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Identification of 16S rRNA genes in bacteriocin-producing bacteria

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Abstract. *Sukmawati S, Sipriyadi, Rosalina F, Ratna, Dewi NK, Zakariah MIB. 2024. Identification of 16S rRNA genes in bacteriocin-producing bacteria. Biodiversitas 25: 3720-3727.* The use of safe and effective biopreservatives in the food industry is highly important to extend the product shelf life and maintain the product quality without posing health risks. Bacteriocins, antimicrobial compounds produced by bacteria, offer biopreservatives potential that can inhibit the growth of pathogens and spoilage microorganisms in food products. Bacteriocins as biopreservatives in the food industry due to their antimicrobial properties and potential to enhance food safety and shelf life. Bacteriocins as biopreservatives are stable at high temperatures, which makes them suitable for various food processes. They are also stable across a broad pH range, ensuring their effectiveness in different food environments. This stability under various conditions instills confidence in their effectiveness. This study aimed to identify the 16S rRNA gene of bacteriocin-producing bacteria as an initial step in developing biopreservative candidates. This research method is a descriptive method that describes the types of bacterial species that are capable of producing bacteriocins. Bacteria were isolated from shrimp paste (cincalok), and genetic analysis was performed using PCR and sequencing techniques to identify the 16S rRNA gene. The bacterial samples derived from shrimp paste were identified as *Bacillus paramycoides* strain MCCC 1A04098 BSH 1, *Bacillus albus* strain MCCC 1A02146 BSH 2, and *Bacillus cereus* strain IAM 12605 BSH. All three samples are Gram-positive with bacilli-shaped cells, leading to the conclusion that these bacteria have the potential as biopreservatives.

Keywords: Bacteria potential, bacteriocin, biopreseravtive, 16S rRNA

INTRODUCTION

Food preservatives are added to increase the product's shelf life and retain its nutritional quality. However, not all preservatives are beneficial for the product or consumer health, particularly synthetic preservatives. The use of synthetic preservatives in seafood and meat can negatively impact food quality and safety. Synthetic preservatives contain chemical elements and other additives that may have adverse effects on human health. Some may trigger allergic reactions, a potential danger that consumers should be cautious of, or disrupt the digestive system (Dwivedi et al. 2017). The use of synthetic preservatives in food can leave toxic chemical residues in food products (Teshome et al. 2022), which could pose health risks if consumed for an extended period (Kumari et al. 2019).

Some synthetic preservatives can alter the original taste and aroma of food products, reducing sensory quality and making them less appealing to consumers. Synthetic preservatives can interact with other food components, forming undesirable chemical compounds (Jabeen et al. 2023), which can affect product quality and produce harmful compounds (Dey et al. 2022). These compounds can lead to carcinogenic properties when exposed to heat or certain processing conditions, posing serious health risks to consumers (Gupta et al. 2021; Flórez-Méndez and Mndez 2022). Additionally, discarded synthetic preservatives contribute to environmental pollution (Chaudhari et al. 2019).

Given this background, synthetic preservatives can be replaced with natural preservatives such as bacteriocins. Bacteriocins are proteinaceous antimicrobial peptides synthesized by lactic acid bacteria, which can be used as bio-preservatives to address pathogenic issues (Sulthana et al. 2021). Bacteriocins are increasingly being explored as natural preservatives in the food industry due to their antimicrobial properties and potential to enhance food safety and shelf life. Bacteriocins are stable at high temperatures, which makes them suitable for various food processes. They are also stable across a broad pH range, ensuring their effectiveness in different food environments (Ng et al. 2020; Lahiri et al. 2022). Bacteriocins are proteolytically active, which helps them to inhibit microbial growth effectively (Lahiri et al. 2022). Bacteriocins exhibit broad-spectrum antimicrobial activity, particularly against Gram-positive bacteria, which are common food spoilage

microbes. They work by disrupting the cell membrane of target bacteria, leading to their death or inhibition (Surati 2020). Examples of bacteriocins used. Nisin, one of the most widely used bacteriocins, is effective against a range of foodborne pathogens. Pediocin PA1 is known for its activity against *Listeria*, a common pathogen in dairy products. Enterocin and leucocin are also used in food preservation due to their broad-spectrum activity (Ng et al. 2020). Bacteriocins must be safe for human consumption and not harm the intestinal microflora. They must remain active during food production, storage, and distribution processes. The approval process for bacteriocins involves a rigorous process of identification, characterization, and ensuring they meet safety standards before commercial use (Kamarajan et al. 2015).

Therefore, this review highlights recent developments and research related to the use of nanoparticles and bacteriocin conjugates to address the resistance crisis and their application in the food industry. The research problem formulated in this study is how to identify the 16S rRNA gene of bacteriocin-producing bacteria as natural preservative candidates in fishery products. The problem-solving approach recognizes that the use of synthetic preservatives is not in line with sustainable food principles. Thus, utilizing natural preservatives like bacteriocins supports future sustainable food practices. The advantages of using bacteriocins as preservatives or bio-preservatives include their non-toxic nature, ease of degradation by proteolytic enzymes, safety for gut microbiota, and stability across a range of pH and temperatures. However, before these benefits can be realized, this study first identifies the 16S rRNA gene of bacteriocin-producing bacteria. The novelty of this research lies in the screening of bacteriocin-producing bacterial isolates from shrimp paste (*cincalok*), which represents the first study conducted based on the location of the raw material.

MATERIALS AND METHODS

Research time and location

This research was conducted from March to September 2024. It took place in the Microbiology Laboratory of Muhammadiyah University of Sorong and the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Bengkulu University. The samples used in this research were bacteria isolated from *cincalok* made from *Acetes* sp. obtained from South Sorong, Southwest Papua, Indonesia.

Procedures

Sterilization of equipment and materials and availability of supporting facilities

The research tools used, such as petri dishes, test tubes, micropipettes, microtubes, beakers, Erlenmeyer flasks, centrifuge tubes, etc., were available in the microbiology laboratory of the Fisheries Faculty, Muhammadiyah University of Sorong. Glassware was sterilized using an oven at 180°C for 2 hours, while Non-glass materials were sterilized in an autoclave for 15 minutes at 121°C. The growth media used in this study were also sterilized using the same autoclaving method.

Preparation of growth media for bacteriocin-producing bacteria

The media used for the growth or re-culture of bacteriocin-producing bacterial isolates was MRS Agar + 0.5% $CaCO_3$, which was subsequently sterilized using an autoclave.

Sample preparation (Re-culture of bacteriocin-producing bacterial isolates)

The bacteriocin-producing bacterial isolates previously identified from *cincalok* made from *Acetes* sp. from South Sorong, West Papua, included three potential isolates (Sukmawati et al. 2022). These bacterial isolates were re-cultured by streaking them onto MRS Agar media, followed by incubation for 24 hours.

Gram-staining test

A gram staining test was conducted to ensure that the bacteriocin-producing bacterial culture was pure. The Gram staining procedure was as follows: the bacterial isolate was suspended on a glass slide and fixed, followed by crystal violet staining and rinsing with distilled water. The slide was then treated with Lugol's iodine, rinsed with distilled water, decolorized with 96% alcohol for 10 seconds, and briefly rinsed with distilled water. Finally, the slide was stained with safranin for 10 seconds, rinsed with distilled water, and observed under a microscope using immersion oil.

DNA isolation of bacteriocin-producing bacteria (Modified from Sambrook 2001)

The bacterial culture was centrifuged, and the pellet was rinsed with STE buffer, followed by another round of centrifugation and three additional washes. The pellet was then re-suspended in STE buffer and lysozyme, and incubated at 55°C for one hour. Afterward, Proteinase-K was added, and the mixture was incubated again at 55°C for another hour. Next, CTAB was introduced, and the mixture was incubated at 65°C for 30 minutes. The solution underwent phenol treatment and centrifugation. The clear phase was transferred to a fresh tube, and isopropanol along with sodium acetate, was added, followed by a 12-hour incubation. The solution was then centrifuged at 12,000 rpm for 10 minutes, and the supernatant was discarded. The pellet was washed with alcohol, air-dried for 1 hour to remove alcohol, and then dissolved in sterile ddH_2O. The DNA isolate was stored at 4°C.

Amplification of 16S rRNA gene from bacteriocin-producing bacterial isolates

The 16S rRNA gene from genomic DNA was amplified using a Polymerase Chain Reaction (PCR) machine with the forward primer 63f (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and reverse primer 1387r (5′-GGG CGG WGT GTA CAA GGC-3′). The PCR conditions were as follows: pre-denaturation at 94°C for 4 minutes, denaturation at 94°C for 45 seconds, annealing at 55°C for 1 minute,

elongation at 72°C for 1 minute and 10 seconds, and post-PCR at 72°C for 7 minutes, with a total of 30 cycles (Marchesi et al. 1998). DNA visualization was performed using a UV transilluminator (gel documentation system, Axygen).

Sequencing and phylogenetic construction

The sequencing data were processed using ChromasPro version 1.5 of the BLAST analysis, which was conducted using the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST/). Further data analysis was conducted with MEGA 7.0 (Kumar et al. 2016) and MEGA 11.0 (Tamura et al. 2021). A phylogenetic tree was constructed using the Neighbor-Joining Tree method with 1000 bootstrap replicates for validation.

RESULTS AND DISCUSSION

Based on the Gram staining results of the three bacterial isolates, the samples labeled BSH 1, BSH 2, and BSH 3 were identified as Gram-positive with bacilli-shaped cells (Figure 1). The bacterial samples isolated from cincalok made of *Acetes* sp. were identified as follows: BSH 1 was identified as *Bacillus paramycoides* strain MCCC 1A04098, BSH 2 as *Bacillus albus* strain MCCC 1A02146, and BSH 3 as *Bacillus cereus* strain IAM 12605. All three samples were found to be Gram-positive and exhibited a bacilli cell morphology. Previous research has shown that bacteriocins from these bacterial strains can inhibit pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium.* These bacteriocins are resistant to proteinase-K, remain stable at both high and low temperatures, and maintain their stability across a broad pH range, unaffected by the presence of NaCl (Sukmawati et al. 2022). *Bacillus paramycoides*, which is in symbiosis with seagrass, has the potential to serve as an antibacterial agent and can be used as a reference for food preservatives (Pringgenies et al. 2024).

The sequence alignment of the 16S rRNA genes from bacterial isolates BSH 1, BSH 2, and BSH 3, which were derived from *cincalok* made with *Acetes* sp., is shown in Table 1. The constructed phylogenetic trees can be viewed in (Figures 2; Figure 3; and Figure 4).

Table 1. Results of 16S rRNA gene sequence alignment of bacterial isolates against NCBI database (BLASTN)

Isolate name	Description	Max score	Total score	Query cover	E value	Per. identity	Accession number
BSH 1	*Bacillus paramycoides* strain MCCC 1A04098	1895	1895	99 %	0.00	93.46%	NR_157734.1
	Bacillus tropicus strain MCCC 1A01406	1892	1892	99 %	0.00	93.39%	NR_157736.1
	Bacillus nitratireducens strain MCCC 1A00732	1890	1890	99 %	0.00	93.39%	NR_157732.1
	Bacillus anthracis strain ATCC 14578	1892	1892	99%	0.0	93.39%	NR_041248.1
	Bacillus luti strain MCCC 1A00359	1890	1890	99%	0.0	93.39%	NR_157730.1
	Bacillus albus strain MCCC 1A02146	1890	1890	99%	0.0	93.39%	NR_157729.1
BSH 2	*Bacillus luti* strain MCCC 1A00359	2362	2362	100%	0.0	99.61%	NR_157730.1
	Bacillus nitratireducens strain MCCC 1A00732	2362	2362	100%	0.0	99.61%	NR_157732.1
	Bacillus albus strain MCCC 1A02146	2362	2362	100 %	0.00	99.61%	NR_157736.1
	Bacillus paramycoides strain MCCC 1A04098	2357	2357	100 %	0.00	99.54%	NR_157734.1
	Bacillus mycoides strain NBRC 101228	2318	2318	100%	0.0	99.00%	NR_113990.1
	Bacillus cereus strain IAM 12605	2357	2357	100 %	0.00	99.54%	NR_115526.1
	Bacillus mycoides strain NBRC 101228	2318	2318	100%	0.0	99.00%	NR_113990.1
BSH 3	*Bacillus tropicus* strain MCCC 1A01406	2364	2364	99%	0.0	99.69%	NR_157736.1
	Bacillus nitratireducens strain MCCC 1A00732	2364	2364	99%	0.0	99.69%	NR_157732.1
	Bacillus luti strain MCCC 1A00359	2364	2364	99%	0.0	99.69%	NR_157730.1
	Bacillus albus strain MCCC 1A02146	2364	2364	99%	0.0	99.69%	NR_157729.1
	Bacillus anthracis strain ATCC 14578	2361	2361	99%	0.0	99.61%	NR_041248.1
	Bacillus cereus strain ATCC 14579	2359	2359	99%	0.0	99.61%	NR_074540.1

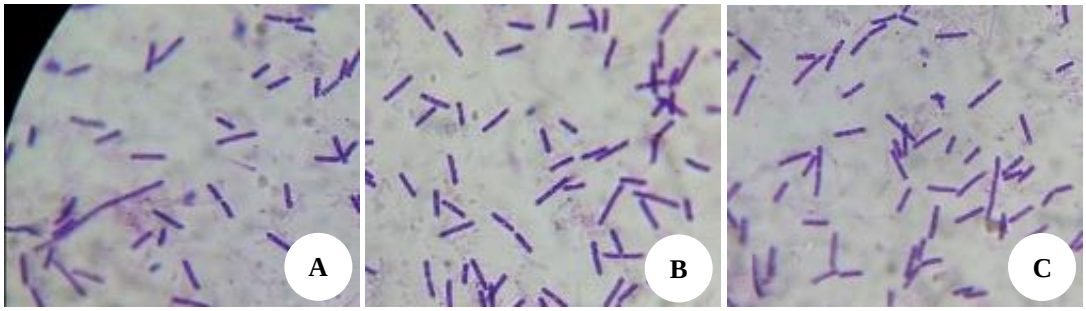

Figure 1. Bacterial cells after Gram-staining. A. BSH 1; B. BSH 2; C. BSH 3

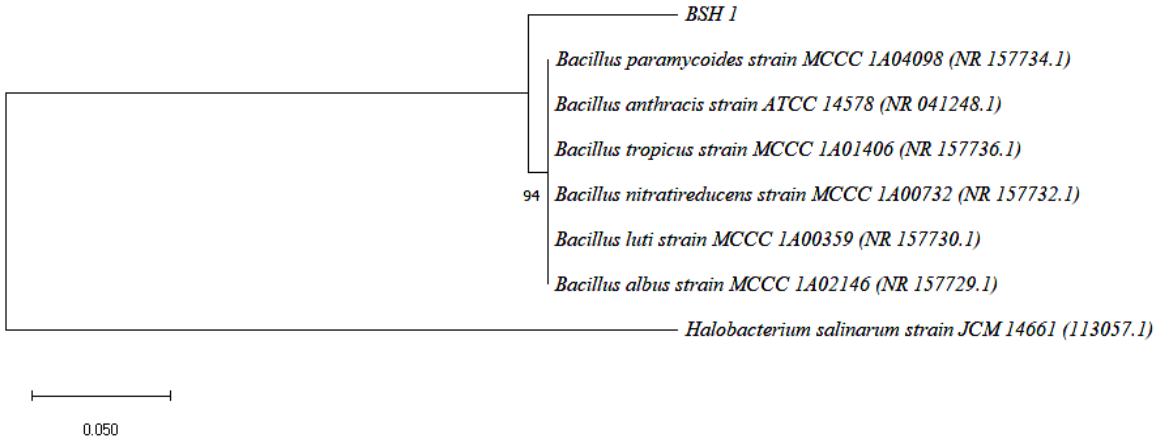

Figure 2. Phylogenetic tree of bacterial sample BSH 1 isolated from *cincalok* made of *Acetes* sp.

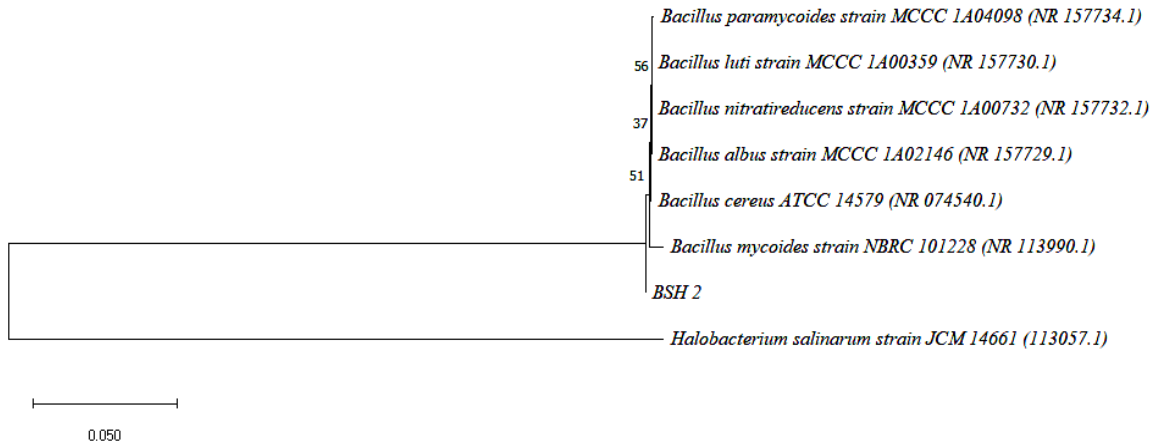

Figure 3. Phylogenetic tree of bacterial sample BSH 2 isolated from *cincalok* made of *Acetes* sp.

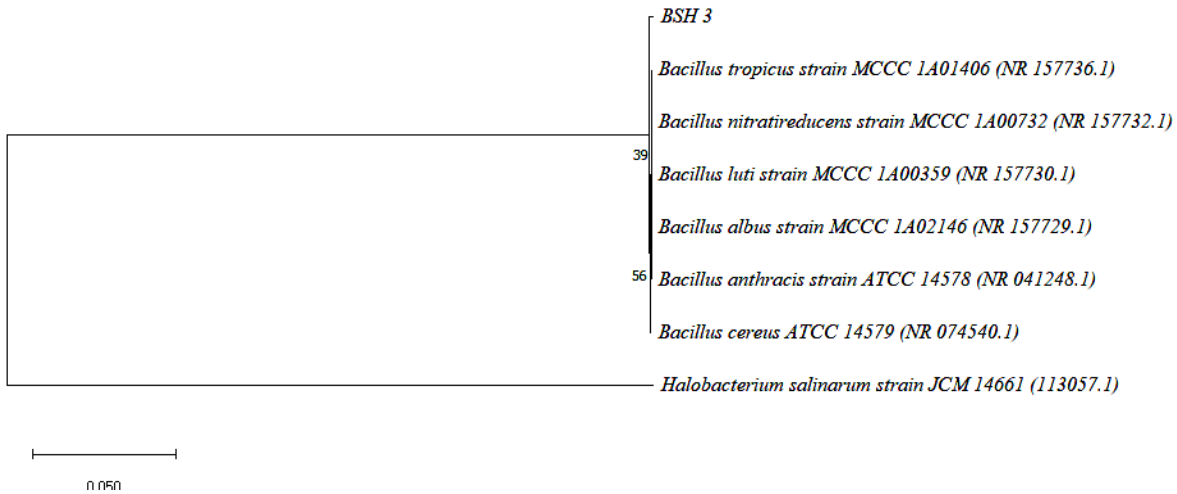

Figure 4. Phylogenetic tree of bacterial sample BSH 3 isolated from *cincalok* made of *Acetes* sp.

The sequences of the 16S rRNA genes for isolates BSH 1, BSH 2, and BSH 3 are as below.

16S rRNA gene sequence of isolate BSH 1

AGCAGGCGGACGGGTGAGTAACGCGTGGGTAACCTGC
CCATAACACTGCATTAAATCCGGGTAACCGGGGCTAATA
CCGGCATAACATTTTGTAACCGCATGGTTCGAATGGCAT
AAGAGGCTTCGGCTGTCACTTATGGATGGAATCGATGCA
CATTAGCTAGCATGGTGAAGTAACGTGCATCACCAAGGC
AACGATGCGTACCATACCTGACAGGGTGATCAGGCACAC
TGGGACTGATACACGGCCCACACTCCTACGGGACGCACC
AGTGGGGAATCTTCGGCAATGGACGAAATGCAGAAGGAT
CAACGCCGCGTGAGTGATGAAAGNGATCAGGGTCGTAAA
ACTCTGTTGTTTACGGNAAGAACAAGTGCTACTTGAACA
AGCTGACCCCCTGACCGTACCTAACCAGAAAGCCACGGC
TAACCACGTGCCTAGCAGCCGCGCTAATACGTAAGTGCC
AAGCGTGATCCGGAATTATTGGGCGTAAAGCGCGCACAC
GAGGTTTCGTAAGTCTGATAGTGATAACGCCCACGGCTC
AACCGCGGAAGGGTCATATGGAAACTGGGAGACTTGAGT
GCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAA
TGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGAC
TTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTT
AGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAG
TACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA
ACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACCA
CCCTAGAGATAGGGCTTTTCCTTCGGGAGCAGAGTGACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGT
TGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATGTTAG
TTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGG
TGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC
ATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGG
GCGGTACAAAGAGTTGCAAGACCGCGAGGTCGAGCTAAT
CTCATAAAGCCTTTCTCAGTTCGGATTGCAGGCTGCAAC
TCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATT
CAAG

16S rRNA gene sequence of isolate BSH 2

CTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAA
CACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGG
GAAACCGGGGCTAATACCGGATAACATTTTGAACCGCAT
GGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACATTAAG
GATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACG
GCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGT
GATCGGCCACACATGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
AAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTT
TCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGC
TAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGA
AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAG
CGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCA
CGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTT
GAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGT
GAAATGCGTAAAGATATGGAGGAACACCAGTGGCGAAGG
CGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG
CCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCC
CTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGG
GGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
AGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTG
ACAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATC
TTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTG
CCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAAT
CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACA
ATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGC
TAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTG
CAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCG
GATTCAGCATG

16S rRNA gene sequence of isolate BSH 3

CTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAA
CACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGG
GAAACCGGGGCTAATACCGGATAACATTTTGAACCGCAT
GGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGG
ATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGG
CTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTG
ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAA
GTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTC
GGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTA
GTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCG
CGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACG
GCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGA
GTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGA
AATGCGTACAGATATGGAAGGAACACCAGTGGCGAAGGC
GACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
CGTAAACGATGAGTGCTAAGTCTTAGAGGGTTTCCGCCC
TTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGG
GAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAA
GCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGA
CAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTG
ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGA
TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCT
TAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGC
CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATC
ATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAA
TGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCT
AATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGC
AACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGG
ATTCAGCAGG

Bacillus paramycoides has demonstrated the capacity to produce antimicrobial compounds capable of inhibiting the growth of various pathogens, including both bacteria and fungi. Specifically, *Bacillus paramycoides* strain MCCC 1A04098 can synthesize selenium oxide nanoparticles (SeONPs), which possess antibacterial, anticancer, and anti-cytotoxic properties. SeONPs exhibit antimicrobial effects against *Bacillus cereus, Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. Additionally, SeONPs have shown anticancer effects on the liver (HepG2) and breast (MCF-7) cancer cell lines. Biogenic SeONPs obtained from *Bacillus paramycoides* strain MCCC

1A04098 demonstrate potential in biomedical applications, particularly in vitro antimicrobial and anticancer activities. They also exhibit antioxidant, anti-hypercholesterolemia, and anti-cytotoxic activities in vivo, offering potential as a promising nutraceutical agent (El Refai et al. 2024). Magnesium oxide nanoparticles (MgO NPs) synthesized by this strain are capable of killing both Gram-positive and Gram-negative bacteria and disrupting biofilm formation, making them potential candidates for in vivo applications such as medical implant coatings (Gomaa et al. 2022).

Bacillus paramycoides strain MCCC 1A04098 can be incorporated into concrete to enhance compressive strength and self-healing of cracks. Ureolytic bacterial strains like this one can be used to protect water structures from frequent crack exposure (Mokhtar et al. 2021). Furthermore, *Bacillus paramycoides* has the potential to be a bioremediation agent that degrades or neutralizes environmental pollutants. It is particularly effective in degrading pesticides like atrazine, which pose significant environmental risks. *Bacillus paramycoides* strain MCCC 1A04098 has demonstrated capabilities for efficient bioremediation of atrazine-contaminated aquatic environments or wastewater (Alattas et al. 2023). This condition is partly attributed to its capability to produce biological hydrogen (Chung et al. 2023).

Sample BSH 2, identified as *Bacillus albus* strain MCCC 1A02146, and BSH 3, identified as *Bacillus cereus* strain IAM 12605, have both shown potential as biocontrol agents for managing plant pathogens, reducing the need for chemical pesticides, and supporting sustainable agriculture (Trinh et al. 2023). Both strains can induce resistance to downy mildew disease caused by *Peronosclerospora philipinensis* (Djaenuddin et al. 2020). *Bacillus albus* is a facultative anaerobic bacterium (Liu et al. 2017); this was also proven because the sample used was the product of fermented shrimp (*cincalok*).

Bacillus albus also has the ability to break down organic or inorganic pollutants in the environment, making it useful in bioremediation processes to clean up waste or pollutants (Kumar et al. 2022). Additionally, it has been reported that *B. albus* strain MCCC 1A02146 and *Bacillus cereus* strain IAM 12605 can be used in conjunction with other rhizobacteria to enhance the exudation of IAA by biofertilizers (Prasad et al. 2023). *B. albus* DM-15 holds potential for further study and exploitation as a carbohydrate polymer in food, cosmetic, pharmaceutical, and biomedical applications (Vinothkanna et al. 2022).

While *Bacillus albus* is not specifically known for producing bacteriocins like some other *Bacillus* species, its ability to produce exopolysaccharides (EPS) with significant flocculation and emulsification activities could contribute to its antimicrobial effects in food systems. For example, the EPS produced by *Bacillus albus* DM-15 has been shown to exhibit antioxidant and anticancer activities, which could indirectly support its use in preserving food by reducing oxidative stress and potentially inhibiting pathogenic microorganisms (Vinothkanna et al. 2022). *Bacillus albus* is known for its ability to tolerate various environmental conditions, including acidic and alkaline pH, which makes it versatile for different food preservation scenarios. This adaptability could help maintain the microbial balance in food products, thereby preserving their quality (Sukmawati et al. 2023).

The strain *Bacillus albus* VKDS9 has been studied for its bioremediation potential, particularly in decolorizing and detoxifying biomethane distillery effluent. This ability to detoxify harmful substances could translate to reducing contaminants in food, thereby enhancing its preservative properties. *Bacillus albus* can be used in the preparation of microbial agents and bio-organic fertilizers. These agents could be formulated to enhance the microbial balance in food products, thereby acting as a biopreservative (Shleeva et al. 2023). While primarily associated with plant growth promotion, *Bacillus albus* LRS2 exhibits plant growth-promoting attributes, including the production of indole-3-acetic acid (IAA), siderophores, and nutrient solubilization. This could indirectly support a healthier microbial environment in food systems (Rajkumar et al. 2024).

Meanwhile, *Bacillus cereus* is not typically considered a biopreservative in the conventional sense. Instead, it is primarily known as a foodborne pathogen that can cause severe gastrointestinal illnesses. However, its unique properties and behaviors make it an interesting microorganism for various applications, including potential uses in biotechnology and environmental remediation. *B. cereus* spores are highly resistant to heat and dehydration, allowing them to survive cooking and dry storage (Navaneethan and Effarizah 2023). *B. cereus* can form biofilms on surfaces such as food processing lines and dairy pipelines, which protects spores and vegetative cells against inactivation by sanitizers (Stenfors et al. 2008). *B. cereus* produces enterotoxins and the emetic toxin (cereulide), which cause gastrointestinal illnesses like vomiting and diarrhea (Ghanyem et al. 2015). *B. cereus* can grow at a wide range of temperatures (4-55°C) and pH levels (6-7), making it adaptable to different environmental conditions (Jessberger et al. 2015). *B. cereus* has been shown to degrade keratin in chicken feathers via hydrolytic mechanisms, indicating its potential for bioremediation. *B. cereus* exhibits antibiotic activity via enzymes like cereins, which impede the quorum sensing ability and exhibit bactericidal activity against Gram-negative bacteria. Its ability to degrade keratin and other organic materials makes it a potential tool for bioremediation projects. Its adaptability to various environments could make it useful in ecological studies and bioremediation efforts. Some harmless strains of *B. cereus* are used as probiotics in animal feed to reduce pathogens like *Salmonella* (Haque et al. 2021).

Bacillus cereus is primarily known for its pathogenic properties rather than its use as a biopreservative. However, its diverse abilities make it a versatile microorganism with potential applications in biotechnology and environmental remediation. Its use in these contexts would require careful consideration of its pathogenic potential and strict control measures to prevent contamination and foodborne illnesses. *Bacillus cereus* DSM 31T DSM is capable of producing bioactive metabolites exhibiting strong antifungal capabilities and generating volatile organic compounds (VOCs) and polyphenols (Abdel-Nasser et al. 2024).

Bacillus cereus strain IAM 12605 has demonstrated the best ability to reduce disease development and promote the

growth and yield of tomatoes (Yanti et al. 2018). *Bacillus cereus* produces secondary metabolites in the form of bacteriocins, which have the potential as antimicrobial agents in the pharmaceutical industry or as biopreservatives (Eze et al. 2019; Wang et al. 2021; Deng et al. 2022). The findings also state that *Bacillus cereus* is also capable of producing bacteriocin, which can be used as a biopreservative (Vaca et al. 2023).

In conclusion, the bacterial samples isolated from *cincalok* made of *Acetes* sp. were identified as *Bacillus paramycoides* strain MCCC 1A04098 BSH 1, *Bacillus albus* strain MCCC 1A02146 BSH 2, and *Bacillus cereus* strain IAM 12605 BSH 3. In the three samples, it can be concluded that these bacteria have the potential as biopreservatives. However, further research is needed to investigate the antimicrobial activity and physiological characteristics of these bacterial strains.

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REFERENCES

Abdel-Nasser A, Badr AN, Fathy HM, Ghareeb MA, Barakat OS, Hathout AS. 2024. Antifungal, antiaflatoxigenic, and cytotoxic properties of bioactive secondary metabolites derived from *Bacillus* species. Sci Rep 14: 16590. DOI: 10.1038/s41598-024-66700-y.

Alattas SG, Zabermawi NM, El Bestawy E. 2023. Biodegradation of atrazine using selected marine bacteria: Possibilities for treating pesticide-contaminated wastewater. J King Saud Univ-Sci 35 (6): 102721. DOI: 10.1016/j.jksus.2023.102721.

Chaudhari AK, Dwivedy AK, Singh VK, Das S, Singh A, Dubey NK. Essential oils and their bioactive compounds as green preservatives against fungal and mycotoxin contamination of food commodities with special reference to their nanoencapsulation. Environ Sci Pollut Res 26: 25414-25431. DOI: 10.1007/s11356-019-05932-2.

Chung HCE, Wee SK, Kansedo J, Lau SY, Lim KH, Dol SS, Lipton AN. 2023. Biological hydrogen energy production by novel strains *Bacillus paramycoides* and *Cereibacter azotoformans* through dark and photo fermentation. Energies 16: 3807. DOI: 10.3390/en16093807.

Deng S, Liu S, Li X, Liu H, Li F, Liu K, Xin B. 2022. Thuricins: Novel leaderless bacteriocins with potent antimicrobial activity against Gram-positive foodborne pathogens. J Agric Food Chem 70 (32): 9990-9999. DOI: 10.1021/acs.jafc.2c02890.

Dey S, Nagababu BH. 2022. Applications of food color and bio-preservatives in the food and its effect on the human health. Food Chem Adv 1: 100019. DOI: 10.1016/j.focha.2022.100019.

Djaenuddin N, Syafruddin S, Patandjengi B, Kuswinanti T. 2020. Potential tests of plant growth bacteria for the control of *Peronosclerospora philipinensis* in corn. Biodiversitas 21 (8): 3886-3892. DOI: 10.13057/biodiv/d210859.

Dwivedi S, Prajapati P, Vyas N, Malviya S, Kharia A. 2017. A review on food preservation: Methods, harmful effects and better alternatives. Asian J Pharm Pharmacol 3 (6): 193-199.

El Refai HA, Mohamed SI, Naser AFA, Saleh AM, Gomaa SK, Zaki RA, Hamed MA. 2024. Biogenic synthesized SeONPs by *Bacillus paramycoides* as antimicrobial, anticancer, antioxidant, apoptotic and hepatorenal treating agent. Biocatal Agric Biotechnol 57: 103080. DOI: 10.1016/j.bcab.2024.103080.

Eze VC, Maduka N, Ahaotu I, Odu NN. 2019. Microbiological quality and shelf life of pickled African walnut (*Tetracarpidium conophorum*) preserved with lactic and citric acids. Microbiol Res J Intl 26 (1): 1-18. DOI: 10.9734/MRJI/2018/45226.

Flórez-Méndez J, López J. 2022. Food additives: Importance, classification, and adverse reactions in humans. Food Addit Contam 17: 1-31. DOI: 10.1007/978-3-031-17346-2_1.

Ghanyem HR, Mohamed W. 2015. Effect of some preservatives on *Bacillus cereus* isolated from some meat products. Assiut Vet Med J 61 (146): 1-7. DOI: 10.21608/avmj.2015.166650.

Gomaa SK, Zaki RA, El-Behery RR, El-Refai HA. 2022. Eco-friendly biogenic synthesis of metallic MgO nanoparticles as potent antibacterial and antibiofilm. Res J Pharm Technol 15 (1): 63-70. DOI: 10.52711/0974-360X.2022.00012.

Gupta R, Yadav, RK. 2021. Impact of chemical food preservatives on human health. Palarch's J Archaeol Egypt 18 (15): 811-818.

Haque MA, Quan H, Zuo Z, Khan A, Siddique N, He C. 2021. Pathogenicity of feed-borne *Bacillus cereus* and its implication on food safety. Agrobiol Record 3: 1-16. DOI: 10.47278/journal.abr/2020.05.

Jabeen M, Murtaza I, Zafar AM, Aslam N, Hassan SU. 2023. A mini review on food preservatives. Intl J Nat Med Health Sci 2 (4): 6-11.

Jessberger N, Dietrich R, Granum PE, Märtlbauer E. 2020. The *Bacillus cereus* food infection as multifactorial process. Toxins 12 (11): 701. DOI: 10.3390/toxins12110701.

Kamarajan P, Takayuki H, Bibiana M, Yang L, Theodora D, Ayyalusamy R, Francis W, Sunil K, Yvonne K. 2015. Nisin ZP, A Bacteriocin and food preservative, inhibits head and neck cancer tumorigenesis and prolongs survival. PLoS One 10: e0131008. DOI: 10.1371/journal.pone.0131008.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33 (7): 1870-1874. DOI: 10.1093/molbev/msw054.

Kumar V, Agrawal S, Shahi SK, Motghare A, Singh S, Ramamurthy PC. 2022. Bioremediation potential of newly isolated *Bacillus albus* strain VKDS9 for decolourization and detoxification of biomethanated distillery effluent and its metabolites characterization for environmental sustainability. Environ Technol Innov 26: 102260. DOI: 10.1016/j.eti.2021.102260.

Kumari PK, Akhila S, Rao YS, Devi BR. 2019. Alternative to artificial preservatives. Syst Rev Pharm 10: 99-102.

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

Liu Y, Du J, Lai Q, Zeng R, Ye D, Xu J, Shao Z. 2017. Proposal of nine novel species *Bacillus cereus* of the group. Intl Syst Evol Microbiol 67 (8): 2499-2508. DOI: 10.1099/ijsem.0.001821.

Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, Wade WG. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol 64 (2): 795-799. DOI: 10.1128/AEM.64.2.795-799.1998.

Mokhtar G, Ahmed AA EA, Reyad, AM. 2021. The effect of isolated *Bacillus ureolytic* bacteria in improving the bio-healing of concrete cracks. Beni-Suef Univ J Basic Appl Sci 10: 55. DOI: 10.1186/s43088-021-00142-7.

Navaneethan Y, Effarizah ME. 2023. Post-cooking growth and survival of *Bacillus cereus* spores in rice and their enzymatic activities leading to food spoilage potential. Foods 12 (3): 626. DOI: 10.3390/foods12030626.

Ng ZJ, Zarin MA, Lee CK, Tan JS. 2020. Application of bacteriocins in food preservation and infectious disease treatment for humans and livestock: A review. Royal Soc Chem Adv 10 (64): 38937-38964. DOI: 10.1039/D0RA06161A.

Prasad B, Sharma D, Kumar P, Dubey RC. 2023. Biocontrol potential of *Bacillus* spp. for resilient and sustainable agricultural systems. Physiol Mol Plant Pathol 128: 102173. DOI: 10.1016/j.pmpp.2023.102173.

Pringgenies D, Soenardjo N, Setyati WA, Pramesti R. 2024. Secondary metabolites from symbiotic bacteria of seagrass and molluscs as a

reference for natural food preservatives. Trends Sci 21 (5): 7404-7404. DOI: 10.48048/tis.2024.7404.

Rajkumar M, Narayanasamy S, Uthandi S. 2024. A root-associated *Bacillus albus* LRS2 and its metabolites for plant growth promotion and drought stress tolerance in little millet (*Panicum sumatrense* L.). Plant Stress 12: 100446. DOI: 10.1016/j.stress.2024.100446.

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

Shleeva MO, Kondratieva DA, Kaprelyants AS. 2023. *Bacillus licheniformis*: A producer of antimicrobial substances, including Antimycobacterials, which are feasible for medical applications. Pharmaceutics 15: 1893. DOI: 10.3390/pharmaceutics15071893.

Stenfors ALP, Fagerlund A, Granum PE. 2008. From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Rev 32 (4): 579-606. DOI: 10.1111/j.1574-6976.2008.00112.x.

Sukmawati S, Ratna R, Sipriyadi S, Yunita M. 2023. Characterization and molecular identification of bacteria from mackerel bekasam in Sorong City, Southwest Papua Province, Indonesia. Biodiversitas 24 (9): 4967-4977. DOI: 10.13057/biodiv/d240940.

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.

Sulthana R, Archer AC. 2021. Bacteriocin nanoconjugates: Boon to medical and food industry. J Appl Microbiol 131 (3): 1056-1071. DOI: 10.1111/jam.14982.

Surati S. 2020. Bacteriocin, antimicrobial as a new natural food preservative: its potential and challenges. Eruditio: Indones J Food Saf 1 (1): 63-82. DOI: 10.54384/eruditio.v1i1.34.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

Teshome E, Forsido SF, Rupasinghe HP, Olika Keyata E. 2022. Potentials of natural preservatives to enhance food safety and shelf life: A review. Sci World J 23: 2022. DOI: 10.1155/2022/9901018.

Trinh LL, Le Nguyen AM, Nguyen HH. 2023. Root-associated bacteria *Bacillus albus* and *Bacillus proteolyticus* promote the growth of peanut seedlings and protect them from the aflatoxigenic *Aspergillus flavus* CDP2. Biocatal Agric Biotechnol 47: 102582. DOI: 10.1016/j.bcab.2022.102582.

Vaca J, Ortiz A, Sansinenea E. 2023. A study of bacteriocin like substances comparison produced by different species of *Bacillus* related to *B. cereus* group with specific antibacterial activity against foodborne pathogens. Archives Microbiol 205 (1): 13. DOI: 10.1007/s00203-022-03356-0.

Vinothkanna A, Sathiyanarayanan G, Rai AK, Mathivanan K, Saravanan K, Sudharsan K, Sekar S. 2022. Exopolysaccharide produced by probiotic *Bacillus albus* DM-15 isolated from ayurvedic fermented *Dasamoolarishta*: Characterization, antioxidant, and anticancer activities. Front Microbiol 13: 832109. DOI: 10.3389/fmicb.2022.832109.

Wang J, Xu H, Liu S, Song B, Liu H, Li F, Xin B. 2021. Toyoncin, a novel leaderless bacteriocin that is produced by *Bacillus toyonensis* XIN-YC13 and specifically targets *B. cereus* and *Listeria monocytogenes*. Appl Environ Microbiol 87 (12): e00185-21. DOI: 10.1128/AEM.00185-21.

Yanti Y, Warnita, Hamid, Hasmiandy. 2018. Development of selected PGPR consortium to control *Ralstonia syzygii* subsp. *indonesiensis* and promote the growth of tomato. Biodiversitas 19 (6): 2073-2078. DOI: 10.13057/biodiv/d190612.