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Harnessing the Antimicrobial Potential: A Comprehensive Review of Bacteriocin Production in Lactic Acid Bacteria

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ABSTRACT

Lactic acid bacteria (LAB) are the most studied bacterial genera with respect to bacteriocins, since some LAB strains have a good potential for production by this kind of antimicrobial peptides. This review presents an extensive analysis of the bacteriocin production system within LAB, focusing on — classification and specificity; biosynthesis machinery (including immunity); action mechanisms against target microorganisms [antibacterial spectrum]; prospected applications in food preservation as well as control/therapy treatments. This review highlights the genetic and environmental factors that influence bacteriocin production, recent progress in optimization strategies as well hurdles and potential of using these natural antimicrobials to full extent. The review also highlights the synergistic effects of bacteriocins with other antimicrobial compounds and their role in modulating gut microbiota.

Figures : 02 References : 25 Tables : 06
KEY WORDS : Antimicrobial peptides, Bacteriocins, Bioengineering, Food preservation, Lactic acid bacteria, Microbiome Modulation

Introduction

Lactic acid bacteria (LAB) are a heterogeneous group of gram-positive, cocci or rods shaped, non-spore forming and catalase negative aerobic /facultatively anaerobic bacteria that have been extensively used in food fermentations more than centuries. Until today LAB are well known for being capable of excreting several kinds of antimicrobials, mainly bacteriocins[1]. Bacteriocins are ribosomally synthesized AMPs or proteins, which kill the bacteria closely related to producer[2].

Bacteriocins are important production for LAB which has numerous applications in food preservation, biomedical, and gut health modulation. This calls for natural antimicrobials and bacteriocins hold a potential as suitable alternative bio-preservatives to conventional chemical preservative, but also antibiotics are loaded to inhibit the growth of microorganisms due to growing concerns over antibiotic resistance and consumers demand related with food safety[3].

This review aims to comprehensively analyse bacteriocin production in LAB, covering their classification, biosynthesis, genetic determinants, and environmental factors affecting their production. In this review, the mode of action and food preservation applications as well as interventions for therapies these bacteriocins displayed have been highlighted. Finally, we will discuss the difficulties and opportunities in exploiting LAB bacteriocins to their maximum extent.

Classification of Bacteriocins

Bacteriocins produced by LAB are typically classified based on their structural characteristics, molecular weight, and mode of action. While several classification schemes have been proposed over the years, the most widely accepted classification divides bacteriocins into four main classes. The LAB produce bacteriocins which generally are classified according the structural description, molecular weight and mode of action. Although a variety of classification schemes have been proposed over the years, according to their structure and functionality, bacteriocins may be classified into four major classes[4]:

Biosynthesis of Bacteriocins in LAB

The biosynthesis of bacteriocins in LAB is a complex process involving multiple genes and regulatory mechanisms. The general steps in bacteriocin biosynthesis include:

1. Transcription of structural genes
2. Translation of precursor peptides

TABLE-1 : Classification of Bacteriocins Produced by Lactic Acid Bacteria

Class	Characteristics	Examples	Producing LAB
I (Lantibiotics)	Small (<5 kDa), heat-stable peptides containing lanthionine or β-methyllanthionine	Nisin, Lacticin 3147	Lactococcus lactis, Lactobacillus plantarum
II	Small (<10 kDa), heat-stable, non-lanthionine-containing peptides	Pediocin PA-1, Sakacin A	Pediococcus acidilactici, Lactobacillus sakei
III	Large (>30 kDa), heat-labile proteins	Helveticin J, Enterolysin A	Lactobacillus helveticus, Enterococcus faecalis
IV	Complex bacteriocins containing lipid or carbohydrate moieties	Leucocin S, Lactocin 27	Leuconostoc mesenteroides, Lactobacillus helveticus

3. Post-translational modifications (if applicable)
4. Export of the mature bacteriocin
5. Immunity protein production

Transcription of the structural genes encoding precursor peptides represent the first step in bacteriocin biosynthesis. These genes are frequently arranged in operons that encode immunity, transport and regulation as well[5]. For example, the precursor peptides of lantibiotics (Class I bacteriocins) are modified post translationally to a significant extent. The introduction of thioether bridges, dehydrated amino acids and other unusual structural modifications are generally performed by specialized enzymes[6]. Compared to class I bacteriocins, relatively smaller modifications are required for Class II bacteriocins other than some disulfide bond formation or additional circularization.

Upon processing, bacteriocin precursors secrete out the cell using dedicated transporters. These germination-killing effectors are generally secreted *via* T1SS, which requires ATP-binding cassette (ABC) transporters and accessory proteins to remove the leader sequence during export[7].

Meanwhile, bacteria that produce bacteriocins also synthesize immunity proteins to protect themselves from the activity of their own toxin. Immunity mechanisms include sequestration of the bacteriocin by specific proteins that bind to it or modification of the producer's cell envelope so as to prevent its binding or pore formation[8].

Genetic Determinants of Bacteriocin Production

Traits that determine the production of bacteriocins in LAB are usually genetic, these traits present as gene clusters or operons. The operons carrying genes for structural peptides, immunity proteins, repressor-like[9] regulatory elements and transporter proteins are located on MGEs. Genetic organization differs in bacteriocin classes according to the producing organism.

The structural gene encodes the bacteriocin precursor peptide, which consists of an N-terminal leader sequence and a C-terminal core peptide. The leader sequence plays crucial roles in post-translational modification, transport, and keeping the bacteriocin inactive within the producer cell[10].

Immunity genes encode proteins that protect the producing bacteria from their own bacteriocin. These can act by binding to the bacteriocin, modifying the cell envelope, or pumping the bacteriocin out of the cell[11].

Transport genes encode proteins involved in the export of the mature bacteriocin. For many bacteriocins, this involves ABC transporters and accessory proteins that cleave off the leader sequence during export[12].

Modification genes are particularly important for Class I bacteriocins (lantibiotics). These genes encode enzymes that catalyze the post-translational modifications necessary for the bacteriocin's final structure and activity[13].

Regulatory genes control the expression of the bacteriocin operon. Many bacteriocins are regulated by quorum sensing mechanisms, where the accumulation of an autoinducing peptide triggers increased expression of the bacteriocin genes[14].

The genetic organization of bacteriocin operons

TABLE 2: Key Genetic Elements Involved in Bacteriocin Production

Genetic Element	Function	Example
Structural gene	Encodes the bacteriocin precursor	nisA (nisin)
Immunity gene	Protects the producer from its own bacteriocin	nisI (nisin immunity protein)
Transport genes	Facilitate export of the mature bacteriocin	nisT, nisP (nisin transport)
Modification genes	Catalyze post-translational modifications	nisB, nisC (nisin modification)
Regulatory genes	Control bacteriocin production	nisR, nisK (nisin regulation)

can provide insights into their evolution and potential for horizontal gene transfer. Some bacteriocin gene clusters are located on plasmids, facilitating their spread among different bacterial species[15].

Environmental Factors Affecting Bacteriocin Production

The production of bacteriocins by LAB is influenced by various environmental factors. Understanding these factors is crucial for optimizing bacteriocin production in both natural and industrial settings. The main environmental factors affecting bacteriocin production include:

1. Nutrient availability
2. pH
3. Temperature
4. Oxygen levels

Bacteriocin production is dependent on nutrient availability. The carbon and nitrogen source type and content may largely affect bacteriocin production. For example, it is known that glucose increases the production of a wide range of bacteriocins such as nisin and pediocin[16].

Another important point directly associated with bacteriocin production is pH. Bacteriocins are usually produced by LAB at a pH close to neutrality, although the optimal range is between 5.5–6.5 for most of them. Similarly, the production at pH 6.0 is most ideal for nisin of Lactococcus lactis[17]. Temperature affects bacterial growth and gene expression, also affecting bacteriocin formation. The best bacteriocin production temperature often corresponds to the one at which growth of the producing strain occurs optimally. However, the production of pediocin by Pediococcus acidilactici was found to peak at 30°C[18]. Oxygen levels can significantly affect bacteriocin production in LAB. While most LAB are facultative anaerobes and prefer microaerophilic conditions, the optimal oxygen level for bacteriocin production can vary. For example, plantaricin production by certain Lactobacillus plantarum strains is enhanced under anaerobic conditions[19]. In some cases, co-culture with specific strains has been shown to enhance bacteriocin production, possibly as a competitive strategy[20].

The growth phase of the bacteria is often linked to bacteriocin production. Many bacteriocins are produced during the late exponential or early stationary phase, suggesting a possible role in competition for limited resources[21].

Mode of Action of Bacteriocins

The bacteriocins exert their antimicrobial activity by destruction of the integrity of target cell membranes. Nevertheless, the mechanism of action could be very different according to pair bacteriocin class and form. Knowledge of how resistance is obtained forms a key factor in predicting the potential usefulness of bacteriocins and provides strategies for preventing its occurrence.

The primary modes of action for each bacteriocin class are as follows:

1. Class I Bacteriocins (Lantibiotics): Class I bacteriocins also referred to as lantibiotics such as nisin corner cell wall synthesis by binding its target lipid II, a precursor molecule for constructing the peptidoglycan. b) They perforate the cell membrane, thereby causing release of intracellular constituents and collapse of proton motive force[22].
2. Class II Bacteriocins: Action of these bacteriocins usually involves permeabilization or disruption the cell membrane from target bacteria. This works as: a) Initial docking is to some receptor at the surface of cell. Insertion into the membrane and

TABLE-3: Environmental Factors Influencing Bacteriocin Production in LAB

Factor	Effect on Bacteriocin Production	Example
Nutrient availability	Carbon and nitrogen sources can modulate production	Glucose often enhances production
pH	Optimal pH varies by bacteriocin; generally 5.5-6.5	Nisin production optimal at pH 6.0
Temperature	Affects growth and gene expression	Pediocin production highest at 30°C
Oxygen levels	Most LAB prefer microaerophilic conditions	Plantaricin production enhanced under anaerobic conditions
Competing microorganisms	Can induce or suppress bacteriocin production	Co-culture with certain strains can increase production
Growth phase	Production often linked to specific growth phases	Many bacteriocins produced in late exponential phase

pore formation or disruption of membrane integrity[23].

3. Class III Bacteriocins: This class includes large, heat-labile bacteriocins with diverse mechanisms like a) those that can act as muramidases and thereby directly lysed the cell wall of target bacteria. b) Some others may be non-lytic which might include functions like inhibition of enzyme activities or triggering autolysis[24].
4. Class IV Bacteriocins: Only few, very large bacteriocins fulfill the requirement for class IV (die uncommonness and high complexity makes understanding their mode of action pretty difficult. They are likely multifactorial in nature because they contain lipids or carbohydrates[25].

Applications of Bacteriocins

Bacteriocins from LAB present antimicrobial activity, which has motivated the promotion of its use in different fields mainly related to food conservation and therapeutic strategies. Their naturalistic source provides them as an attractive outlet away from conventional synthetic preservatives and antibiotics.

TABLE-4 : Examples of Bacteriocin Applications in Food Preservation

Bacteriocin	Producing LAB	Food Application	Target Organisms
Nisin	Lactococcus lactis	Dairy products, canned foods	Listeria monocytogenes, Clostridium botulinum
Pediocin PA-1	Pediococcus acidilactici	Meat products	Listeria monocytogenes
Lacticin 3147	Lactococcus lactis	Cheese	Staphylococcus aureus, Listeria monocytogenes
Enterocin AS-48	Enterococcus faecalis	Fruit juices	Alicyclobacillus acidoterrestris
Sakacin P	Lactobacillus sakei	Fermented sausages	Listeria monocytogenes

TABLE-5: Potential Therapeutic Applications of Bacteriocins

Bacteriocin	Producing LAB	Potential Therapeutic Application	Target
Nisin	Lactococcus lactis	Treatment of MRSA infections	Methicillin-resistant Staphylococcus aureus
Lacticin 3147	Lactococcus lactis	Treatment of C. difficile infections	Clostridioides difficile
Plantaricin	Lactobacillus plantarum	Oral health	Streptococcus mutans
Microcin J25	Escherichia coli	Potential anti-cancer agent	Various cancer cell lines
Enterocin AS-48	Enterococcus faecalis	Treatment of skin infections	Propionibacterium acnes

Food Preservation

Bacteriocins have been of significant interest in food industry as a natural antimicrobial preservative. Food systems benefit in several ways from their use:

1. Intrinsic antimicrobial activity: Bacteriocins can be effective against spoilage and pathogen bacteria, this effect could extend the shelf life of a product resulting in an increase in food safety;
2. Heat stability (trend towards class I and II bacteriocins): Many of the currently available bacteriocin formulations, particularly the Class I-like bacteriocins Duccocin® or Aviguard®, are heat stable, meaning they can be used in pasteurized and high moisture/heat-process foods.

Nisin, produced by *Lactococcus lactis*, is the most well-known and widely used bacteriocin in food preservation. It has been approved as a food additive (E234) in many countries and is particularly effective against gram-positive bacteria, including *Listeria monocytogenes* and *Clostridium botulinum.*

Optimization Strategies for Bacteriocin Production

For successful use of bacteriocins in food preservation and therapeutics optimization is required to improve their production. Many efforts have been made to improve the yield and effectiveness of bacteriocins including:

1. **Strain Selection and Improvement:**
   - o Screening for high-producing strains

TABLE-6: Examples of Optimization Strategies for Bacteriocin Production

Strategy	Bacteriocin	Producing LAB	Outcome
Media optimization	Nisin	Lactococcus lactis	3-fold increase in yield
pH control	Pediocin PA-1	Pediococcus acidilactici	40% increase in production
Co-culture system	Plantaricin	Lactobacillus plantarum	2-fold increase in yield
Gene overexpression	Enterocin A	Enterococcus faecium	5-fold increase in production
Fed-batch fermentation	Lacticin 3147	Lactococcus lactis	10-fold increase in yield

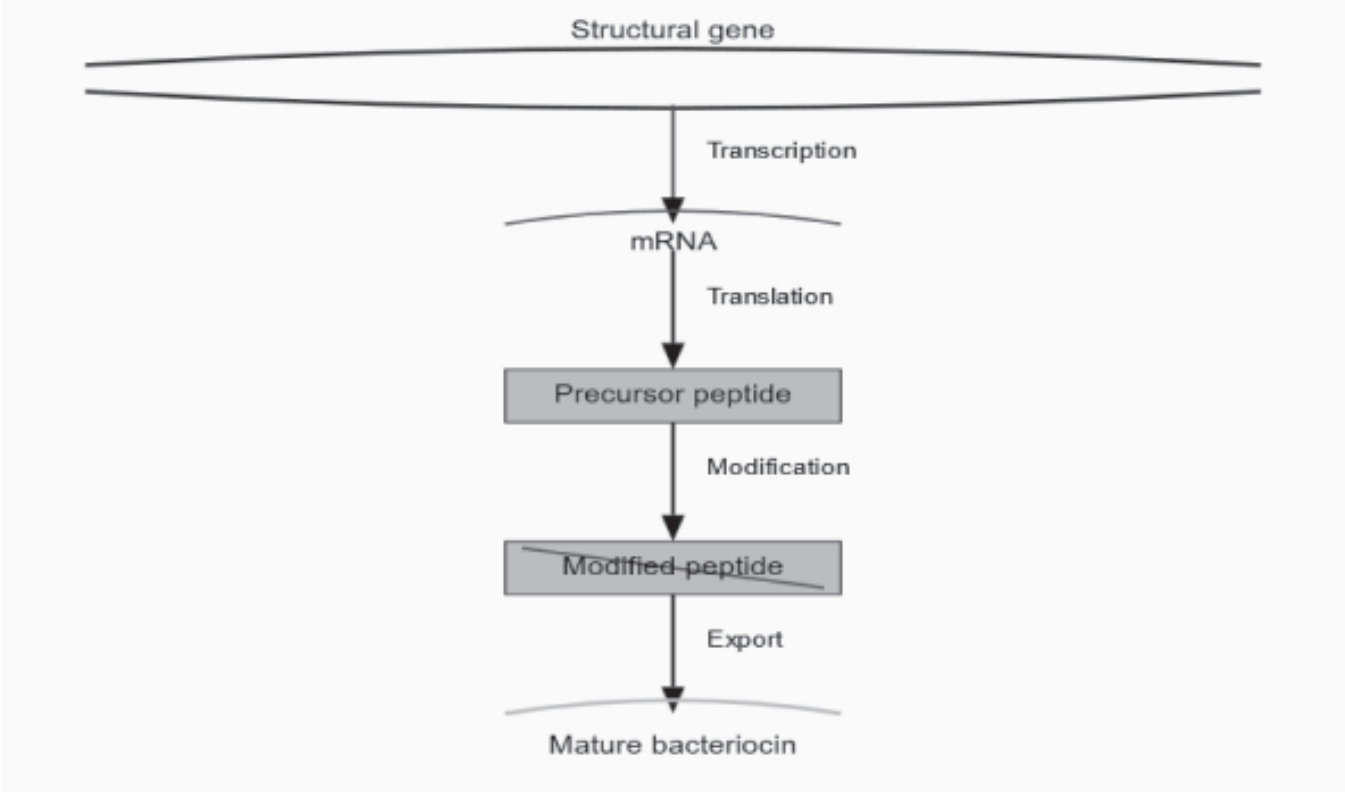

Fig. 1 : Schematic representation of the bacteriocin biosynthesis pathway in lactic acid bacteria.

- o Genetic modification to enhance bacteriocin synthesis
- o Adaptive laboratory evolution to select for improved producers

2. **Media Optimization:**
   - o Tailoring nutrient composition for specific bacteriocins
   - o Supplementation with growth factors or precursor molecules
   - o Use of complex media or food-grade ingredients
3. **Fermentation Process Optimization:**
   - o pH control strategies
   - o Temperature modulation

Some of the optimization strategies recently proposed in literature are listed as follows:

1. Systems biology: A systems-based approach should be used by the researchers which encompasses omics technologies (genomics, transcriptomics, proteomics and metabolomcis to gain insight on complete bacteriocin production pathways) By taking a broad approach, it is possible to recognize bottlenecks and areas for potential optimization.
2. Bioinformatics & Computational Biology: Machine Learning and Artificial Intelligence are being used to predict the optimal conditions of bacteriocin production by analyzing massive datasets containing various parameters of fermentation and yields. Such an approach would save huge time and resources in optimizing.

Synergistic Effects of Bacteriocins

When combined with some other antimicrobial substances or preservation techniques, bacteriocin finds a profound contribution to extend the range of its uses *via* increased killing activity. This combined strategy enhances the overall antimicrobial activity and also prevents resistance meanwhile using lower concentrations of single antimicrobials.

Challenges and Future Prospects

Despite their potential for distinct purposes, bacteriocins face numerous obstacles that should be solved to unleash the full inborn antimicrobial nature. First, since most bacteriocins have a narrow spectrum of activity that is targeted against closely related species the practical application becomes limited due to their non-broad applicability. This is, of course, less common but important because bacteriocins are being pursued as a safer alternative to antibiotics. In addition, bacteriocins tend to require a high production cost due the difficulty associated with its large-scale manufacturing and purification process.

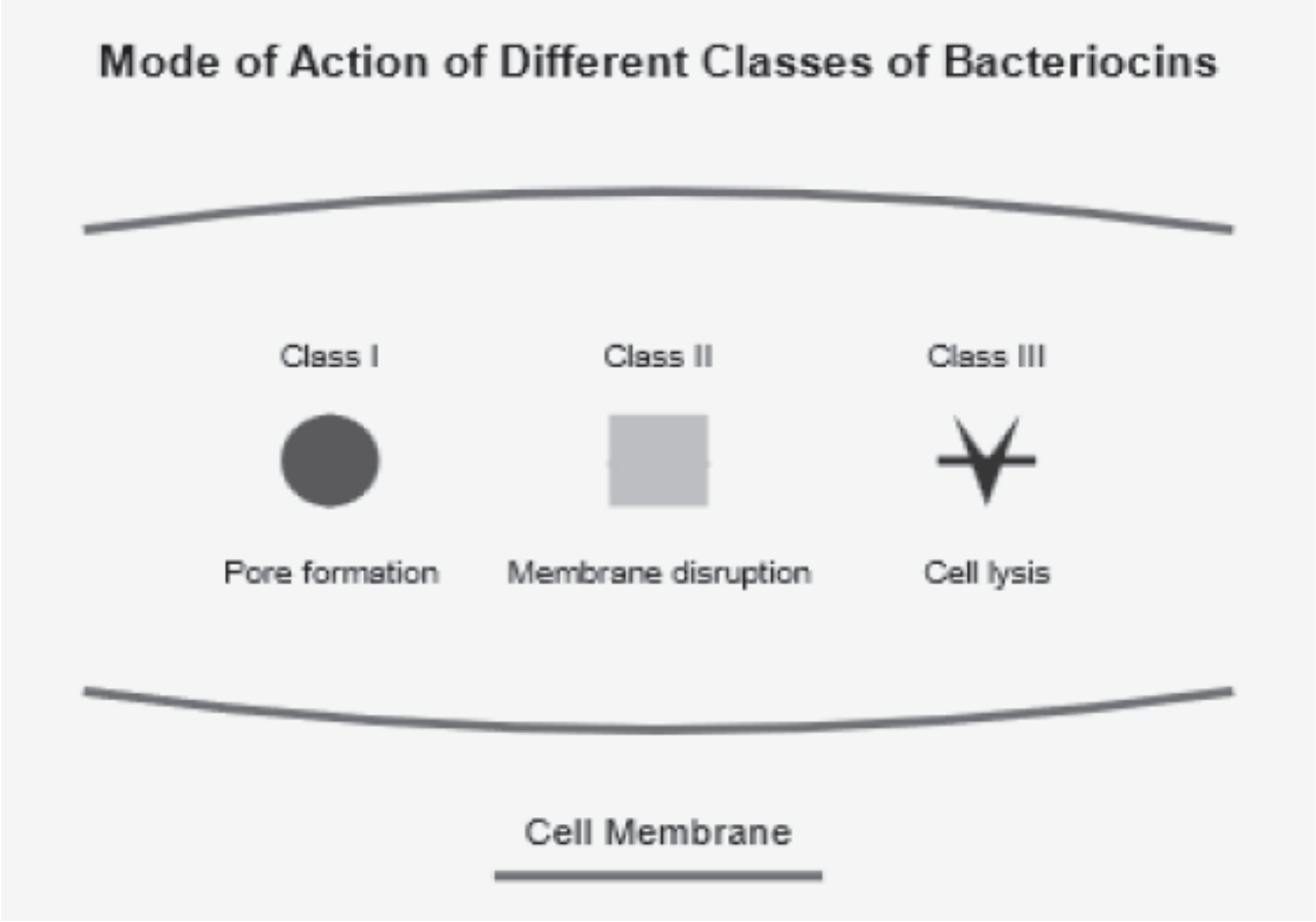

Fig. 2 : Schematic representation of the mode of action of different classes of bacteriocins.

Conclusion

Lactic Acid Bacteria (LAB) bacteriocins constitute a heterogeneous group of molecules with considerable potential for applications as antimicrobials *e.g.* in food preservation, therapeutics and microbiome manipulation. Natural source, specificity and high activity spectral make them promising new substitute for chemical preservatives and antibiotics.

Resumed biosynthesis and modes of action bacteriocins are discussed, focusing upon the genetic surrounding factors which modulate their production. Here, we highlight the usefulness of bacteriocins as bio-preservatives to extend shelf life and improve food safety along with their therapeutic applications for future research in combating antibiotic-resistant pathogens as well manipulating gut microbiome.

We anticipate that as our knowledge of bacteriocins and the complex manner in which they interact with microbial communities and host systems expands, new applications would emerge. These include individualized microbiome interventions, sophisticated food preservation systems and precision antimicrobial therapies.

Current ongoing research in this area is expected to bring ground-breaking solutions to some of the biggest problems linked with antimicrobial resistance, and those associated with microbiome-related conditions.

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