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APPLICATION OF CRUDE BACTERIOCINS BASED ON LC_{50} VALUES TO PROLONG THE SHELF LIFE OF WHITE SHRIMP

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Abstract

Fishery products, including white shrimp, are highly perishable due to microbial contamination during postharvest handling. To address this, natural preservatives, such as bacteriocins, which are antimicrobial compounds produced by bacteria, including those from fermented shrimp paste, are being explored. This study aimed to evaluate the toxicity and preservative potential of three bacteriocin samples (TRS1, TRS2, and TRS3) using a 24-hour LC_{50} test on *Artemia* sp. and antibacterial activity based on total plate count (TPC) in shrimp during storage. A completely randomized design (CRD) was used. Toxicity levels were assessed using probit analysis with log-linear regression to determine LC_{50} values, and TPC was measured at 3-hour intervals over 24 h. Data were analyzed using the Kruskal-Wallis test and Dunn's post-hoc test. TRS1 exhibited the highest toxicity, with an LC_{50} of 0.22 µL/mL, followed by TRS3 (2.48 µL/mL) and TRS2 (4.40 µL/mL). Despite its higher toxicity, TRS1, along with TRS2, effectively suppressed microbial growth in shrimp, maintaining TPC values below the Indonesian National Standard (5×10^5 CFU/g) throughout 24 h. In contrast, TRS3 maintained TPC below the threshold only up to 12 h, with counts nearing control levels (8×10^6 CFU/g) by 24 h. TRS1 and TRS2 treatments initially showed fluctuating TPC patterns, followed by a sharp decline, possibly due to delayed bacteriocin activation and microbial adaptation; however, the exact reason remains unclear. Overall, TRS1 and TRS2 exhibited strong potential as natural preservatives for extending the shelf life of fresh white shrimp by effectively controlling bacterial growth during storage.

Keywords: food safety, microbe, natural preservative, probit analysis, TPC

Aplikasi Bakteriosin Kasar Berdasarkan Nilai LC_{50} untuk Memperpanjang Daya Simpan Udang Putih

Abstrak

Produk perikanan, khususnya udang putih, mudah mengalami penurunan mutu akibat kontaminasi mikrob pascapanen sehingga diperlukan pengawet alami yang aman dan efektif. Bakteriosin merupakan senyawa antimikrob hasil metabolit bakteri yang berasal dari fermentasi terasi udang rebon dan berpotensi sebagai kandidat bioaktif. Penelitian ini menganalisis toksisitas bakteriosin melalui uji LC_{50} pada *Artemia* sp. selama 24 jam, serta efektivitasnya menekan angka lempeng total (ALT) bakteri pada udang putih segar. Sampel bakteriosin (TRS1, TRS2, TRS3) diuji. Nilai LC_{50} dihitung dengan analisis probit berbasis regresi linear logaritmik, sedangkan efektivitas dihitung dari pengamatan ALT selama 24 jam (interval 3 jam) dan dianalisis dengan uji Kruskal-Wallis dilanjutkan uji Dunn's. Hasil menunjukkan TRS1 memiliki LC_{50} terendah (0,22 µL/mL), diikuti TRS3 (2,48 µL/mL) dan TRS2 (4,40 µL/mL), menandakan TRS1 paling toksik terhadap Artemia sp. Dari sisi efektivitas, TRS1 dan TRS2 mampu mempertahankan ALT di bawah ambang SNI (5×10^5 koloni/g) hingga jam ke-24. TRS3 hanya efektif hingga jam ke-12, lalu ALT meningkat mendekati kontrol (8×10^6 koloni/g pada jam ke-24). Pola ALT TRS1 dan TRS2 berfluktuasi di awal sebelum menurun drastis, menunjukkan adaptasi mikrob dan aktivitas bakteriosin yang optimal setelah jam ke-

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6, hal ini disebabkan oleh aktivasi bakteriosin dan adapatasi mikrob, tetapi belum ada bukti kuat untuk menyatakannya. Kesimpulannya, TRS1 dan TRS2 memiliki potensi terbaik sebagai pengawet alami udang putih segar dengan kemampuan menekan pertumbuhan bakteri secara efektif selama penyimpanan.

Kata kunci: ALT, analisis probit, mikrob, pengawet alami, keamanan pangan

INTRODUCTION

The search for natural antimicrobial agents has gained increasing attention over the past few decades, driven by the rising resistance of microorganisms to synthetic antibiotics and growing concerns over chemical residues in food products and aquaculture systems (Amábile *et al*., 2024). Common chemical preservatives, such as sodium sulfite, sodium nitrite, sodium benzoate, tert-butylhydroquinone (tBHQ), and butylated hydroxyanisole (BHA), have raised public health concerns because of their potential carcinogenicity, genotoxicity, and allergenic effects when consumed over the long term (Kalairaj *et al.,* 2024). One group of bioactive compounds with promising potential is bacteriocins, antimicrobial peptides naturally produced by bacteria, including lactic acid bacteria (LAB) (Hernández *et al.,* 2021). Bacteriocins exhibit specific inhibitory activity against microorganisms, particularly food spoilage and pathogenic bacteria, without harming eukaryotic cells (Banerji *et al.,* 2022). These properties make them ideal candidates for use as natural preservatives in the food industry.

Traditional fermented products, such as *terasi* (fermented shrimp paste made from *Acetes* spp.), are rich in complex microbial ecosystems that remain largely underexplored. *Terasi* is a fermented product of shrimp or fish with the addition of salt or other ingredients as a food flavoring due to its distinctive aroma and taste (Sumardianto *et al*., 2022; Jatmiko *et al*., 2025). *Terasi,* a product of spontaneous fermentation, may harbor bacterial isolates with high biosynthetic capabilities, including bacteriocin production. The raw material, rebon shrimp (*Acetes* spp.), has a high nutrient content that supports the growth of proteolytic microorganisms and LAB during fermentation (Herlina *et al.,* 2024). Several studies have reported that fermented products, including shrimp paste, have the potential to produce bacteriocins with strong antibacterial activity. For example, bacteriocins produced by lactic acid bacteria (LAB) demonstrate inhibitory activity against pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* (Sukmawati *et al.,* 2023). These bacteriocins were stable across a wide range of pH values, temperatures, and NaCl concentrations, indicating their robustness as biopreservatives.

Similarly, Herlina *et al.* (2024) reported that Indonesian traditional shrimp paste (*terasi*) produced LAB-derived bacteriocins that effectively inhibited *E. coli*, *Vibrio parahaemolyticus*, and *S. aureus*. Elayaraja *et al.* (2014) isolated *Virgibacillus salexigens* from fermented shrimp paste, which produced stable bacteriocins with strong antibacterial activity. Consistent with previous findings, *Virgibacillus salexigens* isolated from fermented shrimp paste (*terasi*) also produces heat- and pH-stable bacteriocins with broad-spectrum antibacterial activity, notably against *Listeria monocytogenes* (Kobayashi *et al.,* 2016). These findings confirm that fermented seafood products, such as *rebon* shrimp paste, are rich sources of bacteriocin-producing bacteria. Therefore, bacteriocins derived from fermented products possess significant antibacterial potential and can be further developed as natural food preservatives to extend the shelf life and maintain the quality of food products. A nutrient-rich environment creates optimal conditions for the synthesis of various bioactive compounds (Chai *et al.,* 2020).

In the context of seafood, particularly white shrimp (*Litopenaeus vannamei*), post-harvest quality degradation is a major concern, especially when handling and storage are inadequate (Berty, 2020). The primary factors contributing to spoilage include: a) endogenous enzyme activity (autolysis) – upon death, natural enzymes such as proteases begin breaking down muscle tissue, leading to softening and structural damage

that accelerate spoilage (Das *et al.,* 2023); b) microbial spoilage – spoilage bacteria such as *Pseudomonas* and other Gram-negative species proliferate rapidly under moist, room-temperature conditions, producing off-odors such as trimethylamine (TMA), ammonia, and sulfur compounds (Luqman *et al.,* 2024); c) improper storage temperature – suboptimal temperature accelerates microbial growth and enzymatic reactions. Even under cool conditions without ice, shrimp quality declines significantly within 24–48 h (Gökoğlu, 2021). d) Lipid oxidation: Although the fat content in shrimp is low, existing lipids are prone to oxidation, generating rancid odors and degrading sensory quality (Feng *et al.,* 2021); and e) cross-contamination: Poor hygiene during harvesting, processing, or transportation may introduce microbial contaminants that accelerate product spoilage (Akinsemolu *et al.,* 2024).

Shrimp spoilage is commonly characterized by abnormal discoloration (e.g., grayish or reddish tints), strong offensive odor, excessive surface slime, and soft, non-elastic texture (Whittle *et al.,* 2002). Given the health risks associated with synthetic preservatives and the growing need to preserve seafood quality, exploring safer and more environmentally friendly alternatives, such as bacteriocins, is critical. Bacteriocins offer several advantages: they are natural and safe for use in seafood products, do not alter the taste or aroma when used at optimal concentrations, and can be applied via spraying, immersion, or coating. Moreover, they are effective when combined with cold storage using a hurdle technology approach (Lahiri *et al.,* 2022). Bacteriocins can extend shelf life, control foodborne pathogens, and contribute to public health (Parada *et al.,* 2025). Their environmentally friendly nature and favorable safety profiles position them as promising candidates for future food preservation, with minimal adverse effects on humans or animals (Madoramae *et al.,* 2025). These compounds also demonstrate significant thermal stability, maintain antimicrobial activity in the acidic to neutral pH range, and are effective against *Salmonella typhimurium* (Makhlouf *et al.,* 2024).

Previous research successfully isolated and identified three bacteriocin-producing bacteria from shrimp paste: TRS1 (*Bacillus tropicus* strain MCCC 1A01406), TRS2 (*Bacillus nitratireducens* strain MCCC 1A00732), and TRS3 (*Bacillus paramycoides* strain MCCC 1A04098). These isolates produced bacteriocins with varying antimicrobial potencies, as reflected by their minimum inhibitory concentration (MIC) values, which ranged from 0.19% to 0.78% (Sukmawati *et al*., 2025). Based on this background, this study aimed to: (1) characterize bacteriocins produced by bacterial isolates from *terasi udang rebon* by determining their LC_{50} values to assess their relative toxicity against *Artemia* sp.; and (2) evaluate the efficacy of bacteriocins as a natural preservative on white shrimp based on LC_{50} findings

Artemia sp. (brine shrimp) is widely recognized as a reliable model organism for the preliminary toxicity testing of bioactive compounds, including bacteriocins and bacteriocin-like inhibitory substances. Its high sensitivity, short life cycle, low maintenance cost, and lack of ethical restrictions make it suitable for determining LC_{50} values in ecotoxicological assessments. Previous studies have demonstrated that *Artemia salina* exhibits consistent and quantifiable responses to both natural and synthetic toxicants, making it an efficient bioindicator for evaluating environmental and food safety (Chan *et al.,* 2021). Recent studies have confirmed the validity of this method for assessing the toxicity of marine natural products and microbial metabolites (Chan *et al.,* 2021; Mahmud *et al.,* 2023).

MATERIALS AND METHODS

Time and Location of the Study

This study was conducted from September 2024 to January 2025 at the Microbiology Laboratory of the University of Muhammadiyah Sorong, Indonesia. The samples used were bacteriocins produced by bacterial isolates obtained from *terasi udang rebon*, a traditional fermented shrimp paste produced at a household industry scale in Southwest Papua, Indonesia. The bacterial

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strains used in this study were previously identified as *Bacillus tropicus* strain MCCC 1A01406 TRS1, *Bacillus nitratireducens* strain MCCC 1A00732 TRS2, and *Bacillus paramycoides* strain MCCC 1A04098 TRS3 (Sukmawati *et al.,* 2025). White shrimp (*L. vannamei*) used for bacteriocin application were sourced from South Sorong through PT UD Piala in Sorong City.

Bacteriocin Production

Each bacteriocin-producing bacterial isolate was inoculated into 500 mL of MRS Broth (Perez *et al.,* 2022), supplemented with 0.5% $CaCO_3$ (Ooi *et al*., 2015), and incubated at 28°C for 24 h. After incubation, the culture was centrifuged at 4,500 rpm for 15 min to separate the supernatant from the bacterial pellet. The supernatant was then filtered through a 0.22 µm Millipore membrane filter to obtain a crude cell-free bacteriocin extract (Hoover & Steenson, 2014). The presence of bacteriocin in the supernatant was supported by its characteristic stability under heat treatment, varying concentrations of NaCl, and a wide pH range, consistent with findings reported in previous studies. This evidence strongly suggests that the observed antimicrobial activity is attributable to bacteriocins rather than other compounds (Sukmawati *et al.,* 2022).

LC_{50} Toxicity Test

The lethal concentration for 50% mortality (LC_{50}) was determined using a 24-hour exposure assay on 2-day-old *Artemia* sp. larvae. Ten larvae were used per replicate for each concentration of the test substance. Toxicity was evaluated using probit analysis, applying the regression equation $y = ax + b$. Probit analysis determines the relative toxicity of a substance based on the relationship between the logarithm of the concentration and the mortality percentage (expressed in probit units) of the test organism, which follows a linear function (de Almieda *et al.,* 2023). The LC_{50} value was calculated from the mortality data using Microsoft Excel, probit values derived from standard probit tables (Raj *et al*., 2013).

Application of Bacteriocin as a Natural Preservative on White Shrimp

Bacteriocin application was based on the LC_{50} evaluation results. Crude bacteriocin samples with potential as natural preservatives were applied to fresh white shrimp. Shrimp samples (550 g) were immersed in bacteriocin solution (e.g., TRS1 at 158.4 µL/720 mL) for 10 min to allow diffusion into the shrimp tissue and inhibit microbial activity (Franciska *et al*., 2018). After immersion, the samples were drained to remove excess filtrate, sealed in sterile packaging, and stored at room temperature (28°C) for 24 h. Microbial contamination on the shrimp surface was monitored every 3 h (at 11:00, 14:00, 17:00, 20:00, 23:00, 02:00, 05:00, and 08:00) using the total plate count (TPC) method according to the Indonesian National Standard (SNI, 2008). Each treatment was performed in triplicates. The same procedure was applied to TRS2 (3168 µL/720 mL), TRS3 (1785 µL/720 mL) bacteriocin samples, and control as well as to the control. The control treatment consisted of white shrimp samples processed under identical conditions but without the addition of crude bacteriocins.

Total Plate Count (TPC) Analysis

Shrimp samples (25 g) were aseptically placed in sterile containers. Each sample was then mixed with 225 mL of sterile buffered peptone water (BPW) 0.1% in a sterile stomacher bag and homogenized using a stomacher for approximately one–two minutes. This initial mixture represented a 10^{-1} dilution of the sample. Subsequently, 1 mL of the homogenate was aseptically transferred into a test tube containing 9 mL of sterile BPW to obtain a 10^{-2} dilution. Further serial dilutions were prepared up to 10^{-3}, depending on the required analysis range.

From each dilution, 1 mL of suspension was pipetted into sterile Petri dishes in duplicate. Approximately 15–20 mL of plate count agar (PCA), which had been cooled to 45±1°C, was poured into each dish containing the suspension. The Petri dishes were then gently rotated in a forward–backward motion or a figure-eight pattern to ensure that the

sample and medium were thoroughly mixed. The agar was then allowed to solidify. The solidified plates were incubated in an inverted position at a temperature of 34–36°C for 24 to 48 hours. After incubation, the number of colonies formed on each dilution plate was counted, excluding the plates with spreader colonies. The results were recorded, and the TPC value was marked with an asterisk to indicate that the count was obtained outside the standard counting range (SNI, 2008).

Data Analysis

Data for the LC_{50} values were analyzed descriptively. To assess differences in the effectiveness of bacteriocins in preserving white shrimp, the Kruskal-Wallis test at a 95% confidence level ($\alpha = 0.05$) was used with the help of SPSS software. When significant differences were detected, Dunn's post-hoc test was applied to identify the treatment groups that differed.

RESULTS AND DISCUSSION

The 24-hour LC_{50} toxicity test of bacteriocins produced by TRS1 (*Bacillus tropicus* strain MCCC 1A01406), TRS2 (*Bacillus nitratireducens* strain MCCC 1A00732), and TRS3 (*Bacillus paramycoides* strain MCCC 1A04098) revealed distinct LC_{50} values, representing the lethal concentration of bacteriocins required to cause 50% mortality in the test organisms within a 24-hour period (Table 1). The bioassay was performed using *Artemia* sp. as the test organism, which is recognized as a reliable biological model for the preliminary toxicity evaluation of bioactive compounds. This organism was selected because of its high sensitivity and consistent response to a wide range of natural and synthetic toxic substances. This parameter is commonly used to assess the acute toxicity of bioactive compounds and provides a quantitative measure of their safety for potential applications in food and environmental systems.

Regression analysis indicated that the LC_{50} value of the bacteriocins was concentration-dependent and followed a linear relationship. The coefficient of determination (R^2), which indicates the goodness of fit of the linear regression model between the logarithm of concentration and probit values, the higher the R^2 value, the more accurate and reliable the LC_{50} is (Figure 1; Figure 2; Figure 3).

The 24-hour LC_{50} toxicity assay of crude bacteriocins produced by TRS1 (*Bacillus tropicus* strain MCCC 1A01406), TRS2 (*Bacillus nitratireducens* strain MCCC 1A00732), and TRS3 (*Bacillus paramycoides* strain MCCC 1A04098) was conducted to evaluate their relative toxicity toward *Artemia* sp.. The LC_{50} value represents the lethal concentration of bacteriocins required to cause 50% mortality in the test organisms within a 24-hour exposure period, serving as an important parameter for assessing the safety of bioactive compounds. The results revealed distinct LC_{50} values among the three isolates, indicating differences in their bioactivities and toxicological profiles. Bacteriocins from TRS1 exhibited the lowest LC_{50} value, suggesting stronger biological potency with relatively low acute toxicity, whereas TRS2 and TRS3 showed higher LC_{50} values, indicating lower toxicities. These variations are likely associated with differences in the structural and functional characteristics of the bacteriocins produced by each Bacillus species. Following the LC_{50} assessment, the antibacterial effectiveness of

Table 1 LC_{50} values of bacteriocin samples

Bacteriocin sample type	LC_{50} 24-hour value (µL/mL)
TRS1	0.22
TRS2	4.40
TRS3	2.48

TRS1 = bacteriocin produced by *Bacillus tropicus* strain MCCC 1A01406; TRS2 = *Bacillus nitratireducens* strain MCCC 1A00732; TRS3 = *Bacillus paramycoides* strain MCCC 1A04098.

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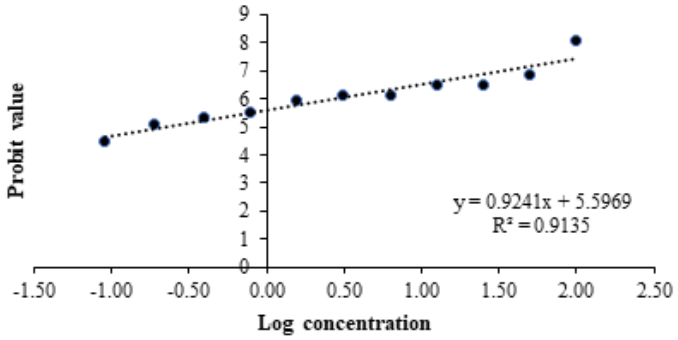

Figure 1 Regression between log concentration and probit value of LC_{50} (24 h) for TRS1 bacteriocin

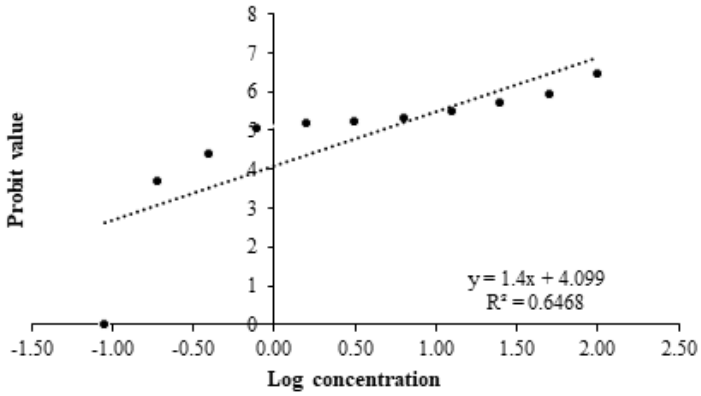

Figure 2 Regression between log concentration and probit value of LC_{50} (24 h) for TRS2 bacteriocin.

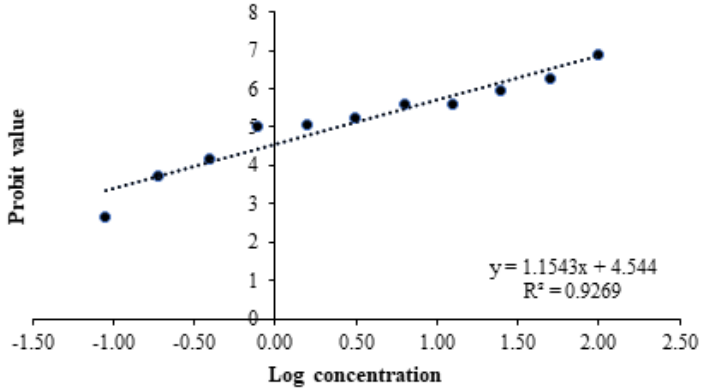

Figure 3 Regression between log concentration and probit value of LC_{50} (24 h) for TRS3 bacteriocin

TRS1, TRS2, and TRS3 was further evaluated by testing their ability to inhibit spoilage bacteria in white shrimp (*L. vannamei*). Fresh shrimp samples were treated with bacteriocins based on their respective LC_{50} concentrations, ensuring safe yet effective exposure levels.

The results demonstrated that bacteriocin-treated shrimp exhibited a slower increase in total bacterial count than the untreated control, indicating that bacteriocins effectively suppressed the proliferation of spoilage-associated microorganisms. Among the treatments, TRS1 showed the most pronounced inhibitory effect, consistent with its stronger antimicrobial potency observed during the minimum inhibitory concentration (MIC) test. These findings confirmed that bacteriocins derived from *Bacillus* isolated from fermented shrimp paste could prolong the shelf life of white shrimp by inhibiting

spoilage-associated microorganisms, thereby maintaining product freshness and safety (Sukmawati *et al.,* 2025). The results showed significant bacterial suppression at various time points over a 24-hour observation period (Table 2).

The total bacterial count (TBC) analysis revealed distinct inhibitory effects of each bacteriocin treatment on microbial growth in white shrimp during 24 h of storage (Table 2). Among the treatments, the TRS1 bacteriocin exhibited the most pronounced antimicrobial activity, maintaining bacterial counts well below the SNI threshold (5.0×10^5 CFU/g) throughout the observation period, with only a modest increase from 4.4×10^4 CFU/g at hour 3 to 1.3×10^5 CFU/g at hour 24. Similarly, the TRS2 bacteriocin treatment demonstrated effective bacterial suppression, although slightly higher counts were recorded compared to TRS1, reaching 1.5×10^5 CFU/g at hour 24. In contrast, the TRS3 bacteriocin-treated samples exhibited a steady increase in TBC from 7.9×10^4 CFU/g at hour 3 to 8.0×10^5 CFU/g at 24 h. Although TRS3 initially inhibited microbial growth, maintaining bacterial levels below the SNI limit until hour 12-its overall antimicrobial performance was less effective than that of TRS1 and TRS2. Meanwhile, the untreated control group showed exponential microbial proliferation, with bacterial counts exceeding 3.1×10^5 CFU/g as early as hour 3 and reaching 8.0×10^6 CFU/g by hour 24, far surpassing the acceptable microbial limit. Based on the Kruskal-Wallis test (Table 3), a significant difference ($p<0.001$) was observed among the bacteriocin treatment groups, indicating strong statistical evidence to reject the null hypothesis. These findings confirmed that the three bacteriocins (TRS1, TRS2, and TRS3) had significantly different effects on bacterial growth inhibition, with TRS1 showing the highest preservative efficacy in maintaining shrimp quality during storage. This finding is supported by the data patterns presented in Table 3.

Owing to the significant difference, a follow-up analysis using Dunn's post hoc test was conducted. The results indicated that all bacteriocin treatments (TRS1, TRS2, and TRS3) showed significant differences compared with the control group, confirming the effectiveness of bacteriocin application. However, no significant differences were observed between TRS1 and TRS2 or between TRS2 and TRS3, suggesting that these treatments elicited relatively similar responses in the plants. Significant differences were primarily evident between TRS1 and TRS3, as well as consistently across all treatments compared to the control, highlighting the variation in effects among treatments. These findings suggest that while all bacteriocin

Table 2 Total bacterial counts (TBC) in white shrimp preserved with bacteriocins

Observation time (h)	Mean of total bacterial count (CFU/g)			Control	SNI[a]
	TRS1	TRS2	TRS3		
3	4.4×10^4	9.0×10^4	7.9×10^4	3.1×10^5	5.0×10^5
6	1.2×10^5	1.3×10^5	9.8×10^4	6.2×10^5	
9	1.0×10^5	1.2×10^5	1.0×10^5	7.4×10^5	
12	9.1×104	1.1×10^5	3.4×10^5	1.7×10^6	
15	5.1×104	8.7×10^4	6.2×10^5	2.6×10^6	
18	6.8×104	1.1×10^5	6.5×10^5	4.1×10^6	
21	1.2×10^5	1.3×10^5	7.2×10^5	6.1×10^6	
24	1.3×10^5	1.5×10^5	8.0×10^5	8.0×10^6	

[a]SNI (Indonesian National Standard)

TRS1 = bacteriocin produced by *Bacillus tropicus* strain MCCC 1A01406;

TRS2 = *Bacillus nitratireducens* strain MCCC 1A00732;

TRS3 = *Bacillus paramycoides* strain MCCC 1A04098.

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Table 3 Results of Kruskal-Wallis test on the effectiveness of bacteriocin treatments on total bacterial count in pacific white shrimp

Total N	96
Test statistic	57.234[a]
Degree of freedom	3
Asymptotic	<.001

treatments effectively inhibited bacterial growth compared to the untreated control, the degree of inhibition varied depending on the bacterial strain that produced the bacteriocin. TRS1, derived from *Bacillus tropicus*, exhibited the strongest antimicrobial activity, likely due to its lower LC_{50} value and higher potency, as observed in previous toxicity and MIC assessments. In contrast, TRS3, produced by *Bacillus paramycoides*, showed weaker inhibition, possibly due to differences in peptide structure, molecular weight, or stability under ambient conditions. The absence of significant differences between TRS1 and TRS2 indicates that the bacteriocins produced by *Bacillus tropicus* and *Bacillus nitratireducens* may share similar functional characteristics or antimicrobial mechanisms. Overall, the statistical results support the hypothesis that bacteriocins can serve as effective natural preservatives by suppressing spoilage bacteria and maintaining shrimp quality during storage.

The Kruskal-Wallis test on the TRS1 of bacteriocin effectiveness based on observation time (every 3 h until 24 h) showed a significant difference ($p = 0.042$) (Table 4).

The results of the analysis indicated that bacteriocin significantly affected bacterial colony counts across different time points. That is, TRS1 was effective in inhibiting bacterial growth at 3, 6, 9, 12, 15, 18, 21, and 24 h. However, no differences were observed among the time points of observation, as shown by Dunn's post hoc test.

The results of Dunn's post hoc test on the effectiveness of bacteriocin TRS1 in *Pacific white shrimp* across different observation times showed that most pairwise comparisons were not statistically significant. Although some differences were observed at the early observation points, particularly between 3 and 6, 9, 21, and 24 h, as well as between 15 and 21 and 24 h ($p < 0.05$), these results did not remain significant after adjustment (Adj. Sig. > 0.05). These findings indicate that the effectiveness of bacteriocin TRS1 remained relatively stable throughout the observation period, with variations across time points not strong enough to be considered statistically significant.

The Kruskal-Wallis test results showed that the effectiveness of the TRS2 bacteriocin was similar across the time points

Table 4 Kruskal-Wallis test on the effectiveness of bacteriocin TRS1, TRS2, and TRS3 in pacific white shrimp across observation time

	Type of bacteriocin		
	TRS	TRS2	TRS3
Total N	24	24	24
Test statistic	14.59[a]	13.11[ab]	22.20[a]
Degree of freedom	7	7	7
Asymptotic	<.042	.070	.002

TRS1 = bacteriocin produced by *Bacillus tropicus* strain MCCC 1A01406;
TRS2 = *Bacillus nitratireducens* strain MCCC 1A00732;
TRS3 = *Bacillus paramycoides* strain MCCC 1A04098.

of observation (every 3 h until 24 h) (Table 4). Meanwhile, TRS3 bacteriocin showed differences in its effectiveness across the time points of observation (Table 4). However, the differences existed only between hours 3 and 23 and hours 3 and 24, as indicated by Dunn's post hoc test.

The results of Dunn's post hoc test indicated that the differences in the effectiveness of TRS3 bacteriocin across observation times were largely not significant after adjustment (Adj. Sig. >0.05). However, significant differences were observed at certain time comparisons, particularly between 3 and 21 h ($p = 0.042$) and between 3 and 24 h ($p = 0.010$). This finding suggests that the effectiveness of TRS3 undergoes a notable change during the early observation period (3 h) compared to later periods (21–24 h).

Overall, although some variation in effectiveness was observed across different times, most differences were not statistically significant after correction, suggesting that the activity of TRS3 bacteriocin remained relatively stable throughout the observation period, with significant changes only evident between the earliest and latest time points.

TRS1 bacteriocin showed an LC_{50} value of 0.22 µL/mL against *Artemia*, indicating a very high level of toxicity. Meanwhile, TRS2 and TRS3 had LC_{50} values of 4.40 and 2.48 µL/mL, respectively, which also fall into the highly toxic category, although they are less toxic than TRS1. These LC_{50} values are classified as highly toxic based on toxicity categories, where compounds with LC_{50} values <100 µL/mL are considered highly toxic; 100–500 µL/mL toxic; 500–1,000 µL/mL moderately toxic; 1,000–2,500 µL/mL slightly toxic; and >2,500 µL/mL are regarded as practically non-toxic (Meyer *et al.,* 1982; USEPA, 2022; Pohan *et al.,* 2023).

Among the tested bacteriocins, TRS1 (produced by *B. tropicus*) exhibited the lowest concentration (0.22 µL/mL), indicating the highest toxicity to *Artemia* sp. In contrast, the bacteriocins of TRS2 and TRS3 had much higher LC_{80} values (4.40 and 2.48 µL/mL, respectively). These LC_{50} findings are critical for the development of bacteriocins as natural preservatives in fishery products, particularly shrimp products. Ideally, bacteriocins used for preservation should exhibit high toxicity against microorganisms to enable the use of very low concentrations but with high effectiveness. The three bacteriocins isolated from the shrimp paste in this study are promising candidates for further applications, either as antimicrobial coatings or as preservatives/additives in shrimp processing.

The observed toxicity was much higher than that reported in previous studies. For example, bacteriocin from *Lactobacillus plantarum* exhibited inhibitory activity against *Listeria monocytogenes,* with a minimum inhibitory concentration (MIC) ranging from 2–4 µL/mL when applied to processed meat products (Sobrino-Lopez & Martin-Belloso, 2008). Meanwhile, a liquid filtrate containing bacteriocin-like substances from *Bacillus subtilis* inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at concentrations of 1–3 µL/mL (Wahyudi *et al.,* 2010). Enterocin, a bacteriocin produced by *Enterococcus faecium*, showed an LC_{50} value against *Artemia salina* in the range of 5–10 µL/mL (Delgado *et al.,* 2007). Bacteriocins from *Streptomyces* isolated from the gut of milkfish (*Chanos chanos*) demonstrated extracellular metabolite activity against *Salmonella enterica* serovar Typhimurium, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*, with an LC_{50} value of 0.227 µL/mL (Kurnianto *et al.,* 2021). Bacteriocin P7 from *Bacillus velezensis*, isolated from a mangrove endophyte, has an MIC value of 30.35 µL/mL (Li *et al.,* 2025). Another study found that a crude extract from *Bacillus velezensis* strain PD9 was effective against MRSA, with an MIC value of 125 µL/mL (Baharuddin *et al*., 2021). Bacteriocins produced by *Lactococcus* spp.. HY449 inhibited the growth of *S. epidermidis* ATCC 12228, *S. aureus* ATCC 65389, *Streptococcus. pyogenes* ATCC 21059, and *P. acnes* ATCC 6919, with an estimated LC_{50} of approximately 50 mg/mL (Oh *et al.,* 2006).

Following the LC_{50} assessment, the effectiveness of bacteriocins isolated from TRS1, TRS2, and TRS3 in inhibiting spoilage bacteria in white shrimp was further evaluated. This involved applying bacteriocins to shrimp and monitoring the total bacterial

count (TBC) at regular intervals. The results showed significant effectiveness in bacterial suppression at various time points over a 24-hour observation period (Table 2). TRS1 demonstrated the highest effectiveness in inhibiting bacterial growth. Even at the 24-hour mark, the TBC remained relatively low (1.3×10^5 CFU/g), far below both the control (8.0×10^6 CFU/g) and the Indonesian National Standard (SNI) threshold (5.0×10^5 CFU/g). Bacteriocins from TRS2 and TRS3 also exhibited inhibitory effects. At 24 h, the TBC of TRS2 bacteriocin reached 1.5×10^5 CFU/g, whereas that of TRS3 exceeded the SNI limit with a TBC of 8.0×10^5 CFU/g. In contrast, the control group showed a sharp increase in TBC from 3.1×10^5 CFU/g at 3 h to 8.0×10^6 CFU/g at 24 h, illustrating rapid microbial growth in the absence of bacteriocins. Only TRS1 and TRS2 bacteriocins maintained TBC values below the SNI threshold throughout the 24-hour period. The TRS3 was effective only up to 12 h, after which the TBC values surpassed the permitted maximum limit.

During the 24-hour observation period, the TRS1 and TRS2 bacteriocin-treated shrimps showed fluctuations in their total bacterial counts. Initially, there was an increase in TBC from 4.4×10^4 and 9.0×10^4 CFU/g, respectively, at hour 3 to 1.2×10^5 and 1.3×10^5 CFU/g, respectively, at hour 6. This early increase may be attributed to the adaptive phase of microorganisms adjusting to the bacteriocin environment (Negash & Tsehai, 2020), during which not all microbes are immediately inhibited (Todorov *et al.,* 2022). The diffusion and penetration times required for bacteriocins to act within shrimp tissues may also influence their initial effectiveness (Silva et al., 2018). From hours 6 to 15, a gradual decline in TBC was observed, indicating optimal antimicrobial activity. Bacteriocins likely disrupt bacterial cell membranes, interfere with metabolic processes, and ultimately lead to cell death (Sharma *et al.,* 2021; Soltani *et al.,* 2021). Interestingly, a rebound in bacterial growth occurred from 18 to 24 h, possibly due to the development of microbial resistance or regrowth from previously suppressed or dormant populations (Telhig *et al.,* 2020).

In contrast, the TRS3 bacteriocin-treated samples exhibited a steady increase in TBC from 7.9×10^4 CFU/g at hour 3 to 8.0×10^5 CFU/g at hour 24. Although TRS3 showed some early inhibition (with TBC under the SNI threshold at hour 12), its overall antimicrobial performance was less effective than that of TRS1 and TRS2. The untreated control group showed exponential microbial growth, confirming the necessity of antimicrobial interventions for shrimp preservation.

CONCLUSION

This study concluded that crude bacteriocins TRS1, TRS2, and TRS3 exhibited 24-hour LC_{50} values of 0.22, 4.40, and 2.48 µL/mL, respectively, indicating that all three were highly toxic to *Artemia* sp. larvae based on standard toxicity categories. In terms of application, crude bacteriocins of TRS1 and TRS2 effectively inhibited microbial growth in fresh Pacific white shrimp over a 24-hour storage period, with total bacterial counts (TBC) consistently remaining below the SNI threshold. In contrast, the TRS3 crude bacteriocin effectively suppressed microbial growth only up to 12 h, after which the TPC exceeded the acceptable limit. Therefore, TRS1 and TRS2 are the most promising candidates for use as natural preservatives in white shrimp, offering superior microbial inhibition during storage. On the other hand, the TRS3 crude bacteriocin might require a higher concentration to sustain long-term efficacy, which requires further analysis.

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