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The potential of antagonistic bacteria from shrimp paste as inhibitors of spoilage bacteria in fishery products

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ABSTRACT

Spoilage bacteria present a considerable obstacle in the preservation of seafood items, including shrimp and fish, leading to economic losses, health hazards, and degradation of product quality. Prevalent spoilage bacteria in seafood comprise *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. This study seeks to assess the inhibitory potential of bacterial isolates obtained from shrimp paste against rotting spoilage bacteria. A descriptive approach was employed to assess the antibacterial activity of four bacterial isolates—designated TRS 1, TRS 2, TRS 3, and TRS 4—against Gram-positive and Gram-negative spoilage bacteria. The inhibitory effect was measured by the diameter of the clear zones surrounding the isolates. The findings indicated that isolate TRS 1 exhibited the most significant inhibition against *Staphylococcus aureus* (19.0 mm) and *Klebsiella pneumoniae* (9.5 mm), whereas isolate TRS 4 displayed the greatest inhibition against *Escherichia coli* (12.0 mm). The findings indicate that some bacterial isolates from shrimp paste exhibit significant antagonistic activity and may function as natural preservatives in seafood products. Additional research is required to validate bacteriocin synthesis by molecular and biochemical techniques and to assess their practical implications in seafood preservation.

Conflict of interest: The authors declare that they have no conflicts of interest.

Introduction

The food industry has a very important role in providing safe and quality products for consumers. However, a significant issue this industry faces is the spread of spoilage microorganisms, which can lead to product damage and consumer health problems. Growing consciousness of the significance of food safety has prompted extensive research to create novel approaches for managing spoilage and pathogenic microorganism in food products.

The problem with the growth of spoilage bacteria, in many cases, is that pathogenic or spoilage microorganisms can infect or contaminate food products, causing a decrease in quality, damage, or even food poisoning[1]. This is a serious problem in the food industry, as it can cause economic losses and health risks for consumers[2]. So far, chemical antibiotics have been used to control bacterial growth in food products. However, excessive use of antibiotics in the food industry can lead to increased antibiotic resistance and become a serious public health problem[3]. Therefore, other alternatives such as bacteriocins are needed. Bacteriocins are antimicrobial compounds produced by bacteria in response to competition with other species[4]. They have potential as safe and effective microbial controls in food applications, due to their activity against various pathogenic and spoilage bacteria without increasing antibiotic resistance.

Lactobacillus acidophilus is one of the several types of bacteria that are recognized as producers of bacteriocin. This bacterium is a member of the lactic acid bacteria family, which is frequently present in the intestines of humans. They produce a bacteriocin known as acidophilin[5], which prevents the growth of harmful bacteria like *Salmonella* and *Escherichia coli*, hence assisting in the maintenance of the microbial balance in the digestive tract.

Lactococcus lactis produces the bacteriocin known as nisin. It is also a type of lactic acid bacteria commonly used in cheese making and other fermented dairy products. Nisin has broad antimicrobial activity against various types of bacteria[6], including *Staphylococcus aureus* and *Listeria monocytogenes*.

The bacteriocins produced by these bacteria are an example of how microorganisms use these compounds to compete in the microbial environment and provide them with an evolutionary advantage. The above background underlies our previous research regarding the search for sources of bacteriocins, and screening tests for bacteriocin-producing isolates have also been carried out[7]. This current study aimed to evaluate the activity of bacterial isolates as potential sources of bacteriocin production to control the growth of spoilage bacteria in food products. By gaining a better understanding of the bacteriocin potential of selected bacterial isolates, it is hoped that innovative and effective solutions can be found to improve the safety and quality of food products, especially fishery products, in the future.

Method

This study is a descriptive analysis that assesses the antibacterial activity of bacterial isolates derived from shrimp paste. Bacterial isolates were designated as TRS 1, TRS 2, TRS 3, and TRS 4. Bacterial isolates were evaluated against various spoilage bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, representing Gram-negative bacteria, and *Staphylococcus aureus*, representing Gram-positive bacteria.

Procedures

The bacterial isolation technique entails the aseptic collection of shrimp paste samples with sterile instruments, which are subsequently deposited in sterile containers and sent to the laboratory. Approximately 1 gram of the shrimp paste sample was dissolved in 9 mL of physiological saline solution (0.85% NaCl) to create a 10^{-1} dilution. Subsequent serial dilutions were executed by transferring 1 mL from the preceding dilution into 9 mL of physiological saline solution, continuing this process until a 10^{-3} dilution was achieved. From each dilution (10^{-1}, 10^{-2}, and 10^{-3}), 0.1 mL was uniformly distributed on the surface of MRSA (Man, Rogosa, and Sharpe Agar) media. The inoculated media were incubated at 30-37°C for a duration of 24 to 48 hours. Following incubation, the developed colonies were examined, and those displaying distinct morphologies were chosen as potential bacterial isolates. These selected colonies were subsequently subcultured onto fresh media utilizing the streak plate technique to achieve pure isolates. The pure isolates were ultimately preserved on agar slants

or in preservative solutions, such as 20% glycerol, at -20°C for subsequent examination[7]. The bacterial isolates were characterized by several methods to ascertain their features.

This research commenced with the formulation of De Man, Rogosa, and Sharpe (MRS) Broth media to reculture bacterial isolates of TRS 1, TRS 2, TRS 3, and TRS 4. De Man, Rogosa, and Sharpe (MRS) is a standard medium for cultivating lactic acid bacteria (LAB)[8] and is the most appropriate medium for growth and bacteriocin synthesis[9].

One-loop test bacteria were inoculated on MRSB (de Man, Rogosa, Sharpe Broth) media and incubated for 24 hours. The second stage entails the preparation of nutrition broth (NB) media for the recultivation of spoilage bacteria (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*). This is accomplished by inoculating a single dose of each spoilage bacterium into NB media and thereafter incubating for 24 hours. The third stage entails the preparation of MRSA media for an antagonist assay utilizing bacterial isolates TRS 1, TRS 2, TRS 3, and TRS 4 against spoilage bacteria. The fourth stage involves evaluating the bacterial inhibition of the sample against spoilage bacteria via the disc method[10]. 0.1 mL of spoilage bacteria was inoculated onto solid MRSA (Man, Rogosa, and Sharpe Agar) media in petri dishes via the spread plate technique. The inoculum was thereafter distributed uniformly with a hockey stick and allowed to dry. Subsequently, the paper disc, which had been immersed in the bacterial culture for 15 minutes, was aseptically positioned on the media surface and incubated for 24 hours. *Data Analysis*

The exact zone data generated surrounding the disc was computed utilizing the following formula I.

$$Inhibition\ zone\ diameter\ (mm) = Inhibition\ zone\ diameter\ (mm) - paper\ disk\ diameter\ (mm) \quad \text{(I)}$$

Results and Discussion

The results of research evaluating the potential of antagonistic bacteria from shrimp paste as inhibitors of spoilage bacteria and the potential sources of bacteriocin production to control the growth of spoilage bacteria showed that sample TRS 4 had the highest value (12.0 mm) in inhibiting the growth of *Escherichia coli*. In comparison, sample TRS 1 had the highest value in inhibiting *Klebsiella pneumoniae* and *Staphylococcus aureus,* with drag power values of 9.5 mm and 19.0 mm, respectively (Table 1).

Table 1 | Inhibitory activity of metabolite compounds produced by bacterial isolates from shrimp paste against spoilage bacteria

	Inhibition zone of the bacterial sample against spoilage bacteria (mm)					
Sample	***Escherichia coli***	**Inhibition Category**	***Klebsiella pneumoniae***	**Inhibition Category**	***Staphylococcus aureus***	**Inhibition Category**
TRS 1	10.5	Strong	9.5	Moderate	19.0	Strong
TRS 2	9.0	Moderate	9.0	Moderate	10.5	Strong
TRS 3	9.0	Moderate	9.0	Moderate	10.0	Strong
TRS 4	12.0	Strong	7.5	Moderate	14.5	Strong

Based on Table 1, sample TRS 1 exhibited the most significant inhibitory activity against *Staphylococcus aureus*, measuring 19.0 mm, categorized as strong; 10.5 mm against *Escherichia coli*, also classified as strong; and 9.5 mm against *Klebsiella pneumoniae*, categorized as moderate. Sample TRS 2 exhibited the most potent inhibitory activity against *Staphylococcus aureus*, measuring 10.5 mm, classified as strong, and 9.0 mm against *Escherichia coli* and *Klebsiella pneumoniae*, categorized as moderate. Sample TRS 3 had comparable values against *Staphylococcus aureus,* measuring 10.0 mm, categorizing it as strong, and 9.0 mm against *Escherichia coli* and *Klebsiella pneumoniae*, categorizing them as

moderate. Sample TRS 4 exhibited the most significant inhibitory activity against *Staphylococcus aureus* at 14.5 mm, 12.0 mm against *Escherichia coli*, categorized as strong, and 7.5 mm against *Klebsiella pneumoniae,* categorized as moderate.

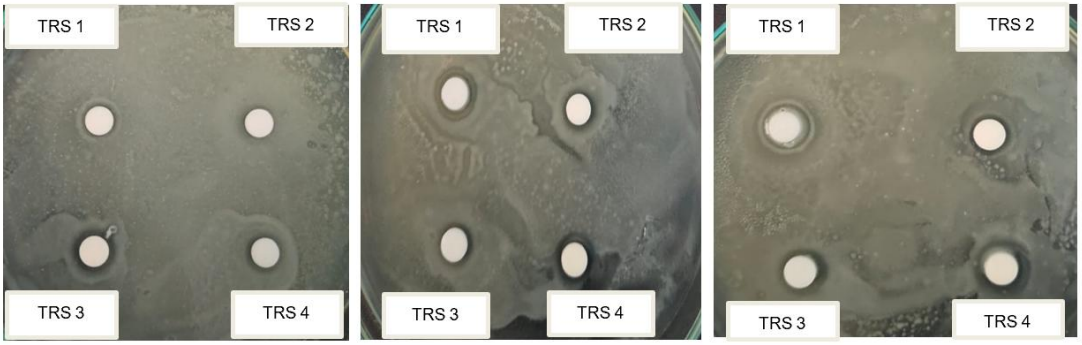

Fig. 1 | Visualization of Inhibitory activity of metabolite compounds produced by bacterial isolates from shrimp paste against spoilage bacteria (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*).

The capacity of bacterial samples derived from shrimp paste to inhibit the proliferation of spoilage bacteria (Fig. 1) is ascribed to the nature of shrimp paste as a fermented food product. Multiple research findings corroborate this outcome. Strains of lactic acid bacteria derived from shrimp intestines possess significant potential for bacteriocin production, which are metabolic chemicals that allow these bacteria to suppress harmful bacteria and foodborne pathogens[11]. Strains of lactic acid bacteria from the genera *Bifidobacterium* and *Lactobacillus*, derived from food, can synthesize bacteriocins or antibacterial proteins that are highly effective against foodborne pathogens, including *Staphylococcus aureus*, *Pseudomonas fluorescens*, *P. aeruginosa, Salmonella typhi, Shigella flexneri, Listeria monocytogenes, Escherichia coli* O157:H7, and *Clostridium botulinum*[12]. Lactic acid bacteria are typically employed as protective starting cultures in food products. These bacteria can produce ribosomally synthesized peptides, termed bacteriocins, which eliminate or inhibit food-spoilage bacteria and pathogens, including *Listeria*[13]. *Lactiplantibacillus pentosus* CF-6HA, derived from traditionally fermented Aloreña table olives, demonstrated the capacity to synthesize bacteriocins exhibiting antibacterial activity against human, animal, and plant pathogens, including *Pseudomonas syringae* and *Verticillium dahliae*[14].

The inhibitory activity of bacterial samples derived from shrimp paste, a prospective source of metabolic components like bacteriocin, against spoilage bacteria indicates that these samples demonstrate significant inhibitory activity against *Staphylococcus aureus.* Only samples TRS 1 and TRS 4 exhibited moderate activity against *Escherichia coli*, whilst samples TRS 2 and TRS 3 also showed medium activity against the same microorganism. Samples TRS 1, TRS 2, TRS 3, TRS 4 exhibited moderate inhibition of *Klebsiella pneumonia* (Table 1; Fig. 1). The findings of this study indicate that the test samples were more efficacious in inhibiting the proliferation of spoilage bacteria from the Gram positive category compared to those from the Gram negative category. This occurs because the cell walls of Gram-positive bacteria do not include a lipopolysaccharide (LPS) structure, while Gram-negative bacteria possess LPS as the principal component of their cell walls. Antimicrobial compounds are typically more efficacious in suppressing the proliferation of Gram-positive bacteria than Gram-negative bacteria, attributable to the disparities in cell wall structure between the two bacterial classifications. Gram-positive bacteria possess comparatively thick cell walls abundant in peptidoglycan, a polymer composed of glycans interconnected by peptides. The cell wall of Gram-negative bacteria is comparatively thinner and comprises a bilayer, with lipopolysaccharide (LPS) situated external to the cell membrane. This distinction

renders the cell walls of Gram-positive bacteria more susceptible to penetration by antimicrobial compounds[15]. The outer membrane of Gram-negative bacteria, consisting of a lipid bilayer of phospholipids and lipopolysaccharides, serves as an extra barrier to antimicrobial compounds. This intricate structure hinders the penetration of antibiotic chemicals, preventing them from accessing the bacterial cytoplasmic membrane[16].

In addition to the intricate outer membrane structure of Gram-negative bacteria, a further distinction is seen in the composition of the membrane phospholipids. Gram-positive bacteria possess a higher concentration of long-chain fatty acids in their phospholipid membranes, which may affect membrane permeability to antimicrobial compounds. This indicates that antimicrobial compounds can more readily infiltrate the membranes of Gram-positive bacteria in contrast to those of Gram-negative bacteria[17]. Gram-negative bacteria typically contain enzymes, such as beta-lactamase, that can neutralize several antimicrobial compounds, including beta-lactam antibiotics[18]. This presents a barrier to the efficacy of antimicrobial compounds against Gram-negative bacteria.

Certain Gram-negative bacteria exhibit supplementary resistance mechanisms, including modifications in membrane porins and the formation of efflux pumps that expel antimicrobial compounds from the cell[19]. Gram-negative bacteria frequently possess a greater number of genes that regulate the production of membrane proteins. This can affect the quantity and variety of porins synthesized by bacteria, as well as alter the sensitivity to antimicrobial compounds. Variability in gene expression can result in differences in sensitivity to antimicrobial compounds among species or even within the same strain of Gram-negative bacteria. This genetic variation can enhance the resistance of Gram-negative bacteria to numerous antimicrobial compounds[20]. The molecular size is a determinant that affects the activity of an antimicrobial compound. Certain antimicrobial compounds possess greater molecular sizes or intricate structures. They may encounter greater challenges in traversing the dense lipopolysaccharide layer of the outer membrane of Gram-negative bacteria[21]. Certain Gram-negative bacteria possess an outer capsule that functions as an extra protective layer. This capsule can inhibit the penetration of antimicrobial compounds into bacterial cells. Conversely, Gram-positive bacteria typically possess few to no capsules, rendering them more vulnerable to the effects of antimicrobial compounds[22].

The metabolic pathways of Gram-positive and Gram-negative bacteria frequently exhibit variations. This may influence bacterial responses to antimicrobial compounds. For instance, Gram-positive bacteria possess metabolic pathways that render them more susceptible to specific compounds. Conversely, Gram-negative bacteria possess mechanisms that enhance their defense against environmental stressors, including assaults by antimicrobial compounds[23]. Gram-negative bacteria can interact with environmental components, such as endotoxins, hydrolytic enzymes, or other compounds, which can influence the activity of antimicrobial compounds[24]. This interaction may modify the efficacy of antimicrobial compounds against Gram-negative bacteria. The interplay of these characteristics results in antimicrobial compounds being more efficacious in inhibiting the proliferation of Gram-positive bacteria than Gram-negative bacteria.

Conclusion

The research found that all bacterial isolates derived from shrimp paste exhibited significant inhibitory effects on *Staphylococcus aureus*. where TRS 1 and TRS 4 isolates displayed a pronounced inhibition zone against *Escherichia coli*, whereas TRS 2 and TRS 3 isolates exhibited a mild inhibition zone against *E. Coli.* All bacterial isolates showed a moderate inhibitory effect on *Klebsiella pneumoniae*.

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Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by **[Sukmawati]**, **[Metusalach]**, **[Syahrul]**, and **[Christy Radjawane]**. The first draft of the manuscript was written by **[Sukmawati]** and **[Metusalach]**, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.