



Isolation, Characterisation and Presumptive Identification of Lactic Acid Bacteria from Matoa (*Pometia pinnata*) Fruit

Rafli Zulfa Kamil¹, Dimas Anugrahillah¹, Heni Rizqiaty¹, Fariz Nurmita Aziz¹, Rangga Wasi Hastrawirid²

¹Department of Food Technology, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Indonesia

²Department of Global Agricultural Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

*Corresponding author (rafli.zulfa@live.undip.ac.id)

Abstract

Each fruit has a distinct nutritional composition physicochemical characteristics, creating specific ecological niches for microbial growth. To the best of our knowledge, this study is the first to report the isolation and phenotypic characterisation of native LAB from matoa fruit, thereby providing baseline microbiological data for this underexplored fruit matrix. This research aims to isolate and characterise native LAB from matoa. The Matoa sample was inoculated into de Man-Rogosa-Sharpe (MRS) Broth and isolated using the streak plate method. The isolate was then characterised based on its morphological, physiological, and biochemical characteristics. Results shows that isolated LAB exhibit cream-coloured, circular colony morphology with clear edges, convex elevation, an opaque internal structure, and varying colony sizes. Cellular characteristics of the isolate included rod-shaped morphology, Gram-positive staining, non-catalase activity, and non-motility. The isolate survived at pH 5 and 6, 6.5% salt content, and at 25°C, 37°C, and 45°C. The isolate also fermented glucose, sucrose, maltose, fructose, and galactose, and also did not produce H₂S or indole. The LAB isolate was identified as belonging to the genus *Lactobacillus* and is presumably identified to be *Lactobacillus plantarum*.

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Introduction

Indonesia is recognised as a megabiodiversity nation, abundant in tropical fruit genetic resources, among which matoa (*Pometia pinnata*) is notable. This unique fruit, indigenous to Papua, is esteemed not only for its distinctive flavour profile, reminiscent of rambutan, durian, and longan, but also for its content of various bioactive compounds, including polyphenols, flavonoids, and vitamin C (Oktavia et al., 2023). Beyond its nutritional attributes, the matoa fruit's skin and flesh, which are rich in natural sugars, present a conducive environment for the proliferation of beneficial microorganisms (Barbosa et al., 2022), particularly LAB. LAB are well-established as a group of bacteria integral to the food and health industries, capable of fermenting carbohydrates into lactic acid (Abubakr and Al-Adiwish, 2017). This process naturally reduces environmental pH, thereby inhibiting the growth of pathogenic bacteria (Mezaini et al., 2009). Furthermore, numerous LAB strains are recognised as probiotics, conferring health benefits to their host, such as enhancing the immune system, balancing gut microbiota, and exhibiting

antioxidant and antimicrobial properties (Gul and Durante-Mangoni, 2024).

The investigation of LAB derived from natural sources or exotic fruits has emerged as a significant area of global research, primarily because their characteristics are profoundly influenced by the isolation source, imparting niche-specific properties (Barbosa et al., 2022). Therefore, fruits that grow in unique tropical ecosystems, such as matoa from Papua, may harbour distinctive LAB populations with adaptive traits not found in conventional fermented substrates or commercially exploited fruits. From both scientific and industrial perspectives, uncovering such indigenous microbiota is important for expanding the diversity of starter cultures, identifying robust strains adapted to tropical stress conditions, and developing locally sourced probiotic candidates. Despite the promising potential of matoa, there remains a paucity of information regarding the diversity of LAB species associated with this fruit. Current research predominantly focuses on the phytochemical content of matoa's skin or leaves, with limited attention given to its microbiological exploration

(Oktavia et al., 2023). There is currently no comprehensive baseline information regarding the diversity and phenotypic characteristics of LAB naturally present in matoa fruit. This represents a significant knowledge gap, particularly considering Indonesia's rich but under-characterised microbial biodiversity. Without such foundational data, the potential utilisation of matoa-derived LAB for functional food development or biotechnological applications cannot be scientifically justified.

This study aims to isolate potential LAB strains from matoa fruit and to characterise their morphological and biochemical properties. The findings of this research are anticipated to provide scientific insights into the germplasm of local microorganisms and to explore the potential applications of matoa-derived LAB as fermentation agents or prospective probiotic candidates.

Materials and Methods

Materials

Matoa was obtained from the local shop *Keboen Buah* in Tembalang, Semarang, and sampling was conducted in September 2024. Matoa fruits were processed by homogenising the matoa flesh and diluting the homogenate with 0.85% sterile NaCl. The chemicals used in this research were analytical reagent grade, including de Man-Rogosa-Sharpe (MRS) Broth (HiMedia), MRS Agar medium (Merck KGaA), skim milk powder, Bacteriological Grade Agar (HiMedia), Tributyrin Agar Base (HiMedia), Sulphide Indole Motility (SIM) Medium (HiMedia), Tween (Polysorbate) 80, soluble starch (Merck KGaA), red phenol broth base (HiMedia), sucrose (Xihua), maltose (Nitra Kimia), lactose (Nitra Kimia), glucose (Merck KGaA), galactose (HiMedia), fructose (Nitra Kimia), 85% glycerol (Merck KGaA), salt, hydrogen peroxide, reagents I (Crystal Violet), II (Lugol), III (Acetone Alcohol), IV (Safranin) for Gram staining (IndoReagen).

Methods

Enumeration and Isolation of LAB

The enumeration and isolation of LAB following the method described by Rahmah et al. (2021). Matoa fruit samples (1 g) were homogenised and inoculated into 9 ml of MRS Broth medium, followed by incubation for 24 hours at 37°C. Subsequent serial dilutions were performed in 0.85% NaCl, and 1 ml from the final three dilutions was plated using the pour plate method on MRS Agar supplemented with 1% (w/v) CaCO₃, with incubation lasting 24–48 hours. Colonies exhibiting clear zones, round shapes, and a cream colour were enumerated to determine the total population and selected based on optimal visual characteristics. Among the 16 potential isolates identified, purification was achieved using the streak plate method for macro- and microscopic morphological assessments. Pure isolates were preserved in agar slant media, from which 4 superior isolates were selected for further characterisation.

Characterisation of LAB Isolate

Macro morphology characterization

The macro-morphological characteristics of the LAB isolate were assessed following the methodology

outlined by Delvia et al. (2015). Visual observations were conducted on the morphology of colonies cultivated on agar media. The parameters evaluated included colour, colony shape, elevation, margin, and colony internal structure.

Micromorphology characterization

Microscopic characterisation was conducted using Gram staining, following the methodology outlined by Dhameliya et al. (2020), to determine the cell morphology and Gram characteristics of the isolates. The procedure employed 24-hour-old fresh cultures and a series of reagents: crystal violet (Gram A), Lugol's iodine (Gram B), acetone-alcohol (Gram C), and safranin (Gram D). Observations were made under a light microscope at magnifications of up to 1000x, utilising immersion oil. The parameters assessed included cell colour (indicating Gram-positive or Gram-negative), cell shape, and colony arrangement.

Biochemistry Characterisation

Biochemical characterisation involves assessing indole and H₂S production and evaluating the capacity of LAB to ferment various carbohydrates. The assessment of indole and H₂S production is conducted following the methodology of Giyatno and Retnaningrum (2021), utilising Sulfide Indole Motility (SIM) medium. Isolates are inoculated by the stab method and incubated for 24–48 hours. Indole production is determined by the addition of three drops of Ehrlich's reagent, with a positive result indicated by the formation of a red ring on the medium's surface. Conversely, the ability to produce H₂S is identified by the appearance of a black precipitate along the stab line.

The procedure for carbohydrate fermentation testing, as outlined by Kodba et al. (2024), employs Phenol Red Broth as the medium. The carbon sources evaluated in this process include glucose, sucrose, fructose, galactose, lactose, and maltose. A positive result is characterised by a colour change in the medium from red to yellow, indicating a decrease in pH due to the LAB isolates' fermentation activity.

Physiology Characterisation

Physiological characterisation of the isolates was carried out referring to the methods of Giyatno and Retnaningrum (2021) and Simanjuntak and Naibaho (2023), which include:

Catalase Test

A 24-hour-old isolate is immersed in 5 ml of H₂O₂ (v/v). A positive result is indicated by the formation of gas bubbles at the tip of the inoculating needle, demonstrating the isolate's ability to produce catalase.

Motility Test

The isolate is inoculated vertically (stab) into semi-solid SIM medium and incubated for 24–48 hours. Motile bacteria are characterised by growth spreading away from the stab area, while non-motile bacteria grow only along the line of inoculation.

Temperature Resistance Test

Isolates are inoculated into MRS Broth medium

Table 1. Macromorphological Characteristics of LAB Isolates from Matoa

LAB Isolate	Colony Morphology					
	Colour	Shape	Edge	Elevation	Internal Structure	Size
MTA-1	Cream	Circular	Clear	Convex	Opaque	Small
MTA-2	Cream	Circular	Clear	Convex	Opaque	Small
MTA-3	Cream	Circular	Clear	Convex	Opaque	Small
MTA-4	Cream	Circular	Clear	Convex	Opaque	Small

and incubated at 10, 25, 37, 45, and 55°C for 24–48 hours. Bacterial growth is indicated by turbidity in the medium.

pH Tolerance Test

Tolerance to acidity levels was tested using MRS Broth adjusted to pH 5, 6, 9, and 10 with HCl or NaOH. Positive growth was observed as a change in the medium's turbidity after 24–48 hours of incubation.

Salt Tolerance Test

Results and Discussion

Population of LAB in Matoa

Enumeration of LAB from matoa (*Pometia pinnata*) resulted in $3,8 \times 10^4$ CFU/mL. These results are deemed to be appropriate according to Ashiwini et al., (2022), who stated that the number of LAB in fresh fruits is usually in the range of 10^4 CFU/mL to 10^7 CFU/mL. The observed microbial load suggests that matoa provides a suitable ecological niche for LAB survival, likely supported by its natural sugar content ranging from 25.95 to 26.69 °Brix and favourable moisture conditions (Tua et al., 2025). Although the count was at the lower end of the reported range, it remains sufficient to enable further isolation and characterisation of potential functional strains.

Macro and Micromorphology Characteristic of LAB Isolates

Macromorphological characterisation is the initial stage of bacterial colony identification through visual observation on growth media. This observation aims to provide a preliminary overview of the bacterial type based on the colony's visible appearance. The parameters observed include colour, shape, edge, elevation, structure, and colony size. The results of these observations are presented in Table 1 and Figure 1. All LAB colonies obtained exhibited uniform macromorphological characteristics: they were cream-colored, circular in shape, had well-defined edges, convex elevation, opaque colony structure, and were small in size. These characteristics are consistent with the report by Amelia et al. (2021), which stated that LAB generally has a round shape, smooth surface, and a whitish-yellow colour.

Morphological characterisation of bacterial isolates was followed by micromorphological identification of cell morphology using the Gram staining method. The purpose of Gram staining is to classify the isolated bacteria into the Gram-positive or Gram-negative groups. The results of Gram staining in the micromorphological characterisation of LAB isolates from matoa fruit are presented in Figure 2. The results of micromorphological observations showed that all bacterial isolates had bacillus (rod-shaped) cell forms

and were classified as Gram-positive bacteria. These characteristics are consistent with the general traits of LAB. Miladiarsi and Wahdaniar (2024) reported that LAB are typically bacillus or rod-shaped, belong to the Gram-positive group, and do not have endospores. In addition, the morphological forms commonly found in LAB are rod-shaped (bacillus) and spherical (coccus) (Cirat et al., 2024). Staining bacterial cells purple indicates that they are Gram-positive. The purple color is produced because Gram-positive bacteria have a thicker cell wall with a high peptidoglycan content, so the crystal violet–iodine complex is not washed away by alcohol during the Gram staining process (Giyatno and Retnaningrum, 2021).

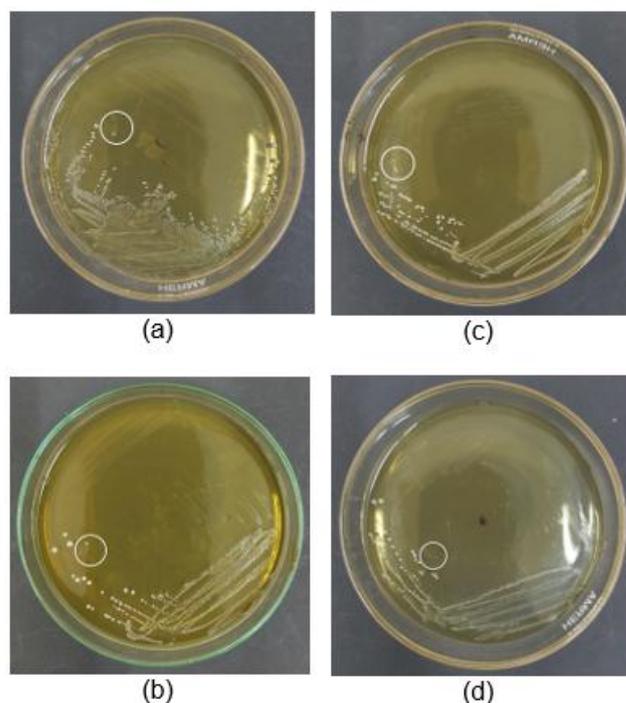


Figure 1. Colony Growth on MRS Agar Plates.

(a) Isolate MTA-1, (b) Isolate MTA-2, (c) Isolate MTA-3, (d) Isolate MTA-4

Biochemical Characteristic of LAB Isolates

Biochemical property testing is conducted to determine the physiological characteristics of bacterial isolates based on their metabolic activity and responses to chemical compounds (Sipahutar et al., 2024). The carbohydrate fermentation test uses phenol red broth media with various carbon sources, namely sucrose, glucose, fructose, galactose, lactose, and maltose. A positive result is indicated by a change in the color of the medium from red to yellow due to a decrease in pH during fermentation (Dhameliya et al., 2020). All LAB isolates showed positive results for all tested sugars (Table 2), indicating carbohydrate fermentation, a

Table 2. Biochemical Characteristics of LAB Isolates from Matoa

Biochemical Characteristics	Isolate Name			
	MTA-1	MTA-2	MTA-3	MTA-4
Fructose	√	√	√	√
Galactose	√	√	√	√
Glucose	√	√	√	√
Lactose	√	√	√	√
Maltose	√	√	√	√
Sucrose	√	√	√	√
H ₂ S	X	X	X	X
Indole	X	X	X	X

Note : (√) means it can ferment/produce; (X) means it cannot ferment/produce.

characteristic of LAB, whether homofermentative or heterofermentative (Meruvu and Harsa, 2023).

Testing for indole and H₂S production in LAB isolates from matoa fruit showed negative results in all isolates, indicated by the absence of a red ring or black precipitate in the medium (Table 2). This is consistent with the report by Nasri et al. (2021), which states that LAB are naturally unable to produce indole and H₂S. The inability to produce indole is related to the absence of the enzyme tryptophanase, which is generally found in pathogenic bacteria such as *Escherichia coli* (Saridewi et al., 2016; Santosa and Retnaningrum, 2020). The absence of black precipitate also indicates that the isolates lack the ability to transform sulfur compounds into sulfides (Khushboo et al., 2023).

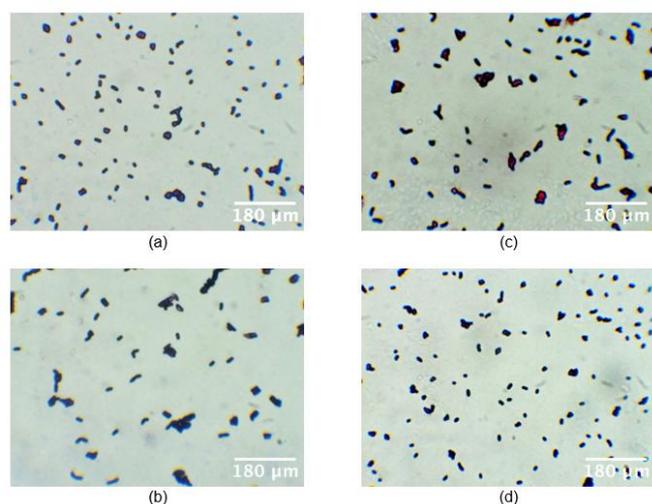


Figure 2. Gram Staining Result.
(a) Isolate MTA-1, (b) Isolate MTA-2,
(c) Isolate MTA-3, (d) Isolate MTA-4

Physiology Characteristic of LAB Isolates

The determination of the physiological characteristics of LAB isolates was carried out through catalase tests, motility tests, and resistance to temperature, pH, and salt to assess the bacteria's adaptability and metabolism. The results of the physiological characterisation serve as the basis for predicting the appropriate genus of LAB. Test indicators include bubble formation in the catalase test, growth patterns in the motility test, and medium turbidity in the environmental resistance tests (Table 3). The test results showed that all isolates had uniform characteristics, namely, catalase-negative, non-motile, able to grow at

temperatures of 25°C, 37°C, and 45°C, grow at pH 5 and 6, and are tolerant of 6.5% NaCl, but do not grow at alkaline pH and high salt concentrations. These characteristics are consistent with the physiological traits of LAB as reported by (Sharma, 2016).

The catalase test showed a negative result (Table 3), indicated by the absence of oxygen bubbles, suggesting that the isolate does not produce catalase to break down H₂O₂ (Trawczyńska, 2020). The motility test showed that all isolates were non-motile (Table 3), as growth occurred only along the stab line, consistent with reports by Abidin et al. (2024) and Haro et al. (2020), which state that LAB are generally non-motile due to limited biosynthetic capabilities. The temperature tolerance test demonstrated that the isolates grew within a mesophilic to near-thermophilic range, specifically 25–45°C, consistent with the classification of LAB based on their optimum temperature (Haro et al., 2020). In the pH tolerance test, the isolates grew at pH 5 and 6 but not at pH 9 or 10. This ability is related to LAB's adaptation mechanisms to acidic environments through cytoplasmic pH regulation, cell membrane composition, and amino acid metabolism that produce NH₃ to reduce acid stress (Putri et al., 2018; Priadi et al., 2020; Wang et al., 2021). Salt tolerance testing showed that the isolates grew at 6.5% NaCl but not at 18%, indicating the osmotic tolerance limit of LAB. High salt concentrations exert osmotic pressure, triggering plasmolysis and thereby inhibiting microbial growth (Puspita et al., 2019).

Presumptive Identification of LAB Isolates

Based on the results of morphological, physiological, and biochemical characterisation, the genus of the LAB isolate was predicted with reference to Bergey's Manual of Systematic Bacteriology (Vos et al., 2009). The isolate exhibited macromorphological colony characteristics, including a circular shape, cream colour, well-defined edges, convex elevation, an opaque structure, and a small size. These traits align with common colony appearances of LAB species observed in isolates from fermented foods and dairy products (Menconi et al., 2014; Ismail et al., 2018). As well as micromorphological characteristics of rod-shaped (bacilli), Gram-positive cells that fit the *Lactobacillus* genus profile are frequently reported in diverse fermented foods, including dairy and vegetables (Monika et al., 2017). Biochemically, all isolates fermented various carbon sources but did not produce indole or H₂S, further supporting typical LAB metabolic patterns: they generally ferment carbohydrates to produce lactic

Table 3. Physiological Characteristics of LAB Isolates from Matoa

Test	Isolate Name			
	MTA-1	MTA-2	MTA-3	MTA-4
Catalase	Negative	Negative	Negative	Negative
Motility	Non motile	Non motile	Non motile	Non motile
10°C	-	-	-	-
25°C	+	+	+	+
37°C	+	+	+	+
45°C	+	+	+	+
55°C	-	-	-	-
pH 5	+	+	+	+
pH 6	+	+	+	+
pH 9	-	-	-	-
pH 10	-	-	-	-
6,5% NaCl	+	+	+	+
18% NaCl	-	-	-	-

Note : (+) means isolate able to grow; (-) means isolate unable to grow

acid but do not produce indole or hydrogen sulfide (Monika et al., 2017). The physiological characteristics of the isolate included being non-motile, catalase-negative, able to grow at 25–45°C and pH 5–6, and tolerant of NaCl concentrations up to 6.5%. Based on the presence of these characteristics and in accordance with Bergey's Manual of Systematic Bacteriology (Vos et al., 2009), the isolate is suspected to belong to the genus *Lactobacillus*. This assumption is also supported by the sample type, matoa fruit, because plant-based sources such as fruits and vegetables are generally dominated by LAB, specifically *Lactobacillus plantarum*.

The catalase-negative trait in the isolate is a characteristic feature of the *Lactobacillus* genus, which detoxifies H₂O₂ through peroxidase without producing oxygen (Vivekanandan et al., 2024). The growth of the isolate within the temperature range of 25–45°C corresponds to the optimal growth temperature of *Lactobacillus*, which is reported to be between 30–40°C, with some species able to grow at extreme temperatures of 2–53°C (Ślizewska et al., 2020). In addition, the ability to grow at acidic pH (pH 5–6) aligns with the optimal pH range for *Lactobacillus*, 5.5–6.2, with some species able to grow at pH 4.5–6.5 (Ślizewska et al., 2020).

The isolate's tolerance to 6.5% NaCl also supports the prediction that it belongs to the genus *Lactobacillus*, as several LAB species in this genus are known to survive at such salt concentrations (Anindita et al., 2022). In fact, *Lactobacillus plantarum* has been reported to withstand up to 10% NaCl (Singhal et al., 2021). This assumption is further supported by the study of Giatno and Retnaningrum (2021), who isolated LAB from kersen fruits and found similar morphological, physiological, and biochemical characteristics, such as small colonies with a milky-white to cream colour, round shape with convex elevation, rod-shaped cells, Gram-positive, non-motile, catalase-negative, and able to ferment various sugars, which further presumptively identified as *Lactobacillus plantarum*.

Conclusion

Matoa, an indigenous Indonesian fruit, was selected for the isolation of lactic acid bacteria (LAB) due to its rich nutrient composition. Based on morphological, biochemical, and physiological characterization in accordance with Bergey's Manual of Systematic

Bacteriology, the isolates were assigned to the genus *Lactobacillus* and are presumptively identified as *Lactobacillus plantarum*. However, definitive identification at the species or strain level requires molecular analysis, such as gene sequencing and alignment with reference sequences in the NCBI database.

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