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# The Effect of pH on the Activity of Bacteriocin from *Lactobacillus acidophilus* on the Growth of *Salmonella typhi*

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***Abstract*—Typhoid fever is one of the most serious infectious diseases in Indonesia. Antibiotic resistance is one of the problems associated with the treatment of typhoid fever. Bacteriocin is an antimicrobial substance produced by *L. acidophilus*. Bacteriocin has bactericidal and bacteriostatic activity. The aim of this study was to determine the activity of bacteriocin from *L. acidophilus* against *Salmonella typhi* as antimicroba. The design of this study was laboratory experimental with posttest only control group design using the Kirby-Bauer agar diffusion method on Mueller Hinton Agar (MHA). The bacteria of this study were *S. typhi* ATCC 6539 and bacteriocin from *L. acidophilus* ATCC 4356 in various pH (2, 4, 6, 8, 10, and 12). The result of observation was carried out by measuring the inhibition zone in each treatment. The result showed bacteriocin from *L. acidophilus* at pH 2 had an inhibition zone 4.91 ±1.70 mm. Statistical calculation using the One Way Anova test showed that p-value was 0.000 then continued with the Post Hoc Tukey test showed bacteriocin from *L. acidophilus* at pH 2 inhibited the growth of *S. typhi* very significant compared to chloramphenicol. *L. acidophilus* can inhibit the growth of *S. typhi* and has an optimal pH, which is at pH 2 with a weak category of inhibitory strength. The bacteriocin from *L. acidophilus* ATCC 4356 could not compete with chloramphenicol as the first line drug for typhoid fever.**

***Keywords—Bacteriocin, Lactobacillus acidophilus, Salmonella typhi***

## I. INTRODUCTION

Typhoid fever is a serious infectious disease caused by *Salmonella typhi* (*S. typhi).* Based on data from World Health Organization (WHO) in 2020, cases of typhoid fever globally reached 11,000,000 to 20,000,000 cases with 128,000 – 161,000 deaths each year [1]. The prevalence of typhoid fever in Indonesia was 300 to 810 per 100,000 population each year and the Case Fatality Rate (CFR) reached 2% [2]. Treatment for typhoid fever is classified into first line and second line drug. Resistance often occurs in connection with the treatment of typhoid fever. Resistance is a condition where bacteria are no longer sensitive to antimicrobials commonly used. The resistance of chloramphenicol as the drug of choice for typhoid fever has been reported. This resistance can develop to Multi Drug Resistance Salmonella Typhi (MDRST) [3]. *L. acidophilus* produces antimicrobial such as lactic acid, hydrogen peroxide, and bacteriocin. Bacteriocin is an active protein substance and has bactericidal and bacteriostatic activity [4].

Previous studies have been conducted to examine the effect of bacteriocin from *L. acidophilus* on the growth of *S. typhi* and the result had no inhibition zone [5]. There are several factors that can affect activity of bacteriocin such as pH , temperature, and enzyme [6]. Each bacteriocin has different optimum pH. As the pH increases closer to alkaline pH, the activity of bacteriocin will decrease [7]. Research by Zhao, bacteriocin from *L. acidophilus* has an optimum pH at pH 2 to 3 against *Escherichia coli* growth [8].

In this study, we controlled the pH of bacteriocin because previous study did not use optimization. Optimization is important to determine the optimal conditions of bacteriocin antimicrobial activity in order to obtain the largest inhibition zone. The aim of this study was to determine the effect of pH on the activity of bacteriocin from *L. acidophilus* against *Salmonella typhi* as antimicroba.

## II. MATERIALS AND METHODS

The study design used a post-test only control group design with the Kirby-Bauer agar diffusion method on Mueller Hinton Agar (MHA). The subject of this study was bacteriocin from *L. acidophilus* ATCC 4356 and the object was *S. typhi* ATCC 6539. In this study, the sample used was bacteriocin *L. acidophilus* with six different pH treatments (pH 2, 4, 6,8,10, and 12). There were two control groups, chloramphenicol as a positive control and aquades as a negative control. The bacteriocin sensitivity was tested to proteolytic enzymes (trypsin enzymes).

The materials used in this study were cultures of *S. typhi* ATCC 6539 and *L. acidophilus* ATCC 4356 from the Eyckman Laboratory of Universitas Padjajaran, aquades, sterile aquades, 0.9% NaCl, 0.5 Mc Farland solution, crystal violet, lugol, ethanol 96. %, safranin, spritus, de Man Rogosa Sharpe Agar (MRSA) media, de Man Rogosa Sharpe Broth (MRSB) media, sodium hydroxide (NaOH), hydrochloric acid (HCL), Tryptic Soy Agar (TSA) media, Mueller Hinton Agar (MHA) media.

### *A. Bacterial Identification*

Rejuvenation of bacteria *S. typhi* was performed on TSA media and *L acidophilus* on MRSA media, afterwards they were incubated at $37^0$C for 24 hours. Bacterial suspension added 0.9% NaCl solution until the turbidity was the same as the 0.5 Mc standard solution. The suspension of *S. typhi* was inoculated on MHA media [5]. Identification of bacteria in this study by macroscopic and microscopic examination. Macroscopic examination by looking at the colonic form and microscopic examination with Gram stain [9].

### *B. Extraction of Bacteriocin*

Bacterial suspension *L. acidophilus* 0.5 ml was put into 4.5 ml MRS broth media then homogenized and made in several tubes. The material was incubated at 37 ° C for 24 hours. The turbidity of the inoculum was equated with a 0.5 Mc Farland solution. Cultures were centrifuged using an ultra-centrifuge at a speed of 10,000 rpm at 4 ° C for 18 minutes. The filtrate was sterilized with a 0.22 µm Millipore filter [9]. Supernatant of bacteriocin was inserted into several tubes and adjusted to pH 2,4,6,8,10 and 12 using NaOH or HCL solutions.

### *C. Bacteriocin Sensitivity Test*

750 µL trypsin enzymes concentration of 1 mg / mL dissolved in a phosphate buffer pH 7,6 then added with a bacteriocin supernatant of 250 µL. The filtrate was sterilized using a Millipore 0.22 µm filter. Disc paper is soaked in the extract for 5 minutes then placed on the MHA media containing *S. typhi.* [9]**.**

### *D. Bacteriocin Test Against Salmonella typhi*

The bacteriocin activity test against *S. typhi* used the agar diffusion method. Disc paper was soaked for 5 minutes in bacteriocin extract then placed on MHA media containing *S. typhi* bacteria then incubated for 24 hours at 37 ° C. The diameter of the clear zone generated around the disc paper is measured using a caliper in mm [9].

## III. RESULTS AND DISCUSSION

In bacterial identification, the result was *L. acidophilus* is a Gram positive bacteria and *S. typhi* is a Gram negative bacteria accordance with the theory. See figure 1 below.

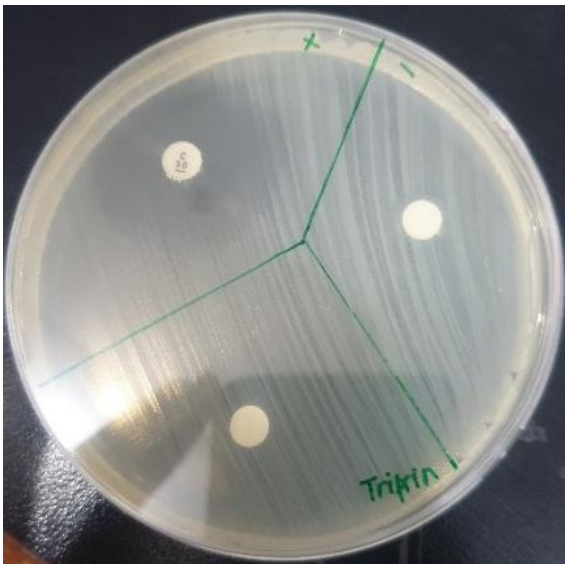


Fig. 1. Bacteriocin sensitivity test to trypsin enzymes.

The bacteriocin sensitivity test in Figure 1 showed that there was no inhibition zone. Trypsin enzyme is a proteolytic enzyme which is able to break down protein [10]. Bacteriocin is active protein substances with small molecular weight and synthesized in ribosomes [7]. Based on the result of this test, the extract taken from *L. acidophilus* is bacteriocin.

The pH of the bacteriocin was adjusted to pH 2, 4, 6, 8, 10 and 12 to test bacteriocin activity against *S. typhi*. Chloramphenicol as a positive control and aquades as a negative control. The aim of this study was to determine the activity of bacteriocin as antimicrobial at various pH, especially acidic to alkaline pH. Based on the diagram below, the bacteriocin from *L. acidophilus* at pH 2 can inhibit the growth of *S. typhi* with average 4.91 ± 1.70 mm. The bacteriocin at pH 4, 6, 8, 10, and 12 no inhibition zone was formed (0 mm). The inhibition zone of chloramphenicol as a positive control was 24.76 ± 0.31 mm and in aquades as a negative control was 0 mm. see figure 2 below.

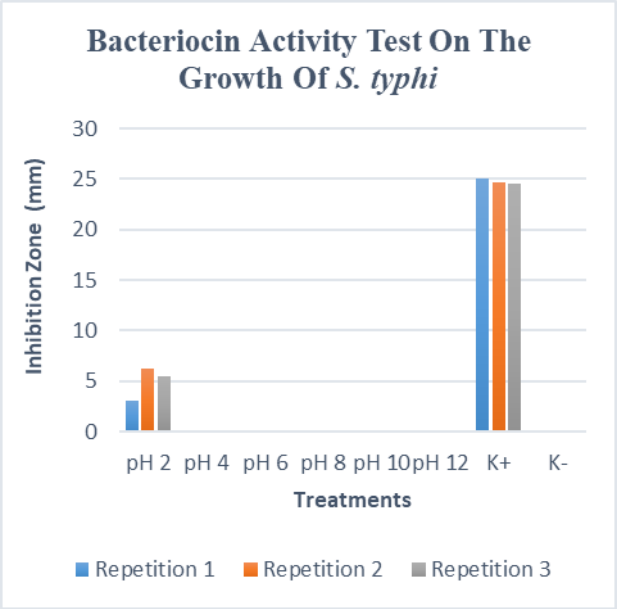


Fig. 2. Bacteriocin activity test on the growth of S. typhi.

The mechanism of action of bacteriocin is to form pores in the cell membrane which will damage the permeability of the target cytoplasmic membrane. The disruption of cytoplasmic permeability causes nutrient leakage within the cell wall and eliminates the Proton Motive Force (PMF). PMF is the electrochemical gradient of the cytoplasmic membrane that regulates the synthesis of Adenosine Triphosphate (ATP). The decrease of PMF will cause cell death due to the cessation of energy formation [11].

The result of this study are appropriate from the research by Zhao that the bacteriocin from *L. acidophilus* has an optimum pH at pH 2 to 3. There are differences in result, at pH 1 to 5 there is an inhibition zone against *Escherichia coli* [8]. Research by Saad, the bacteriocin from *L. acidophilus* has an optimum pH at pH 2 to 8 against the growth of *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* [6]. Factors that can affect the antimicrobial test are the population of bacteria, the concentration of antimicrobial, the composition of the culture media, the type of bacteria, incubation time, and temperature [12].

The number of bacteria can be controlled by comparing the bacterial suspension whose turbidity is equalized with a standard solution of 0.5 Mc Farland. The number of *L. acidophilus* in this study affected the bacteriocin concentration. The higher the concentration of bacteriocin, the more effective it is in inhibiting bacteria. The bacteriocin concentration in this study was insufficient so that it could not inhibit *S. typhi* at various pH. *S. typhi* is a Gram negative pathogenic bacteria. *Salmonella* grows at pH 4 to 9 with an optimum pH between 6.5 and 7.5 [5]. Based on this, it can strengthen the reasons for the inhibition zone at pH 2. *S. typhi* does not grow optimally at pH less than 4.

The media in this study was MHA which is the best media for antibiotic sensitivity test recommended by the Clinical and Laboratory Standard Institute. MHA contains good nutritional content and neutral so that it does not affect the antimicrobial test procedure [13].

Based on the criteria Davis and Stout, the inhibition of bacteriocin from *L. acidophilus* was categorized as weak and the inhibition of chloramphenicol was categorized as very strong. In One Way Anova test results show the value of p=0.000. Therefore, it can be concluded that there is a very significant difference between the diameter of inhibition zone on the growth of *S. typhi* from at least two treatment groups. The results of Post Hoc Tukey test showed that the bacteriocin from *L. acidophilus* at pH 2 could significantly inhibit the growth of *S. typhi* compared to the negative control (p=0.000). The bacteriocin at pH 2 which had the highest percentage of inhibition was still significantly different compared to chloramphenicol as positive control (p=0.000), so that the antimicrobial activity of the bacteriocin *L. acidophilus* ATCC 4356 could not be compared to chloramphenicol which is the first line antibiotic in the treatment of typhoid fever.

## IV. Conclusion

Bacteriocin from *L. acidophilus* ATCC 4356 has been tested for sensitivity to proteolytic enzymes and there is resistance or no inhibition zone so that the bacteriocin can be extracted. Bacteriocin from *L. acidophilus* can inhibit the growth of *S. typhi* with the largest inhibition zone 4.91 ± 1.70 mm at pH 2. The inhibition zone bacteriocin from *L. acidophilus* ATCC 4356 was different and smaller than the inhibition zone chloramphenicol.

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