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Research Article

PROBIOTIC POTENTIAL OF LACTIC ACID BACTERIA *Lactobacillus fermentum* NBRC 15885 ISOLATION FROM TEMPOYAK IN PADANG PARIAMAN DISTRICT, WEST SUMATERA (INDONESIA) TO ACID CONDITIONS, BILE SALTS AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

This study aims to determine the potential of probiotic lactic acid bacteria *Lactobacillus fementum* NBRC 15885 from tempoyak isolates in Padang Pariaman, West Sumatera (Indonesia) against acidic conditions, bile salts and antimicrobial activity. In this study we analyzed the probiotic resistance to low pH and bile salts, antimicrobial activity against pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogene*). The results showed that *Lactobacillus fermentum* NBRC 15885 from tempoyak isolates in Padang Pariaman Regency, West Sumatera (Indonesia) has probiotic properties because it has the ability to resist acidic condition with pH 3.0 with viability of 34%, against bile salts 0.3% with a viability of 97%, and has antimicrobial properties which able to inhibit pathogenic bacteria with a 16 mm inhibition zone in *Escherichia coli*, 14 mm in *Staphylococcus aureus,* 10 mm in *Listeria monocytogene*.

Keywords: probiotic, antimicrobial, acidic condition, bile salts

INTRODUCTION

Probiotic bacteria are a group of lactic acid bacteria that play important role for health, especially for the digestive tract. Lactic acid bacteria play a role in fermentation, mainly for fermented food products such as yogurt[1]. Probiotics are needed to support the digestive health of the body. The benefits of taking probiotics in humans include reducing the possibility of allergies[2]. However, not all lactic acid bacteria are probiotic bacteria. The characteristics of probiotics must be able to reach the intestines that has antimicrobial activity, it must resistant to acidic conditions and bile salts. Probiotics are characterized as living microorganisms that can provide medical benefits to the host if managed in a certain amount[3,4].

In this case probiotics can act as natural antibiotics. Some types of lactic acid bacteria can produce organic acids, hydrogen peroxide and bacteriocin. This compound, especially bacteriocin can cause death in other bacteria, resistant to the selection of digestive tract systems such as stomach acid with pH 2-3, bile fluid with pH 7-8.6. If bacteria do not have these characteristics, then the bacteria will die before reaching the intestine[4,5].

Traditional Indonesian fermented foods have been studied as potential probiotics, for example dadih from West Sumatera are made by fermenting buffalo milk in bamboo tubes. One of the traditional fermented foods from Sumatera is tempoyak. Tempoyak is a fermented product derived from durian fruit meat which is classified as traditional fermented food.

But the potential of probiotics isolated from tempoyak in Padang Pariaman District, West Sumatra (Indonesia) has not been studied.

MATERIALS AND METHODS

Previously lactic acid bacteria *Lactobacillus fermentum* had been isolated from tempoyak in Padang Pariaman District, West Sumatra (Indonesia) and molecularly identified using I6S rRNA with Forward (27F AGAGTTTGATCCTGGCTGAG) and Reverse primer (1492R; GTTTACCTTACGACTT)[6].

Resistance Test against Acid

Bacterial culture of 1 mL was inoculated on MRS Broth media 9 mL and incubated at 37ºC for 24 hours. Then, as much as 1 mL of bacterial culture from MRS Broth was put into a reaction tube containing 9 mL MRS Broth without pH control (control) and on MRS Broth pH 3 (pH regulated by the addition of HCl 5N) and incubated for 90 minutes. Next, pH 3 and control culture were diluted to 10^{-6} then planted using the spread method to the MRS media to be incubated at 37ºC for 48 hours. The number of bacteria that can survive was calculated by the Colony Forming Unit (CFU). Comparison of cell numbers before and after incubation will be expressed in the form of viability (%). The higher percentage of viability produced indicates the more resistant the bacteria to low pH. Calculation of total colonies growing was done using the CFU/mL formula[5]:

$$\text{LAB (Lactic Acid Bacteria) Colony Amount x } \frac{1}{\text{dilution factor}} \times \frac{1}{\text{sample weight factor}}$$

Calculation of LAB viability was done using formula:

$$\text{Decrease in colony number (\%):} \quad \frac{\text{Total colony of LAB control – total colony of LAB with pH settings}}{\text{Total LAB control}} \times 100$$

Viability (%) = 100 - decrease in colonies number (%)

Resistance Test against Bile Salts

Bacterial culture of 1 mL was inoculated on MRS Broth media 9 mL and incubated at 37ºC for 24 hours. Then, as much as 1 mL of bacterial culture from MRS Broth was put into a reaction tube containing 9 mL MRS Broth without oxgall control (control) and on MRS Broth with oxgall 0.3% then incubated for 24 hours. Next, oxgall 0.3% and control culture were diluted to 10^{-6} then planted using the spread method to the MRS media to be incubated at 37ºC for 48 hours. The number of survival bacteria was calculated by the Colony Forming Unit (CFU). Comparison of cell numbers before and after incubation will be expressed in the form of viability (%). The higher percentage of viability produced indicates the more resistant the bacteria to bile salts. Calculation of total colonies growing was done using the CFU/mL formula[5]:

$$\text{LAB (Lactic Acid Bacteria) Colony Amount x } \frac{1}{\text{dilution factor}} \times \frac{1}{\text{sample weight factor}}$$

Calculation of LAB viability was done using formula:

$$\text{Decrease in colony number (\%):} \quad \frac{\text{Total colony of LAB control – total colony of LAB with oxgall settings}}{\text{Total LAB control}} \times 100$$

Viability (%) = 100 - decrease in colonies number (%)

Antimicrobial Activity Test

Antimicrobial resistance test using well diffusion method was carried out on three test bacteria (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* O157; H7 and *Listeria monocytogene* EP01). As much as 1 mL of lactic acid bacteria culture was centrifuged at 10,000 rpm for 5 minutes at 27ºC, the supernatant was used for antimicrobial resistance. Then, as much as 20 ml of Mueller Hinton Agar (MHA) media were added 0.2% of test bacteria that had been rejuvenated, then it left until they hardened in a petri dish. Next, a hole was made on the MHA media with diameter ± 6.5 mm. Furthermore, antibiotics (penicillin, kanamycin, and ampicillin) were added as a positive control to compare the inhibition zones formed in pathogenic test bacteria. Antibiotics were given using paper disks that already contain antibiotics to determine the resistance and sensitivity of pathogenic test bacteria using positive antibiotic controls. Next, 50 µl of supernatant of lactic acid bacteria was injected. After that, it was incubated at 37ºC. Observations were made on clear zones after 24 hours[7].

RESULTS AND DISCUSSION

Resistance against acid

The ability of *Lactobacillus fermentum* NBRC 15885 from tempoyak isolates in Padang Pariaman, West Sumatra (Indonesia) against acidic condition with the number of control living bacterial (without pH 3 settings) was 250 x 10^7 CFU/ml after the pH 3 settings. The number of living bacterial cell was 87x 10^7 CFU/ml and produced 34% of the lactic acid bacteria viability. Viability of lactic acid bacteria is a comparison of the number of living cells before and after the acidity setting, which stated in percentage (%) form. The higher percentage produced indicates that the bacteria is more resistant to low pH.

Exposure to very acidic conditions can cause membrane damage and the loss of intracellular components such as Mg, K and fat from cells, which can cause death for the bacteria that are not resistant to acid. While acid resistant bacteria have a greater resistance to membrane damage due to a decrease in extracellular pH compared to the bacteria that are not resistant to acid. Bacterial resistance at low pH occurs because its ability to maintain internal pH to be more alkaline than external pH and it has cell membranes that are more resistant to cell leakage due to exposure of low pH. Probiotic bacteria will have an effect on the intestinal environment if the population of the bacteria reaches a minimum of 10^6-10^8 CFU/mL in a state of acid able to maintain the level of cytoplasmic acidity so that proteins and enzymes inside the cell can still work optimally[7,8].

Resistance against Bile Salts

The ability of *Lactobacillus fermentum* NBRC 15885 from tempoyak isolates in Padang Pariaman District, West Sumatra (Indonesia) against bile salts with the number of control living bacterial (without addition of 0.3% oxgall) was 390 x 10^7 CFU/ml after oxgall addition 0.3% with the number of living bacterial cells of 387x 10^7 CFU/ml produced the highest viability of lactic acid bacteria at 97%. Viability is a comparison of the number of living cells before and after being given an arrangement of the addition of oxgall 0.3% which expressed in terms of percent (%). The higher the percentage produced indicates the more resistant the bacteria to bile salts.

The resistance of lactic acid bacteria towards bile salts is related to the Bile Salt Hydrolase (BSH) enzyme which helps to hydrolyze conjugated bile salts, thereby reducing the toxic effects on cells. The tolerance of bile salts is allegedly caused by the role of polysaccharides as one of the constituent components of gram-positive bacterial cell walls. The most serious obstacle to the resistance of probiotics in the small intestine is bile salt. In vitro studies of probiotic resistance to bile salts can be divided into two types which are the study of resilience and growth[9,10].

Antimicrobial Activity

Antimicrobial test was done using the diffusion well method with antibiotics as a positive control. Antibiotics were administered using a paper disc containing 2 µg/disk of ampicillin concentration, kanamycin 30 µg/disk and 3 µg/disk of penicillin. Positive antibiotic control was used to determine the resistance and sensitivity of pathogenic test bacteria. The results of the inhibitory zone can be seen in Figure 1 and Table 1.

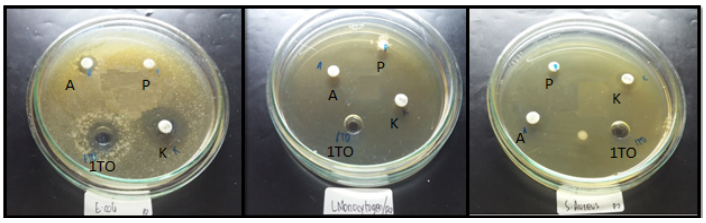

Figure 1: Annotation- Clear zone of LAB isolate against pathogenic bacteria using positive control of antibiotics (A) Ampicillin, (K) Kanamycin, (P) Penicillin, (1TO) *Lactobacillus fermentum* NBRC 15885. (A) Clear zone formed against *Escherichia coli* O157 (B) Clear zone formed against *Listeria monocytogenes*, (C) Clear zone formed against *Staphylococcus aureus.*

Table 1: Diameter of Clear Zone from Antimicrobial Resistance Test with Positive Control

Sample Code	Clear zone diameter (mm)		
	Escherichia coli O157	*Staphylococcus aureus*	*Listeria monocytogenes*
Lactobacillus fermentum NBRC 15885	16	14	10
Ampicillin	13	-	-
Kanamycin	14	-	-
Penicillin	-	-	-

Data from the research results in Figure 1 and Table 1 showed that *Lactobacillus fermentum* NBRC 15885 was able to inhibit the three pathogenic bacteria tested with a 16 mm inhibition zone on *Escherichia coli*, 14 mm in *Staphylococcus aureus*, 10 mm in *Listeria monocytogene*. Positive control of antibiotics on the *Escherichia coli* O157 by ampicillin was produced an inhibition zone of 13 mm diameter, kanamycin with a diameter of 14 mm, penicillin 0 mm. While in *Staphylococcus aureus* and *Listeria monocytogenes* the antibiotics kanamycin, ampicillin, and penicillin did not produce clear zone at all, indicating the resistance of antibiotics.

Bacterial resistance toward antibiotics can be influenced by several things including the ability of bacterial enzymes that can cause inactivation of antibiotics. Generally bacterial resistance toward antibiotics can be caused by changes in antibiotic binding sites by bacteria so that antibiotics are no longer able to bind to bacteria in an effort to stop the bacterial growth[7,11].

Lactic acid bacteria include the production of organic acids, hydrogen peroxide, diacetyl, and broad-spectrum antimicrobial compounds such as reuterin and bacteriocin. The antimicrobial effect is directly given by the presence of organic acids including lactate, acetate and propionate. Antimicrobial properties are produced because of the acidic effect on bacterial cytoplasmic membranes that affect active transport and membrane potential. During growth most of the sugar is converted by lactic acid bacteria into lactic acid which provides inhibition to other microorganisms. When lactic acid is produced, pH is decreased, resulted in organic acids not decomposing or dissociating. These non-dissociated acids are the main antimicrobial properties of lactic acid bacteria. These non-dissociated acids target and attack bacterial membranes causing weak acid anions to gather in the cytoplasm which further affects the metabolic process. There is an inhibition of growth and it will slowly die[5,11].

CONCLUSION

This study shows that *Lactobacillus fermentum* NBRC 15885 lactic acid bacteria from tempoyak isolates in Padang Pariaman, West Sumatra (Indonesia) has potential as probiotics, because it has antimicrobial properties, resistant to acidic conditions and bile salts. *Lactobacillus fementum* NBRC 15885 is resistant to acidic conditions of pH 3.0 with 34% viability, 0.3% bile salt with 97% viability, having antimicrobial properties that can inhibit pathogenic bacteria with a 16 mm inhibition zone in *Escherichia coli*, 14 mm in *Staphylococcus aureus*, 10 mm in *Listeria monocytogene*.

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