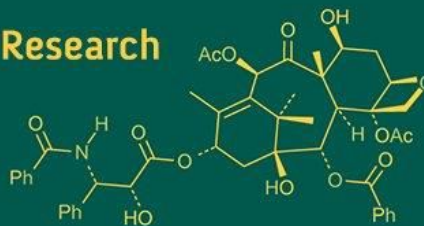
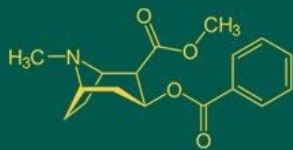


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Isolation, identification, characterization of potential marine bacteria and extraction of bacteriocins

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Abstract

Marine ecosystems are a rich source of microbial diversity with potential bioactive compounds compared to non-marine, including bacteriocins natural antimicrobial peptides with applications in food preservation and pharmaceuticals. The marine bacteria were isolated from coastal seawater samples, identified using morphological, biochemical, and molecular approaches, and screened for bacteriocin production. In the present study, marine bacterial strains were isolated from coastal seawater samples collected from different sites to explore their bacteriocin-producing potential. The isolates were subjected to morphological characterization such as colony morphology, Gram staining, and motility. The biochemical assays such as catalase, oxidase, carbohydrate fermentation tests and molecular identification using 16S rRNA gene sequencing were done. The selected strains were characterized, and bacteriocins were extracted and evaluated for antimicrobial activity against foodborne pathogens. The results demonstrated that several marine bacterial isolates exhibited significant inhibition zones against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*, suggesting their potential for application in antimicrobial formulations.

Keywords: Marine bacteria, bacteriocins, antimicrobial activity, characterization, marine microbiology

Introduction

Marine habitats harbour diverse bacterial communities that produce bioactive molecules capable of inhibiting several human pathogens. Among these, bacteriocins are ribosomally synthesized peptides with potent antimicrobial effects. Their applications range from food preservation to medical therapeutics, offering an eco-friendly alternative to chemical preservatives and antibiotics. The study focuses on isolating marine bacterial strains from seawater samples collected along the coastal region of Cuddalore district. Bacteriocins are proteinaceous compounds produced by bacteria that inhibit the growth of closely related or even unrelated bacterial strains (Zhao *et al.*, 2016) [8]. Unlike traditional antibiotics, they are biodegradable, non-toxic, and can be easily degraded by digestive enzymes, making them safe for use in food and medical applications (Wadekar, 2018) [7]. Moreover, bacteriocins from marine bacteria exhibit superior stability under a wide range of temperature, pH, and salinity conditions compared to terrestrial counterparts, highlighting their potential industrial significance (Balakrishnan and Subramanian, 2020) [1].

Marine ecosystems represent one of the most diverse habitats on Earth, containing a wide variety of microorganisms that possess unique metabolic and adaptive capabilities (Rahman *et al.*, 2023) [5]. These marine microbes thrive under extreme environmental conditions such as high salinity, pressure, and low nutrient availability, which often lead to the synthesis of novel bioactive metabolites (Saha and Ghosh, 2022) [6]. Among these metabolites, bacteriocins ribosomally synthesized antimicrobial peptides—have attracted considerable attention due to their natural origin, safety, and potential as alternatives to chemical preservatives and antibiotics (Cotter *et al.*, 2005) [2].

Bacteriocins are proteinaceous compounds produced by bacteria that inhibit the growth of closely related or even unrelated bacterial strains (Zhao *et al.*, 2016) [8]. Unlike traditional antibiotics, they are biodegradable, non-toxic, and can be easily degraded by digestive enzymes, making them safe for use in food and medical applications (Wadekar, 2018) [7]. Moreover, bacteriocins from marine bacteria exhibit superior stability under a wide range of temperature, pH, and salinity conditions compared to terrestrial counterparts, highlighting

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their potential industrial significance (Balakrishnan and Subramanian, 2020) [1].

The discovery of marine-derived bacteriocin producers, particularly from genera such as *Bacillus*, *Lactobacillus*, and *Pseudomonas*, has opened new avenues for developing natural antimicrobial agents (Kim *et al.*, 2021) [4]. Several studies have reported *Bacillus* species from marine sediments and seawater producing thermostable bacteriocins effective against foodborne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* (Zheng *et al.*, 2020) [9]. These findings emphasize the importance of marine bacteria as an untapped source of new bacteriocins for application in food preservation, aquaculture, and pharmaceutical formulations (Dawood *et al.*, 2022) [3].

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Materials and Methods

Sample Collection

Seawater samples were collected from three coastal sites at cuddalore district viz, parangipettai, Pichavaram and killai at a depth of 1 meter using sterile glass bottles. Samples were transported to the laboratory and processed within 4 hours.

Isolation of Marine Bacteria

Serial dilution was performed, and aliquots were plated on marine agar supplemented with 2% NaCl. Plates were incubated at 28 °C for 48 hours. Morphologically distinct colonies were selected and purified through repeated streaking.

Identification of Isolates

Morphological characters such as Gram staining and colony characteristics were studied based on the standard procedure. The Biochemical tests such as Catalase, oxidase and carbohydrate utilization carried out based on Cappuccino, J. G., and Sherman, N. (2014). The Molecular identification 16S rRNA gene sequencing was performed on selected isolates.

Screening for Bacteriocin Production

Screening was done by using agar well diffusion assay was used. The Pathogens tested were *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*. The Inhibition zones were measured in millimetres after 24 hour incubation at 37 °C.

Extraction and Purification of Bacteriocins

The Culture supernatant was obtained by centrifugation at 10,000 rpm for 15 min. Filtered through 0.22 µm membrane. Ammonium sulfate precipitation (80% saturation) was used to concentrate the proteins. Dialysis against phosphate buffer to remove salts.

Experimental Results

The biochemical characterization of the marine bacterial isolate MB 1 revealed that the organism was Gram-positive and rod-shaped. It shows positive for catalase and oxidase activities, indicating its aerobic nature and ability to degrade hydrogen peroxide. The isolate was negative for indole and methyl red tests, suggesting the absence of tryptophan degradation and stable acid production from glucose fermentation. It showed a positive Voges-Proskauer reaction, The isolate exhibits positive citrate utilization. The results were presented in Table 1.

The bacteriocin activity of the marine bacterial isolate MB1 was evaluated against major dairy pathogens using the agar well diffusion assay. The isolate produced clear inhibition zones of 18 mm against *Listeria monocytogenes*, 15 mm against *Staphylococcus aureus*, and 12 mm against *Escherichia coli* after incubation at 37 °C for 24 hours. These results indicate that the bacteriocin produced by MB1 is effective against both Gram-positive and Gram-negative bacteria, showing the strongest activity against *Listeria monocytogenes*. The results were presented in Table-2.

Table 1: The potential isolate MB 1 was subjected to standard biochemical tests.

S. No	Test	Observation	Result
1	Gram staining	Rod-shaped, Gram-positive	+
2	Catalase test	Effervescence	+
3	Oxidase test	Purple coloration	+
4	Indole test	Negative	-
5	Methyl red test	Negative	-
6	Voges-Proskauer test	Positive	+
7	Citrate utilization	Positive	+
8	Glucose fermentation	Acid production	+

Table 2: Inhibitory effect of Potential Isolate MB 1 against major dairy pathogens

Isolates	Pathogen	Zone of Inhibition (mm)	Incubation Conditions
MB1	<i>Listeria monocytogenes</i>	18	37 °C/24 h
MB1	<i>Staphylococcus aureus</i>	15	37 °C/24 h
MB1	<i>Escherichia coli</i>	12	37 °C/24 h

References

- Balakrishnan S, Subramanian G. Bioactivity evaluation of bacteriocin from marine biofilm-forming bacteria. J Mar Microbiol. 2020;12(3):145-156.
- Cotter PD, Hill C, Ross RP. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol. 2005;3(10):777-788.
- Dawood MAO, El Basuni MF, Van Doan H. Marine bacteriocins: promising alternatives for sustainable aquaculture. Aquac Res. 2022;53(4):1420-1432.
- Kim SH, Lee HJ, Park SY. Exploration of marine-derived *Bacillus* species for bacteriocin production and antimicrobial potential. Mar Drugs. 2021;19(2):95-108.

5. Rahman M, Ahmed N, Singh R. Diversity of marine microorganisms and their biotechnological potential. *Front Mar Sci.* 2023;10:112345.
6. Saha S, Ghosh R. Marine microbial metabolites: current trends and future perspectives. *Microb Biotechnol.* 2022;15(3):765-779.
7. Wadekar BP. Production of bacteriocin from marine *Lactobacillus pentosus* B25 and applications. ResearchGate. 2018.
8. Zhao X, Zhou L, Zhang Q. Characterization of heat-stable bacteriocins from marine *Bacillus* species. *Appl Biochem Biotechnol.* 2016;178(7):1421-1435.
9. Zheng J, Gänzle MG, Lin X. Marine *Bacillus subtilis* strains as sources of novel bacteriocins for food preservation. *Food Microbiol.* 2020;89:103412.