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Potential of Lactic Acid Bacteria Isolated from Mackerel *Bekasam* as Antibacterial and Probiotic Candidates

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ABSTRACT— Mackerel is one type of seawater fish belonging to the pelagic group which has a distinctive delicious taste so that it is favored by the community. Mackerel fish can be found in Indonesian waters, especially in West Papua. Mackerel can be preserved through drying or fermentation processes, including *Bekasam*. The *Bekasam* is a fermented fish product that has a sour taste. During the fermentation process, there is the growth of lactic acid bacteria (LAB), and it is potential to be used as a probiotic candidate. The objective of this study was to determine the characteristics of LAB as probiotic candidates, and to determine the inhibition activity of LAB against pathogenic bacteria. This study is a descriptive study, which describes the characteristics of LAB cells as candidates for probiotic bacteria and also describes the ability of LAB to inhibit the growth of pathogenic bacteria. The results showed that of the six samples investigated, there were 3 samples of LAB that were potential as probiotic candidates, namely samples FST 1, FST 2, and FNT2. Furthermore, sample FST 2 showed the highest inhibition activity against *Escherichia coli*, while sample FNT 3 was not able to inhibit *E. coli* at all. Meanwhile, sample FNT 1 had the highest inhibition activity in inhibiting the growth of *Salmonella typhimurium*, while sample FST 2 was unable to inhibit *S. typhimurium*. Furthermore, sample FST 1 had the highest inhibition activity in inhibiting the growth of *Staphylococcus aureus*.

KEYWORDS: LAB, *Bekasam*, Mackerel, inhibition activity, Probiotic

1. INTRODUCTION

Mackerel is a type of seawater fish that belongs to the pelagic marine fish group and has a distinctive delicious taste that is favored by the community. Mackerel is commonlyn found in Indonesian waters, especially in West Papua [1]. Mackerel can be preserved through drying and fermentation processes including *Bekasam*. The *Bekasam* is a fermented fish product that has a sour taste [2]. During the fermentation process, lactic acid bacteria (LAB) grow, so that the *Bekasam* products generally contain a lot of LAB [3].

Lactic acid bacteria are a group of Gram positive bacteria with bacil or coccus-shaped cells and do not have spores [4]. Lactic acid bacteria have been widely used in food preservation, the presence of lactic acid bacteria in food is able to give a distinctive aroma, taste and texture to fermented products. Lactic acid bacteria also have an important role in the world, including the chemical industry for the synthesis of chemical compounds and the formulation of drugs. Until now, LAB has been widely used as a starter culture, a source of probiotics, vitamins, enzymes and antimicrobial agents, which are useful for human health [5]. Lactic acid bacteria are also used in dealing with food safety problems, because LAB is able to produce organic acid components such as lactic acid and acetic acid. In addition, LAB is able to produce active compounds such as hydrogen peroxide and bacteriocins that can act as antimicrobials [6].

Several studies have examined the potential of LAB originating from *Bekasam*, including as a probiotic agent [7], as a biopreservative [8], producer of lipase enzymes [9], and as antibacterial [10]. Lactic acid bacteria are dominantly found in fermented products and are reported to have antimicrobial activity and have potential as probiotic candidates.

Probiotics are defined as live microorganisms that are capable of providing health benefits to their hosts in sufficient quantities [11]. The criteria that must be possessed by probiotic bacteria are being able to survive when passing through the stomach and small intestine, tolerant of acidic conditions and bile salts [12], having the ability to stick to the intestines [13], and being able to compete with pathogenic microbes [14].

Probiotics have many health benefits. The benefits of probiotics for humans include reducing the risk of lactose intolerance, increasing immune system response, protecting against inflammation/arthritis, preventing hypertension, preventing cancer [15], and preventing diarrhea [16]. In addition, probiotics are also beneficial for fish digestion due to their ability to increase digestibility in the fish body which is useful for increasing feed efficiency, and being able to control the growth of pathogenic bacteria [17]. In line with the increasing consumer demand for probiotics, we have conducted extensive research on sources of Lactic Acid Bacteria as antibacterial and probiotic candidates.

Exploration studies of LABs which have potential as probiotics continue to be carried out by many researchers in various countries. Studies on probiotic sources in Indonesia are still being carried out on dairy products and their derivatives, beef [18], and tempoyak [19]. However, studies on the potential of LAB have never been conducted on *Bekasam* products made from mackerel fish, especially fermented products originating from West Papua. This underlies the importance of studies on the potential of lactic acid bacteria as antibacterial and probiotic candidates. The aims of this study are as follows, firstly, to determine the characteristics of lactic acid bacteria from mackerel *Bekasam*. The second objective was to determine the potential of lactic acid bacteria as probiotic candidates in inhibiting pathogenic bacteria.

2. Material and Methods

2.1 Research Design

This study is a descriptive study that describes the characteristics of lactic acid bacteria (LAB) cells and the inhibitory ability of LAB as probiotic candidates against pathogenic bacteria. *Bekasam* samples of mackerel fish were obtained by making *Bekasam* as fermented products from mackerel fish. The research was conducted in July – September 2022 at the Microbiology Laboratory, Faculty of Fisheries, Universitas Muhammadiyah Sorong.

2.2 Method

2.2.1 Isolation of Lactic Acid Bacteria (LAB)

A total of 1 gram of each sample of mackerel *Bekasam* was taken, then serial dilutions were carried out starting from $10^1 – 10^3$, 1 mL of suspension was inoculated on MRS + 0.5% $CaCO_3$ medium, then incubated at 37 °C for 24 hours. Macroscopic observations were performed on the growing bacterial colonies including colony color, colony shape, and colony elevation. Lactic acid bacterial colonies with different morphological characteristics were selected for further testing.

2.2.2 Gram Staining

The LAB isolate was suspended on an object glass and fixed over a Bunsen flame so that it became a preparation. Crystal violet was dripped on bacterial preparations and rinsed with distilled water, the preparations were then dripped with lugol's iodine and drained with distilled water, the preparations were then rinsed with 96% alcohol for 10 seconds. The preparations were drained with distilled water and dripped with safranin for 10 seconds. The preparations were then rinsed with distilled water. The preparations were observed under a microscope using immercy oil.

2.2.3 Motility test

The bacterial isolates were inserted into the Nutrient Agar medium in a test tube and then incubated at 35 ^oC for 24 hours. Bacteria are motile if their growth appears to be spreading and bacteria are non-motile if their growth does not appear to be spreading.

2.2.4 Catalase test

One loop of bacterial isolate was transferred to an object glass, then dripped with 3% H_2O_2 solution. Catalase enzyme is characterized by the formation of gas bubbles.

2.2.5 Oxidative-fermentative test

One loop of bacterial isolate was inserted into Baird Parker agar medium in a test tube. Each test bacteria was inserted into two tubes as follows: the first tube was covered with 3-5 ml paraffin and the second tube was without paraffin. All tubes were incubated at 30 ^oC for 48 hours. If yellow color is formed in both tubes, it is fermentative. If the tube without paraffin is yellow, it is oxidative. If there is no color change in both tubes, it is negative oxidative-fermentative.

2.2.6 Citrate test

Bacterial isolates were inoculated on Simmons citrate slant agar, then incubated at 30 ^oC for 48 hours. If the media changes color from green to yellow, the result is positive.

2.3 Inhibition test against pathogenic bacteria

One loop of bacterial isolate was spotted on Mueller Hinton Agar medium [20] which had been inoculated with pathogenic bacteria (*S. typhImurium, E. coli,* and *S. aereus*), then incubated at 37 ^oC for 24 hours. The clear zone formed reflects the antibacterial activity. The inhibitory activity of LAB was calculated [21], [22] with the formula:

$$\text{BAL Activity} = \frac{a-b}{b}$$

Description:
a : Clear zone area (mm^2)
b : BAL colony area (mm^2)

3. Results

The results of screening and characterization of lactic acid bacteria from mackerel Bekasam as probiotic candidates are presented in Table 1.

Table 1. Results of screening and characterization of lactic acid bacteria (LAB) as probiotic candidates

Sample	Average Colony	Colony Characteristics	Cell shaped and Gram	Catalase Test	Motility Test	Oxidative-Fermentative	Citrate Test

	Number (CFU/g)	Shape	Elevation	Margin	Staining			Test	
FST 1	$1.8x10^4$	Round	arise	Slick	Rod, +	+	√	Fermentative	√
FST 2	$4.6x10^4$	Round, Coiled	Embossed flat	Slick, Wavy	Rod, +	+	√	Fermentative	√
FST 3	$5x10^4$	Round, Coiled	Embossed flat	Slick, Wavy	Rod, -	+	√	Fermentative	√
FNT 1	$6.5x10^4$	Round, Coiled	Embossed flat	Slick, Wavy	Rod, -	+	√	Fermentative	√
FNT 2	$3.8x10^4$	Round, Coiled	Embossed flat	Slick, Wavy	Rod, +	+	√	Fermentative	√
FNT 3	$7.6x10^4$	Round, Coiled	Embossed flat	Slick, Wavy	Rod, -	+	-	Fermentative	√

Inhibition activity of lactic acid bacteria (LAB) as probiotic candidates against pathogenic bacteria (*Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aereus*) (Table 2, Figure 1; Figure 2).

Table 2. The results of inhibition activity of BAL against pathogenic bacteria

Sample	*S. typhimurium* (mm)			*E.coli* (mm)			*S. aereus* (mm)		
	U.1	**U.2**	**U.3**	**U.1**	**U.2**	**U.3**	**U.1**	**U.2**	**U.3**
FST 1	0.13	9.00	16.5	20.88	0.00	0.00	2.83	2.10	3.88
FST 2	0.00	0.00	0.00	27.33	28.17	44.00	0.00	0.00	0.00
FST 3	24.00	12.75	5.25	35.43	35.43	1.05	0.53	0.63	0.16
FNT 1	10.00	21.14	42.33	5.54	0.20	22.75	0.04	0.36	6.00
FNT 2	20.43	17.13	0.00	4.19	8.44	14.00	0.43	0.38	0.50
FNT 3	15.00	17.17	36.50	0.00	0.00	0.00	1.00	0.96	1.20

Description: U: Replication

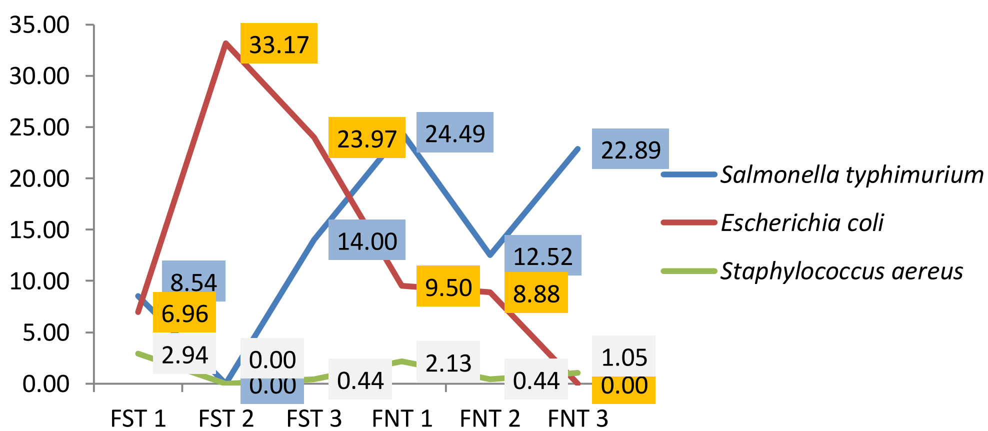

Figure 1. Inhibition activity of LAB against pathiggenic bacteria

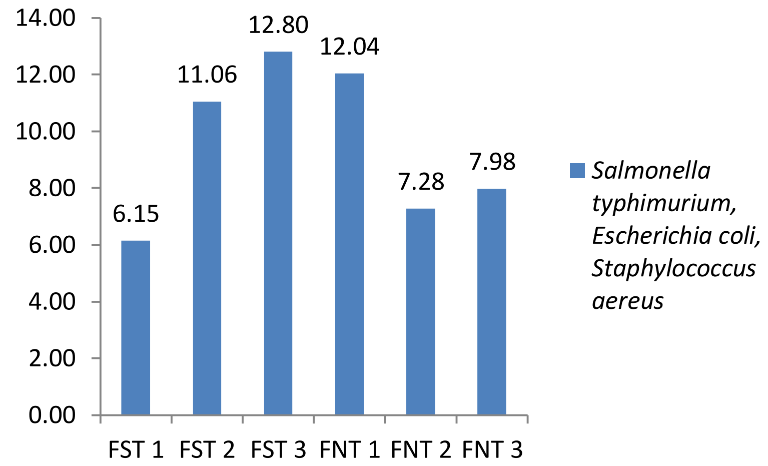

Figure 2. inhibition activity of BAL against pathogenic bacteria

4. Discussion

Six samples of *Bekasam* products investigated showed the presence of lactic acid bacteria. The number of LAB populations from each gram of *Bekasam* samples varied, with the highest total population obtained by sample FNT 3 (7.6x104 CFU/g), followed by sample FNT 1 (6.5x104 CFU/g), sample FST sample 3 (5x104 CFU/g), sample FST 2 (4.6x104 CFU/g), sample FNT 2 (3.8x104 CFU/g), and sample FST 1 (1.8x104 CFU/g). The characteristics of the colonies found were bacil-shaped cell and coiled, raised elevation, and smooth and wavy margin. The LAB isolated from samples FST 1, FST 2, FNT2 had rod-shaped cells, Gram positive, and these samples indicated that LAB isolated were potential candidates for probiotic bacteria because they belonged to the group of gram-positive bacteria. While the samples of FST 3, FNT 1, FNT 3 had rod-shaped cells but were included in the Gram negative group. Furthermore, the six studied samples were detected to have the catalase enzyme. Meanwhile, almost all of the samples tested positive for the motility test except for the sample FNT 3. Meanwhile, for the results of the oxidative-fermentative and citrate tests, all samples showed a fermentative reaction and were able to use citrate as an energy source. *Bekasam* samples that were positive for catalase indicated that LAB was aerobes or facultative anaerobes. However, in this study, it was suspected that the bacteria were facultative anaerobes because the *Bekasam* samples were produced by fermentation procces which was strengthened by the positive fermentation test results.

Aerobic bacteria have superoxide dismutase enzymes that can break down free radicals, and are able to break down H2O2 to produce non-toxic compounds [23]. Facultative anaerobic bacteria also possess the enzyme superoxide dismutase, and some have the enzyme catalase. The enzyme is able to catalyze the reaction of H_2O_2 and organic compounds, resulting in non-toxic compounds [24].

Obligate anaerobic bacteria do not have superoxide dismutase and catalase enzymes, so the 'presence of oxygen is toxic to bacteria [25]. Baird Parker’s oxidative-fermentative test aims to determine the metabolic properties of the tested bacteria, whether they are oxidative or fermentative. Of the six samples tested, they were positive for the fermentation test because the media turned yellow in both the samples with and without paraffin treatment.

Bacterial metabolism is fermentatively classified as facultative anaerobic, because the fermentation process occurs under anaerobic conditions [26]. Fermentation is a reduction-oxidation reaction that produces energy in which organic compounds act as electron donors and acceptors. Organic compounds that are usually used are carbohydrates in the form of glucose. Under anaerobic conditions, these compounds are converted into acidic compounds by oxidation-reduction reactions with the help of enzymes as catalysts. Cells that carry out fermentation have enzymes that will convert the results of the oxidation-reduction reaction into a compound that has a more positive charge so that it can capture electrons or act as the final electron acceptor to produce energy [27], [28]. The citrate test is used to see the ability of microorganisms to use citrate as an energy source for cell metabolism. The medium used for this test is Simmons citrate which is a synthetic medium with Na citrate as the only carbon source, NH^{4+} as the N source. If microorganisms are able to use citrate, the acid will be removed from the medium, causing an increase in pH and changing the color of the medium from green to yellow. This means that these bacteria have the ability to use citrate as an energy source [29].

Sample FST 2 showed the highest inhibition value against *Escherichia coli*, while the sample FNT 3 was unable to inhibit *E. coli*. Meanwhile, sample FNT 1 had the highest value in inhibiting *Salmonella typhimurium*, sample FNT 1 had the highest inhibition activity compared to the other four samples (FST 1, FST 3, FNT 2, and FNT 3), while sample FST 2 was unable to inhibit *S. typhimurium*. Furthermore, sample FST 1 had the greatest inhibition value against *Staphylococcus aereus*. Based on the average value, the highest inhibition activity of BAL against the three pathogenic bacteria was sample FST 3, while the lowest was sample FST 1.

Several studies have shown that lactic acid bacteria that have the potential as probiotics and are able to inhibit pathogenic bacteria consist of four genera namely *Micrococcus, Staphylococcus, Bacillus*, and *Hafnia* [30], other researchers reported that probiotic bacteria are Gram positive bacteria with rod or round-shaped cells and belongs to the genus *Lactobacillus* [31].

According to a research conducted [32], LAB isolates from *Bekasam* fermentation have the ability to inhibit the growth of five types of pathogenic bacteria (*Escherichia coli*, *Salmonella typhimurium* ATCC 14028, *Bacillus cereus, Staphylococcus aureus* and *Listeria monocytogenes*) and are potential probiotic candidates. In addition, LAB isolates from cow intestines showed antibacterial ability against *E.coli* and *Shigella* sp. The results showed that LAB isolates with code sp.1 had an inhibition zone against *E. coli* and *Shigella* sp. of 7.5 mm and 6.8 mm respectively, while LAB isolates with code sp.2 had an inhibition zone of 8.9 mm for *E. coli* and against *Shigella* sp. of 8.0 mm [33]. Panjaitan also reported that lactic acid bacteria from tempeh and tape may be candidates for probiotic bacteria capable of inhibiting pathogenic bacteria [34].

Another probiotic from LAB isolated from the indigenous goldfish (*Cyprinus carpio*) showed the ability to produce antibacterial compounds against *A. hydrophila* and *E. tarda* [35]. Furthermore, LAB had also been isolated from tilapia, water, sediment, and several types of traditional fermented foods and it is known that *L. plantarum* strain CR1T5 had the ability as a probiotic [36], [37].

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