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Biopreservation strategies using bacteriocins to control meat spoilage and foodborne outbreaks

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Abstract
Fresh meat is highly perishable, presenting challenges in spoilage mitigation and waste reduction globally. Despite the efforts, foodborne outbreaks from meat consumption persist. Biopreservation offers a natural solution to extend shelf life by managing microbial communities. However, challenges include the effective diffusion of bacteriocins through the meat matrix and the potential inhibition of starter cultures by bacteriocins targeting closely related lactic acid bacteria (LAB). LAB, predominant in meat, produce bacteriocins – small, stable peptides with broad antimicrobial properties effective across varying pH and temperature conditions. This review highlights the recent advances in the optimization of bacteriocin use, considering its structure and mode of action. Moreover, the strengths and weaknesses of different techniques for bacteriocin screening, including novel bioengineering methods, are described. Finally, we discuss the advantages and limitations of the modes of application of bacteriocins toward the preservation of fresh, cured, and novel meat products.

Introduction
Meat is a source of high-quality proteins, minerals, and vitamins (Geiker *et al*., 2021). However, it is especially prone to spoilage, as it undergoes microbial (*e.g*., microbiological spoilage), chemical (*e.g*., autolytic enzymatic reactions), and physical (*e.g*., slime and liquid formation) deterioration; in fact, estimates show that as much as 23% of the annual production in the meat sector is lost and wasted (Luong *et al*., 2020; Odeyemi *et al*., 2020; Karwowska *et al.*, 2021).
Regarding the safety of meats, recent cases (2016-2021) of foodborne outbreaks in the European Union have been linked to the consumption of contaminated meats, for instance, with *Salmonella (S.)* spp. (n=289), *Clostridium perfringens* (n=102), *Staphylococcus aureus* toxins (n=34), and *Listeria monocytogenes* (n=17), resulting in a total of 1363 hospitalizations and economic losses of up to US$90 billion annually (Scharff, 2020; EFSA, 2023).
Research on meat preservation considers not only the extension of the product's organoleptic features but also its microbiological safety (European Commission, 2005 - EC No. 2073/2005). In the domain of biopreservation, natural or controlled microbial communities and their antibacterial products are used as an approach for controlling microbial growth. An integral component of the initial microbial community of meat is lactic acid bacteria (LAB), which rapidly develops under chill-stored, post-processed, and vacuum-packed/modified atmosphere conditions (Nauman *et al*., 2022).
LAB are classified as Gram-positive, catalase-negative, anaerobic, with a varied morphology (rods or cocci), and play a crucial role in fermentation. Moreover, LAB can be used as biocontrol agents by the generation of metabolites with antimicrobial properties against pathogens, including organic acids (*e.g*., lactic acid, acetic acid), short-chain fatty acids, proteases/peptidases, and bacteriocins (Ibrahim *et al*., 2021).
This review highlights the recent advances in the optimization of bacteriocin use, considering the bacteriocin's structure and mode of action. Moreover, the strengths and weaknesses of different techniques for bacteriocin screening, including novel bioengineering methods, are described. Finally, we discuss the advantages and limitations of different bacteriocins' mode of application toward the preservation of fresh, cured, and novel meat products.

General overview
Bacteriocins are small peptides synthesized in the ribosome and can be categorized into four groups, according to their size, structure, and function: class I or lantibiotics (<5 kDa), class II (<10 kDa), class III or bacteriolysins/non-lytic (>30 kDa), and class IV (reclassified as bacteriolysins) (Simons *et al*., 2020; Barcenilla *et al*., 2023). These peptides play a crucial role in the competition for colonization sites and are able to influence the dynamics of the microbiome (Umu *et al.*, 2017). For instance, bacteriocinogenic LAB inhibit target bacteria by interacting with the negatively charged cell membrane, a process mediated by their cationic and amphiphilic motifs (Lei *et al*., 2019; Heilbronner *et al*., 2021).

Mode of action

The bioactivity of bacteriocins can be either of a narrow spectrum (if the inhibition is exclusive to species that are closely related) or a broad spectrum, which is the case for lantibiotics (Woraprayote *et al*., 2016); in addition to presenting either bacteriostatic (inhibition) or bactericidal (killing) effects (Negash and Tsehai, 2020). The general mode of action of these antimicrobial peptides is given by their specialized functional domains: substrate binding site, translocation, and catalytic site (Gillor *et al.*, 2008). The binding domain attaches to specific receptors on bacterial membranes, the translocation domain interacts with specific proteins integral to the cell membrane, and the effector domain executes lethal action: DNA degradation and/or induction of pores in the membrane (Davidson *et al.*, 2008).

Nisin

Lantibiotics act by blocking the lipid II cycle (Figure 1) (Hsu *et al.*, 2004), preventing correct cell wall synthesis, and inducing pore formation by interacting with the outer membrane (Diep *et al*., 2009). Nisin (grey) is composed of 34 amino acids and has a positive charge (+4), which allows it to interact with the anionic lipid II that constitutes the peptidoglycan layer in the bacterial cell membrane. The peptidoglycan is formed by a chain composed of N-acetylglucosamine (green), N-acetyl muramic acid (MurNAc; red), a pentapeptide (not shown), two pyrophosphate molecules (blue), and a prenyl chain (black).

The lipid II mediated pathway of nisin starts with the recognition of lipid II MurNAc and isoprene units. The dehydrobutyrine and α-aminobutyric acid of nisin then establish a hydrogen bond with the pyrophosphate molecules of lipid II. The assembly of nisin occurs without a canonical secondary structure, where two lanthionine rings fold to form a cage-like structure with the nisin backbone amides and the lipid II pyrophosphate, with a molar ratio of 8:4 for nisin and lipid II, respectively. Finally, the cage-like structure induces pore formation, resulting in disruption of the cell membrane.

Pediocin

On the other hand, class II or non-lantibiotic bacteriocins (Figure 2), divided into four subclasses, utilize the mannose phosphotransferase system (man-PTS) to permeabilize the membrane, disrupt proton motive force, and deplete adenosine triphosphate pools (Diep *et al.*, 2007). Class IIb bacteriocins activity depends on two-component, α and β subunits, which fold into α-helical structures and insert themselves into target bacterial membranes to alter their permeability, resulting in ion leakage and cell death (Nissen-Meyer *et al*., 2011; Proutière *et al*., 2023).

Pediocin PA-1 is composed (Figure 2) (Zhu *et al*., 2022) of a hydrophilic and cationic N-terminal (brown) which consists of a three-stranded-β-sheet (containing the pediocin-box) linked by disulfide bridges (black). The region between N- and C- terminal is a flexible region composed of Asp/Asn in residue 17. The C-terminal is hydrophobic and is organized in an α-helix (blue), coupled with a hairpin-like tail (light pink).

The N-terminal β-sheet of pediocin PA-1 links with the extracellular man-PTS core domain (red). Normally, this transporter protein composed by a v-motif (green) and a core domain (red) switches from position depending on the transport of mannose. The change in conformation occurs by an elevator movement.

The binding occurs with the effector domain of pediocin PA-1 (pediocin-box and positively charged Lysn II and His 12) and the core domain of the man-PTS (Val 7, Cys 9, Cys 14, Tyr 3) establishing a linkage that blocks the elevator movement of man-PTS, with a molar ratio of pediocin PA-1 and man-PTS at 3:3. The hairpin-like tail of pediocin makes pi-stacking interactions with Trp 18 (white) of man-PTS and stabilizes the pediocin-manPTS structure, leading to disruption of the cell membrane (Zhu *et al*., 2022).

Screening of bacteriocins

The screening of bacteriocins can be divided into three stages: search for the presence of bacteriocin-encoding genes, evaluation of bacteriocin expression, and assessment of the bacteriocin antimicrobial activity. For cultivable bacteria, the search for a bacteriocinogenic LAB strain begins with the isolation of autochthonous strains from the meat matrix. Then, the bacteriocin-encoding genes can be amplified by PCR (polymerase chain reaction), and their expression evaluated in real-time using real-time quantitative PCR (RT-qPCR). After purification, the bacteriocin's antimicrobial activity can be assessed by antagonism tests (Figure 3) against indicator microorganisms.

For instance, the *in vitro* assessment of bacteriocins or bacteriocin-like substances entail methodologies such as agar well diffusion (Bungenstock *et al*., 2020), agar spot (Moraes *et al*., 2010; Selman *et al*., 2021), turbidimetric (Yang *et al*., 2018; Piazentin *et al*., 2022), enzyme-linked immunosorbent assay (Martínez *et al*., 2000; Surati, 2020) and RT-qPCR (Dortu *et al.*, 2009; Balutis, 2014; Wan *et al*., 2015). Moreover, the bacteriocin does not have an antimicrobial effect on the producer strain. These mechanisms are constituted of self-immunity proteins that competitively antagonize the putative bacteriocin receptors by anchoring the membrane surface (antagonism), being embedded in the membrane (repulsion), or producing metalloproteases that degrade the bacteriocin (Deegan *et al*., 2006; Bastos *et al*., 2015).

Culture-based methods for assessing antimicrobial activity mostly relying on outdated protocols persist in being the most used (Balouiri *et al*., 2016), despite recent advancements in genomics, namely the whole genome sequencing (WGS), RNA sequencing (RNA-seq), and PCR-based techniques. While agar-based tests offer cost-effectiveness and simplicity, particularly at an initial sample screening, they lack the depth necessary to fully explore a strain's bacteriocinogenic potential. Moreover, utilizing different agar-based tests within a single study may introduce intra-study variability and errors due to differing experimental conditions and subjective interpretation of results (Hossain, 2024).

In the case of unculturable microbes, the use of metagenomics tools is utilized since it's estimated that 99% of microorganisms are not possible to culture in isolation (Ayrapetyan and Oliver, 2016). The DNA of the food sample is extracted, followed by library preparation and then sequencing of all of the DNA present in the sample. This sequencing data enables the visualization of the microbial community composition within the food matrix, providing insights into the relationship between meat preservation and 16S rRNA diversity analysis, as the microbiota continually changes during storage (Dorn-In *et al*., 2024).

Shotgun metagenomics enables the discovery of the microbial profile of samples, allowing for the detection of spoilage and pathogenic organisms, as well as differentiation at the strain level (Srinivas *et al*., 2022). This method can be used to monitor the supply chain for agents of concern, providing valuable insights that incentivize food manufacturers to invest in preventative control measures (Imanian *et al*., 2022).

The development of new *in silico* techniques has made it possible to analyze bacteriocins in a high-throughput manner (Nedyalkova *et al*., 2024). In contrast with classical assays for antimicrobial activity determination, the current methods for predicting bacteriocin gene clusters are high-speed and can be automated. BAGEL is a web mining tool that uses whole-genome sequence data to analyze the technological potential of bacterial strains (van Heel *et al*., 2018).

Mining genomes with automated software (Wosinska *et al*., 2022; Sowers *et al.*, 2023) for identification of bacteriocins reveal gene loci that can be functional or not. The data generated from this type of analysis has the potential to be exploited by bioengineering, including *de novo* design of novel bacteriocins (Deo *et al.*, 2022; Kordi *et al*., 2024).

Recent increase in RNA-seq data, which describes the presence and quantity of RNA in a biological sample, demonstrates that RNA-seq can be used to follow survival of target bacteria in the presence of the bacteriocin, by measurement of the expression of genes in food systems. Moreover, it is useful for analyzing the influence displayed by various environmental conditions on gene expression and fine-tune them for achieving optimal conditions (Yang *et al*., 2024).

Representative genomes on the NCBI platform have been used in comparative genomics to predict peptide expression and secretion by the bacteria (Marques *et al*., 2023). These tools in combination with molecular dynamics analysis allow automated assessment of the binding mechanism of action performed by bacteriocins (Walsh, 2017; V A *et al*., 2021; Rodrigues Blanco *et al*., 2022;). The computational tools include docking software and three-dimensional structure modelling of the putative peptides (Frederix *et al*., 2018; Nain *et al.*, 2020; Xin *et al*., 2020; Das *et al*., 2021; Palmer *et al*., 2021; Krishnamoorthi *et al*., 2022; Wang *et al*., 2023;).

The development of these novel methodologies enables the fast discovery of structural and functional characteristics of specific amino acid residues, which can be associated with binding sites in the bacterial membrane (Bindu and Lakshmidevi, 2021; Chen *et al*., 2022; Marques *et al.*, 2023; Oftedal, 2023). By understanding the mode of action of bacteriocins, given the varied nature of their structures, strategies to enhance and potentiate the bacteriocin's antimicrobial activity can be further developed (Amarh *et al*., 2023).

Lantibiotics, for instance, are subjected to post-translational processing (Figure 4) (Hsu *et al*., 2004), featuring compact structures with modified amino acids (*e.g*., lanthionine bridges, β-methyllanthionine, and dehydrated amino acids) and thioether ring structures. In the case of nisin, its structure comprises a globular chain consisting of lanthionine and dehydrated serine residues. These elements undergo post-translational modifications and proteolytic cleavage during the peptide processing phase, contributing to the unique structure of nisin.

Lactocin S, however, is prone to oxidation due to the sulfide bonds in its α and β rings which results in its inactivation; therefore, the synthesis of this bacteriocin occurs under anaerobic conditions, which could be improved by replacement of the sulfide bonds with hydrocarbon chains in analogs of lactocin S leading to more oxidative stability (Ross *et al*., 2012; Tsukano *et al*., 2024).

Lantibiotics active site include the amino acids residues such as the catalytic site and the substrate biding site, with the F(ND)L(DEN)(LVI) motif (Figure 5) being conserved across different bacteriocins from this class. There is a gap in the literature on the correlation between the conserved motifs of bacteriocins and their mode of action considering their 3D structure and its influence on the bacteriocin's activity. Figure 5 depicts predicted 3D structures produced by the α Fold algorithm and visualized with Chimera X (Jumper *et al*., 2021; Varadi *et al*., 2022; Meng *et al.*, 2023;), N- and C-terminus indicated in the figure, region highlighted in red indicates the conserved region FNDLV, the conserved region of the three peptides appear differently across the space on the 3D structure format.

Class IIa bacteriocins are characterized by a conserved sequence at the N-terminal (YGNGV), also known as pediocin-box (Figure 6) (Waterhouse *et al*., 2009), a sequence related to strong anti-listerial activity first described in pediocin PA-1 (Cui *et al*., 2012). Class IIb is composed of two-short chains. Class IIc are circular bacteriocins that lack the leader peptide sequence and are dependent on the general secretion pathway (sec) for transportation across the cytoplasmic membrane (Perez *et al*., 2014; Choi *et al*., 2023). Class IId bacteriocins are typically constituted of NGY residues at the N-terminus and central YxVTK motifs (Yoo *et al*., 2023); this class covers the remaining single-peptide and non-pediocin-like bacteriocins (Iwatani *et al*., 2011).

Class II (pediocin-like) suffers cleavage of a leader peptide (Figure 6) (Waterhouse *et al*., 2009) in the N- terminus in order to turn into a mature bacteriocin, which can be seen from the representation of class IIa bacteriocins isolated from meat aligned by the CLUSTAL W algorithm (Thompson *et al*., 1994; Drider *et al*., 2006; Lee and Kim, 2011). Sequences were fetched from UniProt. The pediocin-box is highlighted in a black square, hydrophobic (blue), positive charge (red), polar (green) glycine (orange), prolines (yellow), aromatic (cyan) residues.

3D primary structures of class IIa bacteriocins (Figure 7), generated using the α fold algorithm and visualized with Chimera X, show that the conserved region YGNGV exhibits distinct spatial arrangements within the 3D structures of the different class IIa bacteriocins. This variation suggests that although the sequence is conserved, the spatial positioning and orientation of this region can differ significantly from one peptide to another (Figure 7). Such differences in the three-dimensional conformation could influence how these bacteriocins interact with their target receptors, potentially

affecting their antimicrobial activity and specificity (Jumper *et al*., 2021; Varadi *et al*., 2022; Meng *et al.*, 2023).
Bacteriocins exhibit robust resistance to high temperatures and low pH due to their specific amino acid composition, a high number of disulfide bridges, and ion pairs (Szilágyi and Závodszky, 2000). The solubility of these peptides increases at low pH due to a net charge change that facilitates greater diffusion through bacterial membranes (Yu *et al*., 2023). Lantibiotics, in particular, demonstrate strong resistance under extreme conditions. Additionally, owing to their proteinaceous nature, bacteriocins are susceptible to proteolytic enzymes—such as pancreatin complex, trypsin, and chymotrypsin—found in the gastrointestinal tract (Aljohani *et al*., 2023). The characteristic nature of bacteriocins can be determined by testing their sensitivity to an array of proteolytic enzymes, producing a pattern of protease sensitivity (Bromberg *et al*., 2004).
WGS has made it possible to identify conserved open reading frames and understand the organization of gene loci encoding the bacteriocin and its immunity genes, in addition, it allowed to predict the promoter and terminator sequence of the peptide from the DNA data by predicting the RNA-polymerase biding motif, which can be useful for improving expression of bacteriocin encoding genes (Ruiz Puentes *et al*., 2022).
Promotion of bacteriocin synthesis can be obtained by constitutive expression of genes or by regulating gene expression as a response to the metabolite production from competing strains (González and Keshavan, 2006; Ng and Bassler, 2009). The bacteriocin synthesis gene clusters have been found to be located in the chromosome and in mobile elements such as plasmids and/or transposons (Achemchem *et al*., 2005; Lahiri *et al*., 2022). These clusters encode genes for the expression of the bacteriocin itself, enzymes, self-immunity regulators of bacteriocin production and are organized in operons and/or regulons that undergo rapid evolution and are susceptible to high rates of horizontal transfer and spontaneous loss (Mørtvedt and Nes, 1990; Noda *et al.*, 2018; Almeida-Santos *et al*., 2021).
A series of databases have been developed specifically for information on bacteriocins, for instance, the open-access database BACTIBASE (http://bactibase.hammamilab.org) with information on bacteriocins based on published literature extracted from PubMed (Hammami *et al*., 2010). LABiocin (https://labiocin.univ-lille.fr/) a database of LAB bacteriocins containing 517 entries extracted from literature searches on Scopus, PubMed/Medline and ScienceDirect with articles published up until 2017 (Kassaa *et al*., 2019). These databases provide valuable information on structure, amino acid sequence, gene sequence, purification, and physicochemical characteristics of bacteriocins; homology search is an additional feature comprised in these databases which allow for sequence alignment using algorithms such as BLAST (McGinnis and Madden, 2004).

Meat application
Gram-positive bacteria, particularly LAB, are the most studied source of bacteriocins from the meat environment (da Costa *et al*., 2019). The bacteriocins isolated from LAB in meat and meat products belong to different species, for instance, *Lactobacillus sakei* from vacuum-packed lamb meat (Holck *et al*., 1994), *Carnobacterium piscicola* from spoiled meat (Jack et al., 1996), *Leuconostoc gelidum* from processed packaged meat (Hastings *et al*., 1991), *Leuconostoc carnosum* from packaged meat (Raimondi *et al*., 2021), *Enterococcus faecium* from dry fermented sausages (Casaus *et al*., 1997), *Carnobacterium divergens* from vacuum-packed meat (Zhang *et al*., 2019) and *Carnobacterium maltaromicus* from vacuum-packed chilled meat (Quadri *et al*., 1994).
In the last 30 years, bacteriocins have been screened and applied in meat for controlling microbial decay and spoilage, acting as natural inhibitors and extending the shelf life of meat products (da Costa *et al*., 2019). However, there are still challenges associated with this approach, including the variability in peptide function depending on the nature of the meat matrix – especially in more fibrous matrices, effective inhibition of target microorganisms, resistance development and compatibility with surrounding LAB (Sionek *et al*., 2024).

Regarding the nature of the meat matrix, challenges arise when applying bacteriocins in meat products primarily due to the hydrophobic nature of the meat and its instability at neutral pH, its interaction with phospholipids derived from meat products and other emulsifiers make it difficult to distribute and solubilize the bacteriocin at pH values higher than 6.0. For instance, in fresh meat, three glutathione molecules are able to conjugate with one nisin molecule resulting in activity loss. However, it is possible to regulate the amount of free sulfhydryl groups present in the matrix, such as with the process of cooking the meat which reduces the free sulfhydryl groups and prevents the formation of the nisin-glutathione complex (Rose *et al*., 2002).
Strains resistant to a specific class of bacteriocins express immunity genes that confer protection against antimicrobial peptides from different classes, for instance, nisin resistant strain of *L. monocytogenes* has been reported to show cross resistance to pediocin PA-1 and leuconocin S (Crandall and Montville, 1998; Darbandi *et al*., 2021).
To overcome the resistance development related to bacteriocins in food systems, it is advisable to apply a combined strategy of peptides in multi-hurdle strategies, for instance, in combination with additives, pH and atmosphere control, recipe modifications with spices and condiments, natural extracts and essential oils as ingredients (Kaur *et al*., 2013; Soltani *et al*., 2021).
When classifying bacteriocins isolated from fresh and processed meat products, two main classes based on biochemical structure emerge: lantibiotics and pediocin-like peptides (Woraprayote *et al*., 2016). There are different methods regarding their practical application as biocontrol agents (Figure 8) and each comes with their set of advantages and disadvantages; for instance, crude preparations are tasteless, colorless and odorless, however, their activity may be limited by a narrow spectrum reach, limited diffusion in solid matrices, and cross-resistance generation (Morata, 2015; Urso *et al.*, 2006). In the next section, we describe the types of bacteriocin application regarding meat preservation.

Mixed starters
The use of LAB as inoculum is the most commonly used mode of application for preservation of foodstuffs. For meat preservation, bacteriocins can be applied as an inoculum of pure or mixed cultures (Baillo *et al*., 2023), as a crude bacteriocin preparation (Xin *et al*., 2023) and as a purified or semi-purified formulation (da Costa *et al*., 2019).
A selection of two autochthonous LAB strains (6.3 log CFU/g) isolated from spontaneously fermented Spanish sausages (salchichón) – *Lactiplantibacillus paraplantarum* BPF2 (producer of leucocin K) and *P. acidilactici* ST6 (producer of pediocin PA-1) were used as starter and reduced levels of rancidity in aroma and taste and improved the intensity and the persistence of the sausage's characteristic flavor (García-López *et al*., 2023).
Commercial bacteriocin products such as Nisaplin© (Aplin and Barrett Ltd.) and Bactoferm© (Chr. Hansen AS) often consist of a mixture of crude preparations and organic acids in bioactive powder form. These products offer a mix of lyophilized starters for use in meat processing industries, such as for the production of salami, pepperoni, dry and cured meats, which are frequently used combined in multi-hurdle strategies with curing, drying and smoking preservative methods (Soltani *et al*., 2021).
Traditional Iberian cold-smoked fermented sausages underwent testing to assess the antilisterial effect of Bactoferm F-LC©. While Bactoferm F-LC© exhibited bacteriostatic activity at 10ºC, *L. sakei* CTC494 showed a more pronounced and rapid inhibition of *Listeria*. This resulted in a significant reduction of the pathogen by 2 log counts (Ortiz *et al.*, 2014).
The application of mixed starters is beneficial because it combines strains that shorten fermentation time with those that enhance the meat's organoleptic properties. Additionally, strains that produce antimicrobial metabolites can be included to combat hazardous and pathogenic bacteria, thereby increasing the food's microbial shelf life.
However, compatibility between strains remains a significant issue, as the antimicrobial metabolites produced by biocontrol strains can be detrimental to the survival of strains promoting fermentation. Therefore, the choice of strains must consider their competitiveness with surrounding bacteria, the

expression levels of bacteriocin synthesis, and their compatibility with the fermenting culture (Zacharof and Lovitt, 2012).

Pure culture

A novel multi-hurdle strategy was developed to extend the shelf life of the Portuguese fermented sausage *alheira*, combining mild high-pressure processing (300 MPa, 5 min at 10 ºC), 0.1% (v/w) *Pediococcus acidilactici* (producer of pediocin PA-1), and 0.1% (v/w) phage Listex. This approach displayed no significant differences in color, texture or lipid peroxidation between unprocessed and minimally processed samples (Komora *et al*., 2023). In this study, homogenization was manually performed by gently massaging the sample for approximately 3 minutes, which did not impact the texture. Although the acidifying capacity of LAB can increase the firmness of sausages, the selected LAB in this case was not a strong acidifier, which may explain why the texture remained unchanged. In contrast, The *E. lactis* Q1, producer of enterocin P, added as a pure culture (10^7 CFU/g) on raw beef reduced *L. monocytogenes* counts by 6 log units after one week of cold storage and improved the sensorial characteristics such as color, odor and appearance, especially since Enterococci are good acidifiers, particularly in meat products (Ben Braïek *et al*., 2020). When comparing mixed and pure cultures, mixed cultures offer greater advantages due to their diverse LAB, which can be fine-tuned for better product quality. Moreover, while pure LAB cultures are effective in reducing pathogen growth in meats, their efficacy is enhanced when used alongside other hurdle strategies.

Crude preparation

The application of the peptide (1280 AU/g) onto the surface of ham resulted in a 1.74 log reduction of *L. monocytogenes* counts ($p<0.05$); therefore, potentially increasing the ham shelf life to one month in refrigerated storage (4ºC). Incorporating the peptide in the meat paste produced an inhibition of background spoilage bacteria resulting in significantly lower counts ($p<0.01$). However, *L. monocytogenes* strains were reported to develop resistance to plantaricin UG1 in subsequent generations (Enan, 2006).

A combination of lactocin and high hydrostatic pressure treatments increased *L. monocytogenes* cell death in chilled vacuum-packed pork loin slices (Dallagnol *et al*., 2017) by a 6 log CFU/g reduction. By applying 200 AU/mL of the pure bacteriocinogenic culture in the salami batter, *L. monocytogenes* was reduced by 2 log CFU/g (de Souza Barbosa *et al*., 2015).

Pediocin PA-1 reduced the counts of *L. monocytogenes* inoculated in raw chicken meat from 5 log CFU/g to 3.8 log CFU/g when stored at 4 ºC for one month, however re-growth was observed after this period, which could be due to the actions of proteases derived from the meat (Kiran and Osmanagaoglu, 2014).

Choosing between a pure culture of bacteriocin-producing bacteria and a crude bacteriocin preparation involves a nuanced assessment of their respective advantages and disadvantages. A pure culture offers the benefit of continuous bacteriocin production, potentially increasing antimicrobial activity over time and reducing initial preparation costs. However, using live bacteria can lead to compatibility issues, where different strains may inhibit each other's growth and compete for nutrients, resulting in inconsistent efficacy. Additionally, regulatory concerns arise with live cultures, particularly in food applications, due to potential health risks and spoilage.

In contrast, a crude bacteriocin preparation provides predictable and controlled antimicrobial effects, as the activity of the bacteriocins is well-characterized and targeted against specific bacteria. This method bypasses compatibility issues and is generally more acceptable from a regulatory perspective. However, the process of purifying bacteriocins is labor-intensive and costly, with challenges in achieving effective concentrations across different food matrices and target microorganisms. Moreover, maintaining the stability of crude bacteriocin preparations requires careful handling and storage. Ultimately, the choice depends on the specific application, balancing cost, regulatory considerations, and the need for precise antimicrobial activity.

Encapsulation

Challenges with the bacteriocin adsorption in the matrix can be addressed by using immobilized preparations, such as encapsulation on gel coatings, films, silica particles, or liposomes (Gálvez *et al.*, 2007). Encapsulation of bacteriocins involves incorporating these antimicrobial peptides into a protective matrix or carrier to enhance their stability, control their release, and improve their effectiveness in various applications.

The application of encapsulated bacteriocins combined with citrus extract, and thyme essential oil led to a synergistic antimicrobial activity for meatballs protection against *L. monocytogenes* and all tested LAB; however, there was no effect observed on the inhibition of *S.* Typhimurium (Sarmast *et al*., 2023). Moreover, the anti-listerial activity of cell-adsorbed bacteriocins combined with oregano essential oil had a synergistic effect on the reduction of *L. monocytogenes* counts and delayed the growth rebound by two weeks in pork meat during storage at 4 ºC (Ghalfi *et al*., 2007).

Smart packaging represents an advanced approach to packaging design, integrating technology to augment the functionality, safety, and user experience of packaged products. A recent advancement in this domain is the incorporation of bacteriocins into packaging materials, utilizing natural antimicrobial agents to enhance food safety and preservation. Typically, this method employs crude bacteriocin preparations rather than inoculum. Nevertheless, significant gaps remain in the current field, particularly concerning the varying characteristics of different matrices for the establishment of effective smart packaging solutions.

Edible films

For instance, whey protein-based edible films, enriched with the cell-free supernatant of *Lactobacillus sakei* strains were applied in beef, resulting in a decrease from 3.5 log + 0.2 CFU/g to 0.3 + 0.1 log CFU/g of *Escherichia coli* counts after 36 h of refrigerated storage. Moreover, sensory evaluation of grilled beef wrapped with the antimicrobial films demonstrated no significant differences in flavor and color as assessed by the panelists, the overall acceptability was high (Beristain-Bauza *et al.*, 2017).

Polythylene-based films

A plantaricin solution applied in an active package made with polyvinylidene chloride film in pork fresh meat inhibited *L. monocytogenes* growth by 1.4 log CFU/g after 7 days of cold storage (Xie *et al.*, 2018). Plantaricin BM-1 solution was used to soak polyethylene-based films applied in meat artificially inoculated with *L. monocytogenes* and exerted antimicrobial activity that inhibited the pathogen growth during storage for 120 days at 25ºC (Zhang *et al*., 2017).

Curvaticin 32Y produced by *L. curvatus* 32Y isolated from dry sausages has been shown to reduce viable counts of *L. monocytogenes* by 1 log when applied in a polythene film by soaking, spray-coating as a preservative for artificially inoculated pork steaks (Mauriello *et al*., 2004). Moreover, in bioactive packaging made of sawdust particles and poly lactic acid biocomposite film, the adsorption of pediocin PA1-AcH enhanced raw sliced pork meat protection against *L. monocytogenes*, with counts reduced by ~2 log units after storage at 4ºC for 14 days (Woraprayote *et al*., 2013).

Cellophane coating

Nisin has been shown to have greater antimicrobial potential in meat preparation when used in combination with organic acids and salts; for instance, nisin reduced the total aerobic bacteria counts in 0.1 log CFU/g from veal meats when applied in a cellophane coating packaging and extended the shelf life of chopped meat under refrigerated storage (Guerra *et al*., 2005).

Alginate matrix

As a component in antimicrobial packaging, nisin had an inhibitory effect against microbial decay and extended the shelf life of refrigerated chicken meat up to 15 days more than the control when applied incorporated in an alginate matrix (Carrión *et al*., 2023). A packaging composed of alginate

films and containing immobilized viable enterocin-producing *E. faecium* Smr18 reduced *S.* Typhimurium counts by 3 log CFU/g in chicken meat after 34 days at cold storage (Rashid *et al*., 2023).

Cultivated meat

Meat grown from animal cells in a laboratory setting is called 'cultivated meat' (CM). CM appears as a new solution to several safety issues with livestock farming such as the zoonotic transfer of viruses and infection through human consumption (Ramani *et al*., 2021). The development of CM is dependent on the retrieval of animal cells by biopsy, creation of a bank of cells, growth and differentiation by reprogramming stem cells into skeletal muscle cells, harvesting the cells and processing them into tissues.

CM meat can become contaminated by bacteria, fungi and viruses, which is managed by the addition of antibiotics during cell growth, as well as by the addition of cryoprotectants during cell storage. Moreover, risk for contamination during further downstream processing is expected to be similar as in case of traditional meat products (Broucke *et al*., 2023).

CM production systems are considered to be more sustainable and safer in comparison to the conventional meat production systems, but it may have a completely different risk profile, such a risk coming from antibiotic application *in vitro* to promote growth of cells still consist of a gap in the field and much attention would require to be paid to the safety of added substrates and other compounds of the culture medium to the human health.

As it will be easier to keep control of pathogenic contamination in cultured meat production, CM is associated with fewer risks with respect to microbial contamination. The application of LAB as starters for CM production is expected to influence the final product similarly than it already affects the meat that incorporates starter cultures, having a positive effect on organoleptic features and on microbial safety (Kolodkin-Gal *et al*., 2024).

Conclusions and future perspectives

Fresh meat is particularly susceptible to spoilage, posing a significant challenge in reducing losses and waste associated with meat and its products on a global scale. Furthermore, despite concerted efforts, foodborne outbreaks linked to the consumption of meat products persist as an unresolved issue.

In this context, biopreservation emerges as a natural alternative for extending the shelf life of meat products by managing their inherent microbial communities; however, proper diffusion of the bacteriocin through the meat matrix remains a challenge, in addition to the inhibition by the bacteriocins against closely related LAB, which could cause loss of starter culture effectiveness.

LAB are the dominant group present in meat and produce a variety of metabolites with antimicrobial effects. Bacteriocins are secondary metabolites produced by LAB with antimicrobial properties, these small peptides are stable in extreme temperatures and pH. While culture-based methods remain prevalent in assessing antimicrobial activity due to their cost-effectiveness and simplicity, they often rely on outdated protocols that may not fully explore the bacteriocinogenic potential of strains. Recent advancements in genomics, such as WGS, RNA-seq, and PCR-based techniques, offer more comprehensive insights into antimicrobial mechanisms. Moreover, computational tools, including docking software and three-dimensional structure modeling, now enable automated assessment of bacteriocin binding mechanisms. This advanced understanding of bacteriocin structures and their modes of action holds promise for developing strategies to enhance and optimize their antimicrobial efficacy in practical applications.

The development for higher efficiency of bacteriocin diffusion from packaging surfaces or application sites into meat, considers multiple factors, including meat texture and the thoroughness of mixing between the bacteriocin and the meat, which significantly influence homogeneous distribution within the meat matrix. The choice of application method is influenced by factors such as the complexity of the food matrix, scalability and cost-effectiveness of production. Looking forward, advancements in

3D printed and CM could profit from integrating LAB and bacteriocins to improve their safety and functionality. Continued research into bacteriocins is crucial for advancing meat safety and expanding functional meat product offerings in the future.

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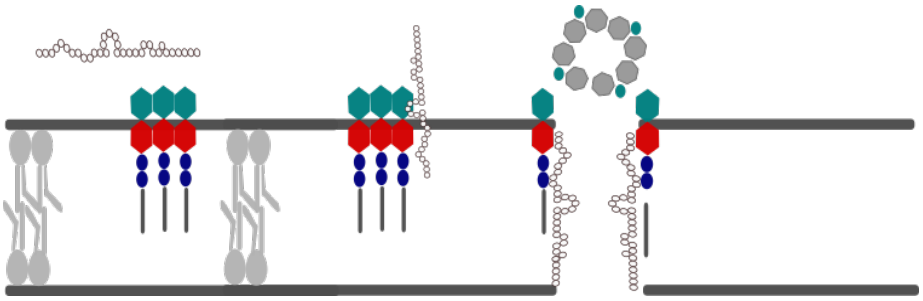

Figure 1. Nisin mode of action.

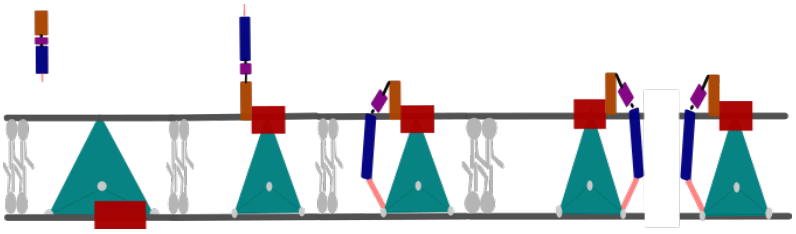

Figure 2. Pediocin-PA-1 mode of action.

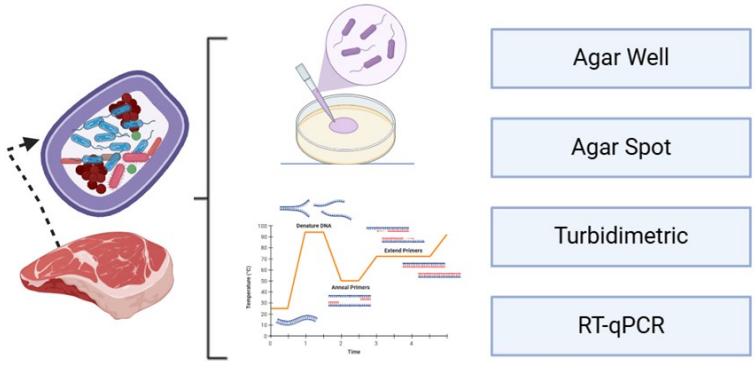

Figure 3. Diagram for different *in vitro* methods for bacteriocin activity screening from foodstuffs.

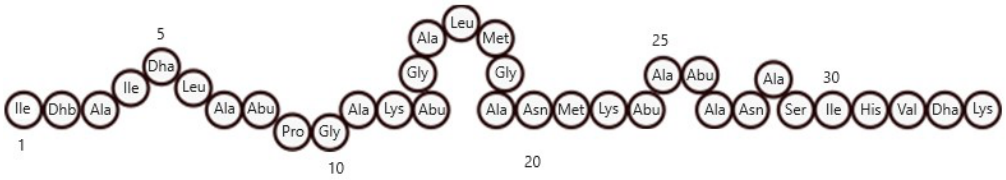

Figure 4. The amino acid composition of nisin.

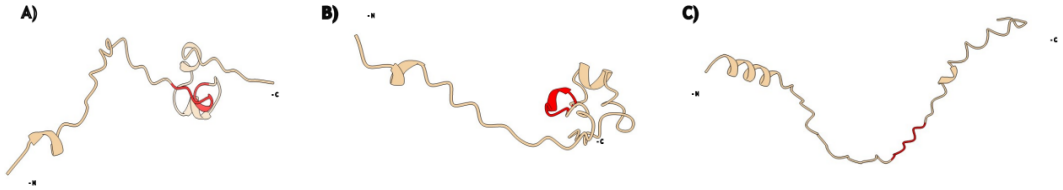

Figure 5. Lantibiotics three-dimensional primary structures. A) Nisin A (P13068); B) Nisin Z (P29559); C) Lactocin S (P23826).

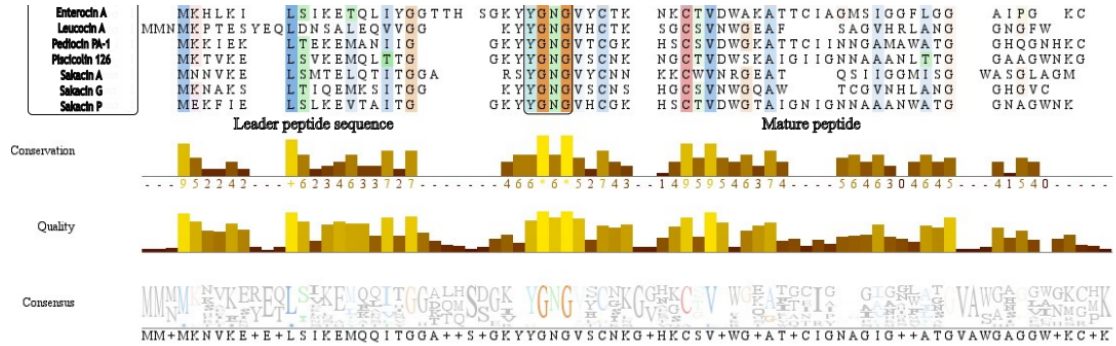

Figure 6. Representation of class IIa bacteriocins isolated from meat aligned by the CLUSTAL W algorithm and visualized in Jalview.

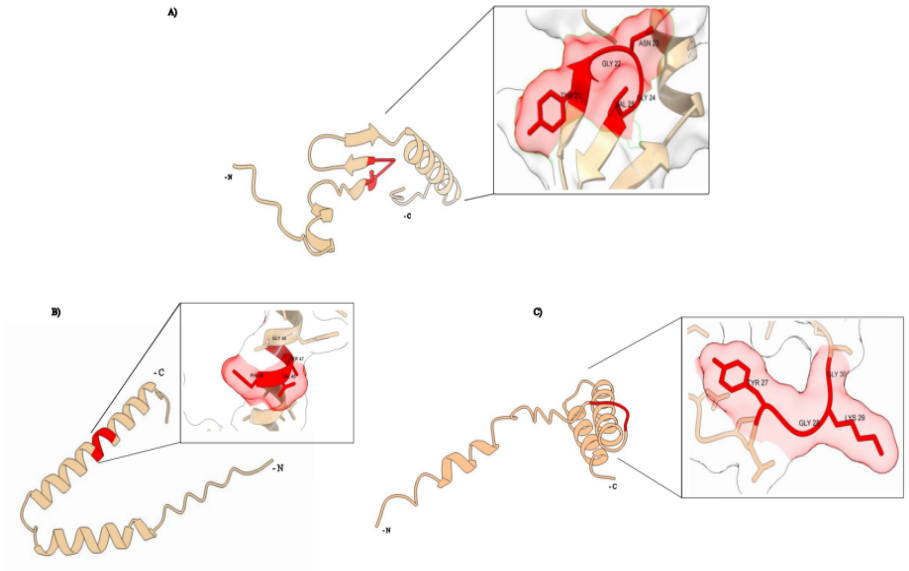

Figure 7. 3D primary structures of class IIa bacteriocins. A) Pediocin PA-1 (P29430); B) Divergicin 750 (Q46597); C) Carnobacteriocin A (P38578).

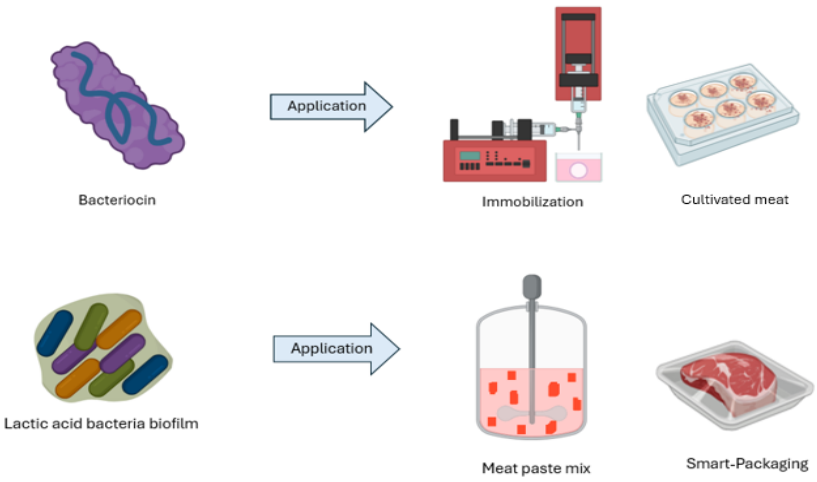

Figure 8. Diagram of lactic acid bacteria biofilm and bacteriocin for different kinds of applications in processing and conservation of meat and meat products.