *P. pentosaceus* K23-2 has a BM of 5 kDa [22], even pediocin ACCEL which produced *P. pentosaceus* ACCEL has a BM of 17.5 kDa. In addition, pediocin is encoded by plasmids, making it possible for genetic manipulation [24]. Pediocin is not a post-translational modification, is a small peptide (<10 kDa), cationic, hydrophobic, heat stable, does not contain lanthionine, and belongs to class IIa bacteriocins, namely bacteriocins with anti-listeria solid effect, and has a sequence Tyr-Gly-Asn-Gly-Val at terminal N.

Pediocin is also stable at hot temperatures, sterilization temperatures, and refrigeration temperatures up to -80°C. Pediocin is reported to have four cysteine residues that form 2 disulfide bonds. This disulfide bond contributes to its heat resistance properties. Its activity persists over a wide pH range but is sensitive to most proteases. Based on its properties, pediocin has the potential to be a natural preservative. Amino acid sequencing results showed that pediocin consists of amino acid residues KYYGNGVTXXGKHSXVDXG, where X is an unidentified residue at positions 9,10,15,18 from the N terminal. The characteristic of pediocin is a near Tyr-Gly-Asn-Gly-Val sequence with the N-terminal characteristic of class IIa bacteriocins. Different pediocins have similar biochemical properties despite having a wide range of molecular weights.

4.3 Purification of pediocin

Purifying pediocin is very important to know its function and evaluate its potential to be applied to various foods. This bacteriocin will be secreted in the growth medium, so the initial step is to concentrate the bacteriocin from the supernatant culture using ammonium sulfate precipitation. The principle of protein deposition using ammonium sulfate was described by Kim *et al.* [42]. Protein solubility will increase in the presence of salt, which is the salting process. At higher salt concentrations, protein solubility generally decreases, resulting in precipitation, which is salting out. Salt that causes a decrease in protein solubility can increase the conformational stability of the protein. A higher salt concentration is required to precipitate proteins with smaller molecular weights than proteins with larger molecular weights. Therefore, the protein deposition process uses saturated ammonium sulfate salt to a concentration of 90%. Ammonium sulfate or $(NH_4)_2SO_4$ has the highest solubility of other phosphate salts, so it is most often chosen as a salting-out reagent. Precipitation of protein with ammonium sulfate has various advantages, including being economical, increasing protein stability to prevent protein denaturation, preventing proteolysis, and reducing sample application time at the chromatographic stage [42].

Although the protein deposition procedure with ammonium sulfate salt aims to reduce the volume of bacteriocins, it does not produce bacteriocins with a high level of purity. Therefore, further purification steps using preparative techniques focusing on isoelectric pH or separation using chromatography, including cation exchange, gel filtration, hydrophobic interactions, and RP-HPLC (reverse-phase liquid chromatography), are deemed necessary to achieve a significant level of bacteriocin purity. Many studies have been carried out on the purification process of pediocin. Bacteriocin purification can be done by precipitation of ammonium sulfate, ion exchange chromatography, hydrophobic interaction, and RP-HPLC [43]. According to Martinez *et al.* [44], as the first choice, the SP (sulfopropyl) sepharose or CM (carboxymethyl) sephadex cation exchange column is the most widely used procedure. Kumar and Srivastava [45] used the pediocin purification method by precipitation of ammonium sulfate and cation exchange chromatography (SP Sephadex) to obtain pure pediocin.

According to Joerger [46], cation exchange chromatography is commonly used for separating and purifying charged proteins, polypeptides, nucleic acids, and other molecules. This technique involves three stages: (1) the occurrence of an equilibrium stage of the ionic group, which is ready to bind charged molecules, (2) the entry of the sample and the binding of the sample to the ion group, which has the opposite charge from the sample. The buffer will wash out the unbound component, and (3) the bound sample is separated by changing the eluent condition, which does not support the molecule's binding based on its ionic strength, and generally involves increasing the ionic strength or changing the pH of the elution buffer. The use of cation exchange gel is only stable at pH 5-8. To facilitate sufficient binding and elution, the required pH for cation exchange chromatography is lower than the isoelectric pH of the sample. Pediocin has an isoelectric pH of 8.67-8.85. The sample begins dissociating from the cation exchange at 0.5-unit intervals from its

isoelectric pH, so the 4 variations used for sample elution are pH 5.5, 6.0, 6.5, and 7.0. Different pediocin purification treatments resulted in different pediocin-specific activities. Purified pediocin has also been characterized to test its stability against temperature, pH, and proteolytic enzymes. According to Karthikeyan and Santosh [6], purified pediocin SM-1 was stable for 60 min at 100ºC, even at 121ºC. Storage for one year at a temperature of -80ºC and -20ºC did not affect its activity. SM-1 pediocin resistance is in the pH 3.0-12.0 and inactive at pH 2.0, 13.0, and 14.0. The purified pediocin SA-1 was stable for 60 min and heat at 121°C. Even though it was storage for four weeks at -80°C, -20°C, 4°C, and 30°C, its antimicrobial activity was not affected. The purified ACCEL pediocin is inactive in the presence of protease enzymes and remains stable at pH 2.0-6.0 at temperatures below 100°C. The activity of as much as 80% persisted after being heated at 121°C for 15 min at pH 2.0-6.0 [47].

4.4 Mechanism of action of pediocin

The mechanism of action of pediocin is almost the same as the mechanism of bacteriocin in general, i.e., inhibiting the target bacteria by damaging the cell membrane and creating pores that cause cell death. The antimicrobial mechanism interferes with the target organism's cell wall or membrane by binding it to cell surface receptors. The cytoplasmic membrane of gram-positive bacteria is a target for pediocin. Pediocin works by changing the permeability of the cytoplasmic membrane through the formation of pores by inserting the C-terminal into the membrane. Pediocin, a hydrophobic molecule, when in contact with target bacteria, can reduce the stability of the cytoplasmic membrane. This disrupts the membrane permeability and potential, causing the cell to lyse. Pediocin kills sensitive bacteria by forming pores in their cell membranes, causing disruption of the transmembrane potential and disrupting the balance maintained by the bacteria with the surrounding environment. Pediocin, like most other bacteriocins, consists of cationic and amphiphilic peptides because it contains excess lysine and arginine residues. The molecular mechanism of cell lysis is still not clearly understood. However, it leads to different or varied modes of action of transmembrane pore formation with detergent-like effects on the membrane. Pediocin SM-1, pediocin SA-1, pediocin CP2, pediocin 05-10 were reported to have bactericidal properties. Pediocin PA-1 was highly active against *L. monocytogenes*, while pediocin SM-1 was more active and effective against LAB and *Listeria* spp. Like other pediocin, pediocin SM-1 is inactive against gram-negative bacteria such as *Salmonella* spp. [44].

Many studies have been carried out on the mode of action of pediocin. Pediocin PA-1 is a bacteriocin produced by LAB that shows potent activity against *L. monocytogenes*, a pathogenic food bacterium of concern to the food industry. The mode of action of pediocin PA-1, which is bactericidal, consists of 3 steps: binding pediocin to the cytoplasmic membrane, then entering the membrane, and forming a pore complex. This process eventually leads to cell death, which can occur with or without cell lysis [48]. The mechanism of action of pediocin PA-1 begins with pediocin molecules approaching the cell surface, followed by specific binding of receptor components on the cytoplasmic membrane. Then pediocin molecules enter the membrane, aggregate, and form an oligomeric structure. In this phase, hydrophilic pores are formed, which can cause the release of small molecules and ions, resulting in cell death. Finally, tryptophan residues from pediocin PA-1 enter the anionic membrane, which involves electrostatic interactions between positive ions from pediocin and negative ions on bacterial membranes that play a role in the binding of pediocin to the target membrane. *In vivo* studies of pediocin PA-1 on *L. monocytogenes* show death. Pediocin induces cytoplasmic ATP depletion, irreversible K^+ ion depletion, and phosphate with cell death depletion. The depletion of ATP is due to the cell having to maintain its proton motive force (PMF) rather than the inability of the cell to form ATP due to the loss of phosphate [49].

The formation of pores by pediocin uses a barrel stave model in which pediocin will bind to specific receptors of the bacterial membrane and oligomerize. The oligomer enters the center of the hydrophobic membrane, and the hydrophilic surface of pediocin creates a pore to the inside. Hydrophilic cells form transmembrane pores. Optimum electrostatic interaction is required, influenced by pediocin concentration, so the peptide can associate to form an active aggregation conformation. The concentration of pediocin determines the size limit of the escape of molecules out of the pores because a higher concentration of pediocin is needed to pass molecules with a molecular weight above 9400 Da. The formation of pores in the cytoplasmic membrane of the target cell can disrupt the transmembrane electrical potential, inhibit amino acid transport, and cause the depletion of small ions [50].

The mechanism of action of pediocin AcH, initially, pediocin AcH binds to a non-specific receptor, which is probably LTA (lipoteichoic acid). When non-specific areas are decomposed (which only occurs in sensitive cells), pediocin AcH binds to specific receptors and disrupts membrane integrity resulting in membrane disintegration and loss of K^+ ions and other small molecules. The target bacteria will lose the ability to replicate. Even for some strains, it can cause loss of structural integrity and cause cell lysis. The specific receptor is absent or cannot be bound in resistant gram-positive bacteria. In gram-negative bacteria, there is no non-specific and specific receptor for pediocin AcH. Pediocin AcH forms a complex with LTA. This LTA is only present in gram-positive bacteria but not in gram-negative bacteria, thus explaining why pediocin AcH is absorbed in gram-positive bacteria, not in gram-negative bacteria.

5. Application of Bacteriocin as a Food Preservative

Contaminating microorganisms during food processing, transportation, and storage can cause food poisoning, disease or infection, and food spoilage. This is a fundamental problem in the industrial world because it causes huge losses. The use of preservatives as additives in food is done to overcome this. Food companies must consider carefully and use as few non-food additives and synthetics as possible. Consumers who are aware of the importance of health are more interested in foods that do not contain preservatives, especially those from non-food ingredients. The orientation of the search for preservatives is that which consumers can accept. It is naturally found in food, for example, derived from plants and animals or produced by microorganisms called biopreservatives. Bacteriocins derived from various lactic acid bacteria (LAB) are natural ingredients that can be used, and they are tested to be safe substances [51]. Bacteriocins produced by LAB have attracted much attention recently due to their potential use as preservatives. This substance is a protein, and it can be degraded in the digestion of humans and animals. LAB bacteriocins have been widely used as food biopreservatives, especially in cheese, milk, and other products. Some bacteriocins are heat stable, thus making them applicable to heat treatment. Bacteriocins are irreversible, easy to digest, positively affect health, and active at low concentrations. Bacteriocins from LAB are readily accepted as additives by health experts and, more importantly, by consumers because LAB is usually naturally present in fermenting food [52].

5.1 Application of lactic acid bacteria and bacteriocins in food to inhibit *L. monocytogenes*

Among the antimicrobial compounds produced by lactic acid bacteria, lately, bacteriocins have been the most widely applied as antimicrobials in foodstuffs. This is because it effectively inhibits and kills several pathogenic bacteria that often contaminate food. In addition, bacteriocins are also capable of being produced. They have a reasonably high activity at low temperatures. They can

work synergistically with low-temperature storage treatments to improve food safety against pathogenic bacteria, especially those resistant to low temperatures, such as *L. monocytogenes*. Another advantage of bacteriocin is that it provides an antimicrobial effect without causing significant changes in the taste and appearance of the resulting product, so it has broader application possibilities.

Some bacteriocins produced by lactic acid bacteria from food have been isolated and are known to have antimicrobial effects against *L. monocytogenes*, including nisin produced by *Lactococcus lactis*, plantaricin UG1 from *Lactobacillus plantarum* isolated from dried sausage, plantaricin D from *Lactobacillus plantarum* strain BFE 905 isolated from salad vegetables, thermophilin 347 produced by *Streptococcus thermophilus* 347 isolated from yogurt, acidocin A produced by *Lactobacillus acidophilus* TK9201, bavaricin A from *Lactobacillus bavaricus* MI401 isolated from sourdough, bavaricin MN from *Lactobacillus sake* MN, pediocin JD from the strain *Pediococcus acidilactici* JD 1-23, enterocin 226NWC produced by *Enterococcus faecalis* 226 isolated from whey culture used as a starter in the manufacture of Mozzarella cheese from buffalo milk, divercin and piscicocin produced by *Carnobacterium* strains from processed fish products. Many studies have been conducted on applying lactic acid bacteria or the compounds they produce to food products to inhibit the growth or kill pathogenic bacteria *L. monocytogenes*, thereby increasing the safety of these foodstuffs. Research on applying lactic acid bacteria and the compounds produced as anti-listeria to foodstuffs has been carried out on fresh and processed foods and animal and vegetable products. Lactic acid bacteria or the compounds produced are applied alone or in combination with other treatments [53].

5.2 Application of bacteriocins in livestock and poultry meat

Reis *et al.* [38] successfully isolated *Lactobacillus bavaricus* MN from meat products which showed antagonistic effects against *L. monocytogenes* in beef. This strain inhibited the growth of *L. monocytogenes* Scott A inoculated on meat products in solution (beef gravy) stored at 4°C. Inhibition was seen at a concentration of *Lactobacillus bavaricus* 10^3 CFU/ml against *L. monocytogenes* inoculated in 10 times more significant amounts. Other strains that also showed anti-listeria effect were five strains of *Lactobacillus sakei*. The five strains up to 10^6CFU/g inhibited the growth of *L. monocytogenes* in cooked sliced meat packaged in a vacuum or with gas and stored at 8°C. Inhibition can be caused by one of these strains or by several strains acting synergistically. There was no sensory quality deviation in these products until storage for 21 days. In addition to being added in the form of bacterial culture, bacteriocins produced by lactic acid bacteria are also widely studied for application to meat products to provide antagonistic effects against *L. monocytogenes*. The antimicrobial effect of commercial nisin (nisaplin) was investigated by Udhayashree *et al.* [30] to inhibit the growth of *L. monocytogenes* in turkey meat. Nisin is added to the hot water used to marinate the turkey. *Leuconostoc monocytogenes* is applied in two ways: in hot water soaking turkey and applied directly to the turkey skin. The addition of nisin caused a decrease in the amount of *L. monocytogenes* by 1 log CFU/ml when cells were added to turkey skin. The decrease continued if the sample was stored at refrigeration temperature. The heat from the washing water showed a synergistic effect with nisin, indicated by a more significant decrease of log 2 after immersion when *L. monocytogenes* cells were given to the washing water. After storage at refrigeration temperature for 48 h, *L. monocytogenes* cells were killed with that treatment.

In addition, the inhibitory activity against *L. monocytogenes* Linll was investigated by two bacteriocins, namely nisin and pediocin AcH, in minced raw pork stored aerobically at 5°C. The bacteriocin was applied to the soaking stage before the meat was minced. The result was that nisin was more efficient than pediocin AcH. However, after two days of storage, bacteria surviving on meat grew at the same rate as controls not treated with lamti-containing bacteriocin. Furthermore,

it was found that bacteria that survived after being treated with nisin became more resistant to the bacteriocin.

In contrast, those who survived after being treated with pediocin AcH remained susceptible. Further observations show that the loss of bacteriocin activity, especially in pediocin AcH, is caused by the rapid degradation of the protease enzyme in pork to the bacteriocin. From this study, it can be concluded that nisin is more suitable to be applied to soaking pork before chopping to inhibit *L. monocytogenes*. However, the concentration given is considered because if there are still bacteria that can survive, they may become a new population more resistant to nisin and other bacteriocins [54].

Ge *et al.* [55] investigated the antimicrobial effect of lactic acid, nisin, and pediocin on *L. monocytogenes* in beef. The meat was sliced into cubes (1 cm^3) and then given lactic acid (2%), nisin ($4x10^4$ IU/ml) and pediocin ($3.2x10^3$ IU/ml). Meat treated with antimicrobials was soaked for 1 min in *L. monocytogenes* cell suspension, then stored at 4°C for 48 h. The results showed that adding lactic acid, nisin, and pediocin significantly reduced the number of *L. monocytogenes* cells at 1.7, 1.1, and 0.6 log CFU/ml of the meat surface (6 cm^2), respectively [20]. In combination with sorbate, nisin also has an anti-listeria effect on beef stored at refrigeration temperature. The sterile beef steak was inoculated with three strains of hemolytic pathogenic *L. monocytogenes* at about 5 log CFU/cm^2, and the surface of the meat was treated with an antimicrobial lamtan consisting of 1.0% sorbate plus 1000 IU/ml nisin. Then the meat is vacuum-packed with 100% CO_2 and stored at 4°C for 4 weeks. In meat not added with nisin and sorbate, the growth of *L. monocytogenes* reached 1.79 log rounds, while in meat packaged with 100% CO_2, the listeria population decreased by 0.54 log rounds [20]. In meat with added nisin and sorbate, with vacuum packaging, the amount of *L. monocytogenes* was 96.5% of the inoculated amount [30]. However, the lag phase of the remaining bacterial cells was not affected. Meanwhile, in meat added with nisin and sorbate and packaged with 100% CO_2, the number of *L. monocytogenes* decreased by 89.3% from the original amount, and the lag phase of the remaining bacteria was extended [20].

The combination of nisin with EDTA was also shown to have an antagonistic effect against *L. monocytogenes* in fresh beef cut into cubes (2.5 cm^3). The meat was inoculated with about 7 log CFU/ml *L. monocytogenes* Scott A and immersed in a solution of nisin or nisin combined with EDTA for 10 min [20, 30]. The samples were then drained for 15 min, vacuum packed, and stored at 4°C for 30 days. The results showed that immersion in the nisin solution reduced the population of *L. monocytogenes* by 2.01 CFU/cm^2. The combination of nisin with EDTA decreased to 0.99 CFU/cm^2 after 30 days of storage compared to controls not dyed with the lamtan [20]. In addition, the antimicrobial effect was also obtained by applying bacteriocins to the films used to package meat products [20]. Cleveland *et al*. [7] used bacteriocins in powder form that were produced from milk-based media and applied to packaging films. Ennahar *et al*. [33] applied pediocin powder to plastic packaging bags at 7.75 mg/cm^2. Beef and poultry samples were then inoculated with *L. monocytogenes* before placing in these bags and the growth of *L. monocytogenes* was inhibited during 12 weeks of storage at 4ºC was reported.

5.3 Applications on sausage

The minimum infectious dose for listeriosis in humans is unclear, so it is essential to control the presence of *L. monocytogenes* in foodstuffs, especially low-Aw fermented foods such as sausages, where *L. monocytogenes* can survive. Joerger [46] investigated the antimicrobial activity of bacteriocin-producing lactic acid bacteria against *L. monocytogenes* in Italian salami sausage. The lactic acid bacteria used were *L. plantarum* MCSl strain-producing bacteriocin, the mutant of this strain that did not produce bacteriocin, and two commercial starter cultures. One of the commercial starter cultures contained a mixture of *Lactobacillus curvatus* and *Pediococcus pentosaceus*, while

the other contained *Lactobacillus* sp., which is not identified. The sausages tested contained 2.5% NaCl, 250 mg/kg NO_2, and 0.3% sucrose. Meanwhile, *L. monocytogenes* were inoculated from SSICA strains 38 and 150, as much as 10^3 to 10^4 CFU/g. In salami sausage that did not contain lactic acid bacteria, the growth of *L. monocytogenes* was detected after 7-14 days of storage. While containing lactic acid bacteria, the number of *L. monocytogenes* decreased during the salami ripening process, but there were still bacteria that could survive until the ripening process ended. Only salami supplemented with *Lactobacillus plantarum* MCSl-producing bacteriocin successfully killed all *L. monocytogenes* cells in salami at the end of ripening. The combination of treatment with adding lysozyme, nisin, and EDTA in bologna sausage also reduced the growth of *L. monocytogenes*. The sausage was made by adding lysozyme 500 mg/kg, nisin 1:3, and EDTA 500 mg/kg, and inoculated with spoilage or pathogenic bacteria, including *L. monocytogenes,* vacuum packed and stored at 8°C for four weeks. This treatment inhibited the growth of *L. monocytogenes* until storage for two weeks.

5.4 Applications to fish and foods of marine origin

Bacteriocins are the main compounds that have antimicrobial effects against *L. monocytogenes* in fish products or foodstuffs of marine origin. The combination of nisin with dehydroacetic acid applied to the catfish cut (*Ictalurus punctatus*) also inhibited and killed *L. monocytogenes*, which were inoculated in the product as much as 10^5 CFU/ml. The fish meat products are packaged and stored at 2°C for six days. Adding nisin alone (0.1%) or in combination with 0.1% dehydroacetic acid significantly reduced the *L. monocytogenes* by 2.2 and 3.1 log CFU/ml, respectively. The application of nisin combined with the addition of NaCl treatment, CO_2 regulation, and storage at low temperatures also showed antagonistic activity against the growth of *L. monocytogenes* inoculated in cold smoked salmon. In general, nisin at a 50 IU/ml concentration showed increased inhibitory activity when used with 100% CO and NaCl. At 5°C, the growth of *L. monocytogenes* was inhibited in vacuum-packed salmon with the addition of nisin at a concentration of 500 or 1000 IU/ml. Storage of salmon in a 100% CO atmosphere gave a lag phase for eight days; after 27 days, *L. monocytogenes* reached 10^6 cfu/g. In the modified atmosphere treatment combined with 500 IU/ml nisin, the lag phase became 8 days, while adding 1000 IU/ml nisin became 20 days [2].

Based on the research of Bharal and Sohpal [19], a combination of nisin addition treatment with soft heating increased the antimicrobial properties of *L. monocytogenes* in cold-packed canned lobster. The addition of nisin to the salt solution filled into cans with lobster as much as 25 mg/kg of the contents of the cans, combined with the process of heating the cans until the internal temperature reaches 60°C for 5 min or 65°C for 2 min, reduces the viability of pathogenic bacteria, *L. monocytogenes*, at 3-5 log CFU/g. The anti-listeria effect of lactic acid bacterial cultures was investigated by Cleveland *et al.* [7]. They proved the ability of *Carnobacterium pisciola* to inhibit the growth of *L. monocytogenes* in cold smoked salmon in vacuum packaging stored at 5°C without causing aberration of sensory properties.

Carnobacterium pisciola cell-free supernatant inhibited *L. monocytogenes* due to the bacteriocin it produces. The bacteriocin-producing *C. piciola* strain extended the growth lag phase of *L. monocytogenes* to 7 days, followed by a decrease in the number of *L. monocytogenes* from 10^3 to less than 10 CFU/ml after 32 days of incubation. In contrast, Cintas *et al.* [4] investigated the inhibition of *L. monocytogenes* by strains. *Carnobacterium* and crude extract of bacteriocin were produced in cold smoked salmon, which was vacuum packed and stored at refrigeration temperature. *Carnobacterium piscicola* V1 was bactericidal against *L. monocytogenes* at 2°C, while *Carnobacterium divergens* V41 had a bacteriostatic effect. *Carnobacterium piscicola* SF668 at a temperature of 8°C prolongs the lag phase, and at 4°C has a bacteriostatic effect on *L.*

monocytogenes. Listeria growth was not affected by non-bacteriocin compounds produced by *C. piscicola.*

5.5 Application on eggs

Bacteriocins produced by lactic acid bacteria also showed antagonistic effects against *L. monocytogenes* in liquid egg products. Arabestani *et al.* [1] investigated the D and Z values of *L. monocytogenes* Scott A in liquid whole eggs with the addition of 90 or 10 mg/mL nisin and NaCl (0 or 10%) by immersion procedure. Adding nisin to unsalted liquid eggs rapidly reduced the number of bacteria by 4 logs in 1 h. Nisin significantly reduced the D value in liquid whole eggs, whether added salt, pasteurized at a temperature of less than 58°C. Meanwhile, for liquid eggs which were pasteurized at the standard pasteurization temperature in Canada and the US (60°C without salt or 63°C with the addition of salt), the addition of nisin 2 h before the pasteurization treatment significantly reduced the D value because the bacteria were already in a wound condition. Because of bacteriocin, they are more sensitive to heat treatment. Nisin works synergistically with heating, so it has the potential to be applied to control *L. monocytogenes* in pasteurized liquid egg products.

5.6 Applications for ice cream

In addition to cheese, a bacteriocin produced by lactic acid bacteria has also been applied to other dairy products such as ice cream, to control *L. monocytogenes* [56]. They investigated the effect of adding nisin on the resistance of *L. monocytogenes* V7 in ice cream made from total fat (10% fat) and reduced fat (3% fat) milk. Ice cream with varying fat composition was inoculated with *L. monocytogenes* during processing and soft frozen, followed by freezing at -18°C for three months. Adding nisin to ice cream made with 3% fat effectively killed *L. monocytogenes*, and at the end of storage, no *L. monocytogenes* cells were found in the product. Meanwhile, in ice cream with 10% fat content, the amount of *L. monocytogenes* was reduced but not significantly. Furthermore, the stability of nisin was relatively constant during storage at -18°C [57].

5.7 Applications in vegetable products

Lactic acid bacteria or their compounds have also been applied as antimicrobials against *L. monocytogenes* in plant foods. For example, Alvarez-Cisneros *et al.* [58] showed the potential of the bacteriocin produced by *Enterococcus mundtii*, mundticin, to inhibit and kill *L. monocytogenes* in minimally processed vegetable products. Bacteriocins, which were applied at the washing step or by coating procedures, prevented the growth of *L. monocytogenes* on bean or mung bean sprouts stored at modified atmospheric conditions (1.5% O_2; 20% CO_2; 78.5% N_2) at a refrigeration temperature of 8°C [59, 60].

6. Conclusions

Bacteriocins are antimicrobial peptide compounds that are easily degraded by proteolytic enzymes in the digestive system of humans and animals. Bacteriocins produced by lactic acid bacteria are very beneficial in the food industry, especially in fermented food products. Their activity can inhibit the growth of several contaminant bacteria that cause spoilage and food-borne illness. The addition of bacteriocins in food is not only to prevent spoilage but also to extend food storage time and inhibit the growth or kill pathogenic bacteria. The number of lactic acid bacteria species have been used in the food industry, but not all species produce bacteriocins. Some bacteriocins from lactic acid

bacteria in the food industry are nisin, diplococcin, acidochilin, bulgarican, helventicin, lactacin, and plantaricin. Bacteriocins derived from lactic acid bacteria and used as biopreservatives have several advantages: a) bacteriocins are not toxic and are readily biodegradable because they are protein compounds; b) the use of bacteriocin does not harm the intestinal microflora because it is easily digested by enzymes in the digestive tract; c) the use of bacteriocins in the food industry can reduce the number of chemicals used as food preservatives; d) the use of very flexible bacteriocins can be in the form of cultured strains of bacteriocin-producing bacteria or purified or semi-purified bacteriocin compounds.

References

[1] Arabestani, M.R., Mousavi, S.M. and Alikhani, M.Y., 2014. Bacteriocins as the alternatives to antibiotics. *Avicenna Journal of Clinical Microbiology and Infection*, 1(2), https://doi.org/10.17795/ajcmi-21086.

[2] Arquest, J.L., Rodriquez, E., Langa, S., Landete, J.M. and Medina, M., 2015. Antimicrobial activity of lactic acid bacteria in dairy product and gut: effect on pathogens. *BioMed Research International,* 2015, http://doi.org/10.1155/2015/584183.

[3] Chen, Y.-S., Yanagida, F. and Srionnual, S., 2007. Characteristics of bacteriocin-like inhibitory substances from dochi-isolated *Enterococcus faecium* D081821 and D081833. *Letters in Applied Microbiology*, 44 (3), 320-325, https://doi.org/10.1111/j.1472-765X.2006.02058.x.

[4] Cintas, L.M., Casaus, M.P., Herranz, C., Nes, I.F. and Hernandez, P.E, 2006. Review: bacteriocins of lactic acid bacteria. *Food Science and Technology International*, 7(4), 281-305, https://doi.org/10.1106/R8DE-P6HU-CLXP-5RYT.

[5] Sarra, M., Taoufik, G., Patrick, L.C., Benjamin, B., Yannick, F. and Khaled, H., 2013. Isolation and characterization of enterococci bacteriocinic strains from tunish fish viscera. *Food Science and Nutrition*, 4, 701-708.

[6] Karthikeyan, V. and Santosh, S.W., 2009. Isolation and partial characterization of bacteriocin produced from *Lactobacillus plantarum*. *African Journal of Microbiology Research*, 3(5), 233-239, https://doi.org/10.1111/1348-0421.12091.

[7] Cleveland, J., Montville, T.J., Nes, I.F. and Chikindas, M.L., 2001. Bacteriocins : safe, natural antimicrobial for food preservation. *International Journal of Food Microbiology*, 71, 1-20, https://doi.org/10.1016/S0168-1605(01)00560-8.

[8] Ferreira, A.E., Canal, N., Morales, D., Fuentefria, D.B. and Corção, G., 2007. Chracterization of enterocin produced by *Enterococcus mundtii* isolated from human feces. *Brazilian Archives of Biology and Technology*, 50(2), 249-258, https://doi.org/10.1590/S1516-89132007000200010.

[9] Franz, C.M.A.P., Van-Belkum, M.J., WHolzapfel, H., Abriouel, H. and Gálvez, A., 2007. Diversity of enterococcal bacteriocins and their grouping in a new classification scheme. *FEMS Microbiology Reviews*, 31, 293-310, https://doi.org/10.1111/j.1574-6976.2007.00064.x.

[10] Ogaki, M.B., Rocha, K.R., Terra, M.R., Furlaneto, M.C. and Furlaneto-Maia, L., 2016. Screening of the enterocin-encoding genes and antimicrobial activity in enterococcus Species. *Journal of Microbiology and Biotechnology*, 26(6), 1026-1034, https://doi.org/10.4014/jmb.1509.09020.

[11] Avaiyarasi, N.D., Ravindran, A.D., Venkatesh, P. and Arui, V. 2016. *In vitro* selection, characterization and cytotoxic effect of bacteriocin of *Lactobacillus sakei* GM3 isolated from goat milk. *Food Control*, 69, 124-133, https://doi.org/10.1016/j.foodcont.2016.04.036.

[12] Nes, I.F., Yoon, S.-S. and Diep, D., 2007. Ribosomally synthesized antimicrobial peptides (bacteriocins) in lactid acid bacteria: a review. *Food Science and Biotechnology*, 16(5), 675-690.
[13] De Vuyst, L. and Leroy, F., 2007. Bacteriocin from lactic acid bacteria: production, purification and food applications. *Journal of Molecular Microbiology and Biotechnology*, 13(4), 194-199, https://doi.org/10.1159/000104752.
[14] Cotter, P.D., Hill, C. and Ross, R.P., 2005. Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology*, 3, 777-788.
[15] Khalid, K, 2011. An overview of lactic acid bacteria. *International Journal of Biosciences*, 1(3), 1-13.
[16] Dimov, S.G., Ivanova, P. and Harizanova, N., 2005. Genetics of bacteriocins biosynthesis by lactic acid bacteria. *Biotechnology and Biotechnological Equipment*, 19(3), 4-10, https://doi.org/10.1080/13102818.2005.10817270.
[17] Nwuche, C.O., 2013. Isolation of bacteriocin producing lactic acid bacteria from ugba dan okpiye, two locally fermented nigerian food condiments. *Brazilian Archives of Biology and Technology*, 56(1), 101-106, https://doi.org/10.1590/S1516-89132013000100013.
[18] Hosseini, S.V., Arlindo, S., Böhme, K., Fernández-No., C., Calo-Mata, P. and Barros-Velázquez, J., 2009. Molecular and probiotic characterization of bacteriocin producing *Enterococcus faecium* strains isolated from nonfermented animal foods. *Journal of Applied Microbiology*, 107, 1392-1403, https://doi.org/10.1111/j.1365-2672.2009.04327.x.
[19] Bharal, A. and Sohpal, V.K., 2013. Evaluation of antimicrobial activity of bacteriocin (*L. acidophilus*) against human pathogenic and food born microorganisms. *International Journal for Innovative Research in Science and Technology*, 2(9), 4221-4225.
[20] Azhar, N.S., Md Zin, N.H. and Hamid, T.H.T.A., 2017. *Lactococcus lactis* strain A5 producing nisin like bacteriocin active againts gram positif and gram negative bacteria. *Tropical Life Sciences Research*, 28(2), 107-118, https://doi.org/10.21315/tlsr2017.28.2.8.
[21] Drider, D., Fimland, G., Héchard, Y., McMullen, L. M. and Prévost, H., 2006. The continuing story of class IIa bacteriocins. *Microbiology and Molecular Biology Reviews*, 70(2), 564-582.
[22] Perez, R.H., Zendo, T. and Sonomoto, K., 2014. Novel bacteriocins from lactic acid bacteria (LAB): varoius structure and aplications. *Microbial Cell Factories*, 13(1), 3-13.
[23] Bharti, V., Mehta, V., Singh, S., Jian, N., Ahirwal, L. and Mehta, S., 2015. Bacteriocin: a novel approach for preservation of food. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(9), 20-29.
[24] Perez, R.H., Perez, M.T. M. and Elegado, F.B., 2015. Bacteriocins from lactic acid bacteria: a review of biosynthesis, mode of action, fermentative production, uses and prospects. *International Journal of Philippine Science and Technology*, 8(2), 61-67.
[25] Oldak, A., Zielinska, D., Rzepkowska, A. and Kolozyn-Krajewska, D., 2017. Comparison of antibacterial activity of *L. plantarum* strains isolated from two differents kinds of regional cheeses from Poland: Oscypek and Korycinski cheese. *BioMed Research International,* 2017, https://doi.org/10.1155/2017/6820369.
[26] Toit, M.D, Franz, C.M.A.P, Dicks, L.M.T. and Holzapel, W.H., 2000. Preliminari characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig feces. *Journal of Applied Microbiology*, 88, 482-494, https://doi.org/10.1046/j.1365-2672.2000.00986.x.
[27] Nespolo, C.R. and Brandelli, A., 2010. Production of bacteriocin-like substances by lactic acid bacteria isolated from regional ovine cheese. *Brazilian Journal of Microbiology*, 41(4), 1009-1018.
[28] Saelim, K., Sohsomboon, N., Kaewsuwan, S., and Maneerat, S., 2012. Producing properties of *Enterococcus faecium* CE5-1 producing a bacteriocin –like substance and its antagonistic

effect againts antibiotic-resistance enterococci *in vitro*. *Czech Journal of Animal Science*, 1, 529-539.

[29] Malik, H., Sur, B., Singhal, N. and Bihari, V., 2008. Antimicrobial protein from *Streptomyces fulvissimus* Inhibitory to methicillin resistant *Staphylococcus aureus*. *Indian Journal of Experimental Biology,* 46, 254-257.

[30] Udhayashree, N., Senbagam, D., Senthilkumar, B., Nithya, K. and Gurusamy, R., 2012. Production of bacteriocin and their application in food products. *Asian Pacific Journal of Tropical Biomedicine*, 2(1), S406-S410.

[31] Perin, L.M. and Nero, L.A., 2014. Antagonistic lactic acid bacteria isolated from goat milk and identification of a novel nisin variant *Lactococcus lactis*. *BMC Microbiology*, 14(36), 2-9.

[32] Rabie, M.A.H., Moustafa, M.G., Abdel-Wahed, E.M., AL-Harby, K.B. and El-Hazar, K., 2018. Bacteriocin-like substances produced by specific strains of lactic acid bacteria isolated from milk products. *Research Journal of Microbiology*, 13(2), 70-83.

[33] Ennahar, S., Werner, D.A., Sorokine, O., Van Dorsselaer, A., Bringel, F., Hubert, J.C. and Hasselmann, C., 1996. Production of pediocin AcH by *Lactobacillus plantarum* WHE92 isolated from cheese. *Applied and Environmental Microbiology*, 62(12), 4381-4387, https://doi.org/10.1128/aem.62.12.4381-4387.1996.

[34] Goh, H.F. and Philip, K., 2015. Purification and characterization of bacteriocin produced by Weissella *confuse* A3 of dairy origin. *Plos One*, 10(10), https://doi.org/10.1371/journal.pone.0140434.

[35] Mohankumar, A. and Murugalatha, N., 2011. Characterization and antibacterial activity of bacteriocin producing lactobacillus isolated from raw cattle milk sample. *International Journal of Biology,* 3(3), 128-143.

[36] Yang, S.-C., Lin, C.-H., Sung, C.T. and Fang, J.-Y., 2014. Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. *Frontiers in Microbiology*, 5, https://doi.org/10.3389/fmicb.2014.00241.

[37] Rattanachaikunsopon, P. and Phumkhachorn, P., 2010. Lactid acid bacteria: their antimicrobial compounds and their uses in food production. *Annals of Biological Research*, 1(4), 218-228.

[38] Reis, J.A., Paula A.T., Casarotti, S.N. and Penna, A.L.B., 2012. Lactic acid bacteria antimicrobial compounds: characteristics and applications. *Food Engineering Reviews,* 4(2), 124-140.

[39] Jack, R.W., Tagg, J.R. and Ray, B., 2015. Bacteriocins of gram positive bacteria. *Microbiological Reviews*, 59(2), 171-200, https://doi.org/10.1128/mr.59.2.171-200.1995.

[40] Lavermicocca, P., Lonigro, S.L., Evidente, A. and Andolfi, A., 1999. Bacteriocin production by *Pseudomonas syringae* pv. *ciccaronei* NCPPB2355. Isolation and partial characterization of the antimicrobial compound. *Journal of Applied Microbiology*, 86(2), 257-265, https://doi.org/10.1046/j.1365-2672.1999.00656.x.

[41] Vimont, A., Fernandez, B., Hammami, R., Ababsa, A., Daba, H. and Fliss, I., 2017. Bacteriocin-producing enterococcus faecium LCW 44: a high potential probiotic candidate from raw camel milk. *Frontiers in Microbiology*, 8, https://doi.org/10.3389/fmicb.2017.00865.

[42] Kim, C.H., Ji, G.E. and Ahn, C., 2000. Purification and molecular characterization of a bacteriocin frim *Pediococcus sp.* KCA1303-10 isolated from fermented flatfish. *Food Science and Biotechnology*, 9(4), 270-276.

[43] Tulini, F.L., Gomes, B.C. and de Martinus, E.C.P., 2011. Partial purification and characterization of a bacteriocin produced by *Enterococcus faecium* 130 isolated from mozzarella cheese. *Food Science and Technology (Campinas)*, 31(1), 155-159, https://doi.org/10.1590/S0101-20612011000100022.

[44] Martinez, B., Rodriguez, A. and Suarez, E., 2016. Antimicrobial peptides produced by bacteria: the bacteriocins. In: T. Villa and M. Vinas, eds. *New Weapons to Control Bacterial Growth*. Cham: Springer, pp. 15-38.

[45] Kumar, M. and Srivastava, S, 2010. Enhanced production of enterocin from *Enterococcus faecium* Lr/6 by statistical optimization of the growth medium. *Trakia Journal of Sciences*, 8(4), 12-21.
[46] Joerger, R.D, 2003. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophage. *Poultry Science Association*, 82, 640-647, https://doi.org/10.1093/ps/82.4.640.
[47] Najim, H.N., Mohammed, Z.A. and Khudir, Z.S., 2012. The antibacterial activity of bacteriocin produced by *Lactobacillus acidophilus* isolates against sensitive reference strain *Lactobacillus acophyllus* R0052 and its stability to different pH, heating and storage temperatures. *The Iraqi Journal of Veterinary Medicine*, 36(OE), 274-279.
[48] Sim, K.Y., Chye, F.Y. and Anton, A., 2012. Probiotic potential and antimicrobial activities of microorganisms isolated from an indigenous fish sauce. *Borneo Science*, 31, 57-63, https://doi.org/10.51200/bsj.v31i.174.
[49] Maky, M.A., Ishibashi, N., Zendo, T., Perez, R.H., Doud, J.R., Karmi, M. and Sonomoto, K., 2015. Enterocin F4-9, a novel O-Linked glycosylated bacteriocin. *Applied and Environmental Microbiology,* 81(14), 4819-4826, https://doi.org/10.1128/AEM.00940-15.
[50] Sharaf, E.F. and Al harbi, R.M., 2011. Isolation, identification and antimicrobial activity of some local isolates of lactic acid bacteria. *Research Journal of Microbiology*, 6(12), 826-838.
[51] Gautam, N., Sharma, N. and Ahlawat, O.P., 2014. Purification and characterization of bacteriocin produced by *Lactobacillus brevis* UN isolated from Dhulliachar: a traditional food product of North East India. *Indian Journal of Microbiology*, 54(2), 185-189.
[52] Egan, K., Fieldy, D., Rea, M.C., Ross, R.P., Hill, C. and Cotter, P.D., 2016. Bacteriocins: Novel solutions to age old spore-related problems? *Frontiers in Microbiology*, 7, https://doi.org/10.3389/fmicb.2016.00461.
[53] Zamfir, M., Calewaert, R., Cornea, P.C., Savu, L., Vatafu, I. and De Vuyst, L., 1999. Pufication and characterization of a bacteriocin produced by *Lactobacillus acidophilus* IBB 801. *Journal of Applied Microbiology*, 87(6), 923-931, https://doi.org/10.1046/j.1365-2672.1999.00950.x.
[54] Yusuf, M.A. and Hamid, T.H.A.T.A., 2013. Lactic acid bacteria: bacteriocin producer: a mini review. *IOSR Journal of Pharmacy,* 3(4), 44-50.
[55] Ge, J., Sun, Y., Xin, X., Wang, Y. and Ping, W., 2016. Purification and partial characterization of a novel bacteriocin synthesized by *Lactobacillus paracasei* HD1-7 isolated from chinese sauerkraut juice. *Scientific Reports*, 6, https://doi.org/10.1038/srep19366.
[56] Zacharof, M.P. and Lovitt, R.W., 2012. Bacteriocins produced by lactic acid bacteria a review article. *APCBEE Procedia*, 2, 50-56, https://doi.org/10.1016/j.apcbee.2012.06.010.
[57] Kuniyoshi, T.M., Mendonça, C.M.N., Vieira, V.B., Robl, D., Franco, B.D.G.D.M., Todorov, S.D., Tome, E., O'Connor, P.M., Converti, A., Araújo, W.L., Vasconcellos, L.P.S.P., Varani, A.D.M., Cotter, P.D., Rabelo, S.C. and Oliveira, R.P.D.S., 2021. Pediocin PA-1 production by *Pediococcus pentosaceus* ET34 using non-detoxified hemicellulose hydrolysate obtained from hydrothermal pretreatment of sugarcane bagasse. *Bioresource Technology*, 338, https://doi.org/10.1016/j.biortech.2021.125565.
[58] Alvarez-Cisneros, Y.M., Espunes, T.R.S., Wacher, C., Fernandez, F.J. and Ponce-Alquicira, E., 2011. Enterocins: Bacteriocins with applications in the food industry. In: A. Méndez-Vilas, ed. *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*. Badajoz: Formatex, pp. 1330-1341.
[59] López, J.R, GrandeBurgos, M.J., De Filippis, F., Pulido, R.P., Ercolini, D., Galvez, A. and Lucas, R., 2023. Determination of the effect of the bacteriocin enterocin AS-48 on the microbial loads and bacterial diversity of blueberries. *Heliyon*, 9(5), https://doi.org/10.1016/j.heliyon.2023.e15921.
[60] Martín, I., Barbosa, J., Pereira, S.I.A., Rodríguez, A., Córdoba, J.J. and Teixeira, P., 2023. Study of lactic acid bacteria isolated from traditional ripened foods and partial characterization of their bacteriocins. *LWT – Food Science and Technology*, 173, https://doi.org/10.1016/j.lwt.2022.114300.