

Antibacterial Activity Test of *Lactobacillus plantarum* Bacteriocin Isolated from Dadiah

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ABSTRACT

Background: Dadiah is a traditional food in West Sumatra, Indonesia which is obtained from the buffalo milk fermentation process. The aim of this study to determine antibacterial activity test of *Lactobacillus plantarum* bacteriocin isolated from dadiah.

Methods: This research was an experimental of completely randomized design with determined minimum inhibitory concentration and minimum bactericidal concentration which determined by using dilution method in some concentrations of 100%, 80%, 60%, 40%, 20%, and 0%. Identification of bacteriocin from *Lactobacillus plantarum* isolates was carried out using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method.

Results: The results of the study known that percentage of Bacteriocin of minimum inhibitory concentration from *Lactobacillus plantarum* against *P. aeruginosa* was 98.57% ($p < 0.001$). Meanwhile, the results of minimum bactericidal concentration cannot be identified. Our result showed that the bacteriocin protein band was in the range of 17-28 kDa.

Conclusion: This analysis confirmed that the bacteriocin compound from *Lactobacillus plantarum* had a bacteriostatic effect on *P. aeruginosa*.

Keywords: Dadiah, *Lactobacillus plantarum*, Bacteriocin, *Pseudomonas aeruginosa*

INTRODUCTION

Dadiah is a traditional food from West Sumatra buffalo milk which is a

traditional food that is served at weddings, inaugurations of marriage and other traditional processions. Prizes are obtained from the results of the buffalo milk fermentation process which is inserted into a bamboo tube and covered with banana leaves for one to two days. This fermentation process will produce dadiah which is milky white with a slippery texture, and has a sour taste and distinctive aroma.^[1] Dadiah contains lactic acid bacteria (LAB) which is the group of bacteria that most produce compounds called bacteriocin.^[2] Bacteriocin compounds from LAB obtained from dadiah is known to have antibacterial activity so that it has the potential to be a natural alternative antibiotic to protect humans from the effects of resistant microbes that cause disease without causing health problems.^[3]

Bacteriocin is an antimicrobial peptide produced by LAB during the exponential growth phase. The large amount of bacteriocin produced can kill or inhibit other bacteria that compete in the same ecology.^[4] Bacteriocin can inhibit the growth of pathogenic microbes and has the potential to be used as an antimicrobial in the food industry. As a synthesis agent, bacteriocin in biopreservatives also contributes greatly to the health sector as a natural antibiotic agent and a probiotic food.^[5]

Previous study known cell-free filtrate from LAB indigenous dadiah isolates (*L. plantarum* D-0 and *L. Lactis* D-

01) are proven to produce antimicrobial substrates that can inhibit the growth of indicator pathogenic bacteria such as *S. enteritidis* ser. Typhimurium ATCC 14028, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 indicated by the clear zone of inhibition in the agar well diffusion method.^[6] Another study it was explained that bacteriocin from *Lactobacillus sp.* 1223 SCG isolated from fresh cow's milk can inhibit the growth of pathogenic bacteria such as *Salmonella thypimurium* and *E. coli*.^[7] Other research showed that *L. casei* has antimicrobial activity (positively inhibits *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis*). *L. casei* which is a local isolate has characteristics that have the potential to be used as probiotic microbes.^[8]

Based on studies that have been done, it is not yet known about the antibacterial activity of bacteriocin from *Lactobacillus plantarum* obtained from dadiah against *P. aeruginosa* so that in this study testing the antibacterial activity of *Lactobacillus plantarum* bacteriocin was isolated from dadiah against *Pseudomonas aeruginosa*.

MATERIALS & METHODS

This research was an experimental of completely randomized design with determined minimum inhibitory concentration and minimum bactericidal

concentration which determined by using dilution method in some concentrations of 100%, 80%, 60%, 40%, 20%, and 0%. Identification of bacteriocin from *Lactobacillus plantarum* isolates was carried out using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method. Bacteriocin filtrate was put into each test tube containing a predetermined volume of nutrient broth and 0.1 ml bacterial suspension. Each treatment combination amounts to 2.1 ml for 100% of the mixture. The lowest concentration that can inhibit more than 90% growth of pathogenic bacterial colonies after 24-hour incubation is determined as the MIC value. While the minimum bactericidal concentration value is determined by assessing the lowest concentration of the filtrate which shows no growth of pathogenic bacteria. The quantitative variables were recorded as mean and standard deviation. Hypothesis test used one-way anova. A two-tailed P-value of <0.05 was considered statistically significant. Data were analyzed using the SPSS version 21.0.

RESULT

In the results of this study showed bacteriocin molecular weights ranged from 17 to 28 kDa (indicated by a red circle given an arrow).

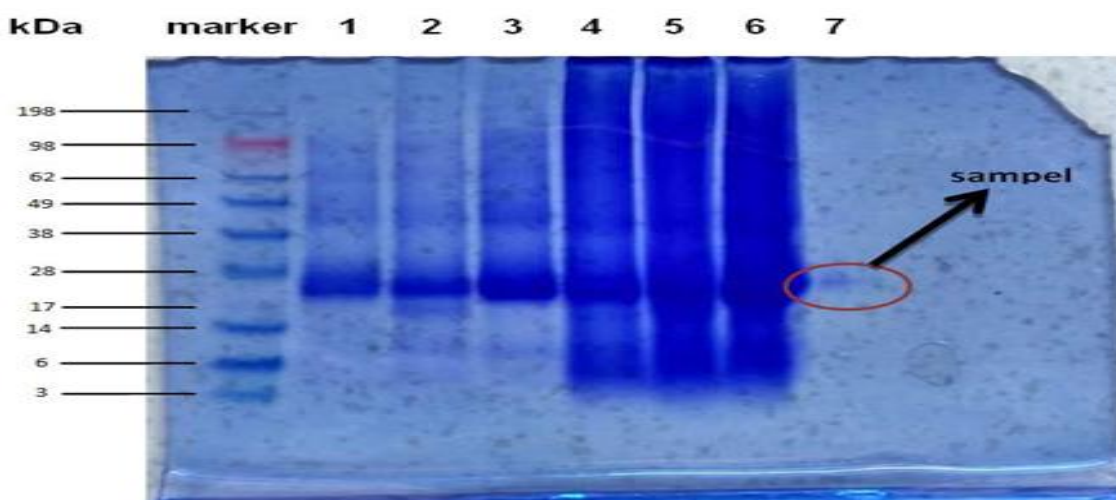


Figure 1: Bacteriocin molecular weights

The effect of bacteriocin filtrate culture on *Pseudomonas aeruginosa* can be seen in Table 1.

Table 1: Antibacterial activity of bacteriocin against some *P. aeruginosa* bacteria

Sample Concentration (%)	Average of <i>P. aeruginosa</i> (log 10 cfu/ml)	p value
100	5.041	<0.001*
80	5.848	
60	6.207	
40	8.153	
20	9.633	
Control (0)	10.021	

*p<0.001, significant

The results showed the average number of *P. aeruginosa* bacteria at a concentration of 100% ie found 1.1×10^5 CFU/ ml, 80% concentration found 7.05×10^5 CFU/ ml, 60% concentration found 1.61×10^6 CFU/ ml, 40% concentration found 1.422×10^8 CFU/ ml, 20% concentration found 4.3×10^9 CFU/ ml and in the control there was 1.05×10^9 CFU/ ml. In this study the minimum inhibitory concentration (MIC) was determined from a decrease in the number of bacterial colonies to 90% of controls. From the results of this study found a decrease in the number of bacterial colonies of more than 90% at a concentration of 60% to 100% compared to controls. Conversely, at a concentration of 20% to 40% a decrease in the number of bacteria is less than 90%. In the results of this study it was found that *L. plantarum* bacteriocin did not have a minimum bactericidal concentration against *P. aeruginosa*. The MIC of bacteriocin *L. plantarum* against *P. aeruginosa* was determined at a concentration of 40%.

DISCUSSION

Determination of bacteriocin molecular weight is done through the SDS-PAGE method using acrylamide gel. The function of determining bacteriocin molecular weight with SDS-PAGE is to separate the purity of a protein. The protein obtained is likely to be contaminated by molecular weights originating from the same molecule, so the SDS-PAGE method must be used to test the purity of a product.

The tape produced in the acrylamide gel via SDS-PAGE is a single protein. [9,10]

In this study, testing the effect of *L. plantarum* bacteriocin filtrate culture on *P. aeruginosa*. Bacteriocin *L. Plantarum* has an influence on the growth of *P. aeruginosa* bacteria. This is because bacteriocin has an antibacterial role. The results of antibacterial test from bacteriocin showed that the smaller the bacteriocin concentration, the lower the ability of bacteriocin to inhibit bacterial growth. The results of this study showed that *L. plantarum* bacteriocin filtrate isolated from dadiah did not have a bactericidal effect on *P. aeruginosa* bacteria. This is because the growth of *P. aeruginosa* bacteria is still found.

Bacteriocin is a metabolite (extracellular) in the form of a protein that is synthesized directly in the ribosome of a bacterium and has a broad spectrum in inhibiting the growth of pathogenic bacteria. Bacteriocin can work on target bacteria in two stages, firstly through absorption on the surface of bacterial cells, and secondly, lethal effects through: (i) bacteriostatic effect; (ii) bactericidal effect without cell lysis or (iii) with cell lysis. The mechanism of action of bacteriocin compounds against bacteria is generally known through the bond between bacteriocin and bacterial cell wall or membrane which can cause instability in the cell membrane. This results in the formation of holes or pores in bacterial cell membranes. [11] The formation of these pores will increase membrane permeability, disrupt the function of proton motive force (PMF) and cause the loss of intracellular material from bacteria. The disruption of PMF will cause disruption in the formation of energy in bacterial cells, resulting in stunted cell growth and lead to bacterial cell death. [12]

Most of the bacteriocins produced by LAB are cationic (because they contain excess lysil and arginil residues) composed of 12-45 amino acids. The electrostatic interaction of positively charged and hydrophobic bacteriocin with a negatively

charged phosphate group on the target cell membrane results in the binding of bacteriocin to the target membrane and this is the initial stage of bacteriocin to initiate cell wall damage. After bonding with the target membrane absorption of helical structures from bacteriocin to the cell membrane through its hydrophobic part, while the hydrophilic part is exposed to solvents. When the bacteriocin concentration threshold is reached, the peptide will unite and form a trans membrane membrane, with the hydrophobic part facing outward, and the hydrophilic part facing inward, thus forming a pore on the membrane. Pore formation causes the loss of intracellular molecules and the entry of extracellular molecules, this causes changes in cellular pH. These events affect the growth of pathogenic bacterial cell growth and can cause the death of bacterial cells that are sensitive to bacteriocin. [13,14]

CONCLUSION

The conclusion of this study confirmed that the bacteriocin compound from *Lactobacillus plantarum* had a bacteriostatic effect on *P. aeruginosa*.

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