ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM RABBIT MEAT BEKASAM ON GRAM POSITIVE AND NEGATIVE BACTERIA

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Abstract

Bekasam is a traditional fermentation product made from rabbit meat that is an adaptation of fish fermentation which are often consume in several regions in Indonesia. The fermentation process is spontaneous and the bacteria growing are dominated by Lactid Acid Bacteria. The aim of this study was to study the antimicrobial activity of lactic acid bacteria isolated from rabbit meat bekasam against gram-positive pathogenic bacteria (Staphilococcus aureus, Listeria monocytogenes) and gram-negative (Salmonella thypimurium, Eschericia coli). Eleven isolates of LAB were isolated from rabbit meat bekasam and the antimicrobial activity was measured during fermentation. The results showed that the highest inhibition of antibacterial activity was obtained from isolate 6.1 which the clear zone were 16.4 mm for S. Aureus, 12.5mm for L. monocytogenes, 15.2 mm for S. thypimurium and 13.1mm for E. coli.

Key words: bekasam, fermentation, lactid acid bacteria, antimicrobial activity

INTRODUCTION

Bekasam is fish fermented food from Indonesia. Bekasam often found in South Sumatra, South Kalimantan and North Sulawesi. Various species of bacteria are involved in the fermentation of, but the dominant species is Lactid Acid Bacteria (LAB). It is prepared by fermenting fish together with rice (50%) and salt (10%) incubated for 7 days at room temperature. Rice is added as a source of carbohydrates to stimulate the growth of lactic acid bacteria [7]. Salt is added to prevent the formation of ammonia that can be produced from nitrogen compounds. Furthermore the salt is used to screening specific characteristics of bacteria, namely bacteriostatic properties for pathogenic and spoilage bacterias [4].

In this study, the raw material used was rabbit meat which had a higher protein content compared to chicken, beef, lamb and pork. The nutritional content of rabbit meat is 20.8% protein, 10% fat, niacin (8.43 mg/ 100 g ingredients, equivalent to 42% of total daily needs), vitamin B12 (8.3 µg / 100 gr ingredients), and selenium (Se) with levels of 38.5 µg / 100 g [1].

During lactid acid fermentation, lactid acid bacteria would convert carbohydrates into lactic acid and other compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins which can inhibit the growth of pathogenic microbes[3]. The aim of this study was to study the antimicrobial activity of lactic acid bacteria isolated from rabbit meat bekasam against gram-positive *(S. aureus, L. monocytogenes*) and gram-negative bacteria (*S. thypimurium, E. coli).*

MATERIAL AND METHOD

Isolation LAB from bekasam

Ten grams of rabbit meat bekasam were mixed with 90 ml sterile salin water. The samples were serially diluted and 100 µl of suitable dilutions was spread on De Man, Rogosa and Sharpe (MRS) agar plates (Himedia, India)with 1% $CaCO_3$. The culture was incubated at 37°C for 24-48 hours. Different colonies growing on agar were purified. The colony was characterized by its shape and color. The stock cultures of LAB was maintained in MRS broth supplemented with 15% glycerol and stored at -80°C.

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Test Microorganisme

Microorganisme used were gram positive and gram negative bacteria. Gram positive bacteria were *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 7644 and gram negative bacteria were *Escherichia coli* ATCC 11229, and *Salmonella Typhimurium* ATCC. All pathogen were grown in Nutrient Agat (NA) at 37ºC for 24 hours. These microorganisme were obtained from the Central Laboratory Padjadjaran University, Jatinangor, Indonesia.

The test microorganisms were standardized by using 0.5 Mc Farland standard which is equivalent to cell density of 1.5 x 108 CFU/ml. Mc Farland Standard was used as reference to adjust the turbidity of microbial suspension. The turbidity was adjusted using steriled distilled water.

Antimicrobial activity of LAB

Antimicrobial activity was test using a agar well diffusion assay according to the method by Tagg [12]. A bacterial suspension of pathogenic bacteria was swab on Nutrient Agar (NA) using sterile cotton bud. Aliquots of supernatants (40 µL) were placed in wells (6 mm diameter). The plates were incubated at 37ºC for 24 hours and diameters of the growth inhibition zones were measured. Standard antibiotics (cloramphenicol 30mg/ml) were used as the negative and positive control.

RESULTS AND DISCUSSIONS

Isolation of LAB were obtained in MRS agar with $CaCO_3$, LAB produces lactic acid and reacts with $CaCO_3$ to produce soluble lactate calcium, characterized by a clear zone around the growing bacterial colonies [13]. Eleven isolates of lactic acid bacteria were isolated from rabbit meat bekasam.

Antimicrobial activity was figure out the ability of isolates to produce antimicrobial substances that are suppressed the growth of pathogenic bacteria. Antimicrobial activity was carried out using gram positive pathogens *(S. aureus, L. monocytogenes)* and gram negative pathogens (*E. coli, S. typhimurium).*

Eleven isolates were found to produce inhibition zones against S. aureus, *L. monocytogenes, E. coli* dan *S. typhimurium* (Table 1). The antimicrobial activity of each isolate is different due to the different antimicrobial components produced. Based on the category of bacterial inhibition zone diameter there are 4 categories of inhibitory strength based on its diameter, diameter 5-9 mm has weak inhibitory strength, diameter 10-14 mm medium inhibitory strength, diameter 15-20 mm strong inhibitory strength,> 21 mm inhibition strength is very strong [8].

Table 1 Antimicrobial Activity of LAB Isolates Againts Various Pathogen

No	Kode Isolat	*S. Aureus*	*L. monocytogenes*	*S. thypimurium*	*E. coli*
		----------mm----------			
1	Antibiotic	27.2	28.0	26.6	27.0
2	3.1	7.3	7.8	6.4	6.2
3	3.2	8.5	8.1	7.2	7.8
4	4.1	8.2	7.3	6.3	6.4
5	5.1	8.1	7.4	7.2	7.0
6	5.2	9.2	10.5	8.3	7.2
7	6.1	16.4	12.5	15.2	13.1
8	6.2	11.5	7.2	11.3	12.2
9	6.3	13.2	13.1	10.2	7.5
10	7.1	15.2	15.3	11.9	10.2

LAB have the ability to inhibit pathogenic bacteria in mammals digestive tract, because LAB produce organic acid such as lactic acid, acetic acid, hidrogen peroxide, bacteriocins, phenyllactic, p-OH-phenyllactic acid, caproic acid or reuterin [14], [11].

Based on the results of the inhibitory test it was shown that all isolates had a medium-strong resistance to pathogen bacteria. Table 1. shows that the clear zone for *S. aureus* ranges from 7.3 - 15.2; *L. monocytogenes* ranges from 6.0 - 15.3; *E. coli* ranged from 6.2 to 15.3 and *S. thypimurium* ranged from 6.3 to 15.2. It is shown that all isolates have the ability to inhibit gram-positive and gram-negative pathogenic bacteria. These findings are in agreement with those obtained by [10] which were isolated LAB from fermented foods, such as glutinous rice dough, corn noodle, chili sauce, potherb mustard pickles, and stinky tofu, in northeast China. Thirty-five strains have antimicrobial activity against *S. aureus* and *Salmonella*. The other research was isolated LAB from the fermented food product, Budu. All isolates were have antimicrobial activity againts *E. coli, Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus and Bacillus cereus* [14].

In general, the inhibitory effect of the isolated antibacterial strains on S. aureus and L. monocytogenes was greater than that on E. coli and Salmonella. The results suggested that the concentration and type of the produced antibacterial substance differed from those of LAB. Thus, different LAB showed various degrees of inhibitory activity against pathogenic bacteria. In general, gram-negative bacteria have better resistance to antimicrobial compounds, because it has more complex cell wall structure which is the outer layer is lipopolysaccharide and the inner layer is peptidoglycan the structure of the cell wall of gram-positive bacteria is simpler making than gram-negatif it easier for antimicrobial compounds to enter the cell.

Chlorampenicol is used as positive standard because it was classified as broad spectrum antibiotic antibiotic, which mean effective for gram positive and gram negative bacteria [5].

Isolates 6.1 and 7.1 have higher inhibitory strength compared to inhibition produced by other isolates. The inhibition mecanism of pathogenic bacteria by isolates, caused by electrostatic compounds produced by positively charged isolae with negatively charge lipid membrane. The hydrophobic part of the bacteriocin will enter the cytoplasmic membrane by forming a pore. Pore formation will cause proton motive force (PMF) failure, which is a proton that forms energy for use in a variety of cell activities including bacterial cell metabolism. Therefore failure of PMF will cause cell death by stopping all reactions that require energy [2]. While the inhibitory mechanism due to lactic acid that is in low pH conditions lactic acid is in the form of undissociated to be lipophilic, so it is able to diffuse into the membrane of pathogenic bacteria. The undissociated form of an antimicrobial component will cause protons to enter the cell more quickly, the undissociated acid will cripple the proton gradient electrochemically or by changing the permeability of the cytoplasmic membrane which will disrupt the transport system of the substrate [9].

CONCLUSIONS

Eleven isolates of LAB were isolated from rabbit meat bekasam and the antimicrobial activity and highest inhibition of antibacterial activity was obtained from isolate 6.1.

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