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Analysis of bacteriocins of lactic acid bacteria isolated from fermentation of *rebon* shrimp (*Acetes* sp.) in South Sorong, Indonesia as antibacterial agents

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Abstract. *Sukmawati S, Sipriyadi, 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.* Bacteriocins are protein compounds that are often used as biopreservative agents due to their antibacterial effects against various types of pathogenic bacteria. Bacteriocins are used as food additives that can control the growth of spoilage bacteria in food. Bacteriocins can be obtained from Lactic Acid Bacteria (LAB) and are generally isolated from fermentation products, such as *rebon* shrimp fermentation. The objective of this study was to analyze the activity of bacteriocins isolated by fermented *rebon* shrimp in South Sorong as antibacterials. The method used in this study is a descriptive study by describing the ability of bacteriocins to inhibit pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*). The results showed that of the seven bacteriocin samples obtained from the fermented *rebon* shrimp paste and acidic shrimp paste were able to inhibit the growth of pathogenic bacteria (*S. aureus*, *E. coli*, and *S. typhimurium*). The sensitivity test of bacteriocin to protease enzymes and its activity against pathogenic bacteria showed that bacteriocins derived from LAB were not sensitive to proteinase-K. The test results of the effect of temperature on bacteriocins and their activity against pathogenic bacteria showed that these bacteriocins were stable at high temperatures, normal temperatures and freezing temperatures. The test results of the effect of pH on bacteriocin activity on pathogenic bacteria showed that bacteriocins were stable in a wide pH range including pH 2.0; pH 3.0; pH 4.0; and pH 5.0; potassium phosphate buffer with pH 6.0; pH 7.0; and pH 8.0; and glycine buffer with pH 9.0; pH 10; and pH 11. Meanwhile, The test results of the effect of NaCl on bacteriocin activity on pathogenic bacteria showed that the bacteriocin sample was not affected by the administration of NaCl. Thus it can be stated that the seven samples had a great ability to inhibit the growth of pathogenic bacteria dan it can be further developed as the basis of natural food preservatives.

Keywords: Bacteriocins, fermentation, LAB, *rebon* shrimp

INTRODUCTION

South Sorong, West Papua is an area that has extensive mangrove cover and river. South Sorong Regency is the area with the highest source of shrimp producer in West Papua. In 2019, shrimp production in South Sorong was 18% of the 421,000 tons of the total production of West Papua province (DKP 2020). The types of shrimp caught by local fishermen are jerbung shrimp (*Penaeus merguiensis*), pink shrimp (*Metapenaeus ensis*), and tiger prawn (*Penaeus monodon*) (Suruan et al. 2020). Based on the potential of local natural resources in southern Sorong, we prepared a fermented product made from *rebon* shrimp paste or known as *terasi* by local community.

The fermented product is capable of producing Lactic Acid Bacteria (LAB). The LAB are able to produce organic acid compounds such as acetic acid, lactic acid, acetaldehyde, and antimicrobials (Rachmawati et al. 2006; Bintsis 2018). Acetaldehyde is also a flavor enhancer in food, while antimicrobials are compounds that can inhibit the growth of pathogenic bacteria. In addition, the LAB have the ability to produce biological preservatives, including bacteriocins (Le et al. 2019). Bacteriocins are a series of peptides, which are secondary metabolites that have potential as natural preservatives (biopreservation) (Zimina et al. 2020). It is known that lactic acid bacteria are capable of producing bacteriocins. Bacteriocins have been used as natural food preservatives in several countries (Savadogo et al. 2006).

Bacteriocin is one of antibacterials, has the ability to inhibit or even kill the growth of pathogenic bacteria, is a sensitive decomposer, particularly if it is derived from Gram-positive bacteria (Cao et al. 2019). Bacteriocins can be extracted from bacterial cells by a propagation process to produce peptide compounds (Zimina et al. 2020). Bacteriocins may function as bactericidal. Bacteriocides are antibacterial compounds that have activity to kill bacteria. Bacteriocins can also be bacteriostatic (Garriga et

al. 2002). Bacteriostatic is an antibacterial substance that is only able to inhibit the growth or the multiplication of the bacterial population, yet non-lethal. Bacteriocins produced by LAB consist of various types which depend on the bacterial strain (Abbasiliasi et al. 2017). A type of bacteriocin that has been widely studied is Nisin which is produced by *Lactococcus lactis*. Nisin produced by the LAB contain amino acids which is important in antimicrobial activities (Özel et al. 2018). The advantage of using bacteriocin as a biopreservative is that it is non-toxic, easily degraded by proteolytic enzymes, is not harmful to gut microbes, and is stable in a wide range of pH and temperature (Usmiati and Marwati 2007).

The bacterial isolate from the fermented Thai plaa-som fish was reported to be able to produce bacteriocins and inhibit the growth of Gram-positive bacteria (Srionnual et al. 2007). *Staphylococcus hominis* KQU-131 from fermented Thai marine fish (pla-ra) produced bacteriocin which was stable to high temperature. *Lactobacillus plantarum* PMU33 isolated from fermented som-fak fish produced bacteriocins that could inhibit the growth of pathogenic bacteria including *L. monocytogenes, B. cereus, and Staphylococcus aureus* (Noonpakdee et al. 2009).

The urgency of our research is that food safety is very important for the public to pay attention to because several diseases are caused by food-borne diseases. In order to prevent contamination of pathogenic bacteria in food, it is by using food preservatives. However, the use of synthetic preservatives has the potential to be detrimental to health. For example, the use of nitrites in meat can produce residues in the form of nitrosamines that cause colon cancer, also benzoates may cause allergies and skin irritation (Daskalopoulou et al. 2015). Therefore, natural preservatives are needed that do not have a bad impact on health, such as bacteriocins. In the future, bacteriocins can be applied as preservatives in seafood and meat products. Based on this background, we analyzed the activity of bacteriocin as an antibacterial from the fermented shrimp paste and acidic shrimp paste in South Sorong, West Papua. The purpose of this study was to analyze the ability of bacteriocins as pathogenic antibacterials.

MATERIALS AND METHODS

Research design

This research is a descriptive study that describes the ability of bacteriocins to inhibit pathogenic bacteria, the sensitivity of bacteriocins against protease enzymes, the effect of temperature on the activity of bacteriocins, the effect of pH on the activity of bacteriocins, and the effect of NaCl on the activity of bacteriocins. The sample used was shrimp paste, which was prepared from fermented *rebon* shrimp and acidic fermented *rebon* shrimp which are fermented products originating from South Sorong, West Papua. The shrimp paste and acidic shrimp paste samples were isolated to obtain lactic acid bacteria (LAB) candidates, then the potential LAB isolates that produced bacteriocin were obtained. Isolate samples from acidic *rebon* shrimp paste (*cincalok*) were coded BSH T1, BSH T2, and BSH T3 while isolate samples from *rebon* shrimp paste were coded TRS P1, TRS P2, TRS P3, and TRS P4.

Bacteriocin production

The LAB candidate isolates were then inoculated into liquid MRS + 0.5% $CaCO_3$ medium, then incubated at 37°C for 24 hours. Furthermore, 1 mL of bacterial culture was separated between the supernatant and pellet using centrifugation at 6000 rpm for 10 minutes. Then, the supernatant was neutralized to pH 6.5 by adding 0.1 M NaOH. The supernatant was then filtered using 0.22 micron millipore so that the filter obtained was a cell-free neutral supernatant (Ogunbanwo et al. 2003).

Selection of bacteriocins as pathogenic antibacterial

The bacteriocin activity test was carried out using the well diffusion agar method (Leeman et al. 2017). A total of 50 L of bacteriocin was inserted into the well on Mueller Hinton Agar medium that had been inoculated by pathogenic bacteria (*S. aureus, Escherichia coli, and Salmonella typhimurium*) with a cell density of 10^6 CFU/mL, then incubated at 37ºC for 24 hours. Furthermore, the clear zone formed around the well was observed. The unit of bacteriocin activity is expressed in Activity Units (AU). 1 AU is defined as the area of inhibition per unit volume of the tested bacteriocin sample (Montoro et al. 2018). Bacteriocin activity was calculated using the following formula (Usmiati and Marwati 2007; Hardianti and Aziz 2019):

$$\text{Bacteriocin activity} = \frac{Lz - Ls}{V}$$

Where:
Lz : Clear zone area (mm^2)
Ls : Well area (mm^2)
V : Sample volume (mL)

Sensitivity test of bacteriocin to protease enzyme

Bacteriocins were analyzed through sensitivity testing to protease enzymes. The test was carried out by adding 10 L of proteinase K (2 mg/mL) to 100 L of cell-free supernatant and then incubated for 2 hours at 37 ºC. Furthermore, the bacteriocin inhibition activity was tested against pathogenic bacteria. The supernatant without the addition of enzymes was used as a control (Yadav et al. 2011).

Determination of the effect of temperature on bacteriocin activity

Bacteriocin activity was tested against several variations of temperature consisting of high temperature, low temperature, and freezing temperature. The bacteriocin activity test against high temperature was carried out by heating 1.5 mL of bacteriocin at 60°C, 70°C, 80°C and 100°C for 30 minutes, at temperature of 121°C for 15 minutes and 5 minutes, respectively. Bacteriocins without heat treatment were used as control. Furthermore, the bacteriocin activity of the various temperature treatments was tested with indicator bacteria. Stability test of bacteriocin on storage at low temperature and freezing

temperature was carried out by storing 1.5 mL of bacteriocin at 4-20°C and -40°C for four weeks, and then the bacteriocin activity was tested (Sharma et al. 2006).

Determination of the effect of pH on bacteriocin activity

The effect of pH on bacteriocin activity was tested by making a suspension of bacteriocin crude extract in 50 mM citrate buffer with pH 2.0; pH 3.0; pH 4.0; pH 5.0; potassium phosphate buffer with pH 6.0; 7.0; and pH 8.0; glycine buffer with pH 9.0; pH 10; and pH 11. Bacteriocins without pH treatment were used as controls. Subsequently, the samples and controls were incubated at 30°C for 4 hours. After incubation, the bacteriocin suspension was neutralized again by the addition of 6 M HCl for acidification and 0.1 M NaOH addition for alkalinization (Hamida 2021).

Determination of the effect of NaCl on bacteriocin activity

The effect of NaCl on bacteriocin activity was tested by adding bacteriocin with NaCl with varying concentrations of 2%, 4%, 6%, 8%, and 10%. Bacteriocin without the addition of NaCl was used as a control. Both samples and controls were incubated at 37°C for 2 hours and then the bacteriocin activity was tested (Hamidah et al. 2019).

Data analysis

Data analysis was carried out descriptively, starting from the production of bacteriocins, the ability of bacteriocins to inhibit pathogenic bacteria, the sensitivity of bacteriocins to protease enzymes, the effect of temperature on the activity of bacteriocins, the effect of pH on the activity of bacteriocins, and the effect of NaCl on the activity of bacteriocins.

RESULTS AND DISCUSSION

Bacteriocin activity derived from lactic acid bacteria (LAB) as an antibacterial pathogen was tested against *S. aureus*, *E. coli*, and *S. typhimurium*. Bacteriocin activity was tested using the well method (Figure 1).

The results of the sensitivity test of bacteriocin to protease enzymes and the activity against pathogenic bacteria (*S. aureus*, *E. coli*, and *S. typhimurium*) were performed using the well method (Figure 2).

The test results of the effect of temperature on the activity of bacteriocins against pathogenic bacteria (*S. aureus*, *E. coli*, and *S. typhimurium)* are presented in Table 1. Furthermore, the test results of the effect of pH (Citrate buffer, Phosphate buffer, Glycine buffer) on bacteriocin activity against pathogenic bacteria are presented in Table 2. While the test results of the effect of NaCl on bacteriocin activity against pathogenic bacteria are presented in Table 3.

Discussion

Bacteriocin activity as antibacterial showed different inhibition values against pathogenic bacteria. Of the seven samples tested, it showed that sample TRS P4 had the highest value (23.23 mm) in inhibiting *S. typhimurium*, then sample TRS P1 was able to inhibit *E. coli* with an inhibition value of 22.45 mm, then the BSH T1 sample had an inhibition value of 15.52 mm against *S. aureus*. Meanwhile, the lowest inhibition activity against *S. typhimurium* was shown by sample BSH T3 with a value of 19.86 mm. *Escherichia coli* was inhibited by sample BSH T1 with an inhibition value of 16.72 mm, and the inhibition value of sample TRS P4 against *S. aureus* was 13.1 mm (Figure 1).

According to the average of the seven bacteriocin samples, the ability of each sample to inhibit the three pathogenic bacteria (*S. typhimurium*, *S. aureus*, and *E. coli*), the highest bacteriocin activity was found in sample TRS P1 with an average inhibition value of 19.60 mm, while the lowest bacteriocin activity in inhibiting the three pathogenic bacteria was found in sample BSH T3 with an average inhibition value of 16.82 mm. The difference in the bacteriocin inhibition zone index against pathogenic bacteria can be influenced by the sensitivity of the indicator bacteria, the concentration of antimicrobial compounds, and the rate of diffusion of antimicrobial compounds (Ming et al. 2015).

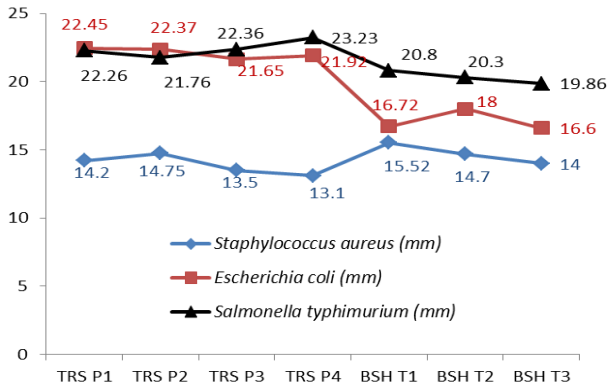

Figure 1. Result of bacteriocin activity as pathogenic antibacterial

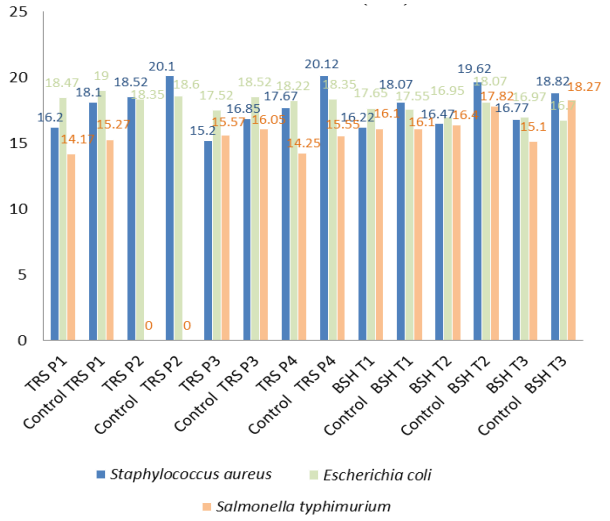

Figure 2. The results of the sensitivity test of bacteriocin to protease enzymes and the activity against pathogenic bacteria

Table 1. Test Results of effect of temperature on bacteriocin activity against pathogenic bacteria

Bacteriocin-producing isolate code	Temp. treatment (°C)	Inhibition zone index (mm)		
		Escherichia coli	*Staphylococcus aureus*	*Salmonella thypimurim*
TRS P1	Control	15.80	7.60	16.15
	60	15.65	3.95	14.75
	70	14.75	4.42	16.35
	80	14.75	4.17	15.8
	100	14.75	4.22	16.05
	121a	14.75	2.97	14.55
	121b	14.75	2.97	15.35
	4	14.05	14.80	15.60
	-20	13.12	13.57	15.20
	-40	12.57	13.65	15.10
TRS P2	Control	15.45	9.50	15.22
	60	15.45	9.50	15.45
	70	15.45	9.50	15.25
	80	15.45	9.50	15.20
	100	15.45	9.50	14.20
	121a	15.45	9.50	13.95
	121b	15.45	9.50	4.50
	4	13.95	14.20	14.20
	-20	13.15	12.90	13.50
	-40	13.12	12.60	14.10
TRS P3	Control	16.80	7.15	16.95
	60	16.30	6.55	16.95
	70	15.80	5.30	16.65
	80	15.85	4.00	17.05
	100	16.35	4.00	17.05
	121a	16.35	4.00	16.75
	121b	16.35	4.00	16.60
	4	13.50	13.62	15.50
	-20	12.90	12.22	14.80
	-40	12.70	12.20	14.90
TRS P4	Control	15.20	13.60	14.8
	60	15.15	13.50	14.00
	70	14.95	13.50	12.75
	80	15.35	11.85	13.00
	100	15.30	11.85	13.40
	121a	16.10	11.85	13.70
	121b	15.90	11.85	13.70
	4	12.70	14.30	16.70
	-20	12.00	13.30	15.50
	-40	12.10	13.20	15.50
BSH T1	Control	15.85	10.30	16.45
	60	15.05	10.25	15.50
	70	13.90	10.25	16.15
	80	14.45	10.25	16.15
	100	13.60	10.25	16.15
	121a	13.15	10.25	15.25
	121b	13.15	10.25	15.60
	4	14.50	13.90	15.20
	-20	13.60	13.20	14.40
	-40	12.50	13.50	14.50
BSH T2	Control	14.65	11.22	11.72
	60	15.45	10.35	16.02
	70	14.75	9.80	14.72
	80	14.75	9.80	15.52
	100	14.75	8.90	15.35
	121a	14.75	9.00	15.35
	121b	14.80	7.05	15.37
	4	13.70	13.10	16.80
	-20	12.20	12.80	15.80
	-40	11.90	12.60	15.50
BSH T3	Control	15.30	13.92	17.15
	60	14.60	13.50	16.20
	70	15.00	13.85	16.50
	80	13.55	13.62	16.50
	100	14.60	13.47	16.20
	121a	14.85	13.47	16.30
	121b	14.85	13.47	15.70
	4	13.40	13.20	16.07
	-20	12.80	14.10	15.65
	-40	12.50	13.80	15.50

Note: temperature of 60 °C, 70 °C, 80 °C, 100°C for 30 minutes; temperature of 121[a] °C for 5 minutes; temperature of 121[b] °C for 15 minutes, dan temperature of 4 °C, -20 °C, -40 °C for 30 days.

The results of the sensitivity test of bacteriocin on protease enzymes and its inhibition activity against pathogenic bacteria had the highest inhibition value in sample TRS P2 18.52 mm against *S. aureus*, sample TRS P1 with an inhibition value of 18.47 mm against *E. coli*, and sample BSH T2 of 16.40 mm against *S. typhimurium*, respectively. While the lowest inhibitory inhibition was found in the sample TRS P3 with a value of 15.2 mm against *S. aureus*, sample BSH T2 with an inhibitory value of 16.95 mm against *E. coli*, and sample TRS P1 with an inhibitory value of 14.17 mm against *S. typhimurium* (Figure 2). The bacteriocin activity in inhibiting the three pathogenic bacteria was found in sample TRS P4 with an inhibition value of 16.71 mm, while the lowest inhibition activity against the three pathogenic bacteria was found in the sample TRS P2 with a value of 12.29 mm.

Based on the results of the study, the seven bacteriocin samples tested were not sensitive to proteinase-K administration. This is indicated by the formation of an inhibitory zone against pathogenic bacteria. However, according to the results reported by Maulidayanti (2018), bacteriocins added with proteinase-K were not able to inhibit the growth of *S. typhimurium* and *L. monocytogenes* which were indicated by the absence of an inhibition zone around the wells, while bacteriocins without the addition of proteinase-K showed inhibitory zone activity because they were able to inhibit the growth of indicator bacteria. The inhibition zone that was not formed in the bacteriocin + proteinase-K sample was caused because the protein derived from bacteriocin had been degraded by proteinase-K so that it was unable to inhibit the growth of *S. typhimurium* and *L. monocytogenes* bacteria. The addition of proteinase-K affects the activity of bacteriocins. Proteinase-K is a proteolytic enzyme that is capable of hydrolyzing peptide bonds and affects the conformation of bacteriocin proteins (Sunaryanto and Tarwadi 2015). Compared with the results of other studies, these differences can be caused by the structure of the different bacteriocin compounds, because the bacterial isolates that produce bacteriocins are also different. In addition, the indicator bacteria used are also different.

Table 2. Test results of effect of pH (citrate buffer, phosphate buffer, glycine buffer) on activity on pathogenic bacteria

Bacteriocin-producing isolate code	pH treatment*	Inhibition zone index (mm)	
		Escherichia coli	*Salmonella thypimurim*
TRS P1	Control	20.87	21.30
	2.0	17.52	17.27
	3.0	17.82	17.82
	4.0	17.07	18.37
	5.0	17.07	18.72
	6.0	17.07	17.82
	7.0	15.65	17.60
	8.0	14.67	17.77
	9.0	15.50	17.97
	10	15.42	16.95
	11	15.00	17.27
TRS P2	Control	20.30	19.30
	2.0	17.32	17.70
	3.0	18.35	18.02
	4.0	17.47	18.40
	5.0	17.67	18.30
	6.0	17.02	16.35
	7.0	16.27	18.92
	8.0	16.27	18.50
	9.0	15.85	18.32
	10	15.72	18.30
	11	15.17	18.22
TRS P3	Control	22.30	20.35
	2.0	17.12	18.65
	3.0	16.75	19.40
	4.0	16.87	19.55
	5.0	18.15	18.55
	6.0	17.12	17.77
	7.0	17.45	19.40
	8.0	15.50	18.60
	9.0	18.70	18.02
	10	17.35	16.75
	11	18.60	16.95
TRS P4	Control	21.95	20.42
	2.0	17.15	18.75
	3.0	17.52	18.75
	4.0	18.65	18.65
	5.0	18.7	18.37
	6.0	18.95	18.75
	7.0	15.7	18.25
	8.0	17.52	17.77
	9.0	16.42	18.97
	10	16.27	19.22
	11	15.75	18.85
BSH T1	Control	20.02	20.02
	2.0	14.80	15.10
	3.0	15.37	15.20
	4.0	16.80	14.30
	5.0	15.70	15.30
	6.0	16.02	15.80
	7.0	15.80	16.20
	8.0	15.50	16.01
	9.0	15.65	15.80
	10	15.82	15.70
	11	14.45	15.10
BSH T2	Control	19.67	21.07
	2.0	14.07	17.90
	3.0	13.52	18.30
	4.0	15.12	18.90
	5.0	15.55	18.37
	6.0	14.70	18.10
	7.0	18.00	18.95
	8.0	13.50	14.60
	9.0	19.17	19.70
	10	17.65	18.82
	11	17.37	18.30
BSH T3	Control	22.02	22.65
	2.0	16.90	19.15
	3.0	17.80	18.90
	4.0	17.80	18.55
	5.0	18.92	18.27
	6.0	18.90	18.55
	7.0	16.70	17.00
	8.0	17.42	19.02
	9.0	17.17	17.97
	10	17.10	17.92
	11	16.72	18.92

Note: *pH: (Citrate buffer: 2.0; 3.0; 4.0; 5.0), (Phosphate buffer: 6.0; 7.0; 8.0), (Glycine Buffer: 9.0; 10; 11).

Table 3. Test results of the effect of NaCl on bacteriocin activity against pathogenic bacteria

Bacteriocin-producing isolate code	NaCl treatment (%)	Inhibition zone index (mm)		
		Escherichia coli	*Staphylococcus aureus*	*Salmonella thypimurim*
TRS P1	Control	14.20	17.05	14.20
	2	14.20	16.10	14.60
	4	14.40	16.00	14.85
	6	14.40	15.60	14.42
	8	14.40	15.45	13.30
	10	14.35	14.60	12.92
TRS P2	Control	13.60	16.57	14.55
	2	13.75	15.20	14.40
	4	13.75	15.30	13.90
	6	13.75	15.25	14.15
	8	13.65	15.20	14.17
	10	13.35	15.20	14.22
TRS P3	Control	13.55	16.60	17.00
	2	13.85	15.82	15.95
	4	13.35	16.35	14.95
	6	14.25	14.80	16.17
	8	14.52	16.30	15.57
	10	14.50	15.95	15.45
TRS P4	Control	15.70	18.75	62.67
	2	15.05	18.05	14.40
	4	15.50	17.75	15.17
	6	15.97	17.40	14.97
	8	15.17	16.25	14.85
	10	15.07	17.00	14.00
BSH T1	Control	14.60	18.52	13.85
	2	14.60	17.25	13.65
	4	15.02	17.30	13.85
	6	15.07	17.10	14.50
	8	15.52	13.95	13.55
	10	14.97	16.95	13.40
BSH T2	Control	14.70	17.80	14.20
	2	14.17	17.17	13.65
	4	14.35	16.40	13.80
	6	14.57	16.45	14.40
	8	14.80	15.90	14.05
	10	14.90	15.95	14.55
BSH T3	Control	14.82	16.15	12.25
	2	14.50	15.22	12.72
	4	14.60	15.40	14.10
	6	14.80	15.50	13.82
	8	14.35	14.95	13.30
	10	14.65	14.70	13.70

The effect of temperature on bacteriocin activity on pathogenic bacteria showed that sample TRS P3 at a temperature treatment of 100-121°C had an inhibitory value of 16.35 mm against *E. coli.* Furthermore, sample TRS P4 with a temperature treatment of 4°C showed an inhibitory value of 14.3 mm against *S. aureus*, and sample TRS P3 with a temperature treatment of 80-100°C had an inhibitory value of 17.05 mm against *S. typhimurium* (Table 1). The highest average inhibition zone index against the three pathogenic bacteria was found in sample BSH T3 (temperature of 121^{a}) with a value of 14.87 mm, while the lowest inhibition zone index against the three pathogenic bacteria was found in sample TRS P2 (temperature of 121^{b}) with a value of 9.82 mm.

The activity of bacteriocins remained stable against heat treatment and freezing temperatures, presumably because bacteriocins are short peptides that are temperature stable, also due to the presence of certain amino acids that are able to maintain the structure of bacteriocins from heat treatment (Gálvez et al. 2007). The stability of bacteriocins to varying temperatures can be attributed to the formation of a small globular structure so that the hydrophobic region is strong, cross-linked stability, and the high cysteine content (Flynn 2021).

Resistance to temperature variations is the main characteristic of some bacteriocins produced by lactic acid bacteria. A study (Kayalvizhi and Gunasekaran 2010) showed that bacteriocin activity was stable against heat after being heat treated at an incubation temperature of 100°C for 15 minutes. Another study showed that bacteriocins derived from BAL PB3.6 and PG1.9 were able to inhibit pathogenic bacteria over a wide temperature range, where bacteriocins derived from the two isolates had inhibitory activity after being heat treated at a temperature of 50°C to 100°C for 10 minutes. Various reports have stated that some bacteriocins are stable to heat treatment and can withstand temperatures from 100°C to 121°C (Gautam et al. 2014). Bacteriocin derived from *L. brevis* MTCC 7539 maintained its activity up to 100°C for 20 minutes and a temperature of 121°C for 10 minutes (Gautam and Sharma 2009). Stability to temperature is an important character if bacteriocins are used as natural food preservatives, because some food preparation methods involve heating (Gavahian et al. 2019). The mechanism of bacteriocin resistance to heat is related to the molecular structure of bacteriocin which is a peptide, the stability of the temperature test is also caused by the presence of a highly hydrophobic region, stable cross-linking, and high glycine content (Carlier et al. 2015). Van et al. (2011) also stated that most of the bacteriocins produced by lactic acid bacteria were described as proteins that have hydrophobic bonds with tertiary structures that cause stability to temperature.

Test results of the effect of pH on bacteriocin activity in *E. coli* showed that sample BSH T2 had the highest value of 19.17 mm (Glycine Buffer; pH 9). Furthermore, the bacteriocin activity of the seven samples with various pH treatments tested on *S. aureus* showed that there was contamination so it could not be analyzed. Meanwhile, sample TRS P3 showed an inhibitory zone index value against *S. typhimurium* with a value of 19.55 mm (Cirate buffer; pH 4) (Table 2). All bacteriocin samples were able to inhibit pathogenic bacteria in a wide pH range as evidenced by the bacteriocin inhibition activity after being treated with pH 2.0; pH 3.0; pH 4.0; pH 5.0 mM, potassium phosphate buffer pH 6.0; pH7.0; pH 8.0 mM, glycine buffer pH 9.0; pH 10; and pH 11.

pH greatly determines the safety of food ingredients (Usmiati and Marwati 2007). The stability of bacteriocins over a wide pH range is very important in the application of various types of food, whether food with high, neutral or low acidity levels (Abanoz and Kunduhoglu 2018). Some bacteriocins have been reported to be stable over a wide pH range. This is related to the solubility of bacteriocins originating from lactic acid bacteria. Bacteriocins have different activities at different pH conditions (Yang et al. 2018). *Lactobacillus rhamnosus* L34 isolated from various animal milks produced bacteriocins that were able to survive in the pH range of pH 2.0; pH 3.0; pH 4.0; pH 5.0; pH 7.0; and pH 8.0 for 24 hours of incubation. Another study showed that the bacteriocins R1333 and ST16 remained stable at pH 2.0; pH 4.0; pH 6.0; pH 8.0; pH 10; and pH 12, while the bacteriocins produced by *L. brevis* OG1 were stable at pH 2.0 to pH 8.0 (Ogunbanwo et al. 2003).

Several studies have shown that bacteriocins are active at neutral, acidic and alkaline pH. The maximum activity of bacteriocin derived from *L. brevis* was active at pH 7.0 (Gautam et al. 2014). Similar results were also reported by Todorov et al. (2013) that the bacteriocins of *L. sakei* ST22Ch, ST153Ch, and ST154Ch were stable at pH 4.0-pH 10. Bacteriocin derived from *L. sakei* MBSa1 was stable at pH 2.0 - pH 6.0 (Barbosa et al. 2014), while bacteriocin derived from ASM1was stable at neutral and alkaline pH conditions (Hata et al. 2010).

The test results of the effect of NaCl on bacteriocin activity on pathogenic bacteria showed that the samples that had the highest activity in inhibiting pathogenic bacteria were sample TRS P4 + NaCl 6% which were able to inhibit *E. coli* with a value of 15.97 mm, sample TRS P4 + NaCl 2% was able to inhibit *S. aureus* with a value of 18.05 mm, and sample TRS P3 + NaCl 6% was able to inhibit *S. typhimurium* with a value of 16.17 mm (Table 3). Furthermore, the results of the bacteriocin activity test against the three pathogenic bacteria showed that sample TRS P4 + 4% NaCl had the highest value of 16.14 mm while sample BSH T3 + 2% had the lowest value (14.15 mm) in inhibiting the three pathogenic bacteria.

It is important to test the effect of NaCl on bacteriocin activity because food processing often involves NaCl or table salt, so it is necessary to know in advance the characteristics of the bacteriocin that will be used whether it has activity in inhibiting the target bacteria when added with NaCl. Several research results show that the activity of bacteriocin is stable against the addition of 2.0 % - 8.0% salt. Another study (Obi et al. 2018) stated that the addition of NaCl (0-10%) to bacteriocins had no effect on bacteriocin activity as seen from the bacteriocin treatment without the addition of NaCl as a control.

Bacteriocins are peptide compounds that are often used as biopreservative agents because they have antibacterial effects against various types of pathogenic microbes. Bacteriocins used in food must meet several criteria, including: derived from strains that are safe for consumption (GRAS), work on a broad spectrum in inhibiting the growth of pathogenic bacteria, are resistant to heat, and do not change the taste and quality of the food (Wu et al. 2021).

Bacteriocins produced by Gram-positive bacteria contain 30 to 60 amino acids with activities ranging from narrow to broad spectrum. Some of the advantages of bacteriocins compared to antibiotics include having a specific target, are able to be degraded by protease enzymes in the digestive system, allowing for genetic engineering, and are safe for consumption (Papagianni and Papamichael 2011). In addition, antibiotics will be toxic to the producing cells when they have reached a certain limit, while bacteriocins are not toxic. Bacteriocins were able to inhibit the growth of *E. coli* atcc 25922, *E. faecalis* atcc 29212, *S. aureus* atcc 25923, and *B. subtilis* atcc 66923. Bacteriocins produced by *Lactobacillus lactis* were reported to be stable against heating up to 70°C and stable in the pH range of 3.0 to pH 7.0 (Sunaryanto and Tarwandi 2015). Lactic acid bacteria isolated from shrimp intestines were reported to be able to produce bacteriocin as an antibacterial agent in fishery products (Romadhon et al. 2012).

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