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Characteristics And Antibacterial Test Of Lactid Acid Bacteria From Sidoarjo Shrimp Petis Against *Vibrio Sp*, Bacteria

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Abstract*— Indonesia as the largest archipelago with abundant potential fishery resources contributes to national foreign exchange (Damayanti & Sugiarto, 2022). One of the main commodities of fishery products commonly exported by Indonesia is shrimp (Dewi et al., 2022). Increasing shrimp production through intensive aquaculture faces the challenge of disease, especially Vibrio sp. bacterial infection. The use of antibiotics as a general solution raises resistance problems, so alternatives such as probiotics are needed. Lactic acid bacteria (LAB) are potential candidates for probiotics because of their ability to produce organic acids that inhibit the growth of pathogenic bacteria. This study aims to analyse the characteristics of LAB from Sidoarjo shrimp petis, a typical fermented product that has potential as a source of LAB, and test its antibacterial ability against Vibrio sp. The stages carried out in the study consisted of 5 stages including the first stage of sampling, the second stage of lactic acid bacteria isolation, the third stage of characterisation of lactic acid bacteria isolates, the fourth stage of lactic acid bacteria antibacterial test, and the fifth stage of data analysis. The results showed the total colonies of Lactic Acid Bacteria (LAB) in the six isolates with a value of 7.08 x 106 colonies.mL. LAB characteristics on the six isolates consisted of macroscopic, microscopic, and biochemical characteristics. Microscopic characteristics of the six isolates showed the same results, namely round, white colour, flat and convex elevation, and smooth edges. Microscopic characteristics of the six isolates showed the same results, namely bacillus and gram-positive cell forms. Biochemical characteristics on the six isolates showed different results. Antibacterial tests were carried out after knowing the type of lactic acid bacteria isolates through several characteristic tests, it can be seen that there are 4 isolates including isolates PTS.5.1, PTS.5.2, PTS.6.1, and PTS. 6. 6. The results of antibacterial tests on 4 isolates have antibacterial compounds in inhibiting the growth of gram negative bacteria (Vibrio sp,). Seen the results obtained isolates that have the greatest antibacterial activity is PTS.5.1 with a final result of 10.2625 mm.

***Keywords*— Shrimp, Vibrio sp, Lactid Acid Bacteria, Antibacterial, Probiotics.**

I. INTRODUCTION

Indonesia is one of the largest archipelagic countries in the world, where the outside waters in Indonesia reach 6.32 million km2 and have a coastline of 99,093 km [1]. Indonesia is geographically an archipelagic country with more than 70 percent of its territory dominated by waters, so the advantage that Indonesia gets is that it has abundant fishery resources, contributing to national foreign exchange income [2]. One of the main commodities of fishery products that Indonesia generally exports is shrimp [3]. According to data from the Ministry of Maritime Affairs and Fisheries, the value of shrimp exports in 2024 has the highest value of 2,919,030.57 USD. Indonesia, as one of the world's main shrimp exporting countries, is also balanced with shrimp production from year to year which has increased so that Indonesia has a great opportunity to continue to improve its export performance [4].

The very high economic potential of shrimp makes pond actors finally carry out intensive cultivation of shrimp, but this intensive cultivation has many challenges and obstacles, one of which is the emergence of diseases in cultivated shrimp [5]. One type of bacteria that often appears in shrimp farming is the type of Vibrio sp bacteria, by attacking shrimp when the shrimp's body condition is weak and environmental conditions are not good, if not prevented or treated, it will have a negative impact until mass death in farmed shrimp [6]. The appearance of bacterial attacks in the cultivation environment can cause death up to 100% and result in economic losses, one of the treatments that is often carried out in overcoming the problem of bacterial infection attacks is through the use of antibiotics, but the use of antibiotics in the cultivation environment leaves a new problem, namely antibiotic resistance to antibiotic residues in body tissues, so the right effort to replace the use of antibiotics in the cultivation environment is through the application of probiotics.

Probiotics are an alternative technology to replace the use of antibiotics and chemical compounds in the aquaculture industry [7]. The use of probiotics in intensive cultivation can provide benefits to cultivated

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organisms, one of which is an increase in immune response [8]. Microbes that are generally used as probiotics are those that belong to the group of Lactic Acid Bacteria [9].

Lactic acid bacteria are known to have the ability to produce organic acids that are able to reduce the pH of the environment and inhibit the growth of pathogenic bacteria, this is what distinguishes them from other types of non-pathogenic bacteria [10]. The use of BAL as a probiotic has several advantages compared to the use of conventional antibiotics, one of which is that BAL is generally safe to use, and does not cause resistance to pathogenic bacteria [11]. Lactic acid bacteria are bacteria that can live in a variety of diverse places in nature, including plants, the digestive tract of living things, fruits, vegetables, processed food products, dairy products, and one of them fermented products [12]. The existence of lactic acid bacteria plays a role in improving the taste of fermented products and has a preservation effect. Various kinds of fermented products in Indonesia are known as a type of food product typical of Sidoarjo, East Java, namely shrimp petis as a processed product from the use of shrimp commodities that have the potential to be a source of BAL.

This study aims to analyze the characteristics of Lactic Acid Bacteria isolated from Sidoarjo shrimp petis and test its antibacterial ability against pathogenic bacteria Vibrio sp,. The research used 4 stages, including sampling, isolation and characterization of BAL, antibacterial test of BAL, and data analysis. The results of this study are expected to provide important information about the potential of BAL from Sidoarjo shrimp petis as a probiotic candidate for shrimp cultivation.

A. Time And Place

Sampling of the research location in Buduran, Sidoarjo, East Java was carried out on January 15, 2025 at a shrimp petis production house. Testing was carried out at the Marine Biology Laboratory, Faculty of Agriculture, Trunojoyo University, Madura from February 10 to 18, 2025

B. Tools And Materials

The tools used in the research included bunsen, analytical scales, Erlenmeyer, test tubes, test tube racks, petri dishes, tips, micropipettes, incubators, ose needles, microscopes, preparation glasses, cover glass, dropper pipettes, vortex mixers, tweezers, and calipers. The materials used in the study included shrimp petis, spirtus, MRS agar, MRS broth, aquades, crystal violet, iodine solution, alcohol 95%, safranin, H2O2 3%, bacterial suspense, disc paper, CaCO3, Nutrient agar, Agar puree media, Nutrient broth, Oxidized paper, and carbohydrate fermentation media (*glucose, sucrose, maltose, lactose, mannitol*).

II. METHOD

This research includes 5 stages including the first stage of sampling, the second stage of lactic acid bacteria isolation, the third stage of characterisation of lactic acid bacteria isolates, the fourth stage of lactic acid bacteria antibacterial test, and the fifth stage of data analysis. Details of the research procedure are as follows:

A. Shrimp Petis Sampling

Sampling was carried out by the researcher himself by taking packaged shrimp petis from industrial owners in the city of Sidoarjo before testing, Petis in the form of a blackish-brown thick paste with shrimp, sugar, and salt as the main ingredients using traditional processing methods.

B. Media Creation

The media used is liquid media and solid media in the gar. The liquid medium uses sterile aquades media and MRS broth. Solid media to use MRS agar and Nutrient agar media. MRS agar as much as 68.2 grams/liter of sterile aquades is dissolved and homogenized, the completely dissolved media is then sterilized using an autoclave at a temperature of 121°C for 15 minutes at a pressure of 2 atm. MRS broth of 52.2 grams/liter of sterile aquades was dissolved and homogenized, the completely dissolved medium was then sterilized using an autoclave at a temperature of 121°C for 15 minutes at a pressure of 2 atm. As much as 20 grams of nutrient agar is dissolved in 1000 ml of aquades and homogenized, the completely dissolved medium is then sterilized using an autoclave at 121°C for 15 minutes at a pressure of 2 atm.

C. Isolation Of Lactic Acid Bacteria from Petis

Microbial isolation is to separate the microbe from its environment and grow it as a pure culture in an artificial medium [13]. Isolation of shrimp petis is carried out by taking aseptically as much as 1 gram of petis added to 9 mL of diluent solution, namely aquades and homogenized (1st dilution). Then a tiered dilution is carried out. Each dilution was carried out by the spread plate method on MRS agar medium which was added with 1% CaCO3. Next, it is incubated at 37°C for 48 hours. The calculation of bacterial abundance using the TPC (Total Plate Count) method which refers to SNI 2332.3:2015 uses the following formula:

$$N = \frac{\Sigma C}{[(1 \times n1)+(0{,}1 \times n2) \times d]}$$

Information:

N = number of product colonies, expressed in colonies per mL or colonies per g
ΣC = number of colonies on all the counted cups
n1 = number of cups at the first diluted calculated
n2 = number of cups at the second dilution calculated
d = the first diluted calculated

The step is taken after knowing the abundance of bacteria, then BAL colonies are taken aseptically to be moved and scratched with the streak method to obtain a single colony, then incubated at a temperature of 37°C for 24 hours [14].

D. Characterization Of Bale Isolates

In addition to being used for identification, this step is used to determine the presence of bacterial types in the growth medium through known characteristics [15]. The steps were taken to find out the existence of lactic acid bacteria that act as research objectives. The characterization test was carried out by observation of the morphology, gram properties, and biochemical physiology of the bacteria.

1) Macroscopic test

Macroscopic characterization by observing the morphology of colonies includes the shape, color, edges, and elevation of bacterial colonies.

2) Microscopic tests

Microscopic characteristics with a series of Gram staining methods of bacterial cells are then observed under a microscope.

3) Biochemical tests

Biochemical tests include motility tests, catalase, oxidation tests, and carbohydrate fermentation tests.

a. Motility test

The motility test was carried out by isolating in Nutrient broth medium containing 0.5% agar (semi-solid) and then incubated for 48 hours at a temperature of 37°C. The motility test is positive if the colony growth is widespread in agar [14].

b. Catalase test

The catalase test is carried out by taking 1 ose isolate then smeared on the glass of the object, then 3% H2O2 is dripped as many as 2-3 drops on top of the preparation. The catalase test is negative if no oxygen bubbles are formed in the preparate [14].

c. Oxidation test

The oxidation test was carried out by taking 1 ose of bacterial isolate on oxidized paper aseptically. Changes in bacterial colonies are observed for about ±5 seconds. When the colonies change deep blue/violet color on the paper, it shows positive oxidation. When the colonies turn red on the paper, it shows negative oxidation [16].

d. Carbohydrate fermentation test

The test was carried out by taking 1 ose of bacterial isolate inoculated into test tubes containing glucose, sucrose, maltose, lactose, and mannitol. Furthermore, it is incubated for 24 hours at a temperature of 37°C [17].

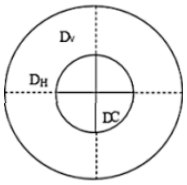
E. Bal Isolate Refresh

It is done by taking 2-4 beads using ose needles on MRSB liquid media. Then it was incubated at 37°C for 48 hours (Adhinugraha *et al*., 2022).

F. Antibacterial Test

Antibacterial testing on BAL isolates was carried out by disc diffusion method. The test bacteria used were Vibrio sp,. The antimicrobial used is lactic acid bacteria from Sidoarjo shrimp petis. Positive control of pathogenic bacteria using ciprofloxacin antibiotics and negative control using aquades diluent solution. The test medium used is Nutrient agar medium.

The test bacteria were suspended into the aquades diluent solution until it obtained the same turbidity as the standard of 0.5 McFarland solution. A total of 25 microliters of pathogenic bacteria were grown on the medium using the diffusion dish method. The disc paper is then soaked in a lactic acid bacteria suspension solution. The disc paper is then placed on the surface of the test media, then incubated at 37°C for 72 hours. Observation of the formation of clear zones is carried out every 24 hours of incubation. The diameter of the clear zone is measured vertically and horizontally using calipers in units (mm). The measurement of the inhibition zone is carried out by calculating the average diameter of the inhibition zone which refers to the following formula [18]



Information:
DV : Vertical Diameter
DH : Diameter Horizontal
DC : Disc diameter

From the results of the formula calculation, the values obtained will be included in the antibacterial effectiveness classification criteria [18].

1. Rated <5mm : Weak inhibition
2. Rated 5-10 mm : medium inhibition
3. Rated 10-20 mm : Strong inhibition
4. Value >20 mm : The inhibition is very strong

$$\text{Inhibition Zone} = \frac{(DV-DC)+(DH-DC)}{2}$$

Figure 1. Technique of measuring the inhibition zone
(Source: Wally *et al*., 2022)

Data Analysis

The data is presented descriptively and displayed in the form of tables and pictures including lactic acid bacterial isolates that have been tested.

III. RESULTS AND DISCUSSION

A. Isolation And Characteristics Of Lactic Acid Bacteria

In the initial stage of isolation, a sample in the form of 1 g of Sidoarjo shrimp petis was diluted and inoculated in MRSA media supplemented with 1% CaCO3. The results of isolation obtained total plate count (TPC) data of all lactic acid bacteria that grew as many as 7.08 x 106 colonies/mL. From the entire bacterial culture, characteristics tests were carried out macroscopicly, microscopicly, and biochemically. The results are shown in Table 1 and Table 2 as follows.

TABLE 1.
RESULTS OF ISOLATION OF LACTIC ACID BACTERIA PETIS SIDOARJO SHRIMP

It	Isolates	Macroscopic				Microscopic	
		Shape	Color	Elevation	Banks	Cell shape	Gram staining
1	PTS. 5.1	Round	White	Flat	Soft	Basil	Positive
2	PTS. 5.2	Round	White	Convex	Soft	Basil	Positive
3	PTS. 6.1	Round	White	Flat	Soft	Basil	Positive
4	PTS. 6.2	Round	White	Flat	Soft	Basil	Positive
5	PTS. 7.1	Round	White	Flat	Soft	Basil	Positive
6	PTS. 7.2	Round	White	Flat	Soft	Basil	Positive

(Source: Primary Data, 2025)

TABLE 2.
HASIO BIOCHEMISTRY OF LACTIC ACID BACTERIA

It	Isolates	Biochemistry							
		Catalase	Motility	Oxidase	Fermentation of carbohydrates				
					Glucose	Sucrose	Maltose	Lactose	Mannitol
1	PTS. 5.1	-	-	+	+	+	+	+	+
2	PTS. 5.2	-	-	+	+	+	+	+	+
3	PTS. 6.1	-	-	+	+	+	+	-	-
4	PTS. 6.2	-	-	+	+	+	+	+	-
5	PTS. 7.1	+	+	-	+	+	+	-	-
6	PTS. 7.2	+	+	-	+	+	+	-	-

(Source: Primary Data, 2025)

The sample used in this test is a sample of Sidoarjo shrimp petis. Results on **Table 1** shows spherical acidic bacteria with a white color. Lactic acid bacteria obtained on the results **Figure 2** It can be seen growing in the media. Lactic acid bacteria are able to grow and secrete acid in MRSA media and bind CaCO3 to soluble Ca-lactate, thus giving rise to clear zones [19]. Similar results were also obtained by Nurhamidah *et al*., (2020) in a journal entitled Isolation and Characterization of Lactic Acid Bacteria (BAL) from Ale-ale and Cincalok which isolated fermented food samples with MRSA had round-shaped isolation results with white and beige colors in 4 isolates. The use of MRSA selective media plays an important role in the isolation of lactic acid bacteria which functions to facilitate the growth of certain microbes and block the growth of other microbes [20].

The results of gram staining in **Table 1** show that the bacterial colony that grows is a type of gram-positive bacterial group that is characterized by purple color on the cells after being observed under a microscope. The results in **Table 1** of the gram staining show the six results of the type of bacterial group with the form of bacillus. This is the same as said by Surbakti *et al.,* (2019) [21] that the lactic acid bacteria group has characteristics as a gram-positive group of bacteria, namely the color of bacterial cells with purple, in contrast to the type of gram-negative bacteria group, namely the color of the bacterial cell is red. BAL is a type of gram-positive bacteria that uses carbohydrates as one of them or the main source of carbon that is used to increase energy. Similar results were also obtained in a journal entitled Isolation of Lactic Acid Bacteria in Infant Feces and Its Potential in Inhibiting the

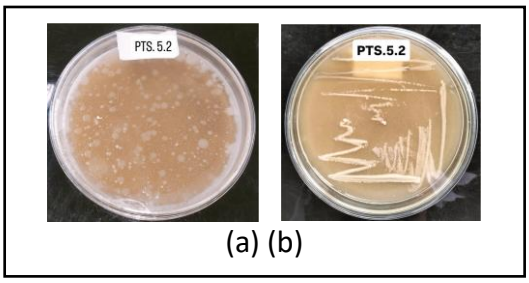


Figure 2. Lactic Acid Bacteria in MRSA Media (a) TPC Isolation Results; (b) Streak results/rejuvenation
(Source: Personal Documentation)

Growth of Escherichia coli by Hasbi *et al*., (2024) at the stage of identification of lactic acid bacteria in microscopic grams, gram staining on 8 isolates showed results with a type of gram-positive bacterial group with purple cells.

The test carried out after the microscopic characteristics test was carried out biochemical tests on the six isolates. The catalase biochemical test was carried out to determine the ability of bacteria to produce the catalase enzyme. The results in **Table 2** of the catalase test showed positive results in 2 isolates, namely PTS. 7.1 and PTS. 7.2 isolates where there were gas bubbles formed in the test conducted and negative in 4 isolates including PTS. 5.1, PTS. 5.2, PTS. 6.1, and PTS. 6.2, where there were no gas bubbles formed in the test as shown in Figure 4. The results of the catalase test are suspected to be 4 isolates, including PTS. 5.1, PTS. 5.2, PTS. 6.1, and PTS. 6.2 in accordance with the characteristics of Lactic Acid Bacteria, namely not catalase positive. This is in accordance with the statement of Suhaeni & Syakur (2016) stating that lactic acid bacteria are catalase negative bacteria because they do not produce catalase enzymes that can break down hydrogen peroxide.

Motility biochemical tests are carried out to observe the movement of bacteria (Falakh & Astri, 2022). The nature of motility or movement of bacteria can be known by looking at the growth of bacteria that propagate around the ose puncture in the medium. Results on **Table 2** The motility test showed positive results in 2 isolates, namely PTS.7.1 and PTS.7.2 isolates, and negative motility results in 4 isolates, including PTS.5.1, PTS.5.2, PTS.6.1, and PTS.6.2 isolates. The result was said to be positive motility indicated by the presence of bacterial growth propagating around the ose puncture in the medium in the test carried out, while the negative motility result was indicated by the absence of bacterial growth propagating around the ose puncture in the medium in the test carried out. The results of 4 isolates, including PTS.5.1, PTS.5.2, PTS. 6.1, and PTS. 6.2 isolates, are in accordance with the characteristics of BAL, which is not motile, meaning that the bacteria only grow around the place where the puncture is carried out on the medium (Simanjuntak & Naibaho, 2023). Lactic acid bacteria have a very limited biosynthesis ability, so they are non-motile [22].

Oxidase biochemical tests are carried out to determine the presence or absence of oxidase enzymes in bacteria (Arfiandi & Tumbol, 2020). The results in **Table 2 of** the oxidation test showed positive results in 4 isolates, namely PTS.5.1, PTS.5.2, PTS. 6.1 and PTS.6.2 isolates, and negative motility results in 2 isolates, including PTS.7.1 and PTS. 7.2 isolates. This result is in accordance with the characteristics of BAL, which is negative oxidase (Surak *et al*., 2024).

Carbohydrate fermentation biochemical tests are carried out to determine the ability of bacteria to ferment carbohydrates using various types of sugar [23]. The results of the positive carbohydrate fermentation test will cause a color change in the test medium to yellow due to the use of sugar as a carbon source to be able to produce acids that reduce the pH of the medium, while the test results produce negative values that will not change the color of the test medium [24]. Results on **Table 2** showed that 6 isolates had positive sugar fermentation with different results. The results showed that PTS. 5.1 isolate was able to ferment glucose, sucrose, maltose, lactose, mannitol, PTS isolate. 5.2 was able to ferment glucose, sucrose, maltose, lactose, and mannitol, PTS. 6.1 isolate was able to ferment glucose, sucrose, and maltose carbohydrates, PTS. 6.2 was able to ferment glucose, sucrose, maltose, and lactose carbohydrates, PTS. 7.1 isolate was able to ferment glucose, sucrose, and maltose, as well as PTS isolates. 7.2 Able to ferment carbohydrates of glucose, sucrose, and maltose. Results on **Table 2** showed that not all lactic acid bacteria isolates could utilize various types of sugars present in the test media. The results are in accordance with the characteristics of lactic acid bacteria, which are able to ferment sugar [25].

B. Lactic Acid Bacterial Antibacterial Test

Lactic Acid Bacteria (BAL) produce antibacterial compounds *Food Grade Microorganism* as an alternative used for preservation in microbial control [26]. The antibacterial test produced by BAL was carried out to assess the ability of lactic acid bacteria to inhibit or kill pathogenic bacteria that can damage product quality. The test was carried out after the type of lactic acid bacteria isolate was known through several characteristic tests, it can be known that there are 4 isolates including PTS.5.1, PTS.5.2, PTS.6.1, and PTS.6.2 isolates. The results of the BAL antibacterial test are presented in the table below.

TABLE 3.
ANTIBACTERIAL TEST RESULTS TO 24 HOURS

It	Isolation Code	Repetition (mm)		Final Result (Average±Stdev) (mm)	
		1	2		
1	Control (-)	0		0	-
	(+) Control	9,55		9,55	Keep
2	PTS. 5.1	10,3	9,95	10,125±0,175	Strong
3	PTS. 5.2	7,675	7,85	7.7625±0.0875	Keep
4	PTS. 6.1	9,15	9,3	9,225±0,075	Keep
5	PTS. 6.2	8,725	8,675	8.7±0.025	Keep

(Source: Primary Data, 2025)

TABLE 4.
ANTIBACTERIAL TEST RESULTS TO 48 HOURS

It	Isolation Code	Repetition (mm)		Final Result (Average±Stdev) (mm)	
		1	2		
1	Control (-)	0		0	-
	(+) Control	10,1		10,1	Strong
2	PTS. 5.1	10,25	10,075	10.1625±0.0875	Strong
3	PTS. 5.2	7,825	7,875	7.85±0.025	Keep
4	PTS. 6.1	9,475	9,35	9.4125±0.0625	Keep
5	PTS. 6.2	8,725	8,725	8,725±0	Keep

(Source: Primary Data, 2025)

TABLE 5.
ANTIBACTERIAL TEST RESULTS TO 72 HOURS

It	Isolation Code	Repetition (mm)		Final Result (Average±Stdev) (mm)	
		1	2		
1	Control (-)	0		0	-
	(+) Control	10,1		10,1	Strong
2	PTS. 5.1	10,4	10,125	10.2625±0.135	Strong
3	PTS. 5.2	7,825	7,9	7.8625±0.0375	Keep
4	PTS. 6.1	9,625	9,375	9.5±0.125	Keep
5	PTS. 6.2	8,75	9,075	8.9125±0.1625	Keep

(Source: Primary Data, 2025)

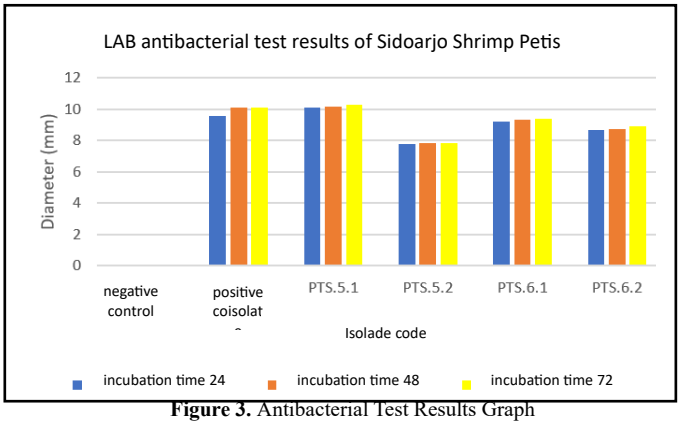


Figure 3. Antibacterial Test Results Graph
(Source: personal documentation)

In the test of the effectiveness of BAL as an antibacterial against *Vibrio sp.* It was carried out by measuring the inhibition zone generated during 3 days of incubation time at 37°C with results compared to negative control. The results showed that the six isolates had antibacterial compounds and were effective in inhibiting the growth of Gram-negative bacteria (*Vibrio sp*,). This can be seen from the clear zone around the isolate that is formed. It can be seen from the results obtained in **Figure 3** that the isolate that has the largest antibacterial activity is PTS.5.1 with a final result of 10.2625 mm. Similar results in the journal entitled Identification and Testing of Antibacterial Activity of Lactic Acid Bacterial Isolate from Shrimp Fermentation (Cincalok) Against Vibrio parahaemolyticus and Listeria monocytogenes by Samboja *et al.,* (2019) on the results of antibacterial testing on 5 isolates showed the formation of an inhibitory zone on the medium with the largest result possessed by C2 isolate with an inhibitory zone area of 0.529 mm.

IV. CONCLUSION

The conclusion of this study is as follows:

1. The total Lactic Acid Bacteria (BAL) in the six isolates showed a value of 7.08 x 106 colonies/mL.
2. The characteristics of Lactic Acid Bacteria in the six isolates consist of macroscopic, microscopic, and biochemical characteristics. The macroscopic characteristics of the six isolates showed results with the same shape, namely round, white, flat and convex elevation, and smooth edges. The microscopic characteristics of the six isolates showed the same results, namely with the shape of bacillus cells and gram-positive. The biochemical characteristics of the six isolates showed different results in all tests while the catalase test showed 2 isolates, including PTS. 7.1 and PTS. 7.2 isolates, showed positive results for catalase, and 4 isolates, including PTS. 5.1, PTS.5.2, PTS. 6.1, and PTS. 6.2 isolates, showed positive results on 2 isolates, namely PTS.7.1 and PTS.7.2 isolates, and negative motility results in 4 isolates including PTS.5.1, PTS.5.2, PTS.6.1, and PTS.6.2 isolates, in the oxidation test showed positive results in 4 isolates, namely PTS.5.1, PTS.5.2, PTS. 6.1 and PTS.6.2 isolates, and negative motility results in 2 isolates including PTS.7.1, and PTS. 7.2 isolates, and in the carbohydrate fermentation test showed that 6 isolates had positive sugar fermentation with different results. The results showed that PTS. 5.1 isolate was able to ferment glucose, sucrose, maltose, lactose, mannitol, PTS isolate. 5.2 was able to ferment glucose, sucrose, maltose, lactose, and mannitol, PTS. 6.1 isolate was able to ferment glucose, sucrose, and maltose carbohydrates, PTS. 6.2 was able to ferment

glucose, sucrose, maltose, and lactose carbohydrates, PTS. 7.1 isolate was able to ferment glucose, sucrose, and maltose, as well as PTS isolates. 7.2 Able to ferment carbohydrates of glucose, sucrose, and maltose.

3. The results of antibacterial tests on 4 isolates, including PTS. 5.1, PTS. 5.2, PTS. 6.1, and PTS. 6.2 isolates, have antibacterial compounds in inhibiting the growth of gram-negative bacteria (*Vibrio sp,*). This can be seen from the clear zone around the isolate that is formed. It can be seen from the results obtained that the isolate that has the largest antibacterial activity is PTS.5.1 with a final result of 10.2625 mm.

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