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Isolation and Characterisation of Lactic Acid Bacteria in Cincalok from Pengujan Village, Riau Islands

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Abstract: Cincalok is a fermented food made from *Acetes sp*. The fermentation process requires the help of lactic acid bacteria. During the fermentation process, lactic acid bacteria function as antimicrobials and flavour enhancers, and as antimicrobials during the fermentation process, extending food storage time. The research aimed to isolate and identify lactic acid bacteria contained in cincalok. Isolation was done using the pour plate technique to get a pure culture of bacteria grown on MRSA. The colony and cell characteristics were also studied, and biochemical characterisation was performed. This study showed that 5 isolates of bacteria were isolated from Cincalok. The morphological and biochemical characterisation results showed that five isolates are lactic acid bacteria.
Keywords: cincalok, fermentation, lactic acid bacteria

1. Introduction

Fermentation is a food processing process that involves microorganisms preserving food ingredients and changing their texture [1]. It is a traditional method with a simple process and economical costs that aims to maintain the product's shelf life. Cincalok is a traditional fermented food widely favoured by the Malay community, especially in the Riau Islands. This product in West Kalimantan is also known as ronto and cencaluk in Malaysian [2].

Cincalok is a fermented product made from shrimp Acetes sp. The cincalok fermentation process involves the role of lactic acid bacteria. Lactic acid bacteria produce acid to inhibit the growth of pathogenic bacteria that cause decay so that they can extend the shelf life of fermented products. Lactic acid bacteria can also produce bacteriocin compounds. The bacteriocin compound can inhibit the growth of pathogenic bacteria [3]. Lactic acid bacteria isolated from Rusip produce peptides that have the potential as antihypertensives [4]. Lactic acid bacteria isolated from tilapia (Oreochromis niloticus) contains lovastatin, which can lower cholesterol levels [4].

Based on this information, research on the isolation and characterization of lactic acid bacteria found in cincalok fish from Pengujan Village is necessary. The lactic acid bacteria isolates obtained can be further studied to determine the bioactive potential contained in the lactic acid bacteria isolates from Cincalok.

2. Material and Methods

2.1. Materials

The laboratory equipment included Petri dishes, micropipettes, vortex, incubator, microscope, laminar flow, stirring rod, hot plate, autoclave, test tubes, and Durham tubes. The main material used were cincalok (a fermented food), MRSA, MRSB, peptone water, TSIA, ethanol 98%, violet crystal, iodin, paraffin, safranin, H_2O_2, and Kovac reagent.

2.2. Methods

2.2.1. Sample collection and preparation

The sample (cincalok) was collected from Pengujan Village, Bintan Regency, Riau Islands. The cincalok used as a sample was fermented for 7 days.

2.2.2. Isolation and Purification of Lactic Acid Bacteria

1 ml of cincalok sample that has been diluted with a micropipette and then 9 ml of distilled water and homogenized using a vortex. After that, multilevel dilutions were carried out from 10^{-1} to 10^{-6}. In the 10^{-6} sample, 1 ml was taken and put into a Petri dish. After that, it was grown on MRSA media (De Man Rogosa Sharpe Agar) using the pour plate technique. Furthermore, the cincalok sample was flattened on the media and homogenized. Then incubated for 24 hours at 37 °C [5]. The purification stage of lactic acid bacteria is done by scraping 4 quadrants on a Petri dish that has been incubated before. Then the Petri dish was incubated again for 24 hours at 37°C. The purification process was carried out 4 times to get pure colonies finally.*Morphological Test of Lactic Acid Bacteria*

Colonies and Cells

2.3.1. Colony Morphological Test

The morphology of bacterial colonies is observed by looking at the bacteria's physical shape, which appears without a microscope. The colony morphological test includes colony shape, elevation type, and colony edges. Observations are divided into colony shapes (circular, irregular, filamentous, and rhizoid); elevation (convex, flat, raised, and umbonate), and margin (entire, undulate, lobate, and erose.

2.3.2. Gram Staining

Gram staining is done by taking 1 loop bacteria from a nutrient agar medium, placed on a glass slide, widening, and bacteria fixation was carried out. Violet crystals were dropped into the sample and allowed to stand for 5 minutes. Then it was washed with running water. Lugol was added and allowed to stand for 60 seconds, then it was washed with running water. The next step is to immerse in a vessel containing alcohol 95% alcohol while shaking for 30 seconds. Wash again with running water. Then, it was stained with three drops of safranin, let stand for 1 minute, washed with running water, and dried. The final step, bacteria were observed using a microscope with a magnification of 400x [6]. Bacterial cell shapes are categorised into cocci, bacilli, and spirals [7].

2.3.3. Biochemical Characterization

Biochemical tests were performed to identify and characterize the lactic acid bacteria isolated from cincalok. The tests were motility, gas production, catalase, oxidative, fermentative, and citrate Simmon tests [5].

3. Results and Discussion

3.1 Morphology of Lactic Acid Bacteria Colonies and Cells

The identification was performed according to the morphological and microscopic characteristics. The morphological characteristics of different isolates studied on MRSA are summarized in Table 1. The result showed there were five isolates. All of the isolates have the same morphology. Lactic acid bacteria isolated from ale-ale and cincalok have a round shape, white with convex elevation, and entire edges [8]. Lactic acid bacteria isolated from Indonesian commercial kefir grain have a circular shape, white with convex elevation, and entire edges[9].

Identification of cell morphology of lactic acid bacteria includes bacterial shape and Gram staining. All isolates were identified as Gram-positive cocci through Gram staining and microscopy. Lactic acid bacteria isolated from fermented catfish (bekasam) also showed gram-positive [10]. Gram-positive bacteria have a thicker cell wall peptidoglycan than gram-negative bacteria. The thickness of the cell wall peptidoglycan in gram-positive bacteria causes the crystal violet colour not to be removed by alcohol. Gram-positive bacteria are shown in purple, while Gram-negative bacteria are shown in red in the Gram-staining [11]. Gram-positive bacteria are shown in Figure 1.

3.2 Biochemical Characteristics of Lactic Acid Bacteria

Several biochemical tests were carried out, namely catalase test, motility, gas production, indole, Simmon citrate, fermentation type, and oxidative fermentative. The results of biochemical characteristics are summarized in Table 2.

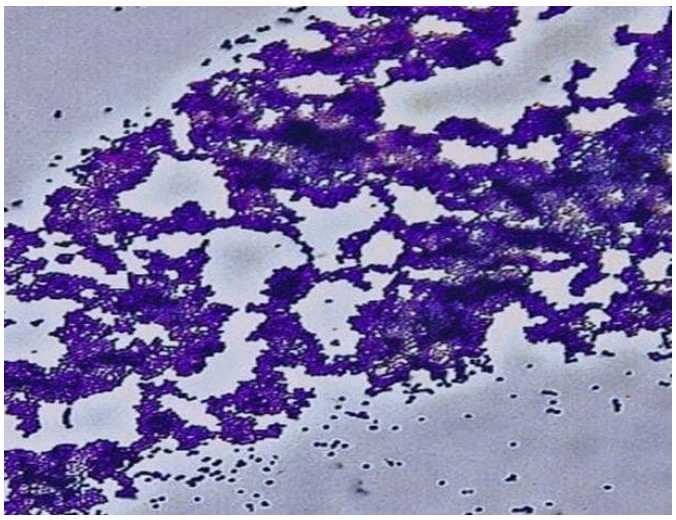

Figure 1. Gram-positive lactic acid bacteria from Cincalok with a magnification of 400x

3.2.1. *Catalase*

In Table 2, it is shown that all isolates of lactic acid bacteria are catalase-negative because they cannot produce air bubbles on the preparation glass dripped with hydrogen peroxide. *Pediococcus acidilactici* from Bekasam is a negative-catalase bacterium [12]. Isolates of lactic acid bacteria in traditional food Denkke Naniura produce negative catalase. Catalase is an enzyme that can decompose hydrogen peroxide (H_2O_2) into water and O_2 [13]. Hydrogen peroxide is formed during aerobic metabolism, so microorganisms that grow under aerobic conditions can decompose these toxic substances. Peroxide compounds that accumulate can cause free radicals, which are toxic by damaging cell membranes and inhibiting bacterial growth [14].

Table 1. Morphological Characteristics of Lactic Acid Bacteria Colonies and Cells

Isolates	Form	Elevation	Margin	Surface	Opacity	Chromogenesis
A1	circular	convex	entire	smooth and glistening	opaque	white
A2	circular	convex	entire	smooth and glistening	opaque	white
A3	circular	convex	entire	smooth and glistening	opaque	white
A4	circular	convex	entire	smooth and glistening	opaque	white
A5	circular	convex	entire	smooth and glistening	opaque	white

Table 2. Biochemical Characteristics of Lactic Acid Bacteria from Cincalok

Isolates	Test parameters						
	C	M	TSIA	I	S	TF	O/F
A1	-	-	k/k	-	-	Ho	F
A2	-	-	k/k	-	-	Ho	F
A3	-	-	k/k	-	-	Ho	F
A4	-	-	k/k	-	-	Ho	F
A5	-	-	k/k	-	-	Ho	F

Notes: (+) Positive reaction; (-) No reaction; (k/k) yellow slant/yellow butt; (Ho) homofermentative; (F) fermentative.

3.2.2. *Motility*

Motility tests are carried out to determine the presence of flagella and the ability of bacteria to move. This test distinguishes bacteria into two groups, namely motile and non-motile. Lactic acid bacteria are non-motile because they do not have flagella. The results show that five lactic acid bacteria isolates from Cincalok are non-motile. The Isolates of Lactic acid bacteria from traditional fermented foods from West Kalimantan (ale-ale and cincalok) are non-motile [8]. Lactic acid bacteria do not have flagella, so their movement is only a straight line along the puncture, while motile bacteria have flagella, so that they grow away from the puncture, which makes the media look cloudy [15].

3.2.3. *TSIA Test*

The TSIA test is based on their ability to ferment these three types of sugars and produce acid and hydrogen sulfide. Lactic acid bacteria can ferment glucose, sucrose, and lactose. The test used TSIA medium. Identification of the ability to ferment sugars based on the color change in the slant and butt of the medium on the test tube. The results of the TSIA test are shown in Table 2. All of the isolates can ferment glucose, lactose, and sucrose which indicated by a change in the color of the medium which turns yellow on the slant and butt. This is indicated by a change in the color of the medium which turns yellow on the slant and butt. Isolation of lactic acid bacteria from cocoa beans can only ferment glucose [5]. Bacteria fermenting sugar will release some acids that change the phenol red indicator in the TSIA media to yellow; if fermentation does not occur, the indicator remains red. If bacteria can ferment glucose will produce acid so that the butt part turns yellow because there is a decrease in pH. However, the slant part remains red because it is alkaline. Conversely, if bacteria can ferment glucose, sucrose, and lactose will produce enough acid so that it can lower the pH in the slant and butt parts which causes the media in both parts to turn yellow.

3.2.4. *Indole Test*

The indole test is a biochemical test used to determine the ability of bacteria to convert the amino acid tryptophan to indole, which accumulates in the medium. Lactic acid bacteria are indole-negative bacteria. Table 2 shows that all of the isolates are indole-negative. Lactic acid bacteria isolated from cocoa beans showed negative indole [5]. Negative indole is indicated by the absence of a red ring layer, while positive indole is indicated red ring on the test tube. When reacting with indole solution, Kovac's reagent will change from yellow to cherry red, caused by Kovac's reagent staining hydrochloric acid and p-dimethylaminobenzaldehyde in amyl alcohol [16] [16]. Because amyl alcohol is not water soluble, the red coloration will form in an oily layer at the top of the broth. Tryptophan is an amino acid that results from oxidation by forming 3 main end products, namely indole, pyruvic acid, and ammonia [17].

3.2.5. Simmon Citrate Test

Simmons Citrate Agar is an agar medium used to differentiate bacteria based on utilising citrate as the sole carbon source and inorganic dihydrogen phosphate as the sole nitrogen source [18]. In Table 2, all of the isolates indicated negative Simmons citrate. Isolates of lactic acid bacteria in tilapia bekasam with the addition of curd produce positive Simmons citrate [18]. Positive Simons citrate is indicated by the ability of bacteria to utilize citrate as a substrate by producing the enzyme citrate permease, which can convert citrate into pyruvate. Citrate metabolism will break down ammonium salts into ammonia and increase alkalinity. The bromthymol blue indicator will change from green to blue if the medium is alkaline. The shift in pH turns the bromthymol blue indicator in the medium from green to blue above pH 7.6 [19].

3.3.6. Fermentation Type

The fermentation type test is carried out to identify bacteria based on their fermentation type. Lactic acid bacteria can be homofermentative and heterofermentative[20]. In Table 2, all of the isolates of lactic acid bacteria isolated from Cincalok are homofermentative. Homofermentative is indicated by the absence of air bubbles in the Durham tube. Bacterial isolates from fish paste are homofermentative [20]. Homofermentative bacteria cannot produce gas due to the absence of the pyruvate oxidase enzyme, which plays a role in converting pyruvate to CO2 and forming H2O2. It causes homofermentative bacteria to only produce lactic acid in the final product without producing CO2 gas [15]. If heterofermentative bacteria can break down pyruvic acid through glycolysis, they will produce lactic acid, acetate, ethanol, and carbon dioxide.

3.3.7. Oxidative-Fermentative Test (OF)

The oxidative-fermentative test is conducted to determine whether bacteria metabolize glucose oxidatively or fermentatively by using 2 test tubes, one of which is covered with paraffin. The addition of paraffin is to cover the media so that there is no air in the test tube. Table 2 shows that all isolates are fermentative because only the paraffin-covered tubes changed color from green to yellow in the medium. Fermentative bacteria can utilize carbohydrates in anaerobic conditions through fermentation, while oxidative bacteria only utilize carbohydrates in aerobic conditions through oxidation [21]. Bacteria are oxidative if the colour of the medium changes to yellow only in tubes without paraffin; fermentative bacteria are indicated by tubes with paraffin that change the colour of the medium to yellow [22].[22].

4. Conclusion

There are 5 isolates of lactic acid bacteria isolated from Cincalok. The characteristics of isolates are gram-positive, catalase-negative, non-motile, indole-negative, able to ferment glucose, lactose, and sucrose, able to use citrate, homofermentative fermentation type, and fermentative oxidative test is fermentative.

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