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Identification of bacteriocin-producing bacteria from shrimp paste using 16S rRNA gene method and assay for MIC of their bacteriocin

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Abstract. *Sukmawati S, Metusalach M, Syahrul S. 2025. Identification of bacteriocin-producing bacteria from shrimp paste using 16S rRNA gene method and assay for MIC of their bacteriocin. Biodiversitas 26: 3367-3377.* Bacteria play a crucial role in various ecosystems, serving as key components in biogeochemical cycles and sources of bioactive compounds. Bacteriocins, a type of secondary metabolite, are antimicrobial compounds produced by bacteria to inhibit the growth of other microorganisms, notable for their specificity, stability, and eco-friendliness. These compounds have significant potential in various applications, ranging from food preservation to medical use as an alternative to antibiotics. This study aims to identify bacteriocin-producing bacteria isolated from shrimp (*Acetes*) paste (*terasi*) and evaluate the Minimum Inhibitory Concentration (MIC) of their crude bacteriocin for its antimicrobial potential. A descriptive method was employed to characterize the bacterial species producing crude bacteriocin from shrimp paste and assess the MIC ability of potential bacteria in inhibiting indicator bacteria. The results revealed that bacteriocin-producing bacteria isolated from shrimp paste, with sample codes TRS1, TRS2, and TRS3 were identified as *Bacillus tropicus* strain MCCC 1A01406, *Bacillus nitratireducens* strain MCCC 1A00732, and *Bacillus paramycoides* strain MCCC 1A04098, respectively. These three bacterial isolates could produce bacteriocins. The results of the Minimum Inhibitory Concentration (MIC) test showed that at the lowest bacteriocin concentration, but with the highest minimum inhibition, sample TRS1 had an inhibition value of 2.3 mm at a concentration of 0.19%, sample TRS3 had an inhibition value of 1.0 mm at a concentration of 0.19%, and sample TRS2 had an inhibition value of 1.2 mm at a concentration of 0.78%. A total of three isolates identified as *Bacillus* spp. demonstrated antimicrobial activity with MIC values ranging from 0.19% to 0.78%.

Keywords: Antibacterial, bacteriocin, food industry, potential, preservatives

INTRODUCTION

Bacteria are ubiquitous and essential to Earth's ecosystems, playing a major role in biogeochemical cycles and serving as a source of bioactive compounds (Wess 2023). Their ability to produce diverse primary and secondary metabolites has attracted significant research interest. These metabolites, particularly bacteriocins, are crucial in bacterial ecological interactions (Gonzalez and Aranda 2023). Bacteriocins are antimicrobial secondary metabolites used by bacteria to inhibit the growth of competing microorganisms. They hold great promise for multiple applications, including as natural alternatives to chemical preservatives in food and as potential substitutes for antibiotics in addressing antimicrobial resistance (Gonzalez and Aranda 2023).

Bacteriocins from Lactic Acid Bacteria (LAB) are effective natural preservatives, inhibiting both Gram-positive and, in recent formulations, Gram-negative bacteria (Lahiri et al. 2022). In shrimp paste fermentation, LAB, yeasts, and halophilic bacteria influence flavor but can cause spoilage if not well-controlled (Li et al. 2021). Growing concerns over synthetic preservatives have increased interest in bacteriocins, especially when combined with controlled fermentation and active packaging to enhance safety (Karnwal et al. 2024). Advances in microbiome research support their broad use without targeting specific species. For example, nisin and pediocin are used as natural preservatives (Khan et al. 2014). Bacteriocins can inhibit the growth of pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella*, thereby enhancing food safety. In medicine, given the increasing resistance to conventional antibiotics, bacteriocins are emerging as promising candidates for new therapeutic agents (Cheruvari and Kammara 2024). Moreover, bacteriocins also act in a highly specific manner, allowing them to target pathogenic bacteria without disrupting the normal human microbiota, unlike broad-spectrum antibiotics (Barbosa et al. 2021).

Bacteriocin-producing bacteria can be identified using the 16S rRNA gene, widely applied for bacterial classification due to its conserved and variable regions, and presence in nearly all prokaryotes (Janda and Abbot 2017). This gene enables phylogenetic tree construction to determine evolutionary relationships. The search for bacteriocin producers is driven by rising antibiotic resistance, a global health threat intensified by the movement of humans, animals, and food (Naghavi et al. 2024). Bacteriocins are promising alternatives, acting through unique mechanisms like pore formation or inhibition of cell wall synthesis. They are selective, eco-friendly, and less toxic than traditional antibiotics (Heilbronner et al. 2021).

Before bacteriocins can be produced, it is crucial to identify potential bacteriocin-producing bacterial species,

enabling their propagation and cultivation for bacteriocin production. The potential bacteriocin-producing bacteria in this study were isolated from *Acetes* shrimp paste. Shrimp paste (*terasi*) is a traditional fermented marine product rich in diverse microbial communities, particularly Lactic Acid Bacteria (LAB) and *Bacillus* spp., which are known producers of bacteriocins. Its high protein content and salt concentration create selective pressure that supports the growth of bacteriocin-producing microbes. The natural fermentation process, without the addition of starter cultures, makes *terasi* a unique and underexplored ecological niche for isolating novel bacteriocin-producing strains.

Several studies have confirmed the presence of bacteriocins in fermented seafood products, such as *Plaa-som* and *Kung-chai*, highlighting their potential as antimicrobial sources (Kim and Austin 2008; Hwanhlem et al. 2011; Jiang et al. 2022). Recent findings also show LAB from fish products inhibiting pathogens like *Listeria monocytogenes* (Elidrissi et al. 2023), with strains from snakehead fish and mud crabs showing strong antimicrobial activity (Pramono et al. 2023; Du et al. 2024).

Research on bacteriocins from Indonesian *terasi* is still limited, and the potential of its endemic microbiota remains underexplored. This study addresses this gap by isolating and identifying bacteriocin-producing bacteria from *terasi* and assessing their antimicrobial activity through Minimum Inhibitory Concentration (MIC) testing. MIC, defined as the lowest concentration that inhibits microbial growth, is crucial for evaluating antimicrobial potential (Darbandi et al. 2022). The use of crude bacteriocins in this study serves as a foundation for future development of purified bacteriocins and natural biopreservatives from traditional Indonesian fermented marine products.

This study aimed to identify bacteriocin-producing bacteria from shrimp paste and evaluate their Minimum Inhibitory Concentration (MIC) values against spoilage bacteria in shrimp. The prospects of this research contribute to advancements in biotechnology and microorganism-based production methods by focusing on the development of bacteriocins as an integral part of global solutions to the antibiotic resistance crisis.

MATERIALS AND METHODS

Research time and location

This study was conducted from September to December 2024. The bacterial samples used in this study were isolated from *terasi*, a type of fermented shrimp paste made from *Acetes* shrimp produced by home scale industry in Sorong, Southwest Papua, Indonesia.

Bacterial isolation

In the bacterial isolation stage, all equipment such as Petri dishes, Erlenmeyer flasks, measuring pipettes, forceps, and others were first sterilized. De Man-Rogosa-Sharpe Agar (MRSA) medium was prepared by dissolving 68.2 grams in 1 liter of distilled water, followed by sterilization using an autoclave at 121°C for 15 minutes. Each shrimp paste (terasi) sample (made from Acetes shrimp) was weighed at 1 g and diluted in 9 mL of 0.9% sodium chloride (NaCl) physiological solution, creating a 10^{-1} dilution. Then, 1 mL of the 10^{-1} dilution was transferred to make further serial dilutions up to 10^{-2} and 10^{-3}. From each dilution factor (10^{-1}, 10^{-2}, 10^{-3}), 1 mL was plated onto Petri dishes containing MRSA medium. The samples were incubated at room temperature (29°C) for 24 hours. After incubation, morphological observations were conducted, and one colony from each sample was selected for further identification (Kanauchi 2019).

Sterilization of equipment and materials, and preparation of growth media for bacteriocin-producing bacteria

To ensure aseptic conditions, all the equipment used was sterilized using an autoclave at 121°C for 15 minutes. The bacterial growth medium used for culturing bacteriocin-producing bacteria was de Man-Rogosa-Sharpe Agar (MRSA). MRSA was used for both initial culturing and reculturing of bacteriocin-producing bacterial isolates. To prepare the medium, 68.2 g of MRSA powder were weighed and dissolved in 1 L of distilled water. The solution was then heated using a hotplate stirrer until fully dissolved, followed by sterilization in an autoclave at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to 45-50°C before being poured into sterile petri dishes.

Bacterial identification

Three bacterial isolates were selected from the bacteria isolated and cultured from *terasi* made from *Acetes* shrimp. The bacterial identification procedure involved DNA isolation, amplification of the 16S rRNA gene, sequencing, and phylogenetic analysis.

Bacterial DNA isolation

Bacterial culture samples were centrifuged at 8,000 rpm for 10 minutes to obtain bacterial DNA. The resulting bacterial pellet was washed with STE (Sodium chloride; Tris-HCl; EDTA) buffer three times, then resuspended in STE buffer and treated with lysozyme enzyme. The mixture was incubated at 55°C for 1 hour. Following incubation, proteinase K enzyme was added, and the mixture was incubated at 55°C for an additional 60 minutes. Cetyltrimethylammonium Bromide (CTAB) was then added to the NaCl solution, followed by incubation at 65°C for 30 minutes. Subsequently, a phenol:chloroform (25:24, v/v) solution was added, and the mixture was centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to a new tube, mixed with 0.6 volumes of isopropanol and 20 µL sodium acetate, and incubated at -20°C for 12 hours. The solution was centrifuged at 12,000 rpm for 10 minutes, and the resulting pellet was washed with 1 mL of 70% ethanol. The DNA pellet was air-dried for 1 hour to remove residual ethanol and then dissolved in distilled deionized water, following a modified method from Sambrook and Russell (2001).

Amplification of the 16S rRNA gene from bacterial isolates

The 16S rRNA gene from the bacterial genomic DNA was amplified using the Polymerase Chain Reaction (PCR) technique with the forward primer 63f (5′-CAG GCC TAA

CAC ATG CAA GTC-3′) and the reverse primer 1387r (5′-GGG CGG WGT GTA CAA GGC-3′), performed using a Bio-Rad T100™ thermocycler (Marchesi et al. 1998). The PCR reaction mixture contained La Taq DNA polymerase, guanidine chloride buffer, deoxynucleoside triphosphates (dNTPs), distilled deionized water, and DNA template.

The PCR conditions consisted of pre-denaturation for 4 minutes, denaturation for 45 seconds, annealing for 1 minute, elongation for 1 minute and 10 seconds, and post-PCR for 7 minutes, performed for 30 cycles. After amplification, the PCR products were electrophoretically-separated on a 1% agarose mini gel at 75 V for 45 minutes. The DNA bands were visualized using a UV transilluminator and stained with Ethidium Bromide (EtBr) in an Axygen gel documentation system (Brzoska and Hassan 2014).

Sequencing and phylogenetic construction

The sequencing results were assembled using ChromasPro software version 1.5 and subsequently compared with genomic data from the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences were analyzed using MEGA 5.0 software (Tamura et al. 2011). A phylogenetic tree illustrating the evolutionary relationships among microorganisms was constructed using the Neighbor-Joining Tree method with 1000 bootstrap replications (Felsenstein 1985).

Culture of bacteriocin-producing bacterial isolates

The selected bacteriocin-producing bacterial isolate was cultured or propagated in MRS Agar medium. MRS Agar medium was weighed at 52 g and dissolved in 1000 mL of distilled water, then sterilized using an autoclave at 121°C for 15 minutes. The sterilized MRS Agar medium was poured into sterile test tubes, each containing 10 mL, then tilted and left to solidify. The bacterial isolate was streaked onto the solidified medium and incubated at 30°C for 24 hours. The bacterial isolate cultures were used to produce crude bacteriocin.

Bacteriocin production

Each bacteriocin-producing bacterial isolate was inoculated into 100 mL of MRS Broth medium (Perez et al. 2022) supplemented with 0.5% $CaCO_3$ (Ooi et al. 2015) and incubated at 37°C for 24 hours. Then 100 mL bacterial culture was centrifuged at 4,500 rpm for 15 minutes to separate the supernatant from the pellet. The supernatant was then filtered using a 0.22 µm Millipore membrane filter to obtain cell-free supernatant, referred to as crude bacteriocin (Hoover and Steenson 2014).

Antibacterial activity assay of bacteriocin

The crude bacteriocin (bacteriocin crude extract solution) was subjected to the Minimum Inhibitory Concentration (MIC) assay. This assay aimed to determine the lowest concentration of crude bacteriocin capable of inhibiting general bacteria or indicator bacteria. In this study, *Escherichia coli* as indicator bacteria referred to bacteria isolated from banana shrimp, which were used to assess the spoilage process in shrimp.

These bacteria were considered major contributors to shrimp spoilage due to their ability to proliferate in shrimp tissue. Crude bacteriocin samples were dissolved in 30% methanol (The use of 30% methanol in bacteriocins aims to enhance solubility, aid in purification, maintain stability, and inhibit bacteriocin-degrading enzymes, ensuring it remains active and effective (modified from Taylor et al. 2007) to obtain bacteriocin concentrations as presented in Table 1.

The MIC assay was performed using the spread plate method on nutrient agar medium. Blank paper discs (6 mm in diameter, 2 mm in thickness) were placed on the agar surface. Subsequently, using the disc diffusion method, 15 µL of each concentration of crude bacteriocin from the broth dilution was pipetted onto each of the blank paper discs, and the assay was performed in triplicate. The plates were incubated at room temperature (29-30°C) for 24 hours. Following incubation, the clear inhibition zones formed around the discs were measured. The antibacterial activity of crude bacteriocin was calculated using the following equation (Sharma et al. 2011):

$$\text{Inhibition Zone Activity (mm)} = \text{Diameter of Clear Zone(mm)} - \text{The diameter of the paper disc (mm)}$$

RESULTS AND DISCUSSION

Three different bacterial isolates obtained from *Acetes* shrimp paste, exhibiting potential bacteriocin production, were identified as belonging to the genus *Bacillus* (Table 2). This identification was based on the alignment results of the 16S rRNA gene sequences against the available data in the National Center for Biotechnology Information (NCBI). The corresponding 16S rRNA gene sequences are listed in Figure 1, Table 2 and Table 3, while the phylogenetic trees among these isolates are visualized in Figures 2, 3, and 4.

Table 1. Crude bacteriocin concentrations for Minimum Inhibitory Concentration (MIC) assay

Concentration (%)	Crude bacteriocin (µL)	30% Methanol solvent (µL)
100	1000	-
50	500	500
25	250	750
12.5	125	875
6.25	62	938
3.125	31	969
1.56	15	985
0.78	7	993
0.39	3	997
0.19	1	999
0.09	0.9	999.1
0	-	1000

Note: Modified from Apriyanto et al. (2014)

Based on phylogenetic analysis, the TRS1 sample showed 99.69% identity with *Bacillus tropicus* strain MCCC 1A01406, as shown in the phylogenetic tree in Figure 2. Meanwhile, the phylogenetic analysis identified the TRS2 sample as *Bacillus nitratireducens* strain MCCC 1A00732 with 99.77% similarity, as presented in the phylogenetic tree in Figure 3. Furthermore, the TRS3 sample was identified as *Bacillus paramycoides* strain MCCC 1A04098 with 99.61% identity (Figure 4).

The selection of *B. tropicus* strain MCCC 1A01406 from TRS1 for MIC (Minimum Inhibitory Concentration) testing was based on its morphological differences compared to *B. nitratireducens* strain MCCC 1A00732 and *Bacillus luti* strain MCCC 1A00359. These differences, including variations in shape, texture, color, and colony edges, were important factors in the selection process. The MIC test results at various concentrations indicated that *Bacillus tropicus* strain MCCC 1A01406 (TRS1), *B. nitratireducens* strain MCCC 1A00732 (TRS2), and *B. paramycoides* strain MCCC 1A04098 (TRS3) exhibited inhibitory activity against indicator bacteria (Table 4).

Discussion

The results from the alignment of 16S rRNA gene sequences (Table 2; Table 3), PCR amplification of the 16S rRNA gene (Figure 1) and the phylogenetic tree (Figure 2) were shown to indicate that the TRS1 isolate was identical to *B. tropicus* strain MCCC 1A01406, *B. nitratireducens* strain MCCC 1A00732, and *Bacillus luti* strain MCCC 1A00359, with the same similarity percentage of 99.69%. The TRS1 isolate was also found to have similarities with *Bacillus cereus* ATCC 14579 at 99.61%, *Bacillus rhizoplanae* strain JJ-63 at 98.06%, and *Bacillus pseudomycoides* at 97.83%. Based on the similarity identity values, it was concluded that the TRS1 isolate was identified as *B. tropicus* strain MCCC 1A01406, despite two other strains having the same identity values. This identification was determined not only based on the percentage of similarity but also on the proximity of the TRS1 isolate to *B. tropicus* strain MCCC 1A01406, as well as the morphological similarity of TRS1 colony characteristics to *B. tropicus* strain MCCC 1A01406. Meanwhile, the similarities with *B. cereus* ATCC 14579, *B. rhizoplanae* strain JJ-63, and *B. pseudomycoides* were found to have lower identity values than the previous three strains (Table 2).

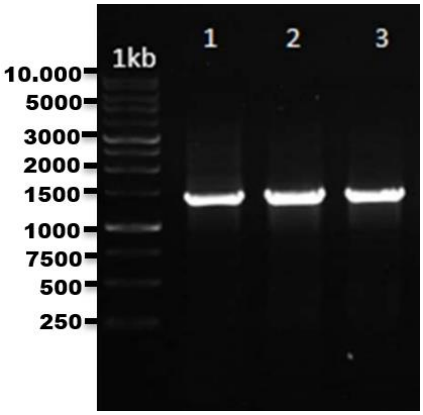

Figure 1. PCR amplification of the 16S rRNA gene using primers 63F and 1387R; M: 1 kb DNA ladder marker: 1. TRS1 sample, 2. TRS2 sample, and 3. TRS3 sample

Table 2. Alignment results of 16S rRNA gene sequences of bacterial isolates against NCBI (BLASTN) database

Isolate name	Description	Max score	Total score	% Query cover	E value	% identity	Accession number
TRS1	*Bacillus tropicus* strain MCCC 1A01406	2359	2359	100	0.00	99.69	NR_157736.1
	Bacillus nitratireducens strain MCCC 1A00732	2359	2359	100	0.00	99.69	NR_157732.1
	Bacillus luti strain MCCC 1A00359	2359	2359	100	0.00	99.69	NR_157730.1
	Bacillus cereus ATCC 14579	2353	2353	100	0.0	99.61	NR_074540.1
	Bacillus rhizoplanae strain JJ-63	2242	2242	100	0.0	98.06	NR_181926.1
	Bacillus pseudomycoides	2239	2239	100	0.0	97.83	NR_114422.1
TRS2	*Bacillus nitratireducens* strain MCCC 1A00732	2350	2350	100	0.00	99.77	NR_157732.1
	Bacillus luti strain MCCC 1A00359	2350	2350	100	0.00	99.77	NR_157730.1
	Bacillus albus strain MCCC 1A02146	2350	2350	100	0.00	99.77	NR_157736.1
	Bacillus thuringiensis strain NBRC 101235	2333	2333	99	0.0	99.53	NR_112780.1
	Bacillus mycoides strain ATCC 6462	2305	2305	100	0.0	99.14	NR_115993.1
	Bacillus bingmayongensis strain FJAT-13831	2298	2298	100	0.0	98.99	NR_148248.1
TRS 3	*Bacillus paramycoides* strain MCCC 1A04098	2357	2357	100	0.00	99.61	NR_157734.1
	Bacillus tropicus strain MCCC 1A01406	2351	2351	100	0.00	99.54	NR_157736.1
	Bacillus nitratireducens strain MCCC 1A00732	2351	2351	100	0.00	99.54	NR_157732.1
	Bacillus cereus strain CCM 2010	2346	2346	100	0.0	99.46	NR_115714.1
	Bacillus proteolyticus strain MCCC 1A00365	2340	2340	100	0.0	99.38	NR_157735.1
	Bacillus thuringiensis strain ATCC 10792	2329	2329	100	0.0	99.23	NR_114581.1

Table 4. Inhibitory activity of crude bacteriocin from potential *Bacillus* isolates derived from *Acetes* shrimp paste against indicator bacteria

Concentration (%)	Crude bacteriocin (µL/mL)	Inhibition zone diameter (mm) Crude bacteriocin activity vs. indicator bacteria					
		TRS1	Stedev	TRS2	Stedev	TRS3	Stedev
100	1000	16.3	2.47	8.5	2.47	13.2	0.64
50	500	12.8	0.78	5.0	0.49	12.3	2.69
25	250	11.7	0.85	4.3	0.57	8.5	1.20
12.5	125	10.5	0.85	3.5	0.49	6.8	1.27
6.25	62	9.3	0.92	2.8	0.57	5.0	0.49
3.125	31	8.0	0.92	2.0	0.21	4.3	0.35
1.56	15	6.7	0.99	1.7	0.35	3.8	0.21
0.78	7	5.3	0.92	1.2	0.85	3.5	0.85
0.39	3	4.0	1.20	0.0	-	2.3	0.92
0.19	1	2.3	1.63	0	-	1.0	0.71
0.09	0.9	0.0	-	0	-	0.0	-
0	-	0.0	-	0	-	0.0	-

Note: TRS1 represents crude bacteriocin produced by *Bacillus tropicus* strain MCCC 1A01406, TRS2 represents crude bacteriocin produced by *Bacillus nitratireducens* strain MCCC 1A00732, and TRS3 represents crude bacteriocin produced by *Bacillus paramycoides* strain MCCC 1A04098. The value 1-7 µL/mL that is circled indicates the MIC (Minimum Inhibitory Concentration)

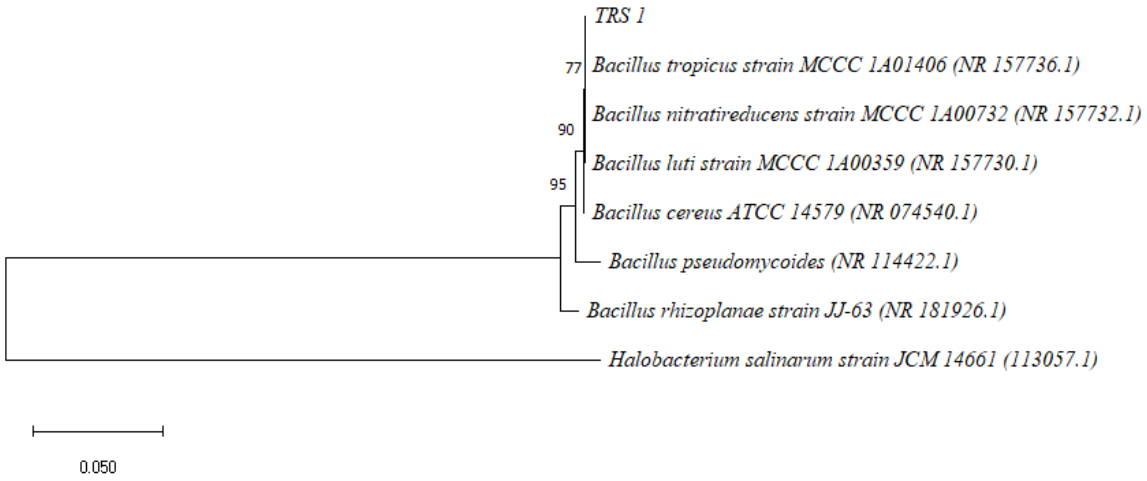

Figure 2. Phylogenetic tree of TRS1 sample, identified as *Bacillus tropicus* strain MCCC 1A01406

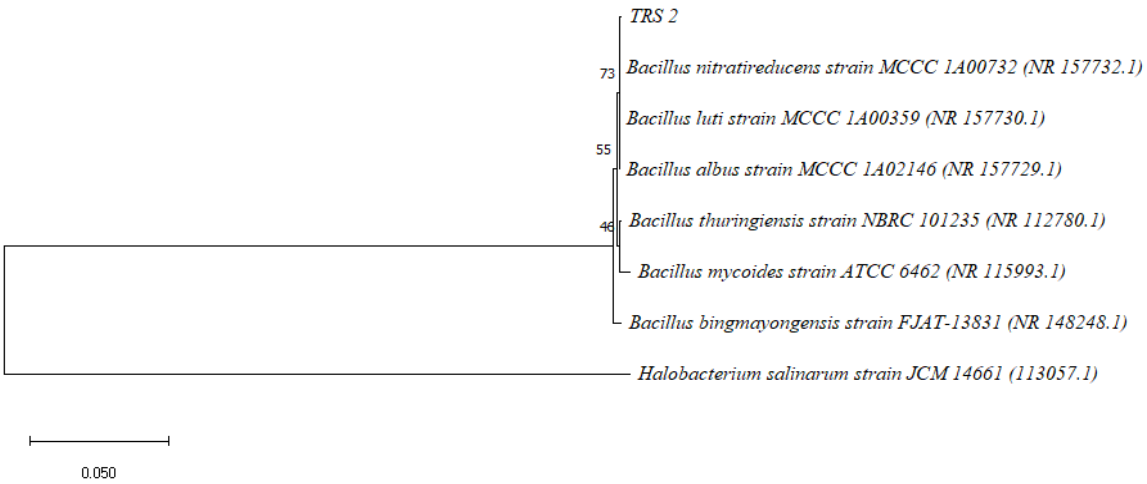

Figure 3. Phylogenetic tree of TRS2 sample, identified as *Bacillus nitratireducens* strain MCCC 1A00732

Table 3. 16S rRNA gene sequences of three potential bacteriocin-producing isolates

Name isolate	Species name	The 16S rRNA gene sequence	Acc. no from NCBI
TRS1	*Bacillus tropicus* strain MCCC 1A01406	TGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG ATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAA GGCGGCTTCGGCTGNNTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACG GCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAAACA CGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGT GCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCA GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAG GTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGA GACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGA GGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGG AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGG TTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGG CTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGC AACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTC GGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT CCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTG CCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCT ACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAA AACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATC GCGGATTCAGC	PV759527.1
TRS2	*Bacillus nitratireducens* strain MCCC 1A00732	TGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTTC CGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCT TCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAA GGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGA CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCG CGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGA ATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCT TAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGT GCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCA GTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAG GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCC TTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAG AACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGA GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG AGCGCAACCCTTGATCTTAGTTGCCATCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACA AACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTG CTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCT CAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATTCA GC	PV759528.1
TRS 3	*Bacillus paramycoides* strain MCCC 1A04098	TGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG ATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAA GGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGC TCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACG GCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC AACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGC TAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGC AGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGT GGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGA CTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGG AACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAG CAAACAGGAGTAGATACCCTGCTAGTAAACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTT TCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCT GAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAA CGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGG GAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC CGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCC GGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTAC ACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAA CCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGC GGATTCAGCATG	PV759529.1

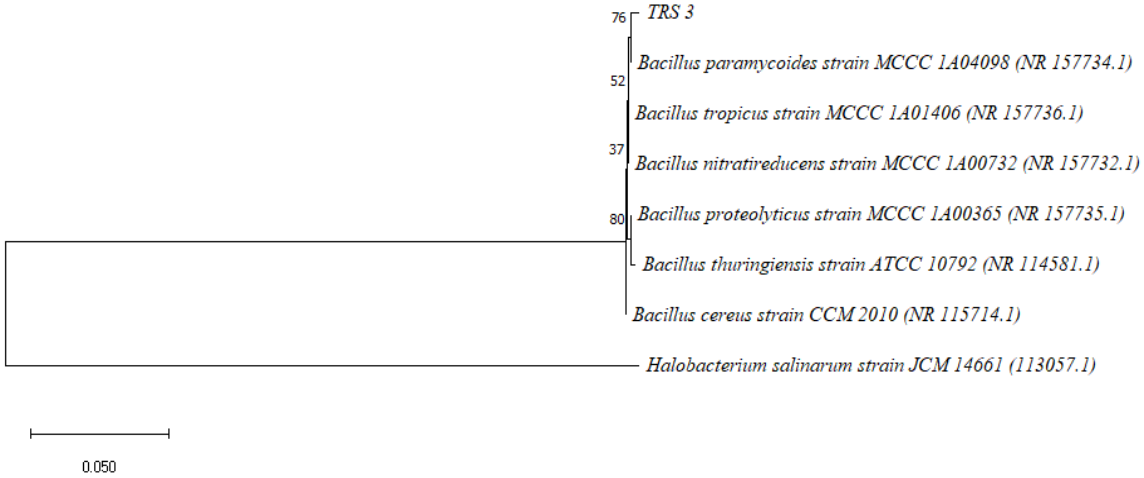

Figure 4. Phylogenetic tree of TRS3 sample, identified as *Bacillus paramycoides* strain MCCC 1A04098

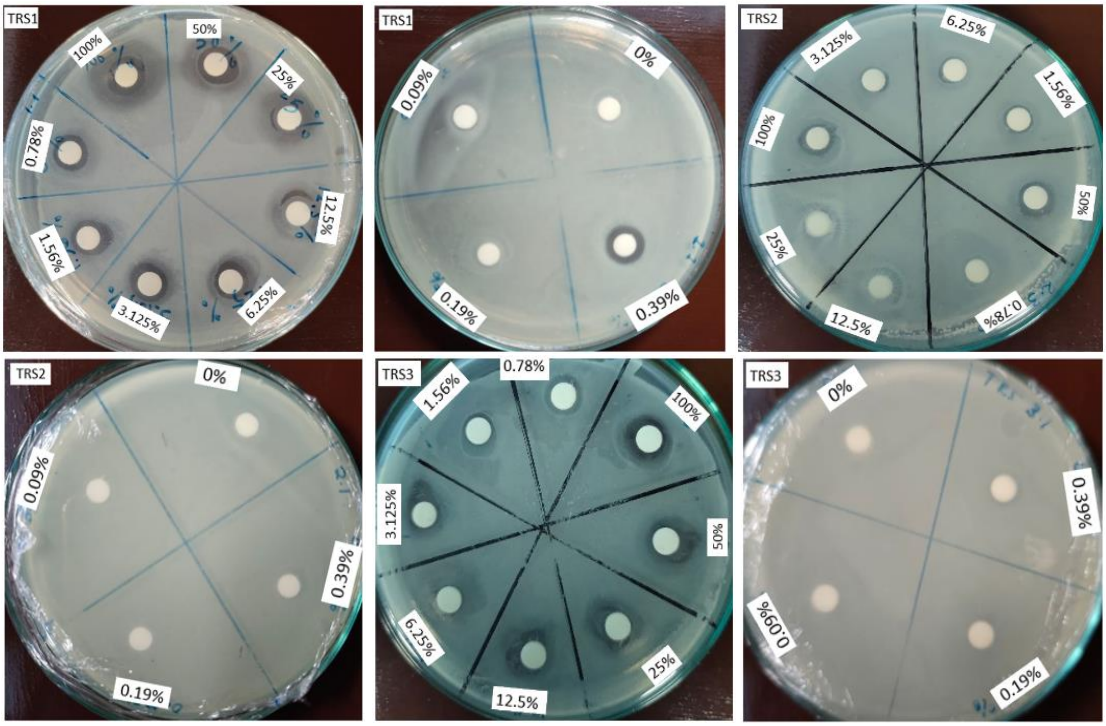

Figure 5. Inhibitory activity of crude bacteriocin produced by *Bacillus tropicus* strain MCCC 1A01406 (TRS1), *Bacillus nitratireducens* strain MCCC 1A00732 (TRS2), and *Bacillus paramycoides* strain MCCC 1A04098 (TRS3) against indicator bacteria (MIC value samples TRS1 and TRS3: 1 µL/mL, TRS2: 7 µL/mL)

The TRS2 isolate was also found to have identical similarity values of 99.77% to three strains, namely *B. nitratireducens* strain MCCC 1A00732, *B. luti* strain MCCC 1A00359, and *B. albus* strain MCCC 1A02146. Meanwhile, three other strains were found to have lower similarity identity values, leading to the conclusion that the TRS2 isolate was identified as *B. nitratireducens* strain MCCC 1A00732. This identification was also based on the consideration of colony morphology similarities, the identical percentage value of 99.77% (Table 2), and the proximity of the TRS2 isolate to *B. nitratireducens* strain MCCC 1A00732 in its phylogenetic tree (Figure 3). The TRS3 isolate was found to have the highest identity similarity value with *B. paramycoides* strain MCCC 1A04098 at 99.61%, compared to the other five strains (Table 2). Similarly, the proximity of the TRS3 isolate to *B. paramycoides* strain MCCC 1A04098 was also observed (Figure 4). Even though the genetic identity (99.69% or 99.7%) is the same for several strains, the decision to select one particular strain as the reference for identification was

based on: The highest score values (Max/Total Score), an E-value of 0.00 across matches, with priority given to the most significant and consistent aligment, and the nomenclatural stability and frequent usage of the strain in previous literature (Table 2).

The characteristics of *B. tropicus* are known to be those of a Gram-positive bacterium with potential applications in biotechnology, particularly in enzyme production and bioactive compound synthesis (Duhan et al. 2020). *B. nitratireducens* is recognized as a bacterium capable of reducing nitrate to nitrite. Several strains of *B. nitratireducens* have also been reported to possess probiotic properties, which may be beneficial in agriculture and aquaculture (Besson et al. 2022). *B. paramycoides* is a species with similarities to *B. cereus* but with distinct genetic characteristics. This bacterium has been reported to produce enzymes and bioactive compounds (Ren et al. 2022).

Then, the results of bacterial isolation from *Acetes* shrimp paste revealed three samples with high potential for bacteriocin production, because these three bacterial isolates are capable of producing bacteriocins that can inhibit pathogenic bacteria such as *Staphylococcus aureus, Escherichia coli,* and *Salmonella typhimurium.* Additionally, these bacteriocins are stable at temperatures ranging from -40°C to 121°C, resistant to pH levels from 2 to 11, and not sensitive to the enzyme proteinase K (Sukmawati et al. 2022). These samples were identified as *B. tropicus* strain MCCC 1A01406 for sample code TRS1, *B. nitratireducens* strain MCCC 1A00732 for TRS2, and *B. paramycoides* strain MCCC 1A04098 for TRS3 (Table 2; Figures 2-4). Notably, these three bacteria demonstrated the ability to produce bacteriocins, which have potential applications as preservatives in food products. The bacteriocins produced by these bacteria exhibited inhibitory effects against indicator bacteria. However, the bacteriocin from *B. tropicus* strain MCCC 1A01406 (TRS1) demonstrated the highest inhibitory capability against indicator bacteria, as it was effective at the lowest crude bacteriocin concentration compared to the other two (Table 4). At a concentration of 0.19%, the crude bacteriocin from *B. tropicus* strain MCCC 1A01406 (TRS1) produced an inhibition zone that was 2.3 mm, *B. paramycoides* strain MCCC 1A04098 (TRS3) 1.0 mm. However, *B. nitratireducens* strain MCCC 1A00732 (TRS2) showed no inhibitory activity against indicator bacteria not only at 0.19% but also at 0.39% concentrations (Table 4). The MIC value of samples TRS1 and TRS3 is 1 µL/mL, while the MIC value of sample TRS2 is 7 µL/mL.

Compared to recently characterized bacteriocins, the MIC values obtained in our study 1 µL/mL for TRS1 (*B. tropicus*) and TRS3 (*B. paramycoides*), and 7 µL/mL for TRS2 (*B. nitratireducens*) are within a promising range. For instance, bacteriocin P7 from *Bacillus velezensis* G7, isolated in 2025 from mangrove plants, exhibited an MIC of approximately 30 µg/mL against *Staphylococcus aureus* (Li et al. 2025). Even when accounting for differences in concentration units, crude bacteriocins from our terasi-derived isolates reach inhibitory levels comparable to purified analogues. Other *Bacillus*-derived bacteriocins, such as thucin A1 from *B. thuringiensis* P86 (2022), have shown potent activity against Gram-positive foodborne pathogens (Zhang et al. 2022), while novel bacteriocins like PCM7-4 characterized in 2025 also demonstrated strong anti-*Listeria* effects (Ma et al. 2025).

According to international standards, the effective concentration range for food preservatives such as propionate, benzoate, and sorbate in inhibiting microbial growth is between 100-1,500 ppm, depending on the type of food product and the target microorganism (Davidson et al. 2012). For example, the MIC values of propionate, benzoate, and sorbate against spoilage microbes in meat and dairy products range from 100-500 ppm, while for eggs, it may reach 1,200-1,500 ppm (Seo et al. 2023). The FDA safety limit for sodium benzoate is ≤0.1% (1,000 ppm) and for potassium sorbate is 0.1-0.3% (1,000-3,000 ppm) (Akolawole et al. 2022; FDA 2023). This indicates that synthetic preservatives must be applied within these thresholds to ensure both microbial inhibition and regulatory compliance.

When compared to the crude bacteriocins obtained in this study, which showed MIC values of 1 µL/mL (~1,000 ppm) for TRS1 and TRS3, and 7 µL/mL (~7,000 ppm) for TRS2, it can be concluded that TRS1 and TRS3 fall within or near the practical threshold for standard preservative use. In contrast, TRS2 exceeds the regulatory safety limit. Therefore, the TRS1 and TRS3 isolates demonstrate potential as natural biopreservatives that are consistent with international standards. Notably, bacteriocins from shrimp paste derived strains remain relatively underexplored. Our findings thus contribute novel data: *B. tropicus* and *B. paramycoides* isolated from fermented shrimp paste are confirmed bacteriocin producers with measurable MICs, expanding the diversity of *Bacillus* species known to yield antibacterial peptides. The activity of TRS1 and TRS3 at 1 µL/mL indicates effective inhibition at low concentrations-criteria aligned with practical thresholds for food-grade biopreservatives. Further purification and quantification will clarify whether these bacteriocins can meet industrial standards in application scenarios.

The classification of inhibition zone diameters, as described by Kowalska-Krochmal and Dudek-Wicher (2021), categorizes inhibition into four levels: weak inhibition (≤5 mm), moderate inhibition (6-10 mm), strong inhibition (11-20 mm), and very strong inhibition (≥21 mm). The inhibition effect of crude bacteriocins from the three bacterial samples exhibited a clear zone, indicating a completely transparent area with no growth of the indicator bacteria, also referred to as total inhibition (bactericidal effect). This phenomenon occurs due to pathogen cell lysis. The clear zones formed due to bacteriocin activity against indicator bacteria (both pathogenic and spoilage bacteria) indicate their antimicrobial potential. Typically, bacteriocin are composed of peptide chains undergoing post-translational modifications, such as disulfide bond formation or cyclization (Sugrue et al. 2024). These structural modifications play a crucial role in enhancing stability and antimicrobial activity (Bahrami et al. 2024).

This study showed that of the three potential bacteriocin-producing bacterial species. *B. tropicus* strain MCCC 1A01406 TRS1 demonstrated the highest inhibition capacity against indicator bacteria (Table 4). This finding aligns with Sardar et al. (2025), highlighting the potential

of *B. tropicus* MCCC 1A01406 as an effective alternative antibiotic in poultry farming. Their study reported significant improvements in growth performance, feed intake, and feed conversion ratio compared to the control group. Additionally, its probiotic properties were associated with enhanced meat quality, increased antioxidant activity, and improved nutritional content with higher protein and fiber content, as well as reduced fat, ash, and nitrogen-free extract levels. Moreover, *B. tropicus* BB-5 has been reported to exhibit strong protein and starch hydrolysis capabilities, leading to increased total acid content, lactic acid, acetic acid, and volatile flavor compounds. These characteristics enhance the fermentation quality of Cupei, improving the overall quality of Sichuan bran vinegar (Wu et al. 2025). Due to these beneficial properties, *B. tropicus* has been proposed as a probiotic bacterium (Shu et al. 2024).

Another potential bacteriocin-producing bacterial strain identified in this study is *B. nitratireducens* strain MCCC 1A00732. Although, its crude bacteriocin derived from this species exhibited a lower inhibition capacity compared to that from strain TRS1, it still demonstrated moderate inhibitory effects (Table 4). Currently, no direct information is available regarding *B. nitratireducens* as a bacteriocin producer. However, insights can be inferred based on the general characteristics and capabilities of *Bacillus* species in bacteriocin production. Several *Bacillus* strains, including *Bacillus* sp. DB407, *B. licheniformis* DB612, and *B. subtilis* DB821, have been reported to produce bacteriocins (Lim 2022). Two specific strains, *Bacillus tequilensis* ST1962CD and *Bacillus subtilis* subsp. *stercoris* ST2056CD, exhibit antimicrobial activity against *Staphylococcus* spp. These strains have been shown to produce antimicrobial agents such as surfactin and subtilosin, which hold potential for treating *Staphylococcus*-related infections. These antimicrobial agents are non-cytotoxic and can be further optimized for efficient biotechnological production (Ye et al. 2023). Recent studies have highlighted the potential of *B. nitratireducens* in improving soil productivity. This bacterium produces Indole-3-Acetic Acid (IAA), siderophores, and ACC deaminase, which contribute to enhanced soil fertility (Susilowati et al. 2024). Additionally, *B. nitratireducens* has been explored for its applications in mining industries and waste management, promoting sustainable and environmentally friendly practices (Rus et al. 2024).

The third bacterial strain identified in this study was *B. paramycoides* strain MCCC 1A04098. This bacterium exhibits potential in bacteriocin production, as indicated by the crude bacteriocin's ability to inhibit the growth of indicator bacteria (Table 4). Supporting evidence from a study on *B. paramycoides* MCCC 1A04098 BSH 1 which was isolated from cincalok - a traditional fermented shrimp product. The bacteriocin produced by this species is thermostable and maintains stability across various pH ranges, making it suitable for applications in environmental and food industries (Sukmawati et al. 2024). *B. paramycoides* is also known to produce bioactive compounds with significant antifungal properties (El Mohammady et al. 2024), which have also been reported to exhibit strong antimicrobial activity against *E. coli* and *Mucor* sp. (Hossain et al. 2024). Furthermore, bioactive compounds produced by *B. paramycoides* shows antioxidant activity, with an IC_{50} value of 0.65 mM for DPPH, while also displaying antimicrobial properties (Aziz et al. 2024). A strain of *B. paramycoides* MCCC 1A04098, isolated from fermented cassava (tape), demonstrated the ability to produce antibiotics (Muhiddin et al. 2024). Moreover, *B. paramycoides* MCCC 1A04098 is capable of synthesizing zinc oxide nanoparticles (ZnONPs), which possess antioxidant, antimicrobial, and anticancer properties ZnONPs exhibited antioxidant and antibacterial activity against various pathogens, as well as cytotoxic and apoptotic effects against liver cancer cells (HepG2) and breast cancer cells (MCF-7) (El-Refai et al. 2024).

In conclusion, this study identified three potential bacteriocin-producing bacteria isolated from *Acetes* shrimp paste. Sample TRS1 was identified as *Bacillus tropicus* strain MCCC 1A01406, TRS2 as *Bacillus nitratireducens* strain MCCC 1A00732, and TRS3 as *Bacillus paramycoides* strain MCCC 1A04098. Among these isolates, the crude bacteriocin extracted from *Bacillus tropicus* strain MCCC 1A01406 (TRS1) exhibited the highest antimicrobial potency, followed by *Bacillus paramycoides* strain MCCC 1A04098 (TRS3), while *Bacillus nitratireducens* strain MCCC 1A00732 (TRS2) demonstrated the lowest inhibitory activity. The results of the Minimum Inhibitory Concentration (MIC) test showed that at the lowest bacteriocin concentration, but with the highest minimum inhibition, sample TRS1 had an inhibition value of 2.3 mm at a concentration of 0.19%, sample TRS3 had an inhibition value of 1.0 mm at a concentration of 0.19%, and sample TRS2 had an inhibition value of 1.2 mm at a concentration of 0.78%. The samples TRS1 and TRS3 showed MIC values of 1 µL/mL, while TRS2 showed an MIC value of 7 µL/mL, it can be concluded that TRS1 and TRS3 fall within or near the practical threshold for standard preservative use. In contrast, TRS2 exceeds the regulatory safety limit. Therefore, the TRS1 and TRS3 isolates demonstrate potential as natural biopreservatives that are consistent with international standards. These findings provide a foundation for further research to explore the optimization of bacteriocin production, purification methods, and potential applications in food preservation or medical treatments. Future studies could also investigate the genetic mechanisms underlying bacteriocin synthesis in these bacterial strains to enhance their industrial applicability.

REFERENCES

Akolawole JS, Okoye PAC, Omokpariola D. 2022. Effect of storage on the levels of sodium benzoate in soft drinks sold in some Nigerian market with exposure and health risk assessment. Environ Anal Health Toxicol 37 (4): e2022030. DOI: 10.5620/eaht.2022030.

Apriyanto H, Harpeni E, Setyawan A. 2014. Utilization of *Rhizophora* sp. fruit extract as an antibacterial agent against freshwater fish pathogenic bacteria. E-Jurnal Rekayasa dan Teknologi Budidaya Perairan 3 (1): 289-296. [Indonesian].

Aziz MI, Hasan MM, Ullah R, Bari A, Khan MA, Ul Hasnain SZ, Baloch R, Akram M, Obaid A, Ullah A, Abbas K, Amin A. 2024. Potential role of *Citrus bergamia* flower essential oil against oral pathogens. BMC Complement Med Ther 24 (1): 157. DOI: 10.1186/s12906-024-04457-7.

Bahrami S, Andishmand H, Pilevar Z, Hashempour-Baltork F, Torbati M, Dadgarnejad M, Rastegar H, Mohammadi SA, Azadmard-Damirchi S. 2024. Innovative perspectives on bacteriocins: Advances in classification, synthesis, mode of action, and food industry applications. J Appl Microbiol 135 (11): lxae274. DOI: 10.1093/jambio/lxae274.

Barbosa AAT, de Melo MR, da Silva CMR, Jain S, Dolabella SS. 2021. Nisin resistance in Gram-positive bacteria and approaches to circumvent resistance for successful therapeutic use. Crit Rev Microbiol 47 (3): 376-385. DOI: 10.1080/1040841X.2021.1893264.

Besson S, Almeida MG, Silveira CM. 2022. Nitrite reduction in bacteria: A comprehensive view of nitrite reductases. Coord Chem Rev 464: 214560. DOI: 10.1016/j.ccr.2022.214560.

Brzoska AJ, Hassan KA. 2014. Quantitative PCR for detection of mRNA and gDNA in environmental isolates. Methods Mol Biol 1096: 25-42. DOI: 10.1007/978-1-62703-712-9_3.

Cheruvari A, Kammara R. 2024. Bacteriocins future perspectives: Substitutes to antibiotics. Food Control 168: 110834. DOI: 10.1016/j.foodcont.2024.110834.

Darbandi A, Asadi A, Ari MM, Ohadi E, Talebi M, Zadeh MH, Emamie AD, Ghanavati R, Kakanj M. 2022. Bacteriocins: Properties and potential use as antimicrobials. J Clin Lab Anal 36 (1): e24093. DOI: 10.1002/jcla.24093.

Davidson PM, Taylor TM, Schmidt SE. 2012. Chemical preservatives and natural antimicrobial compounds. In: Doyle MP, Buchanan RL (eds). Food Microbiology: Fundamentals and Frontiers. 4th eds. ASM Press, Washington, DC.

Du H, Li S, Yao H, Wang N, Zhao R, Meng F. 2024 Bacteriocin mining in *Lactiplantibacillus pentosus* PCZ4 with broad-spectrum antibacterial activity and its biopreservative effects on snakehead fish. Foods 13 (23): 3863. DOI: 10.3390/foods13233863.

Duhan P, Bansal P, Rani S. 2020. Isolation, identification and characterizaton of endophytic bacteria from medicinal plant *Tinospora cordifolia*. S Afr J Bot 134: 43-49. DOI: 10.1016/j.sajb.2020.01.047.

El Mohammady EM, El-Rahim A, Mohamed W, Gohar MR, Abd El Aleem M, Elsayed TR. 2024. Applying wheat-associated bacteria's producing Volatile Organic Compounds (VOCs) antagonistic to plant pathogenic fungi. Egypt J Chem 67 (13): 1103-1115. DOI: 10.21608/ejchem.2024.325246.10557.

Elidrissi A, Ezzaky Y, Boussif K, Achemchem F. 2023. Isolation and characterization of bioprotective lactic acid bacteria from Moroccan fish and seafood. Braz J Microbiol 54 (3): 2117-2127. DOI: 10.1007/s42770-023-01077-0.

El-Refai HA, Saleh AM, Mohamed SIA, Aboul Naser AF, Zaki RA, Gomaa SK, Hamed MA. 2024. Biosynthesis of zinc oxide nanoparticles using bacillus paramycoides for in vitro biological activities and in vivo assessment against hepatorenal injury induced by CCl4 in rats. Appl Biochem Biotechnol 196 (9): 5953-5973. DOI: 10.1007/s12010-023-04817-y.

FDA [Food and Drug Administration] U.S. 2023. Code of Federal Regulations Title 21: Substances Generally Recognized as Safe. https://www.ecfr.gov/current/title-21

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791. DOI: 10.1111/j.1558-5646.1985.tb00420.x.

Gonzalez JM, Aranda B. 2023. Microbial growth under limiting conditions-future perspectives. Microorganisms 11 (7): 1641. DOI: 10.3390/microorganisms11071641.

Heilbronner S, Krismer B, Brötz-Oesterhelt H, Peschel A. 2021. The microbiome-shaping roles of bacteriocins. Nat Rev Microbiol 19 (11): 726-739. DOI: 10.1038/s41579-021-00569-w.

Hoover DG, Steenson LR. 2014. Bacteriocins of Lactic Acid Bacteria. Academic Press, Cambridge, Massachusetts.

Hossain KM, Khan U, Rahman SM, Khan MS. 2024. Potential antimicrobial and fruit juice clarification activity of amylase enzyme from *Bacillus* strains. Biotechnol Rep 44: e00861. DOI: 10.1016/j.btre.2024.e00861.

Hwanhlem N, Buradaleng S, Wattanachant S, Benjakul S, Tani A, Maneerat S. 2011. Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (*Plasom*) and production of *Plasom* from selected strains. Food Control 22 (3-4): 401-407. DOI: 10.1016/j.foodcont.2010.09.010.

Janda JM, Abbott SL. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. J Clin Microbiol 45 (9): 2761-2764. DOI: 10.1128/jcm.01228-07.

Jiang Y-H, Xin W-G, Zhang Q-L, Lin L-B, Deng X-Y. 2022. A novel bacteriocin against *Shigella flexneri* from *Lactiplantibacillus plantarum* isolated From *Tilapia intestine*: Purification, antibacterial properties and antibiofilm activity. Front Microbiol 12: 779315. DOI: 10.3389/fmicb.2021.779315.

Karnwal A, Malik T. 2024. Exploring the untapped potential of naturally occurring antimicrobial compounds: novel advancements in food preservation for enhanced safety and sustainability. Front Sustain Food Syst 8: 1307210. DOI: 10.3389/fsufs.2024.1307210.

Kanauchi M. 2019. Lactic Acid Bacteria. Methods in Molecular Biology. Humana New York, NY. DOI: 10.1007/978-1-4939-8907-2.

Khan H, Flint SH, Yu P-L. 2014. Bacteriocins: The natural food preservatives. In: Rai VR, Bai JA (eds). Microbial Food Safety and Preservation Techniques. CRC Press, Boca Raton.

Kim D-H, Austin B. 2008. Characterization of probiotic carnobacteria isolated from rainbow trout (*Oncorhynchus mykiss*) intestine. Lett Appl Microbiol 47 (3): 141-147. DOI: 10.1111/j.1472-765X.2008.02401.x.

Kowalska-Krochmal B, Dudek-Wicher R. 2021. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathogens 10 (2): 165. DOI: 10.3390/pathogens10020165.

Lahiri D, Nag M, Dutta B, Sarkar T, Pati S, Basu D, Kari ZA, Wei LS, Smaoui S, Goh KW, Ray RR. 2022. Bacteriocin: A natural approach for food safety and food security. Front Bioeng Biotechnol 10: 1005918. DOI: 10.3389/fbioe.2022.1005918.

Li W, Lu H, He Z, Sang Y, Sun J. 2021. Quality characteristics and bacterial community of a Chinese salt-fermented shrimp paste. LWT 136: 110358. DOI: 10.1016/j.lwt.2020.110358.

Li Y, Ma H, Pan R, Long Y, Zhao Y, Yu M, Peng J, Ma Y. 2025. Optimisation of cultivation conditions for *Bacillus velezensis* G7 from mangrove plants and exploration of potential bacteriocins. Front Pharmacol 16: 1530043. DOI: 10.3389/fphar.2025.1530043.

Lim E-S. 2022. Influence of bacteriocin-producing *Bacillus* strains on quality characteristics of fermented soybean product with biogenic amine-forming lactic acid bacteria. Appl Biol Chem 65: 5. DOI: 10.1186/s13765-021-00664-9.

Ma H, Ding Y, Peng J, Li Y, Pan R, Long Y, Zhao Y, Guo R, Ma Y. 2025. Identification and characterization of a novel bacteriocin PCM7-4 and its antimicrobial activity against Listeria monocytogenes. Microbiol Res 290: 127980. DOI: 10.1016/j.micres.2024.127980.

Marchesi JR, Takuichi S, Andrew JW, Tracey AM, John CF, Sarah JH, William GW. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol 64: 795-799.

Muhiddin NH, Herawati N, Ramlawati R, Hidayah N. 2024. Characterization of antibiotic-producing bacteria from cassava tape fermented by traditional ragi tape. Biodiversitas 25 (9): 3359-3368. DOI: 10.13057/biodiv/d250958.

Naghavi M, Vollset SE, Ikuta KS et al. 2024. Global burden of bacterial antimicrobial resistance 1990-2021: A systematic analysis with forecasts to 2050. Lancet 404 (10459): 1199-1226. DOI: 10.1016/50140-6736(24)01867-1.

Ooi MF, Mazian N, Foo HL, Loh TC, Mohamad R, Rahim RA, Ariff A. 2015. Effects of carbon and nitrogen sources on bacteriocin-inhibitory activity of postbiotic metabolites produced by *Lactobacillus plantarum* I-UL4. Malays J Microbiol 11 (2): 176-184. DOI: 10.21161/mjm.13014.

Perez RH, Zendo T, Sonomoto K. 2022. Multiple bacteriocin production in lactic acid bacteria. J Biosci Bioeng 134 (4): 277-287. DOI: 10.1016/j.jbiosc.2022.07.007.

Pramono H, Suciati P, Putra TF, Andika N, Utari S. 2023. Reduction of pathogenic bacteria during fermentation of masin by protease and bacteriocin-producing lactic acid bacteria. Aquasains 7 (1): 629-636. DOI: 10.23960/aqs.v7i1.p629-636. [Indonesian]

Ren W, Li P, Wang X, Che Y, Long H, Zhang X, Cai X, Huang A, Zeng Y, Xie Z. 2022. Cross-habitat distribution pattern of *Bacillus* communities and their capacities of producing industrial hydrolytic enzymes in Paracel Islands: Habitat-dependent differential contributions of the environment. J Environ Manag 323: 116252. DOI: 10.1016/j.jenvman.2022.116252.

Rus AMM, Winarko R, Chaerun SK, Mufakhir FR, Astuti W, Minwal WP. 2024. Bioleaching of Rare Earth Elements (REEs) from Indonesian red mud by the bacterium *Bacillus nitratireducens* strain SKC/L-2. E3S Web Conf 543: 02014. DOI: 10.1051/e3sconf/202454302014.

Sambrook J, Russell DW. 2001. Molecular Cloning a Laboratory Manual. 3rd eds. Cold Spring Harbor Laboratory Pr, New York.

Sardar D, Afsana S, Habib A, Hossain T. 2025. Dietary effects of multi-strain probiotics as an alternative to antibiotics on growth

performance, carcass characteristics, blood profiling and meat quality of broilers. Vet Integr Sci 23 (2): 1-17. DOI: 10.12982/VIS.2025.059.

Seo Y, Sung M, Hwang J, Yoon Y. 2023. Minimum inhibitory concentration (MIC) of propionic acid, sorbic acid, and benzoic acid against food spoilage microorganisms in animal products to use MIC as threshold for natural preservative production. Food Sci Anim Resour 43 (2): 319. DOI: 10.5851/kosfa.2022.e79.

Sharma N, Kapoor RT, Gautam N, Kumari R. 2011. Purification and characterization of bacteriocin produced by *Bacillus subtilis* R75 isolated from fermented chunks of mung bean (*Phaseolus radiatus*). Food Technol Biotechnol 49 (2): 1330-1340.

Shu H, He X, Hong Z, Dong K, Zou Y, Cao M, Wang R, Xu Y, Liao L, Zuo H, Pei X. 2024. Screening and genome analysis of potential probiotic lactic acid bacteria with broad-spectrum antibacterial activity from Sichuan sun-dried vinegar grains (Cupei). LWT 202: 116288. DOI: 10.1016/j.lwt.2024.116288.

Sugrue I, Ross RP, Hill C. 2024. Bacteriocin diversity, function, discovery and application as antimicrobials. Nat Rev Microbiol 22: 556-571. DOI: 10.1038/s41579-024-01045-x.

Sukmawati S, Sipriyadi S, Rosalina F, Ratna R, Dewi NK, Zakariah MIB. 2024. Identification of 16S rRNA genes in bacteriocin-producing bacteria. Biodiversitas 25: 3720-3727. DOI: 10.13057/biodiv/d251034.

Sukmawati S, Sipriyadi S, Yunita M, Dewi NK, Noya ED. 2022. Analysis of bacteriocins of lactic acid bacteria isolated from fermentation of rebon shrimp (*Acetes* sp.) in South Sorong, Indonesia as antibacterial agents. Biodiversitas 23: 3852-3859. DOI: 10.13057/biodiv/d230763.

Susilowati DN, Widawati S, Suliasih S, Syah MF, Radiastuti N, Sofiana I, Anda M. 2024. Characteristics and potency of culturable bacteria from tidal and non-tidal swamps in South Kalimantan and South Sumatra, Indonesia. AIP Conf Proc 2970: 050015. DOI: 10.1063/5.0208354.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28 (10): 2731-2739. DOI: 10.1093/molbev/msr121.

Taylor TM, Davidson PM, Zhong Q. 2007. Extraction of nisin from a 2.5% commercial nisin product using methanol and ethanol solutions. J Food Prot 70 (5): 1272-1276. DOI: 10.4315/0362-028X-70.5.1272.

Wess L. 2023. The Curious World of Bacteria. Greystone Books Ltd, Canada.

Wu Y, Tie Y, Tang Z, Zhu H, Li Y, Zhu W, Wang X, Yin L. 2025. Isolation and identification of functional *Bacillus* strain from Cupei of sichuan bran vinegar and evaluation of its fermentation characteristics. Shipin Gongye Ke-ji 46 (2): 122-131. DOI: 10.13386/j.issn1002-0306.2024010069.

Ye J, Fugaban JII, Dioso CM, Bucheli JEV, Choi G-H, Kim B, Holzapfel WH, Todorov SD. 2023. *Bacillus strains* isolated from Korean fermented food products with antimicrobial activity against *Staphylococci*, an alternative for control of antibiotic-resistant pathogens. Foodborne Pathog Dis 20 (7): 279-293. DOI: 10.1089/fpd.2023.0019.

Zhang X, Xin N, Zhu Z, Li X, Dai D, Pan C, Peng D, Sun M. 2022. Three novel leaderless bacteriocins have antimicrobial activity against gram-positive bacteria to serve as promising food biopreservative. Microb Cell Fact 21 (1): 194. DOI: 10.1186/s12934-022-01912-3.