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Lactic Acid Bacteria That Produce Antibacterial Compunds on Candied Pakoba Fruit (*Syzygium luzonense)*

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Abstract

Lactic Acid Bacteria (LAB) are a group of Gram-positive, cocci or rod-shaped microorganisms characterized by their catalase-negative phenotype. These bacteria are known to produce various antibacterial compounds, including organic acids, hydrogen peroxide, carbon dioxide, and bacteriocins, which exhibit bacteriostatic or bactericidal properties against pathogenic bacteria. The aim of this study is to isolate LAB that produce antibacterial compounds during the fermentation of Pakoba fruit (Syzygium luzonense) into sweets, with a focus on inhibiting the growth of Escherichia coli and Staphylococcus aureus. This research employs a quantitative descriptive method, involving the isolation of LAB from candied Pakoba fruit samples and subsequent testing of their antibacterial activity against the aforementioned pathogens. The barrier zone formed around the wells is then measured. The data from the study showed that BAL isolation from Pakoba fruit candied was obtained from as many as 10 isolates, and antibacterial activity tests showed that 10 BAL isolates produced antibacterial compounds with inhibitory diameters of 7.6 – 15.3 mm. The isolates with the greatest antibacterial activity are LAB isolate MP(1)5.2 for E. coli and LAB isolate MP(3)6.3 isolates for S. aureus. Based on the results of identification using the profile matching method, it was shown that the selected LAB isolates, namely MP(1)5.2 and MP(3)6.3, were classified in the genus Lactobacillus with the characteristics of stem cell shape, single cell arrangement, catalase-negative, non-motile, did not form spots, and did not produce gas from glucose.

Keywords: Lactic acid bacteria, Pakoba fruit, antibacterial, E. coli, S. aureus.

INTRODUCTION

Lactic acid bacteria (LAB) have emerged as a pivotal group of microorganisms, contributing significantly to various sectors, including food production, agriculture, and pharmaceuticals. Their multifaceted benefits encompass probiotic properties, enhancement of organoleptic attributes in food and beverages, and preservation capabilities. Lactic acid bacteria are also known as beneficial microorganisms, where these bacteria are a group of bacteria that do not produce toxic substances and are not harmful to health, in reality, the presence of these bacteria in food provides a good function for health because this bacteria can inhibit pathogenic microbes naturally (Lawalata et al., 2019; Rosyidah et al., 2023).

Lactic acid bacteria (LAB) are beneficial microorganisms that warrant further exploration, with certain

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strains exhibiting potential as probiotic candidates under specific conditions. To be considered effective probiotics, these bacteria must meet several key criteria, including resistance to acidic environments (low pH), tolerance to bile acids and salts, ability to produce antimicrobial compounds, and capacity to thrive in the human gastrointestinal tract, thereby promoting a balanced gut microflora. Additionally, they must demonstrate a satisfactory safety profile for human consumption (Lawalata et al., 2023; Melia et al., 2022; Seveline, 2018).

LAB can be isolated from various food sources such as fruits (Lawalata et al., 2019; Lawalata et al., 2023; Barbosa et al.,2022; Giyatno dan Retnaningrum, 2020), vegetables (Ma'unatin et al., 2020), meat (Charmpi et al., 2020), ikan (Park et al., 2009), fermented foods (Adhinugraha et al., 2022; Setiarto et al., 2018). Fruits are a potential source of LAB because they have a high content of simple carbohydrates, vitamins, minerals, and antioxidants (Fossi et al.,2023). Therefore fruits and lactic acid bacteria have the potential as functional foods.

Pakoba fruit (Syzygium sp.), is a medicinal plant deeply rooted in the traditional knowledge of the Minahasa people and is endemic to North Sulawesi, Indonesia. This fruit is exclusively found in the Minahasa region and enjoys significant popularity among the local population due to its cultural and potential medicinal value. The results of the research conducted by Pongoh et al (2021) show that white pakoba leaves and fruits have antimicrobial, antidiabetic, and antioxidant potential. Pakoba fruit has a very sour and unique taste so it is often used as a commercial food, for example, candied or rujak and pickles. Not only that, the fruits of the pakoba plant can be made into sweets, juices, dodol, and other traditional foods (Lawalata et al., 2023; Walean et al.,2020; Pangemanan et al.,2019)

The results of phytochemical screening on ethanol extracts from Syzygium myrtifolium plants, the content of secondary metabolite compounds in the form of alkaloids, flavonoids, terpenoids, and saponins were found (SIgihartini & Maryati, 2022). Likewise, with the results of the research presented by Kinho et al. (2011), It was found that in the plant type Syzygium sp, there is a content of secondary metabolites in the form of alkaloids, flavonoids, and tannins. Furthermore, the results of the research presented by Sudarmi et al (2017) found that the plant type Syzygium cumini contains alkaloids, phenols, and terpenoids. The benefits of the white pakoba plant (S. luzonense) are very useful in the world of medicine. The function of this plant is as a painkiller, anti-inflammatory, and also anti-fungal. In addition, from the results of the phytochemical screening of this white pakoba fruit extract, it was found that it contained a lot of alkaloid compounds, flavonoids, and tannins. And just like white pakoba, other types of pakoba that are red can also be anti-diabetic drugs and antioxidants (Walean et al., 2020).

Pakoba fruit is widely used for medicine most simply because Pakoba fruit is rich in bioactive compounds and serves as a potential source of probiotics due to its content of beneficial microorganisms, specifically Lactic Acid Bacteria (LAB).Some of the compounds produced by BAL and are antibacterial are organic acids, hydrogen peroxide, reuterine, bacteriocins, and carbon dioxide. Organic acids are compounds produced by lactic acid bacteria during the fermentation process causing the pH to drop. The buildup of acidic end products and decreased pH interfere with the growth of gram-positive or gram-

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negative pathogenic bacteria during the fermentation process. Undissociated lipolytic acids such as lactic acid and acetic acid can penetrate microbial cell membranes. Organic acids (lactate, citrate, fumarate, acetate, and malate) can make the cytoplasm of pathogenic bacteria acidic, preventing substrate transport and transmembrane potential. Hydrogen peroxide (H2O2) is a compound produced by LAB. This compound is antibacterial due to the hyperbaric toxicity produced by the cell membrane lipid peroxide that promotes increased cell membrane permeability (Ardilla et al., 2022).

Candied Pakoba fruit is a special food that is very liked by many people and is one of the foods that goes through many stages of the process to preserve the fruit. The process of soaking fruit in sugar water increases the sugar content contained in the fruit and lowers the water content, thereby slowing down the growth of fruit pathogenic bacteria and making the fruit more durable for up to three months. The process of making candied fruit products can change the taste of fruit from sour to sweet. Fermentation carried out by LAB will increase the content of bacterial cell walls classified as peptidoglycans, lipoproteins, and glycoproteins, thereby inhibiting fruit rot (Lawalata et al., 2023; Pongoh et al., 2021).

While extensive research has been conducted on lactic acid bacteria (LAB) in various food products, studies on LAB in candied fruits remain limited. This study aims to investigate the presence of LAB in candied Pakoba fruit (Syzygium luzonense) and explore its potential to produce antibacterial compounds, similar to other fruits such as nutmeg.

RESEARCH METHODS

The material used in this study is fresh white pakoba fruit (*Syzygium luzonense*) obtained from the Kakaskasen plantation, Tomohon city, North Sulawesi Province

Methods

Sample preparation of Candied Pakoba Fruit

Candied Pakoba Fruit is made by weighing 250 grams of fresh pakoba fruit and 250 grams of sugar, then putting them in a container and storing them at room temperature (20°C - 25°C) for 7 days.

Lactic Acid Bacterial Isolation

The isolation of LAB from candied Pakoba fruit was performed using the pour plate method. A 1 mL sample of the fruit candy was suspended in 9 mL of phosphate-buffered saline (PBS) to achieve a 10^{-2}, dilution, followed by serial dilutions up to 10^{-7}. Aliquots of 1 mL from dilutions 10^{-5}, 10^{-6}, and 10^{-7}, were inoculated into sterile petri dishes, and 1% MRS agar supplemented with CaCO3 was added. The plates were incubated at 37°C for 48 hours under microaerophilic conditions. Colonies exhibiting clear zones around them were selected as potential LAB isolates. Purification of isolated bacterial colonies is carried out by subculturing them on MRSA medium until a uniform bacterial colony is obtained. Single cultures of LAB are stored in colony form in test tubes with slanted MRSA medium at 4°C for further characterization.

Morphological characterization and biochemical assays of lactic acid bacteria

Identification of lactic acid bacteria includes colony shape identification and characterization of cell morphology (cell shape and arrangement, Gram staining), as well as biochemical tests (catalase, motility,

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spore formation, SIM assay, gas production, and pH analysis).

The morphological characteristics observed include the shape, edges, elevation, and color of the colonies. Gram staining was performed using a series of dyes: Crystal Violet (Gram A), Lugol/Iodine Solution (Gram B), Alcohol (Gram C decolorizer), and Safranin (Gram D counterstain). The staining results were observed under a microscope with a magnification of 1000X, allowing for the determination of cell shape and Gram reaction (positive or negative).

In addition to morphological characterization, several biochemical tests were conducted, including catalase tests, motility tests, spore formation tests, gas production from glucose, and pH analysis. The catalase test was performed by applying 2-3 drops of 3% hydrogen peroxide (H2O2) reagent to a bacterial culture on a glass slide. The presence of gas bubbles indicated a positive result (Sari et al., 2012). The motility test was conducted by inoculating bacterial culture into SIM (Sulfide Indole Motility) medium and incubating at 37°C for 48 hours. Motility was determined by observing creeping growth around the puncture site (Aisyah et al., 2014). Spore staining was performed using Malachite green 5% as the primary stain and safranin as the counterstain. Bacterial isolates were smeared on a glass slide, stained with Malachite green, and then counterstained with safranin. The presence of green spores under 1000x magnification microscopy indicated a positive result. Notably, lactic acid bacteria do not produce endospores. The pH test involved incubating 10 grams of fruit flesh samples in 100 mL of MRS broth medium (pH 5.5) at room temperature for two consecutive days.

Antibacterial Activity Test Of LAB

Lactic acid bacterial isolates that had been successfully isolated and grown in MRSA media were tested for their antibacterial activity against pathogenic bacterial isolates, namely *Escherichia coli* and *Staphylococcus aureus*. A total of 0.1 mL of *Escherichia coli* and *Staphylococcus aureus* suspensions were inoculated separately into sterile petri dishes using the pour plate method, and 15 mL of nutrient broth (NB) medium was added. After the medium solidified, wells with a diameter of 0.7 cm were created. Each petri dish contained three wells, and 0.1 mL of antibacterial substance was added to each well. The petri dishes were then incubated at 40°C, for 24 hours. The antibacterial activity was indicated by the presence of clear zones around the wells, and the diameter of the inhibition zones was measured from three different sides and averaged (Sutrisna et al., 2017).

Data Analysis

The identification results were presented in a qualitative descriptive format, focusing on the morphological and biochemical characteristics of each lactic acid bacterial (LAB) isolate derived from the fermentation of Pakoba fruit (Syzygium luzonense). The study also assessed and described the antibacterial activity of the LAB isolates against specific indicator bacteria, providing insights into their potential antimicrobial properties.

RESULTS AND DISCUSSION

Lactic Acid Bacterial Isolation from Candied Pakoba Fruit (Syzygium luzonense)

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Lactic acid bacterial (LAB) isolation was conducted on the first and third days of fermentation of candied Pakoba fruit. Twelve colonies of acid-producing bacteria were successfully isolated, characterized by the formation of clear zones around the colonies on the MRSA-CaCO3 isolation medium (Table 1). These isolates were further screened for lactic acid bacteria based on key characteristics, including (1) Gram staining, (2) Cell shape, (3) Catalase test, (4) Spore formation, (5) Motility, (6) Gas or acid production from glucose.

Lactic Acid Bacteria Selection

The bacterial isolation results from candied Pakoba Fruit showed that not all colonies producing clear zones on MRS-CaCO3 medium were LAB members. Some colonies with clear zones exhibited Gram-negative staining, catalase positivity, and spore formation (Table 2).

Through selection and screening, 10 out of 12 colonies were identified as potential LAB isolates based on the following criteria of (1) Gram-positive, (2) Rod-shaped cells, (3) Catalase-negative, (4) No spore formation, (5) Non-motile, (6) No gas production from glucose.

Table 1. Presents the morphology of acid-producing bacterial colonies from candied Pakoba Fruit.

No	Isolate Code	Color of Bacterial Colonies	Colony Shape	Elevasi	Edges	Inner Structure
1	MP(1) 5.1	Milk-White	Circulate	Convex	Flat edges	Opaque
2	MP(1) 5.2	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
3	MP(1) 6.1	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
4	MP(1) 6.2	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
5	MP(1) 7.1	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
6	MP(1) 7.2	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
7	MP(3) 6.1	Milk-White	Circulate	Convex	Flat edges	Opaque
8	MP(3) 6.2	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
9	MP(3) 6.3	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
10	MP(3) 7.1	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
11	MP(3) 7.2	Yellowwish-white	Circulate	Convex	Flat edges	Opaque
12	MP(3) 7.2	Yellowwish-white	Circulate	Convec	Flat edges	Opaque

Based on the colony morphology results, the 12 isolates exhibited a milky yellowish-white color, circular shape, convex elevation, flat edges, and opaque structures.

Table 2. presents the results of screening for LAB and non-LAB isolates grown on MRSA-CaCO3 medium, obtained from candied Pakoba fruit.

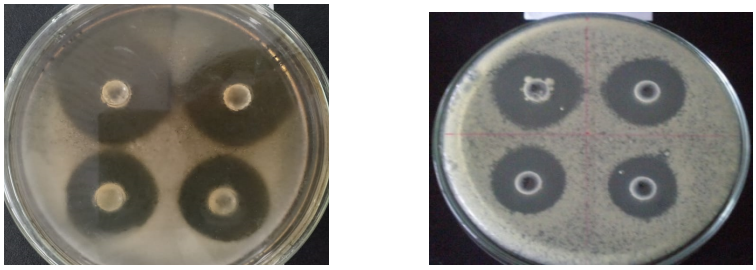
No	Source of Isolation	Isolate Code	Acid Producer (Clear zone)	Gram	Catalase	Spora	Motil	LAB / NON LAB
1	Candied Pakoba Fruit	MP(1) 5.1	+	+	+	-	-	Non LAB
2		MP(1) 5.2	+	+	-	-	-	LAB
3		MP(1) 6.1	+	+	-	-	-	LAB
4		MP(1) 6.2	+	+	-	-	-	LAB
5		MP(1) 7.1	+	+	-	-	-	LAB
6		MP(1) 7.2	+	+	-	-	-	LAB
7		MP(3) 6.1.	+	-	+	-	+	Non LAB
8		MP(3) 6.2	+	+	-	-	-	LAB
9		MP(3) 6.3	+	+	-	-	-	LAB
10		MP(3) 7.1	+	+	-	-	-	LAB
11		MP(3) 7.2	+	+	-	-	-	LAB
12		MP(3) 7.3	+	+	-	-	-	LAB

Test of Antibacterial Activity of LAB Isolate Against Indicator Bacteria

The inhibition power of LAB isolate was tested using the well diffusion method by measuring the diameter of the inhibition zone against indicator bacteria (E. coli and S. aureus). The LAB isolate showed potential in inhibiting pathogenic bacteria, with MP(3) 6.3 isolate exhibiting a 15.6 mm diameter inhibition zone against S. aureus and MP(1) 5.2 isolate showing an 11.6 mm diameter inhibition zone against E. coli (Table 3).

Table 3. presents the inhibition of lactic acid bacteria isolate against the growth of Escherichia coli and Staphylococcus aureus.

Isolate	Average clear zone diameter (mm)	
	Escherichia coli	*Staphylococcus aureus*
MP(1) 5.2	11,6	15,3
MP(1) 6.1	9,6	13,7
MP(1) 6.2	8.5	11,5
MP(1) 7.1	8,0	11,2
MP(1) 7.2	7.6	9,0
MP(3) 6.2	10,4	12,2
MP(3) 6.3	11,1	15,6
MP(3) 7.1	10.0	14.0
MP(3) 7.2	10,3	15,3
MP(3) 7.3	10.1	15,2



A B

Figure 4.1. Diameter of the Inhibition Zone of LAB Isolation to A). *S. aureus* and B). *E. coli*

Identification of Lactic Acid Bacteria Producing Antibacterial Compounds from Candied Pakoba Fruit

The identification of LAB isolates with the greatest antibacterial activity, namely MP(1) 5.2 and MP(3) 6.3, was conducted using the Profile Matching method. Based on the characterization and identification results (Table 4), the two LAB isolates obtained from candied Pakoba fruit samples were identified as members of the genus Lactobacillus. The key characteristics used for genus-level identification included cell shape, cell arrangement, catalase test, motility, spore formation, and gas production from glucose.

Table 4 presents the identification of LAB isolates at the genus level based on the Profile Matching method.

Key Characters*	*Leuconostoc*	*Pediococcus*	*Lactobacillus*	MP(1) 5.2	MP(3) 6.3
Shape of a round cell	+	+	+	-	-
Coloring	+	+	+	+	+
Pairing cell arrangement	-	+	-	-	-
Spore formation	-	-	-	-	-
Catalase reaction	-	-	-	-	-
Gas production	+	-	-	-	-
Motilitas	-	-	-	-	-
Homofermentatif	-	+	+	+	+
Heterofermentatif	+	-	-	-	-

* Key character description genus *Lactobacillus, Leuconostoc* dan *Pediococcus* berdasarkan *Bergey's manual Systematics of Bacteriology* (Sneath *et al*., 2004).

Discussion

The preliminary identification of LAB colonies was based on the formation of clear zones surrounding the colonies on the 1% MRS agar supplemented with CaCO3, indicating potential LAB activity. MRS medium supports the growth of acid-producing bacteria, including LAB, due to the presence of glucose, which serves as a carbon and energy source. The cloudy appearance of calcium carbonate

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(CaCO3) in the medium is dissolved by acids produced by these bacteria, resulting in a clear zone around the colonies. This clear zone formation indicates that the bacteria are acid-producing, a characteristic of LAB. LAB screening involves confirmation tests on suspected LAB isolates, including gram staining, catalase testing, endospore testing, and motility testing. These tests help identify and confirm the presence of LAB.

The results of acid-producing bacteria isolation during the fermentation of Pakoba fruit into sweets showed that not all colonies producing clear zones on MRS-CaCO3 medium were LAB members. Among the colonies producing clear zones, some exhibited Gram-negative, catalase-positive, and spore-forming characteristics (Table 2), suspected to be E. coli, Bacillus, Micrococcus, and Pseudomonas.

In addition to lactic acid bacteria, various other bacteria have been found in fruits and fruit products, including those belonging to the genera Micrococcus, Bacillus, Pseudomonas, Staphylococcus, Salmonella, Shigella, Vibrio, and Klebsiella. These bacteria have been identified in a range of fruits, such as mangoes, avocados, guavas, bananas, papayas, jujube fruits, plums, apples, cantaloupes, watermelons, dragon fruits, and Pakoba fruits (Hasan and Zulkahar, 2018; Babiye, 2017; Sari et al., 2013; Biswas, 2018; Lawalata et al., 2023).

A total of 12 isolates were selected based on clear zone formation around the colonies and confirmation tests, including gram staining, catalase reaction, spore formation, and motility. Out of these, 10 isolates were identified as lactic acid bacteria (LAB). LAB are characterized by their Gram-positive, catalase-negative, non-spore-forming, and non-motile properties, with round or rod-shaped cells. On the first day of Pakoba fruit fermentation, 5 rod-shaped LAB isolates with single-cell arrangements were obtained. Similarly, on the third day of fermentation, 5 rod-shaped LAB isolates with single-cell arrangements were obtained (Table 4.4). Morphology of LAB Isolates is the dominance of rod-shaped bacterial cells during the fermentation process of Pakoba fruit suggests their significant role in the fermentation process.

Ten LAB isolates obtained from the isolation results were selected based on their antibacterial activity against pathogenic and spoilage bacteria. The indicator bacteria used in this study were *Escherichia coli* and *Staphylococcus aureus*, representing Gram-negative and Gram-positive pathogenic bacteria commonly found in fish, the respiratory tract, and human skin surfaces, as well as indicators of sanitation and hygiene. The 10 LAB isolates tested for their ability to inhibit the growth of pathogenic and spoilage bacteria exhibited inhibitory power, with clear zone diameters on Nutrient Agar (NA) medium ranging from 7.6 to 15.3 mm. The diameter of the clear zone formed around the colony represents the inhibition zone diameter (in mm).

Table 3 shows that all LAB isolates can inhibit the growth of indicator bacteria, indicating that the antibacterial compounds produced by LAB are effective against both Gram-positive and Gram-negative bacteria. The sensitivity of bacteria to antimicrobial compounds is influenced by the structure of their cell walls, particularly the peptidoglycan layer. The peptidoglycan layer on Gram-negative bacteria is thinner compared to Gram-positive bacteria. Peptidoglycan Gram-negative bacteria account for only 1% to 2% of

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the dry weight of cells while Gram-positive bacteria account for 20% of the dry weight of cells. The outer membrane of Gram-negative is composed of 30% lipoprotein, 20 – 25% phospholipids, 40 – 45% protein which functions as a defense against the external environment against the action of antibiotics so that penicillin is more difficult to achieve the work target (Lawalata *et al.,* 2019*)*.

According to Pratiwi (2008), the cell wall of Gram-negative bacteria lacks teichoic acid, which is present in Gram-positive bacteria, and contains only a small amount of peptidoglycan. This structural difference makes Gram-negative bacteria more resistant to mechanical damage and potentially more resistant to certain antibacterial agents. Our observation results show that Staphylococcus aureus (Gram-positive) is more susceptible to the antibacterial activity of Lactobacillus than Escherichia coli (Gram-negative). The results of this study demonstrate that Lactobacillus isolates are effective in inhibiting the growth of pathogenic bacteria that can cause infections in the digestive tract of humans and animals. Notably, Lactobacillus is more effective in inhibiting *Staphylococcus aureus* (Gram-positive) than *Escherichia coli* (Gram-negative).

Lactic acid bacterial isolates, which play a role in the fermentation process of Pakoba fruit, have been found to inhibit the growth of pathogenic and spoilage bacteria. This inhibitory ability is attributed to the production of antimicrobial compounds, including organic acids and other metabolites such as diacetyl, hydrogen peroxide, and bacteriocins (Lawalata et al., 2023; Lawalata et al., 2019).

Antimicrobial compounds can alter the permeability of the cytoplasmic membrane, disrupting membrane transport, inactivating essential enzymes, and inhibiting protein synthesis (Davidson et al., 2005). Organic acids, in particular, can diffuse into bacterial cells in their undissociated form. Once inside the cell, the acid dissociates due to the higher pH of the cytoplasm compared to the external environment. To maintain a neutral internal pH and prevent damage to cellular components, bacteria must expel the protons formed as a result of acid dissociation. This process requires significant energy in the form of ATP. The depletion of energy reserves ultimately leads to the death of bacterial cells (Davidson et al., 2005).

Antimicrobial compounds disrupt bacterial cell function by altering the permeability of the cytoplasmic membrane, thereby impairing membrane transport and inactivating crucial enzymes, which ultimately inhibits protein synthesis (Davidson et al., 2005). Specifically, organic acids penetrate bacterial cells in their undissociated form and dissociate within the cell due to the higher internal pH. To maintain cellular homeostasis, bacteria expend significant energy in the form of ATP to expel excess protons resulting from acid dissociation. This energy depletion ultimately leads to bacterial cell death, as the cells are unable to sustain vital functions (Davidson et al., 2005).

Staphylococcus aureus is a Gram-positive, pathogenic bacterium characterized by its spherical shape, with a diameter of 0.5-1 µm, and cluster formation. These bacteria are non-motile. Biochemically, S. aureus can ferment mannitol and glucose, and exhibits positive catalase activity. The production of thermostable nucleases and coagulase are key characteristics of its pathogenicity. S. aureus can cause various infections, including minor skin infections and food poisoning (Hafsan, 2014; Mastuti, 2022).

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Escherichia coli is another pathogenic bacterium that can cause infections in humans. When stained with Gram's method, E. coli appears red, indicating its Gram-negative nature. Biochemical tests show that E. coli is he isolate exhibited a biochemical profile consistent with Escherichia coli, characterized by positive reactions for indole and methyl red tests, and negative reactions for Voges-Proskauer and citrate utilization tests. Can ferment glucose, lactose, and other sugars. Infection with E. coli can lead to symptoms such as diarrhea (Hafsan, 2014; Mastuti, 2022).

The LAB isolates exhibiting antibacterial activity, specifically LAB isolate MP(1)5.2 and LAB isolate MP(3)6.3, were identified using the profile matching method. Based on the characterization and identification results (Table 4.4), the two LAB isolates obtained during the fermentation process of Pakoba fruit (candy) were classified as members of the genus Lactobacillus.The key characteristics used to differentiate the LAB isolates into the genus level include: rounded and yellowish-white colony shape, rod-shaped cell morphology, single cell arrangement, Gram-positive, catalase-negative, and non-spore-forming. These characteristics are consistent with the genus Lactobacillus.

This study demonstrates that Lactic Acid Bacteria (LAB) isolated during the fermentation process of Pakoba fruit into sweets exhibit antibacterial properties against the growth of *E. coli* and *S. aureus*.

CONCLUSION

Lactic acid bacteria (LAB) belonging to the genus Lactobacillus, isolated from candied Pakoba fruit (*Syzygium luzonense*), produce antibacterial compounds that inhibit the growth of *Escherichia coli* and *Staphylococcus aureus.*

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