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Marine Bacteriocins as Natural Antimicrobials: A Sustainable Approach to Safeguard Dairy Products from Pathogenic Microorganisms

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Abstract

Marine Bacteriocins are ribosomally synthesized antimicrobial peptides produced by many bacteria have emerged as promising bio preservatives due to their potent inhibitory effects against the foodborne pathogens. While terrestrial lactic acid bacteria are widely studied for bacteriocin production, marine environments represent an underexplored reservoir of microbial diversity with the potential to yield novel and effective antimicrobial compounds. Marine microorganisms are constantly exposed to harsh environmental conditions such as high salinity, pressure, and temperature variations, driving them to produce unique bioactive substances, including bacteriocins with distinctive structures and mechanisms of action. Recent research has shown that marine-derived bacteriocins exhibit broad-spectrum antimicrobial properties, making them suitable candidates for enhancing food safety, particularly in dairy products. Dairy products are an essential part of the human diet, providing vital nutrients such as proteins, vitamins, and minerals. However, their rich nutritional profile also makes them highly susceptible to microbial contamination during production, processing, and storage. Pathogenic microorganisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. pose significant health risks, contributing to foodborne illnesses and economic losses. Conventional methods to control these pathogens rely heavily on chemical preservatives and antibiotics, which may have adverse health effects and promote antimicrobial resistance.

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LAB plays a critical role in food processing and spontaneous fermentation and is used in a wide range of fermented food. LAB exerts a strong antagonistic activity against many food contaminating microorganisms as a result of the production of organic acids, hydrogen peroxide, diacetyl, inhibitory enzymes and bacteriocins.

Key words : Marine, Bacteriocins, Dairy products, Pathogenic microorganisms, Antibiotics.

Dairy products are an essential part of the human diet, providing vital nutrients such as proteins, vitamins, and minerals. However, their rich nutritional profile also makes them highly susceptible to microbial contamination during production, processing, and storage. Pathogenic microorganisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. pose significant health risks, contributing to foodborne illnesses and economic losses. Conventional methods to control these pathogens rely heavily on chemical preservatives and antibiotics, which may have adverse health effects and promote antimicrobial resistance. Therefore, there is an urgent need for natural and sustainable alternatives to ensure dairy safety. Bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria have emerged as promising bio preservatives due to their potent inhibitory effects against foodborne pathogens. While terrestrial lactic acid bacteria are widely studied for bacteriocin production, marine environments represent an underexplored reservoir of microbial diversity with the potential to yield novel and effective antimicrobial compounds. Marine microorganisms are constantly exposed to harsh environmental conditions such as high salinity, pressure, and temperature variations, driving them to produce unique bioactive substances, including bacteriocins with distinctive structures and mechanisms of action. Recent research has shown that marine-derived bacteriocins exhibit broad-spectrum antimicrobial properties, making them suitable candidates for enhancing food safety, particularly in dairy products. Their stability across various temperatures and pH levels, along with their biodegradability, further supports their potential as eco-friendly alternatives to chemical preservatives. Moreover, their application aligns with global efforts to reduce antibiotic use and promote sustainable food processing. This study aims to explore marine bacteriocins as natural antimicrobials, focusing on their production, characterization, and inhibitory activity against common dairy pathogens. By isolating bacteriocin-producing marine strains and evaluating their protective potential in dairy matrices, this research seeks to contribute to the development of safer, healthier, and environmentally responsible methods to preserve dairy products. Ultimately, harnessing the antimicrobial properties of marine bacteriocins could offer a viable and sustainable approach to safeguarding public health and ensuring the integrity of dairy supply chains.

Bacteriocins are natural peptides secreted by many varieties of bacteria for the purpose of killing other bacteria. This provides them with a competitive advantage in their

environment, eliminating competitors to gain resources. These peptides are ribosomally synthesized, although some are extensively post-translationally modified. Lactic acid bacteria (LAB) are a diverse group of microorganisms that produce lactic acid as the primary end-product of the fermentation of carbohydrates. LAB plays a critical role in food processing and spontaneous fermentation and is used in a wide range of fermented food. LAB exerts a strong antagonistic activity against many food contaminating microorganisms as a result of the production of organic acids, hydrogen peroxide, diacetyl, inhibitory enzymes and bacteriocins.[9]

Based on structural, physicochemical and molecular properties, bacteriocins from LAB can be subdivided into three major classes. Class 1 bacteriocins are lantibiotics, i.e. small, cationic, hydro-phobic, and heat-stable peptides that contain unusual amino acids (*e.g.* the thioether amino acids lanthionine and/or 3-methyl-lanthionine) that are post-translationally formed. Class 2 bacteriocins are small, cationic, hydrophobic, heat-stable peptides that are not post-translationally modified, except for cleavage of a leader peptide from the pre-bacteriocin peptide. Within this class, three subclasses can be distinguished: Subclass 2a or pediocin-like bacteriocins with a strong antilisterial effect, possessing the consensus sequence Tyr-Gly-Asn-Gly-Val in their N-terminus; Subclass 2b or bacteriocins that require two polypeptide chains for full activity; and Subclass 2c or bacteriocins that do not belong to the other subgroups. Class 3 bacteriocins are a group of large, hydrophilic, heat-labile proteins.[4]

Review of Literature :

The search for natural antimicrobial agents in food preservation has gained significant attention in recent years, particularly in the context of rising concerns about synthetic preservatives and antibiotic resistance. Bacteriocins, which are ribosomally synthesized peptides produced by bacteria, have been widely recognized for their antimicrobial activity against a variety of foodborne pathogens[1]. These bioactive molecules are particularly valuable because of their specificity, safety, and potential for integration into food systems.

Bacteriocins and their Role in Food Safety:

Bacteriocins have been extensively studied in terrestrial lactic acid bacteria, such as *Lactobacillus* and *Enterococcus* species, where they have been shown to inhibit the growth of pathogens including *Listeria monocytogenes* and *Staphylococcus aureus*[8]. Their application in dairy products has demonstrated improvements in shelf-life and pathogen control, leading to increased consumer safety and product stability[5].

Marine Microorganisms act as Sources of Novel Bacteriocins :

Compared to terrestrial environments, marine ecosystems remain relatively underexplored for bioactive compounds. Marine bacteria are exposed to extreme environments, which drives them to produce unique secondary metabolites, including novel bacteriocins with enhanced stability and activity[7]. Several studies have successfully isolated marine-derived bacteriocin-producing strains with antimicrobial properties applicable to food safety.

For instance, Wadekar[10] reported the isolation of *Lactobacillus pentosus* from marine sources, demonstrating significant antimicrobial activity against human pathogens such as *E. coli* and *Klebsiella pneumoniae*. The study suggested the feasibility of using marine bacteriocins in probiotic and food preservation applications. Similarly, Elayaraja, *et al*.,[3] purified and characterized bacteriocins from *Lactobacillus murinus* AU06, showing broad-spectrum inhibition against dairy-related pathogens, thereby confirming the protective potential of marine-derived bacteriocins in food matrices.

Need for Sustainable Antimicrobials against dairy pathogens :

Dairy products are vulnerable to contamination by various microorganisms that can lead to spoilage and foodborne diseases[6]. Chemical preservatives, while effective, have raised concerns regarding consumer health and environmental sustainability. The exploration of natural alternatives such as bacteriocins is aligned with the global demand for clean-label and sustainable food solutions.

Research conducted by Twomey (2023) focused on identifying bacteriocins capable of inhibiting dairy pathogens, providing promising data on the application of these compounds in food safety strategies. Additionally, studies by El Ahmadi *et al*.[2] highlight the significance of lactic acid bacteria from raw milk samples as a source of bacteriocins, underscoring the relevance of antimicrobial peptides in dairy preservation.

Stability and Functional Applications :

A critical aspect of bacteriocin application is their stability under varying environmental conditions. Marine bacteriocins have shown resilience to temperature fluctuations, pH changes, and enzymatic degradation, making them suitable candidates for food processing environments[7]. Their integration into dairy products not only addresses pathogen control but also meets the increasing demand for natural preservatives.

Isolation of Bacteriocin-producing Marine Bacteria :

Marine samples were isolated from the coastal areas in parangipettai, cuddalore, Tamil Nadu, India and the samples were serially diluted using sterile saline (0.85% NaCl) and plated on de Man, Rogosa, and Sharpe (MRS) agar supplemented with 2% NaCl to select for halotolerant strains. Plates were incubated at 30°C for 48 hours. Morphologically distinct colonies were selected and purified by streaking on fresh MRS plates.

Bacteriocin production and Extraction:

Isolates exhibiting antimicrobial activity were cultured in MRS broth with 2% NaCl at 30°C for 48 hours under static conditions. The cultures were centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant was filtered through a 0.22 µm membrane filter and subjected to ammonium sulfate precipitation (80% saturation). The precipitate was dissolved in 20 mm phosphate buffer (pH 7.0) and dialyzed against the same buffer overnight.

Culture Conditions for Bacteriocin Production :

Medium : Marine broth was used as

the base culture medium. It was supplemented with additional nutrients like yeast extract and peptone to enhance bacterial growth and peptide synthesis.

Inoculation : A loopful of a fresh pure culture from marine agar slants was inoculated into 100 ml of marine broth under aseptic conditions.

Incubation Parameters :

Temperature: 28 ± 2°C, shaking: 150 rpm to provide sufficient aeration and mixing. Duration: 48 hours to ensure the production of bacteriocins during the late logarithmic phase of growth.

Monitoring Growth : Optical density at 600 nm (OD600) was periodically measured to monitor bacterial growth and determine the optimal harvesting time.

Extraction of Bacteriocins :

The extraction process aims to isolate the bacteriocin peptides from the bacterial culture while removing cells, debris, and other interfering substances.

Preparation of Cell-Free Supernatant:

After incubation, the culture broth was transferred into sterile centrifuge tubes. The broth was centrifuged at 10,000 rpm for 15 minutes at 4°C to pellet bacterial cells. The supernatant containing secreted bacteriocins was carefully decanted without disturbing the pellet.

Filtration :

The supernatant was passed through a sterile 0.22 µm membrane filter to remove any residual bacterial cells and ensure sterility. The filtrate, referred to as the cell-free supernatant (CFS), was used for preliminary antimicrobial assays and further extraction.

Ammonium Sulphate Precipitation :

Solid ammonium sulphate was gradually added to the CFS to achieve 80% saturation while stirring at 4°C to prevent denaturation. The mixture was kept overnight to allow complete precipitation of protein molecules, including bacteriocins. Precipitated proteins were recovered by centrifugation at 12,000 rpm for 20 minutes at 4°C. The protein pellet was dissolved in a minimal volume of phosphate buffer (pH 7.2).

Characterization :

The purified bacteriocin was tested for sensitivity to proteolytic enzymes, stability at different pH values (4–10), temperature tolerance (30–90°C), and resistance to detergents (SDS, Tween 20, Tween 80, Triton X-100).

Screening for Antimicrobial Activity :

The purified isolates were screened for antimicrobial activity using the agar well diffusion method. The target dairy pathogens included: *Listeria monocytogenes, Staphylococcus aureus Escherichia coli,* Salmonella *typhimurium.* Pathogen cultures were grown overnight in nutrient broth and overlaid on nutrient agar plates. Wells (6 mm diameter) were bored into the agar and filled with 100 µL of cell-free supernatant (CFS) from marine bacterial cultures. Plates were incubated at

37°C for 24 hours, and zones of inhibition were measured in millimetres.

The results presented in Table-1 demonstrate the antimicrobial potential of bacteriocins extracted from marine bacterial isolates against common dairy pathogens. The study used a uniform concentration of 100 µg/mL bacteriocin to evaluate its inhibitory effects and determine the minimum inhibitory concentration (MIC) required to prevent pathogen growth.

Activity Against Gram-Positive Pathogens: Among the tested pathogens, *Listeria monocytogenes* and *Staphylococcus aureus*, both Gram-positive bacteria, exhibited larger inhibition zones compared to Gram-negative organisms. Specifically, *Listeria monocytogenes* showed the highest inhibition zone of 18 mm, followed by *Staphylococcus aureus* with 15 mm. *Salmonella enterica* shows the lowest inhibition zone of 13 mm. The MIC values further support this observation, with *Listeria monocytogenes* requiring only 25 µg/mL, and *Staphylococcus aureus* needing 50 µg/mL to inhibit growth. The results were presented in table-1.

Table-1. Effect of marine bacteriocin produced from *lactobacillus lactis* against the dairy pathogenic organisms

Sl. No.	Dairy pathogens	Tested Concentration (µg/mL)	Inhibition Zone (mm)	MIC (µg/mL)
1	*Listeria monocytogenes*	100	18	25
2	*Staphylococcus aureus*	100	15	50
3	*Escherichia coli*	100	12	75
4	*Pseudomonas aeruginosa*	100	10	100
5	*Bacillus cereus*	100	14	60
6	*Salmonella enterica*	100	13	70

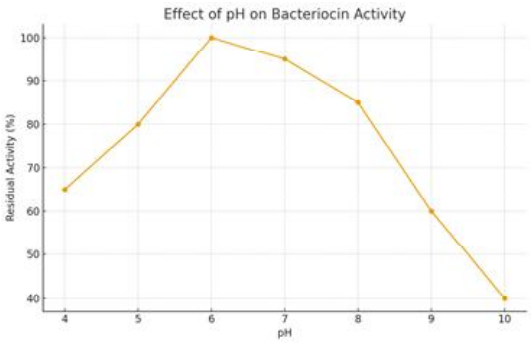

Figure 1. Effect of pH on bacteriocin activity.

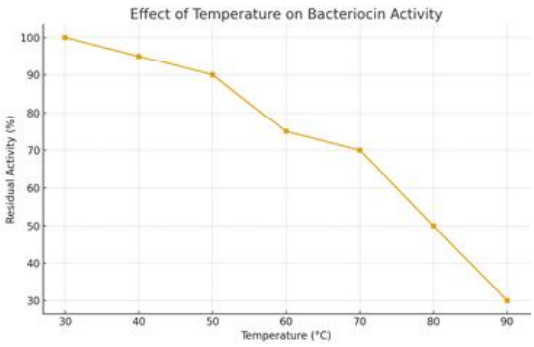

Figure 2. Effect of temperature on bacteriocin activity.

Table-2 Effect of marine bacteriocin produced from *Lactobacillus murinus* against the dairy pathogenic organisms

Sl. No.	Dairy pathogens	Tested concentration (µg/mL)	Inhibition zone (mm)	MIC (µg/mL)
1	*Listeria monocytogenes*	100	16	25
2	*Staphylococcus aureus*	100	15	50
3	*Escherichia coli*	100	13	75
4	*Pseudomonas aeruginosa*	100	11	100
5	*Bacillus cereus*	100	14	60
6	*Salmonella enterica*	100	12	70

Among the tested pathogens, *Listeria monocytogenes* and *Staphylococcus aureus*, both Gram-positive bacteria, exhibited larger inhibition zones compared to Gram-negative organisms. Specifically, *Listeria monocytogenes* showed the highest inhibition zone of 16 mm, followed by *Staphylococcus aureus* with 15 mm. *Salmonella enterica* shows the lowest inhibition zone of 12 mm The MIC values further support this observation, with *Listeria monocytogenes* requiring only 25 µg/mL, and *Staphylococcus aureus* needing 50 µg/mL to inhibit growth. The results were presented in table-2.

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