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Isolation and identification of lactic acid bacteria from traditional food *sarobuong* of Kuantan Singingi District, Riau, Indonesia

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Abstract. *Saryono, Ismawati, Pratiwi NW, Devi S, Sipayung MY, Suraya N. 2023. Isolation and identification of lactic acid bacteria from traditional food* sarobuong *of Kuantan Singingi District, Riau, Indonesia. Biodiversitas 24: 2201-2206.* Lactic acid bacteria (LAB) are commonly used as probiotic agents in fermented processed foods like *sarobuong. Sarobuong* is a traditional food from Kuantan Singingi District made by fermenting bamboo shoots. In this study, the isolation and purification of LAB from *sarobuang* were done using MRS agar media with the streak quadrant method: macroscopic and microscopic observations and biochemical and physiological tests to identify isolates. The antibacterial ability of LAB isolates was used against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. The results showed that 21 isolates were gram-positive bacteria in the form of bacilli and coccus, while carbohydrate degradation was homofermentative. The results of the antibacterial test of 21 isolates indicated by LAB against *E. coli* and *S. aureus* bacteria showed a clear zone ranging from 0.1 to 0.6. The highest antibacterial activity against pathogenic bacteria *E. coli* was observed in isolate RB1, while for pathogenic bacteria *S. aureus*, it was in isolate R15. The results obtained from several isolates that have been tested showed that LAB isolated from *sarobuong* had relatively higher activity than the control and was identified as *Lactobacillus* and *Streptococcus*.

Keywords: Antibacterial, fermented food, LAB, *Sarobuong*

INTRODUCTION

Saraobuong is one of the special foods of Kuantan Singingi District, with the basic ingredients of bamboo shoots processed by fermentation. Bamboo shoot fermentation occurs naturally without the addition of microbes from the outside. Bamboo shoots contain nutritional components such as proteins, carbohydrates, fats, vitamins, minerals, enzymes, coenzymes, reducing and non-reducing sugars, lactic acid, and citric acid as fermentation products (Singhal et al. 2013). The nutritional content of these shoots can be used as a growth medium by lactic acid bacteria (LAB). Several studies have shown that bamboo shoots contain LAB, which has the potential as a probiotic. Qureshi et al. (2014) obtained LAB of the type *Lactobacillus plantarum* EGD-AQ4 isolated from fermented non-alcoholic bamboo shoots from North India and has the potential as a probiotic. Lindayani et al. (2018) isolated and tested the potential of LAB from bamboo shoot fermentation; the results showed that 50% of the isolates had the potential as probiotics. Kong et al. (2020) successfully conducted antifungal and antibacterial tests on bamboo shoots of the Dendrocalamus asper variety (Kong et al. 2020).

Fermented foods usually contain LAB, which can increase the nutritional value of food and can produce antimicrobial, antifungal, and Gamma-aminobutyric acid (GABA) compounds. Therefore, LAB is often used as a probiotic because it can inhibit the growth of bad bacteria in the gastrointestinal tract (Saranraj et al. 2013; Octarya et al. 2021; Octarya et al. 2022;). Furthermore, some LAB exhibit probiotics or other activities, such as lowering cholesterol, that confer health benefits on the host (Jobby et al. 2020). LAB can also be used as a food preservative because it can produce antimicrobial compounds apart from lactic and acetic acids, diacetyl, and bacteriocins (Amelia et al. 2021).LAB can be used as a source of probiotics and has contributed to the developing of fermented foods containing probiotics. Probiotics are living organisms that can provide health benefits if consumed in sufficient quantities by the host (Somashekaraiah et al. 2019). These organisms usually derive from *Lactobacillus* and *Bifidobacterium* (Rahayu et al. 2019). LAB that can be used as probiotics requires several stages of testing, including an antibacterial test. This selection was done to obtain potential LAB as probiotics because not all are probiotics. The pathogenic bacteria test is the first step used to determine the ability of isolates to inhibit the growth of pathogenic bacteria, such as producing antibacterial compounds or lowering the pH of the medium by producing lactic acid or acetic acid.

Research on the isolation and identification of LAB as probiotic candidates have attracted much interest among researchers. Research is usually carried out on fermented

foods because fermentation technology is an effort to increase a food's digestibility and added value. The live microorganisms in fermented foods can improve digestive health and provide health benefits, such as lowering the risk of type 2 diabetes and cardiovascular disease (Rezac et al. 2018). The studies conducted by Linh et al. (2018) and Feng et al. (2017) revealed that the isolated LAB from fermented foods, including fermented cabbage, Chinese cabbage, cucumber, seaweed, and rice bran obtained from a supermarket in Miyazaki, Japan, as well as feces from the intestinal tract of cows. The results showed that of the sixty-five bacterial strains that had been isolated, three could antagonize all pathogens: *Edwardsiella tarda*, *Streptococcus dysgalactiae*, *Streptococcus iniae*, and *Lactococcus garvieae*. The K-C2 strain was selected as the best probiotic candidate based on several tests. Feng et al. (2017) obtained probiotics by isolating LAB from the intestinal mucosa of healthy pigs aged 60 days. The results showed that two isolates of *Enterococcus faecium* WEI-10 and *L. plantarum* WEI-51 had probiotic properties that could be used as potential probiotics in animal feeding supplements.

This research was conducted to obtain LAB isolates from *sarobuong* which have the potential as probiotics, which has never been done before. In addition, the fermentation of LAB from plants such as bamboo has not been widely carried out. Therefore, in this study, isolation and potential probiotic testing of LAB isolates against pathogenic bacteria *E.coli* and *S. aureus* will be carried out. The effect of probiotics is strain-specific, so it is necessary to carry out a safety test based on strain per strain (Rahayu et al. 2019).

MATERIALS AND METHODS

Materials

Sarobuong food samples were obtained from three places in Kuantan Singingi District. The total LAB isolates obtained were 21 isolates. In addition, bacterial isolates for antibacterial tests, namely *E. coli* and *S. aureus*, were obtained from the Enzyme, Fermentation, and Biomolecular Laboratory of the University of Riau.

Isolation and purification of lactic acid bacteria

LAB was isolated from Sarobuong samples by adding 1 g of the sample into a test tube and 9 mL of physiological NaCl solution (10^{-1}). A total of 1 mL of suspension was put into 9 mL of physiological NaCl solution (10^{-2}) until a dilution of 10^{-5}. Furthermore, at a dilution of 10^{-3}–10^{-5}, 100 µL was taken and spread over a petri dish containing MRS media using the spread plate method and incubated at room temperature for 48 hours.

LAB colonies that have grown on MRS media are then purified. Purification was carried out until separate colonies were obtained. Furthermore, the isolate purification was checked by observing the shape and movement of the cells under a microscope. If the characteristics of the colonies examined are the same, then the colony is considered pure.

Morphological identification

Macroscopic test

Microscopic examination was done by observing the colony's shape, the colony's edge, elevation, and the colony's surface. In the morphological test for the LAB form, a test using a microscope was used. Colonies are characterized by uniform morphology (round, single, in pairs, stems, and chains).

Microscopic test

The purified isolates were then subjected to a gram test using the gram staining method. Firstly, the bacterial cultures aged 18-20 hours were smeared on a glass slide; then, the slide was fixed by passing it over a spirit lamp for 5 seconds. Next, for thirty seconds, rinsed using distilled water and dried, then the preparations were added 1-2 drops of iodine, left for 30 seconds, and rinsed with distilled water. Next, the preparations were washed again using 90% ethanol and rinsed with running water. Furthermore, 1-2 drops of safranin are added, allowed to stand for 1 minute, and rinsed with distilled water. Finally, the preparations containing the bacteria were dried, covered with a glass, and observed using a light microscope. Purple indicates the bacterial cell is Gram-positive, while pink indicates Gram-negative (Hardiansyah et al. 2020).

Biochemical and physiological test of lactic acid bacteria

Catalase test

The LAB isolate from MRS agar was taken as much as one ose, then smeared on a glass preparation that had been cleaned with 70% alcohol. Then as much as 1-2 drops of 3% Hydrogen Peroxide (H_2O_2) are added. Furthermore, if air bubbles form, the LAB is positive for the catalase test (Nofiani et al. 2022).

Carbohydrate fermentation test

This carbohydrate fermentation test uses glucose, lactose, fructose, and sucrose. As much as 7.5 g of each carbohydrate source was dissolved in 1000 mL of NB media, and 3-4 drops of phenol red were added. Each solution was put into a test tube of as much as 8 mL, and the Durham tube was inserted into the tube slowly to not form air spaces. Furthermore, the medium was sterilized using an autoclave at 121ºC and a pressure of 15 lbs for 15 minutes. Then the 24 hour-old bacterial culture was inoculated in one ose into each test tube containing the medium and incubated for 48 hours. A medium color change to yellow indicates a positive reaction, and/or gas is formed in the durham tube. The fermentation type is determined by the formation of bubbles in the Durham tube; this indicates that fermentation is heterofermentative. The fermentation is homofermentative if no air bubbles exist in the Durham tube (Nadia et al. 2020).

Salt resistance test

A total of 1 ose of bacterial culture aged 24 hours was inoculated in a test tube containing 8 ml of MRSB media. MRSB media was then dripped with 6.5% NaCl, as much as 3-4 drops, and incubated at 37ºC for 48 hours. If the

medium became cloudy and the presence of sediment arose, it showed a positive result by comparing it with the negative control not inoculated with bacteria (Nurcahyo et al. 2019).

Antibacterial activity assay

An antibacterial test was carried out using the disc diffusion method. First, the LAB isolate was taken and dissolved in 5 mL of sterile distilled water; then, the disc paper was immersed in the LAB isolate solution for 15 minutes. Then the other paper disc was immersed in 5 mL of sterile distilled water as the control. Next, pathogenic bacteria *E. coli* and *S. aureus* were taken 3-4 ose, dissolved in 5 mL of sterile distilled water, and homogenized. Furthermore, 0.1 mL of the pathogenic bacteria solution was taken and inoculated onto the surface of the NA medium on a spread plate and allowed to stand for 5 minutes. Then, paper discs soaked with LAB isolates were placed on the medium surface and incubated for 48 hours at 37ºC. Chloramphenicol was used as the positive control. The appearance of an inhibition zone characterizes isolates that have the potential to produce antibacterial. The measurement of the inhibition zone formed follows the following equation (Verawaty et al. 2020):

$$Dz = \frac{(Dv - Dc) + (Dh - Dc)}{2}$$

Where:

Dz = Diameter of inhibition zone (mm)

Dv = Vertical Diameter of the inhibition zone around the paper disc (mm)

Dh = Horizontal Diameter of the inhibition zone around the paper disc (mm)

Dc = Diameter of disc paper (6 mm)

RESULTS AND DISCUSSION

Isolation and purification of lactic acid bacteria

Lactic acid bacteria (LAB) were isolated and purified using MRS agar media. Isolation was carried out after fermentation for 14 days using the streak quadrant method; the appearance of the colony was milky white. There were 21 isolates from traditional *sarobuong* food from Kuantan Singingi District. The growing colonies were purified to obtain pure isolates. The process of isolating LAB from traditional *Sarobuang* food can be seen in Figure 1.

Morphological identification of LAB isolates from *sarobuong*

Morphological identification was carried out using macroscopic and microscopic observations. The results of macroscopic and microscopic identification of LAB isolates from s*arobuong* are presented in Table 1 and Figure 2.

Figure 1. Isolation of LAB from *Sarobuong*

Table 1. Results of morphological identification of LAB isolates from *sarobuong*

Isolate code	Macroscopic				Microscopic	
	Shape	Edge	Color	Elevate	Shape	Gram stain (+/-)
RB1	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB2	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB3	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB4	Globular	Entire	Milky White	Convex	*Coccus*	+
RB5	Globular	Entire	Milky White	Convex	*Coccus*	+
RB6	Globular	Entire	Milky White	Convex	*Coccus*	+
RB7	Globular	Entire	Milky White	Convex	*Coccus*	+
RB8	Globular	Entire	Milky White	Convex	*Coccus*	+
RB9	Globular	Entire	Milky White	Convex	*Coccus*	+
RB10	Globular	Entire	Milky White	Convex	*Coccus*	+
RB11	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB12	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB13	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB14	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB15	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB16	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB17	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB18	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB19	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB20	Globular	Entire	Milky White	Convex	*Bacilli*	+
RB21	Globular	Entire	Milky White	Convex	*Bacilli*	+

Note: (-) negative; (+) positive

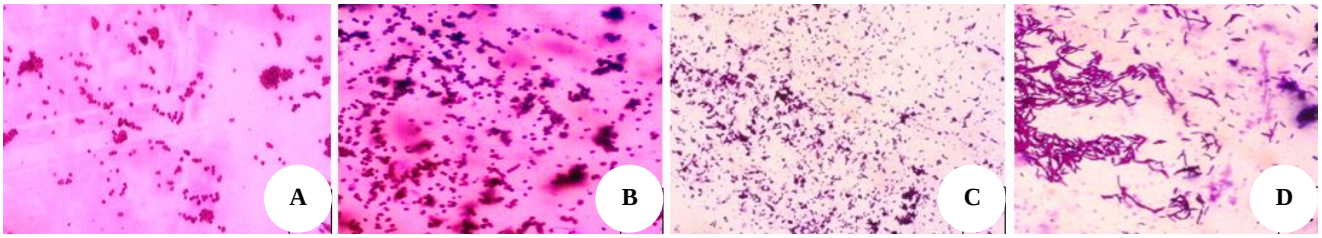

Figure 2. Microscopic identification of several LAB isolates from *Sarobuong* with 400x magnification (A and B) *Coccus*; and (C and D) *Bacilli*

Characteristics of the LAB of *sarobuong*

Characterization of LAB isolated from *sarobuong* was conducted on several tests to determine its characteristics. The tests carried out were catalase test, salt content, and carbohydrate fermentation test (lactose, sucrose, glucose, and fructose). The test results are shown in Table 2.

Antibacterial test

Antibacterial tests were carried out on isolates indicated as LAB based on the results of morphological tests and LAB characterization. The isolates observed that showed the characteristics of LAB were 21 isolates. In addition, antibacterial tests on LAB isolates were conducted on two pathogenic bacteria types, *E. coli* (gram-negative) and *S. aureus* (gram-positive). The result indicated the presence of an inhibitory zone in the form of a clear zone that forms around the disc containing LAB isolates. Antibacterial test results from LAB isolates can be seen in Table 3.

Discussion

The isolation and purification of bacteria from *sarobuong* revealed that 21 isolates were obtained. The isolates obtained were then identified morphologically. The results of morphological identification were carried out using macroscopic and microscopic methods. The bacterial isolates' cell shape, margins, color, and elevation were identified by macroscopic identification. The results showed that as many as 21 isolates had a round shape, flat edges, milky white color, and convex elevation. While the results of microscopic identification showed as many as seven isolates were *coccus*, 14 isolates were *Bacillus*, and all isolates were identified as gram-positive bacteria. These results follow the research of Rezac et al. (2018), who said that the fermentation process involved LAB from several genera, such as *Lactobacillus*, *Streptococcus*, and *Leuconostoc*, but other bacteria and yeasts and fungi also contributed to food fermentation (Rezac et al. 2018).

Table 2. Biochemical and fermentation tests of LAB isolates from *sarobuong*

Isolate code	Biochemical and fermentation test					
	Catalase test (+/-)	Salt level test	Carbohydrate fermentation test			
			Lactose	Sucrose	Glucose	Fruktosa
RB1	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB2	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB3	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB4	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB5	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB6	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB7	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB8	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB9	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB10	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB11	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB12	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB13	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB14	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB15	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB16	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB17	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB18	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB19	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB20	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative
RB21	-	+	Homofermentative	Homofermentative	Homofermentative	Homofermentative

Note: (-) negative; (+) positive

Table 3. Antibacterial test of LAB isolates from *sarobuong*

Isolate code	Positive control (cm)	*E. coli* (cm)	Positive control (cm)	*S. aureus* (cm)
RB1	-	**0.521**	-	0.29
RB2	-	0.470	0.110	0.335
RB3	-	0.103	-	0.463
RB4	-	0.100	-	0.136
RB5	-	0.361	-	0.475
RB6	-	0.138	0.110	0.19
RB7	0.350	0.278	-	0.283
RB8	0.080	0.123	0.170	0.503
RB9	-	0.380	0.460	0.443
RB10	0.090	0.128	0.350	0.613
RB11	0.320	0.313	-	0.63
RB12	0.160	0.308	1.090	0.366
RB13	0.120	0.253	-	0.31
RB14	0.265	0.410	0.050	0.06
RB15	0.350	0.285	1.520	**0.646**
RB16	0.390	0.376	-	0.456
RB17	0.650	0.480	0.110	0.456
RB18	1.110	0.430	0.030	0.166
RB19	-	0.441	0.070	0.313
RB20	-	0.450	2.010	0.626
RB21	0.315	0.205	0.03	0.61

The catalase test (Table 3) was conducted to determine LAB isolates' ability to produce catalase enzymes. The catalase test results showed that 21 LAB isolates were catalase-negative. This is one of the characteristics of LAB, which cannot produce the enzyme catalase. This is indicated by the absence of air bubbles after adding H_2O_2, which indicates the breakdown of H_2O_2 into O_2 (Prastujati et al. 2022). Bacteria that do not release O_2 vesicles indicate that bacteria have peroxidase enzymes that can prevent O_2 production and are expressed as catalase-negative bacteria. In testing the salt content with a concentration of 6.5%, LAB isolates can grow in an environment with a salt content of 6.5%. That shows all isolates obtained are non-halophilic. Ibrahim et al. (2015) reported the same results regarding the identification of LAB. That showed a negative result for the catalase test, meaning that LAB did not produce the catalase enzyme to convert hydrogen peroxide into H_2O and O_2.

The carbohydrate fermentation test in this study was carried out using four carbohydrate sources: lactose, sucrose, glucose, and fructose. In contrast, metabolic test results of various types of sugar showed that all LAB isolates were a homofermentative group with the main end product of lactic acid. Furthermore, the test results showed that all isolates could ferment the carbon source used. Rizqiati et al. (2015) and Ramadhanti et al. (2021) also obtained the same results; glucose testing results from LAB isolates were more homofermentative. This homofermentative is characterized by CO_2 produced during the carbohydrate test. Probiotic LAB is classified as homofermentative and heterofermentative based on their metabolic pathways. However, homofermentative LAB ferments sugar to produce lactic acid, especially under anaerobic conditions. While heterofermentative LAB fermented sugar to produce ethanol, CO_2, and a small amount of lactic acid (Ayyash et al. 2018).

Based on the biochemical and physiological test results, it can be concluded from all the 21 isolates identified as LAB with the genus *Lactobacillus* and *Streptococcus.* That is based on the main characteristics of LAB, which are lack of spores, gram-positive, and catalase-negative (Mokoena 2017). Then, an antibacterial test was conducted to determine the ability of 21 isolates to inhibit pathogenic bacteria *E. coli* and *S. aureus*. In this test, the positive control used was chloramphenicol. The ability of LAB isolates to inhibit pathogenic bacteria was carried out by comparing the zone of inhibition with the positive control. The results showed that LAB from *sarobuong* had a higher inhibitory effect on *E. coli* than *S. aureus.* Morales et al. (2003) stated that inhibition could be grouped into four categories, namely weak (<5 mm), moderate (5-10 mm), strong (>10-20 mm), and very strong (>20-30 mm). Based on these results, most of the inhibition against *E. coli* was categorized as weak (<5 mm), while *S. aureus* was categorized as moderate (5-10 mm). The highest antibacterial activity for pathogenic bacteria *E. coli* was isolated at RB1. While pathogenic bacteria *S. aureus* was isolated at RB15. The results follow the research conducted by Tanaka et al. (2013) and Badwaik et al. (2015), who reported that bamboo shoots contain antibacterial compounds that can inhibit the pathogenic bacteria *E. coli*, and *S. aureus.* Bamboo shoots contain phenolic compounds that can produce antioxidants and anti-inflammatory, anti-allergic, and antimicrobial properties (Ainezzahira et al. 2017). Bamboo is currently widely applied for medicine and health benefits, such as antioxidant, anticancer, anti-aging, anti-free radicals, weight loss, preventing cardiovascular disease, lowering blood pressure, improving digestion, and antimicrobial activity due to different glycosides, and flavones (Thakur et al. 2016).

Based on the ability of lab isolates to suppress pathogenic bacteria *E. coli* and *S. aureus*, these LAB isolates, especially RB1 and RB15, have probiotic properties and the potential to be developed into probiotics. This selection was done to obtain potential LAB as probiotics because not all lactic acid bacteria are probiotics. Therefore, the pathogenic bacteria test is the first step used to determine the ability of isolates to be used as probiotics.

In conclusion, isolation of bacteria from traditional food Kuantan Singingi District, *sarobuong* obtained 21 isolates indicated as LAB. Based on the results of biochemical and physiological tests, 21 isolates were identified as LAB by the genus *Lactobacillus* and *Streptococcus*. While the results of the antibacterial test of 21 LAB isolates were obtained, the highest antibacterial activity against pathogenic bacteria *E. coli* was depicted by isolate RB1, while for pathogenic bacteria *S. aureus* by isolate R15. Thus it was concluded that LAB is isolated, *sarobuong* has a relatively high antibacterial activity and has the potential to be used as a probiotic.

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