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Isolation and Characterization of Lactic Acid Bacteria Isolates from Traditional Sambas Pekasam Based on Lundu Fish and Milkfish

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Abstract

Background: Pekasam, or Bekasam, is a fermented fish product that can potentially produce lactic acid bacteria (LAB). This study aims to isolate and characterize LAB from Pekasam from Sambas Regency. TPC results on MRS media showed that Pekasam from Sambas Regency can potentially contain LAB with a total colony of 107 CFU/g. The isolates were identified for their colony and cell shape so that three selected isolates were obtained, namely SB1(1), SB3(1), and SB3. **Methods:** Isolates were characterized for proteolytic activity, hemolytic activity, low pH tolerance, tolerance to the presence of bile salts, carbohydrate metabolism, and potential antimicrobial activity. All isolates had round colonies, convex or raised surfaces, flat edges, milky white, rod-shaped cells, and were classified as gram-positive bacteria. **Results:** All isolates showed proteolytic activity, ɤ-hemolysis, had low pH tolerance with viability <60% at pH 2 and 3, were resistant to bile salts with viability <73% at NaDC concentrations of 0.2 and 0.4 mmol, were able to metabolize carbohydrates and had antimicrobial activity. The selected isolates were identified as Lactobacillus species and were homofermentative. **Conclusion:** The three selected isolates can potentially be homofermentative LAB of Lactobacillus species.

Keywords: BAL; Fermented Fish; Pekasam.

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Introduction

Pekasam or Bekasam is a traditional food made to preserve fish catches by involving a fermentation process. Generally, Pekasam is made traditionally by mixing cleaned fish with salt and rice and storing it in a closed container for 7 days (Syarifah & Huda, 2016). This traditional food comes from various regions in Indonesia, such as South Kalimantan, South Sumatra, and West Kalimantan. Fish processing into Pekasam is famous in the West Kalimantan community, especially in the Sambas Regency area. This regency is part of the northernmost part of the West Kalimantan province, which directly borders the neighboring country of Sarawak-East Malaysia.

Pekasam from Sambas Regency occurs spontaneously without involving the addition of microorganisms, either in the form of starter or yeast. Microorganisms are naturally found in beneficial and detrimental food ingredients (pathogenic). If environmental and nutritional conditions are suitable, certain microorganisms will grow and become dominant microorganisms under optimum conditions (Navarrete-Bolaños, 2012). The fermentation process in Pekasam can occur due to anaerobic environmental conditions and carbohydrate sources that can stimulate the growth of lactic acid bacteria. Adding carbohydrate sources makes Pekasam potentially a traditional food containing lactic acid bacteria.

Lactic acid bacteria produce lactic acid due to their metabolism, which can form an acidic atmosphere in Pekasam, so pathogenic bacteria will find it difficult to reproduce. Several strains of lactic acid bacteria can also produce proteolytic enzymes consisting of proteinase, peptidase, and specific transport proteins that help the fish fermentation process (Kieliszek et al., 2021). Using salt aims to control the fermentation process so that only obligate halophilic microorganisms can grow (Marantika et al., 2020). Halophilic microorganisms can degrade toxic compounds and produce hydrolytic enzymes such as amylase, protease, cellulase, and lipase, which play an essential role in fermentation (De et al., 2013).

The potential of lactic acid bacteria in fermented food products, especially fermented fish, has been previously studied. In a study conducted by Ida et al. (2017) of 40 isolates obtained from Bekasam from Malaysia, three isolates (L8, L20, and S1) showed antimicrobial effects against pathogenic bacteria, were γ-hemolytic and tolerant to various pH (pH 3, 5 and 7.5) and 0.3% (w/v) bile salts. Another study conducted by Yufidasari et al. (2021) isolated Bekasam from the city of Palembang, South Sumatra, which had antibacterial activity against E. coli, Salmonella, and S. aureus with a total BAL of 58.3%, 66.67%, and 91.67%.

Based on this, Bekasam is a fermented fish product that produces lactic acid bacteria as the dominant microbe (Saithong et al., 1998). However, research on isolating lactic acid bacteria from Pekasam from Sambas Regency has never been conducted, although Pekasam is a local fermentation product in Indonesia. Previous research has focused more on the potential of lactic acid bacteria from Bekasam products from their region and abroad. Therefore, this study was conducted to determine the potential of Pekasam from Sambas Regency by isolating and characterizing lactic acid bacteria.

Method

Sampling

Pekasam samples were obtained from two Pekasam producers in the Sambas area 1.41667°N 109.33333°E, West Kalimantan. Interviews were conducted regarding the fish used, supporting materials, and others with the criteria for Pekasam using fish, namely lundu fish (*Macrones gulio*) and milkfish (*Chanos chanos*). Before testing, samples were put into different sterile ice boxes and taken to the laboratory for further analysis.

Isolation and Purification of Lactic Acid Bacteria

Lactic acid bacteria were isolated in Pekasam using the Pour Plate method using MRSA. One gram of Pekasam was diluted into 9 mL sterile aquadest and homogenized. Dilution was carried out from 10-2 to 10-5. Each dilution was taken as much as 1 mL and then grown on MRSA media. Incubation was carried out for 48 hours at a temperature of 37°C. Purification was done using MRSA slant media in a test tube by scratching the colony and incubating it for 48 hours at 35°C. The colonies that grew were observed for their macroscopic and microscopic morphology. Macroscopic morphology includes shape, margin, elevation, and color. Microscopic morphology includes gram staining and cell shape using a 100× microscope (Ekantari et al., 2017).

Selection of Lactic Acid Bacteria Isolates

a. Proteolytic Test

The selected isolates were grown in SMA media and incubated for 48 hours at 35°C. The colonies on the media were observed for the clear zone formed. The clear zone was measured by comparing the diameter of the clear zone and the diameter of the lactic acid bacteria colony isolate (Matti et al., 2019).

b. Hemolytic Test

Selected LAB isolates were streaked on Blood Agar media containing 5% sheep blood and incubated for 24 hours at 35°C. Hemolytic activity was identified and classified based on the lysis of red blood cells in the medium around the colony. The green zone around the colony

(α-hemolysis), the clear zone around the colony (-hemolysis), and no zone around the colony (γ-hemolysis), only strains with γ-hemolysis are considered safe (Devappa et al., 2021).

c. Low pH Tolerance Test

A total of 2% of the selected LAB isolate starter culture was inoculated into a test tube containing 5 mL of MRS broth media with pH 2, 3, 4, and 6.2 as a control. Incubation was carried out for 3 hours at 37°C. LAB growth expressed by turbidity value (optical density) on a spectrophotometer with a wavelength of 660 nm was measured at 0 and 3 hours; values indicating population growth reaching above 45% were declared resistant to pH (Gupta et al., 2021). Calculation of LAB viability that is resistant to acid using the formula (Zhang et al., 2016):

$$\frac{O\ D\ Value\ End\ of\ Incubation - O\ D\ Value\ Start\ of\ Incubation}{O\ D\ Value\ Control\ End\ of\ Incubation - O\ D\ Value\ Control\ Start\ of\ Incubation}\ X\ 100\%$$

d. Bile Salt Tolerance Test

A total of 2% of the selected LAB isolate starter culture was inoculated into 5 mL of MRS broth media containing bile salts with concentrations of 0.2 mmol, 0.4 mmol, 0.6 mmol, and without bile salt content as a control. Incubation was carried out for 6 hours at 37°C. The growth ability of each strain was measured by absorbance at 600 nm at 0 and 6 hours. Values indicating population growth above 45% were declared salt-resistant (Wasis et al., 2016). Calculation of the viability of LAB resistant to bile salts using the formula (Zhang et al., 2016):

$$\frac{O\ D\ Value\ End\ of\ Incubation - O\ D\ Value\ Start\ of\ Incubation}{O\ D\ Value\ Control\ End\ of\ Incubation - O\ D\ Value\ Control\ Start\ of\ Incubation}\ X\ 100\%$$

e. Carbohydrate Fermentation Ability Test

5% of the selected LAB isolate starter culture was inoculated into 5 mL of a test tube containing peptone water media containing each carbohydrate source: lactose, maltose, sucrose, and glucose of 0.1 g. The Durham tube was inserted into the test tube in an inverted position. Incubation was carried out for 24 hours at a temperature of 40°C. Then, observe the acid and gas production formation in the tube (Gunkova et al., 2021).

f. Antimicrobial Activity Test

The antimicrobial activity test used the disc diffusion method with test bacteria in the form of gram-positive bacteria, including *Bacillus subtilis*, *Staphylococcus aureus*, and negative bacteria including *Salmonella typhimurium*, *Escherichia coli* and *Pseudomonas aeruginosa.* A total of 1 mL of test bacterial suspension was inoculated into MHA media, then homogenized and left to solidify. A disc paper soaked in lactic acid bacteria suspension was placed on the surface of an MHA media sample containing test bacteria. Incubation was carried out for 24 hours at 37°C. Antimicrobial activity was observed by looking at the inhibition zone around the disc and measured using a caliper (Yufidasari et al., 2021).

Result

Sample Characteristics

The Pekasam samples used in this study were Pekasam obtained from Sambas Regency. Pekasam is a fermented fish product that mixes fish, salt, rice, or other carbohydrate sources in a closed container and then leaves for 7 days. The characteristics and formulation of Pekasam samples are presented in Table 1 below.

Table 1. Characterization of Pekasam Samples from Sambas Regency, West Kalimantan

Sample	Characteristics	
	Fish Type	Ingredients
SB1	Lundu Fish (*Macrones gulio*) (±1 kg)	Rice Porridge (±250g) Salt (±30g)
SB3	Milkfish (*Chanos chanos*) (±1 Kg)	Rice (±200g) Salt (±20g) Sugar (±30g)

Based on the Table 1, it can be seen that Pekasam from Sambas Regency is made from two types of fish, namely lundu fish and milkfish, salt, rice, rice porridge, and sugar, so it is classified as a fish-salt-carbohydrate fermentation product. Fish-salt-carbohydrate fermentation is a fish fermentation product that depends on the growth of natural microflora with lactic acid bacteria as the main product. The carbohydrates used are rice porridge, rice, and sugar. Carbohydrates are intended as a carbon source for bacteria to ferment (Eggleston, 2018). When preserved in fresh fish, salt functions as a selective microbial agent and reduces water content to prevent the growth of spoilage microbes. Salt concentration can range from 1% to 20% (w/w) in various forms of fermented fish, which affects microbial growth, fermentation rate, and final product quality (Parajuli, 2018). Lactic acid bacteria can be found in Pekasam because some strains can survive in high salt conditions, such as *Tetragenococcus sp*. can survive and grow in salt at concentrations up to 25% (Justé et al., 2014).

2. Proximate Analysis

Proximate analysis is a quantitative analysis that identifies the nutritional content of a material, such as protein, fat, carbohydrates, water, ash, and fiber. The results of the proximate test of Pekasam samples from Sambas Regency are presented in Table 2 below.

Table 2. Proximate Analysis of Pekasam from Sambas Regency

Sample	**Component (%)**				
	Water Content	**Ash Content**	**Protein Content**	**Fat Content**	**Carbohydrate Levels**
SB1	27,81±1,38	6,46±0,60	14,40±0,42	2,14±0,24	49,19±1,14
SB3	31,86±0,14	7,40±0,63	17±0,28	3,67±0,24	40,07±0,41

Pekasam with sample SB3 has higher water, fat, protein, and ash content than Pekasam sample SB1 except for its carbohydrate content. The different nutritional content of the ingredients used can cause this factor. The types of fish used come from other waters, namely freshwater (lundu fish) and seawater (milkfish). The nutritional content of each fish will differ depending on its living environment. Research conducted by Matondang (2022) shows that seawater fish have a high protein content when compared to freshwater fish. The type of water will also affect the ash content of a material. The ash content of Spirulina platensis cultivated in seawater is higher than in freshwater. Seawater contains more minerals than fresh water (Ekantari et al., 2017). Pekasam from Sambas Regency contains high carbohydrate content due to the addition of carbohydrate sources such as rice porridge, rice, and sugar. The highest carbohydrate content is found in the Pekasam sample SB1, which is 49.19%. Rice porridge contains low levels of amylose because it undergoes a gelatinization process during cooking. This process causes the amylose structure of starch to begin to diffuse out of the granules due to the breaking of hydrogen bonds between amylose and amylopectin. Lactic acid bacteria will efficiently hydrolyze amylose and amylopectin into glucose and maltose. Several types of LAB, such as Lactobacillus, Leuconostoc, and Streptococcus species, have been reported to produce amylase enzymes to degrade starch (Setiarto & Widhyastuti, 2016).

Complex carbohydrates like starch cannot be directly transported into cells. Some microbial cells can produce extracellular hydrolysis enzymes, which are released into the

environment to break down complex carbohydrates into simpler forms and then transported into cells (Lincoln & More, 2017). The hydrolysis process carried out outside the cell will affect the carbohydrate levels in the environment around the fish so that it will increase its carbohydrate levels.

3. Isolation, Morphology, and Characterization of LAB

Each Pekasam sample contains a colony of 10^7 CFU/g, with the highest total in sample SB3, which is 2.20×10^7 CFU/g. A similar study was conducted by Yufidasari et al. (2021), who isolated LAB using MRS media on catfish Bekasam, resulting in a total LAB of 1.2 × 108 CFU/g. The presence of colonies growing on MRS media indicates that Pekasam from Sambas Regency can potentially contain lactic acid bacteria.

Table 3. Total Plate Count (TPC) of Pekasam from Sambas Regency

Sample	TPC (CFU/g)
SB1	27,81±1,38
SB3	31,86±0,14

Colonies that have a clear zone are colonies that can produce acid. Lactic acid will react with CaCO3 and form a new compound, namely calcium lactate (C6H10CaO6), resulting in a transparent color on the media, which is predicted to be a BAL colony. Isolation of two Pekasam samples from Sambas Regency produced three selected isolates: one colony from SB1 and two from SB3. Morphological observations showed differences in the three isolates, as follows.

Table 4. Macroscopic and Microscopic Morphological Characteristics of Three Selected Isolates

Isolation Code	Macroscopic Morphology				Microscopic Morphology	
	Shape	Margin	Elevation	Color	Shape	Gram
SB1(1)	*Circular*	Entire	Convex	Putih Susu	Bacill	+
SB3(1)	*Circular*	Entire	Convex	Putih Susu	Bacill	+
SB3(8)	*Circular*	Entire	Convex	Putih Susu	Bacill	+

Based on the results of macroscopic morphological observations, the three selected isolates had the same colony morphology: round, convex, or raised surface, flat edges, and milky white. The results of this study are similar to those conducted by Saryono et al. (2023), where the selected isolates from sarobuong fermented food showed LAB with the same characteristics, namely round, convex, or raised surface, had flat edges and were milky white. Microscopic observations were carried out using a microscope in the form of cell shape and gram staining. The test results showed that the three selected isolates were classified as gram-positive bacteria because they gave purple results in gram staining and had a rod/bacill cell shape when observed under a microscope. LAB generally has characteristics that include being classified as gram-positive bacteria, not forming spores, and being rod or round in shape. LAB with a rod shape (bacill) is classified as the Lactobacillus species. In contrast, those with a round shape (coccus) are classified as the *Lactococcus, Pediococcus, Streptococcus*, and *Enterococcus* species (Nofiani et al., 2022). The results of morphological observations, both macroscopically and microscopically, show that the three selected isolates have the potential to be LAB belonging to the Lactobacillus species.

3.1 Proteolytic

The three selected isolates showed proteolytic activity, which was marked by forming a clear zone around the colony. Proteolytics are enzymes LAB produces, such as peptidase, protease, or proteinase, which are created to hydrolyze proteins into several bioactive peptide units containing amino acids. The clear zone is formed due to protein hydrolysis activity by extracellular protease enzymes produced by LAB in SMA media containing milk

protein in casein (Matti et al., 2019). The proteolytic index value of the clear zone of each isolate is shown in Table 5 below.

Table 5. Proteolytic Activity Test and Proteolytic Index Value of Selected Isolates

Sample	Proteolytic Activity Test	Proteolytic Index (IP)
SB1(1)	+	1,39
SB3(1)	+	1,09
SB3(8)	+	1,06

The proteolytic index (IP) is the ratio between the clear zone's diameter and the isolated colony's diameter. The IP value in an isolate indicates the ability of a bacterium to hydrolyze protein into bioactive peptide compounds (Saputri et al., 2021). Each selected isolate has a different IP value, with the highest IP value in isolate SB1(1). Differences in IP values can be caused by differences in the ability of each bacterium to produce proteolytic enzymes, so the resulting activity will also differ depending on the species, subspecies, and bacterial strain (Kieliszek et al., 2021).

3.2 Hemolytic

Hemolytic testing is carried out to determine safe isolates for human consumption. The absence of hemolytic activity is considered an essential criterion in evaluating the safety of probiotic strains (Argyri et al., 2013). The results of the hemolytic test on the three selected isolates can be seen in Table 6.

Table 6. Hemolytic Activity Test on Selected Isolates

Sample	Hemolytic Test
SB1(1)	ɣ-hemolysis
SB3(1)	ɣ-hemolysis
SB3(8)	ɣ-hemolysis

Hemolysis is the destruction of red blood cells due to the release of hemoglobin and other intracellular components into the surrounding fluid. One factor that can cause hemolysis is the production of hemolysin, a toxic compound that can be produced by pathogenic microorganisms (Wong et al., 2016). All selected isolates did not have hemolytic activity as indicated by no change in color (forming a greenish zone) on the blood agar media used, so they were classified as ɣ-hemolysis. Bacteria with gamma-type hemolysis are the prerequisite for selecting safe strains for human consumption.

3.3 Low pH Tolerance

In the digestive process system, before entering the large intestine, food containing LAB will pass through the stomach. The stomach produces gastric acid with a pH between 1 and 3, so the pH value will affect the viability of LAB (Megur et al., 2023). Table 7 shows the tolerance of the survival ability of each selected isolate at low pH, namely 2, 3, and 4.

Table 7. LAB Viability at Low pH

Isolate	Viability of LAB (%)			NP
	pH 2	pH 3	pH 4	
SB1(1)	29,13	72,09	17,82	0,3734
SB3(1)	37,22	55,19	49,91	0,9669*
SB3(8)	58,01	35,61	54,18	0,4810

At pH 2, the survival rate of each isolate was <59%, with the highest viability in isolate SB3(8) at 58.01%. At pH 3, the survival rate of each isolate was <73%, with the highest viability in isolate SB1(1) at 72.09%. At pH 4, the survival rate of each isolate was <55%, with the highest viability in isolate SB1(1) at 54.18%. The tolerance level of isolate survival at acidic pH was relatively low during 6 hours of incubation. Jermen et al. (2015) also

indicated that the survival rate of 6 selected LAB isolates was 22.5% at pH 3 for 6 hours. Another study by Bindu & Lakshmidevi (2021) showed different results in selected isolates from fermented foods with high survival rates, namely > 91% at pH 3 and <82% at pH 2. To find out the best-selected isolates to survive at low pH, the Effectiveness Index Value (NP) test was carried out. The effectiveness index value shows that the SB3(1) isolate was selected as the best to survive at low pH with an NP of 0.9669.

3.4 Bile Salt Tolerance

Bile salt tolerance is one of the criteria used in selecting LAB to work effectively in the human digestive tract and carry out metabolic activities. As a probiotic, LAB has the characteristics of being resistant to bile salts and acidic conditions. Resistance to bile salts is significant for LAB viability because the large intestine produces bile salts in high concentrations that are toxic to cells (Mulaw et al., 2019). LAB viability against bile salt tolerance is presented in Table 8 below.

Table 8. Viability of LAB at Various Concentrations of Bile Salts

Isolate	Concentration NaDC (mmol)			NP
	0,2	0,4	0,6	
SB1(1)	62,98	72,09	39,78	0,6644
SB3(1)	51,33	55,19	17,88	1
SB3(8)	18,44	35,61	9,74	0

The Bile Salts (NaDC) concentration was 0.2 mmol, 0.4 mmol and 0.6 mmol. This concentration was chosen because the concentration of human bile salts ranges from 0.14 to 0.93 mmol (Boags et al., 2017). Each selected isolate had a different viability and was insignificant as the concentration increased. At a NaDC concentration of 0.2 mmol, the viability of the three chosen isolates ranged from 18.44 to 62.98%, a concentration of 0.4 mmol ranged from 35.61 to 72.09%, and a concentration of 0.6 mmol ranged from 9.74 to 39.78. The tolerance of the selected isolates to the highest bile salt concentration had a low viability of <50%. Other studies have shown different results where all strains of isolates suspected of being LAB have high tolerance to bile salts, and the survival rate of Lactobacillus strains ranged from 88-92% (Haghshenas et al., 2017). LAB can survive in bile salts because they contain bile salt hydrolase enzymes that form bile acids. Free bile acids can produce exopolysaccharides (EPS). EPS is a protective agent against bile salts (0.15-0.3%) at pH 2-3. However, other studies reveal that several LAB strains can survive at high NaDC concentrations ranging from 1-3% (Reda et al., 2018). 4.5 Carbohydrate Fermentation Ability

The carbohydrate fermentation ability test is used to determine the ability of microorganisms to metabolize carbohydrates as an energy source and the types of end products produced. LAB can metabolize various kinds of carbohydrates. Using carbohydrates as a substrate will provide different metabolic pathways and end products. This ability is crucial for fermentation because it can control metabolic activity in the desired fermentation (Buron-Moles et al., 2019). The results of the test on the LAB fermentation ability of three selected isolates are shown in Table 9.

Table 9. Carbohydrate Fermentation Ability of Selected Isolates

Types of Carbohydrates	**SB1(1)**		**SB3(1)**		**SB3(8)**	
	Acid Formation	**Gas Production**	**Acid Formation**	**Gas Production**	**Acid Formation**	**Gas Production**
Glucose	+	-	+	-	+	-
Sucrose	+	-	+	-	+	-
Lactose	+	-	+	-	+	
Maltose	+	-	+	-	+	

The carbohydrate fermentation ability of each selected isolate showed the ability to metabolize glucose, sucrose, lactose, and maltose with the formation of acid and the absence of air bubbles in the Durham tube as the final product. In another study, LAB isolated from fermented pickles from India showed the highest ability to metabolize carbohydrates in sucrose, lactose, maltose, and dextrose (Monika et al., 2017). Based on these results, the three isolates are homofermentative. In carbohydrate metabolism, there are 2 types of fermentation based on their metabolic results, namely homofermentative and heterofermentative LAB. Homofermentative LAB can metabolize hexose through the Embden-Meyerhof-Parnas (EMP) pathway and produce lactic acid as the final product. In contrast, heterofermentative LAB can metabolize pentose through the phosphogluconate pathway, creating the final product of lactic acid, acetic acid, ethanol, and carbon dioxide (Detha et al., 2019).

4. Antimicrobial Test

Antimicrobial activity was tested on three selected isolates using the disc diffusion method. The antimicrobial compounds produced will form a clear zone around the disc paper, indicating its inhibition zone (Wanger, 2007). This test aims to observe the inhibition zone of selected isolates against two types of bacteria tested, namely gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative bacteria (*Pseudomonas aeruginosa*, *Salmonella typhi,* and *Escherichia coli*) which are classified as pathogenic bacteria. The results of the antimicrobial test on the three selected isolates can be seen in the following Table 10.

Table 10. Antimicrobial Activity on Three Selected Isolates

Isolat	*E. coli*	*S. typhi*	*P. aeruginosa*	*B. subtillis*	*S. aureus*
SB1(1)	2,08 ± 0,06^{a}	1,98 ± 0,15^{a}	2,37 ± 0,33^{a}	3,37 ± 0,13^{a}	1,75 ± 0,36^{b}
SB3(1)	2,55 ± 0,13^{b}	2,83 ± 0,21^{b}	2,60 ± 0,36^{ab}	3,07 ± 0,08^{a}	1,10 ± 0,05^{a}
SB3(8)	2,12 ± 0,06^{a}	2,67 ± 0,40^{b}	3,33 ± 0,45^{b}	3,15 ± 0,15^{a}	2,47 ± 0,08^{c}

Each of the three selected isolates can inhibit the growth of pathogenic bacteria, as indicated by forming an inhibition zone in the form of a clear zone around the disc paper. There is a significant difference between the inhibitory diameter of isolate SB3(1) with isolates SB1(1) and SB3(8) against Escherichia coli bacteria. In Salmonella typHi bacteria, there is a difference in the inhibitory diameter between isolate SB1(1) with isolates SB3(1) and SB3(8). In Pseudomonas aeruginosa bacteria, there is a significant difference between the inhibitory diameter of isolate SB1(1) and SB3(8). In *Bacillus subtilis* bacteria, there is no significant difference between the inhibitory diameter of each selected isolate. In *Staphylococcus aureus*, there is a significant difference in the diameter of each isolate. LAB strains can produce antimicrobial substances such as organic acids (mainly lactic acid and acetic acid), hydrogen peroxide, and also other compounds, such as bacteriocins and sensitivity enzymes in each bacteria are different from antimicrobial compounds due to the structure of the bacterial cell wall (Villa & Veiga-Crespo, 2014). The cell wall of gram-positive bacteria is composed of a thick peptidoglycan layer when compared to gram-negative bacteria, which are composed of lipoproteins, phospholipids, and lipopolysaccharides, which cause the cell wall of gram-negative bacteria to be denatured more easily (Harnentis et al., 2020). The diameter size produced in each of these selected isolates is included in the weak category because the diameter of the zone formed is less than 5 mm. The diameter of the inhibition zone is categorized into four categories, namely weak (<5 mm), moderate (5–10 mm), vigorous (11–19 mm) and very strong (≥ 20 mm).

Conclusions

Three isolates were selected as potential lactic acid bacteria originating from the spontaneous fermentation of Pekasam from Sambas Regency. The three isolates were selected because they had round colonies, convex or raised surfaces, and flat edges. They

were milky white and produced purple in gram staining and bacillus-shaped cells. Each isolate showed proteolytic activity, ɤ-hemolysis, could tolerate low pH and bile salts, could metabolize carbohydrates, and had antimicrobial activity on pathogenic bacteria. Based on these results, the three selected isolates can potentially be BAL-homofermentative Lactobacillus species.

Declaration statement

The authors report no potential conflict of interest.

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