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ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM MANDAI (FERMENTED CEMPEDAK PEEL) IN EAST KALIMANTAN

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Abstract:
Mandai is a traditional fermented food from East Kalimantan made from the peel of cempedak (Artocarpus champeden). To the best of our knowledge, this study is the first to report the isolation and characterization of lactic acid bacteria (LAB) from mandai. The fermentation process was carried out for 14 days using MRS Agar medium, resulting in 17 bacterial isolates, consisting of nine Gram-negative and eight Gram-positive isolates. Among them, eight Gram-positive isolates were dominant and exhibited typical LAB characteristics, including acid and gas production from glucose, negative catalase activity, non-motility, growth at 48°C but not at 16°C, and the ability to hydrolyze starch, fat, and casein. During fermentation, the pH decreased from 6.8 to 3.8, confirming active lactic acid production. These findings demonstrate that LAB play a crucial role in generating the characteristic sour taste and improving the quality of mandai. Importantly, the identified LAB isolates have potential as starter cultures to enhance the safety, consistency, and nutritional value of this traditional food.

Keywords: mandai, lactic acid bacteria, fermentation, cempedak skin, traditional food

Abstrak:
Mandai adalah makanan fermentasi tradisional dari Kalimantan Timur yang terbuat dari kulit cempedak (Artocarpus champeden). Sejauh pengetahuan kami, penelitian ini adalah yang pertama melaporkan isolasi dan karakterisasi bakteri asam laktat (BAL) dari mandai. Proses fermentasi dilakukan selama 14 hari menggunakan media MRS Agar, menghasilkan 17 isolat bakteri, yang terdiri dari sembilan isolat Gram-negatif dan delapan isolat Gram-positif. Di antara mereka, delapan isolat Gram-positif dominan dan menunjukkan karakteristik BAL yang khas, termasuk produksi asam dan gas dari glukosa, aktivitas katalase negatif, non-motilitas, pertumbuhan pada 48°C tetapi tidak pada 16°C, dan kemampuan untuk menghidrolisis pati, lemak, dan kasein. Selama fermentasi, pH menurun dari 6,8 menjadi 3,8, yang mengonfirmasi produksi asam laktat aktif. Temuan ini menunjukkan bahwa BAL memainkan peran penting dalam menghasilkan rasa asam yang khas dan meningkatkan kualitas mandai. Yang penting, isolat LAB yang teridentifikasi memiliki potensi sebagai kultur starter untuk meningkatkan keamanan, konsistensi, dan nilai gizi makanan tradisional ini.

Kata kunci: mandai, bakteri asam laktat, fermentasi, kulit cempedak, pangan tradisional

INTRODUCTION

Indonesia is rich in traditional fermented foods that are widely consumed both in households and local markets. These foods are not only culturally important but also provide significant nutritional benefits, including proteins, carbohydrates, fats, vitamins, and bioactive compounds. Globally, lactic acid bacteria (LAB) isolated from traditional fermented foods have attracted increasing attention due to their potential as probiotics, natural preservatives, and sources of industrially relevant enzymes (Marco et al., 2021; Rezac et al., 2018). Therefore, exploring LAB from local fermented foods is important to expand the diversity of microbial resources for functional food development and to strengthen food biotechnology research in Indonesia.

One of the unique traditional fermented foods from East Kalimantan is mandai, which is produced from the mesocarp of cempedak (Artocarpus champeden) peel. Mandai contributes not only to food security and waste reduction but also represents an underexplored source of beneficial microorganisms. Despite its popularity in Kalimantan, scientific studies on mandai remain scarce, especially those focusing on the role and diversity of LAB during the fermentation process.

Previous studies on Indonesian fermented foods have primarily focused on products such as tempeh, tape, dadih, and pekasam, while mandai remains largely unexplored. Although a recent study investigated the microbiome of cincalok, tempoyak, and mandai (Sari et al., 2025), detailed biochemical characterization, identification of dominant LAB isolates, and evaluation of their potential applications are still lacking. This represents a missed opportunity, since mandai may harbor unique LAB strains with promising probiotic properties or enzyme production capabilities that could be applied in food biotechnology.

Recent studies have demonstrated the probiotic potential of LAB from various traditional foods in Indonesia and other countries. For example, Rubak et al. (2023) reported that LAB isolated from Sui Wu'u (a traditional food from East Nusa Tenggara) survived under low pH and bile salt conditions and showed promising probiotic properties. Similarly, LAB isolated from pekasam in West Kalimantan reached counts of 10^5–10^7 CFU/mL and displayed antimicrobial activity against pathogens such as Staphylococcus aureus and Escherichia coli (Rahman et al., 2023). More broadly, studies on dairy-based fermented foods also highlight the technological and probiotic potential of indigenous LAB isolates (Gupta et al., 2024; Khan et al., 2024). These findings emphasize that traditional fermented foods are valuable reservoirs of LAB with unique traits, supporting the need for further investigation of mandai fermentation.

This study aims to isolate and characterize LAB involved in the fermentation of mandai from East Kalimantan. By examining their morphological and biochemical traits, this research seeks to identify dominant LAB isolates, evaluate their functional potential, and highlight their contribution to the sensory quality of mandai. The findings are expected to provide new insights into the microbiological basis of mandai fermentation and expand the pool of indigenous LAB resources that could be utilized as starter cultures or candidates for probiotic applications.

METHODS

This study used equipment including basic laboratory equipment, namely: anaerobic jars, jars, plastic, refrigerators, basins or pans, heat plates, vortexes, stopwatches, petri dishes, autoclaves, microscopes, analytical scales, spoons, tweezers, scissors, knives, cool boxes, Durham tubes, and reactions.

Research Design

This study used an experimental design with laboratory-based fermentation of mandai, followed by microbiological isolation and biochemical characterization of lactic acid bacteria.

Procedure

The mandai fermentation process began with separating the mesocarp (inner peel) from the exocarp (outer peel) of the cempedak fruit (Artocarpus champeden). Approximately 500 g of mesocarp was weighed and washed with clean water, followed by soaking in boiled water for 30 minutes to remove sap and mucus. The sample was drained and transferred into a sterile glass jar, then soaked in 1.2 L of sterile mineral water with the addition of 25% (w/v) table salt (300 g). The jar was sealed and incubated at room temperature (28 ± 2 °C) for 14 days to produce fermented mandai.

Sampling was conducted on days 0, 2, 4, 6, 8, 10, 12, and 14. For each sampling point, approximately 1 g of mandai was homogenized in 45 mL of sterile 0.9% NaCl solution using a vortex mixer. Serial dilutions were prepared up to 10^{-6}. From each dilution (10^{-5} and 10^{-6}), 0.1 mL of suspension was plated using the pour plate method on De Man, Rogosa, and Sharpe (MRS) agar medium (initial pH 6.2 ± 0.2). The plates were incubated at 37 °C for 24–48 h under anaerobic conditions using anaerobic jars and gas packs to ensure consistent oxygen-free environments.

Each experiment was performed in triplicate (n = 3) to ensure reproducibility. A positive control (reference LAB strain: Lactobacillus plantarum ATCC 8014) and a negative control (uninoculated MRS agar plate) were included in each batch to monitor media sterility and method accuracy. To minimize bias, all plating and colony counts were performed by two independent researchers, and only colonies with consistent morphology were selected for further characterization. Isolates were subcultured on fresh MRS agar until pure colonies were obtained.

Participants / Sample

The materials included distilled water, De Man Rogosa Sharpe (MRS Agar) medium, Nutrient Broth (NB) medium, sugar broth medium, Skim Milk Agar (casein) medium, Dextrose Tryptone Bromcresol Purple Agar (DTBPA) medium, phenol red and thymol blue indicators, agar-agar, starch agar medium, glycerol-based fat medium, 70% alcohol, 96% alcohol, 3% hydrogen peroxide, and a kitchen. The main biological component is the mesocarp skin of the cempedak fruit (Artocarpus champeden), which comes from the Samarinda region of East Kalimantan..

Instruments

Morphological test: Size and edge colonies. Analysis of representative isolate morphology was performed using the Gram-based method. The Gram staining process began with the preparation of smears from bacterial isolates, which were then analyzed using Hucker's crystal violet for one minute. The process began with the preparation of smears from bacterial isolates, which were then analyzed using Hucker's crystal violet for one minute. The stain was then air-dried until clear, followed by the application of Lugol's iodine for one minute, followed by decolorization using 96% alcohol for 10 to 20 minutes, which was then air-dried. The stain is air-dried until clear, followed by the application of Lugol's iodine mordant for one minute, followed by the removal of the stain using 96% alcohol for 10 to 20 minutes, which is then air-dried. It is then prepared with a Safranin solution for one minute and then rinsed again with air before being placed in air. The preparation is examined under a microscope at 1000x magnification.

Biochemical Testing of Bacterial Isolates: According to Cowan (1995), the biochemical characteristics of lactic acid bacteria are determined through various biochemical tests, including the ability to produce acid and gas from glucose, motility tests, the ability to grow at temperatures of 14°C and 48°C, catalase enzyme activity, and the ability to hydrolyze proteins, lipids, and proteins. (1995), the characteristics of lactic acid bacteria (LAB) are determined through various LAB tests, including the ability to produce acid and gas from glucose, motility tests, the ability to grow at temperatures of 14°C and 48°C, catalase enzyme activity, and the ability to hydrolyze proteins and lipids. To improve identification results, supporting tests were also conducted as an acid formation effort. To improve identification results, supporting tests were also conducted as an acid formation effort.

Mandai Proximate Test: proximate analysis of mandai products includes determining the ash content, moisture content, fat content, protein content, carbohydrate content, and total acid content in the sample.

Data Analysis

Data obtained from microbial counts (colony-forming units, CFU/mL), pH measurements, and proximate composition (moisture, ash, crude fat, crude fiber, crude protein, carbohydrate, and total lactic acid) were expressed as mean ± standard deviation (SD). Each experiment was performed in triplicate (n = 3).

For proximate composition and pH values, one-way analysis of variance (ANOVA) was applied to determine significant differences between fermentation times (days 0–14). When significant differences were observed ($p < 0.05$), Tukey's Honest Significant Difference (HSD) post-hoc test was conducted to identify which groups differed.

For microbial counts, log-transformed CFU/mL values were analyzed to assess changes in bacterial populations over the fermentation period. Differences in growth patterns between Gram-positive and Gram-negative isolates were evaluated using independent-samples t-tests.

All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Graphs (pH curves, microbial succession, proximate profiles) were generated using GraphPad Prism 9.0. A significance level of $p < 0.05$ was considered statistically significant.

Component	Salinity 25%	Salinity 2-25%
Water	4,41	6,8
Calx	77,53	4,3
Fat	4,43	7,5
Clude fiber	6,98	8,7
Protein	2,34	9,1
Carbohydrate	14,79	63,6
pH	3,83	3,72

According to Andrestian's study, the water, fat, crude fiber, and carbohydrate content had a higher percentage than the different mandai data over a period of 14 days. Andrestian's study found that the water, fat, crude fiber, protein, and carbohydrate content had a higher percentage than the different mandai data over a period of 14 days. These nutritional differences are caused by the different types of salt used, as salt plays an important role in determining the number of bacteria that can grow during fermentation (Hasrul, 2009). This is caused by the type of salt used, as it is important in determining the number of bacteria that can grow during fermentation (Hasrul, 2009). An increase in salt will lower the pH of cempedak, creating conditions suitable for bacterial growth during the mandai fermentation process. Bacteria that can adapt to environments with low pH levels then produce pH enzymes that help break down nutrients in the mesocarp of cempedak.

According to Hasrul (2009) and Andrestian (2009), fermenting mandai with salt at a concentration of 2-5% (b/v) for 14 days produces a higher nutrient content than fermentation at a concentration of 25% (b/v). High salt levels cause an increase in bacteria during the fermentation process, thereby accelerating the degradation and utilization of nutrients contained in the cempedak mesocarp, thus reducing the nutrient content.

RESULTS AND DISCUSSION

The texture of the cempedak mesocarp weakens due to the fermentation process. Weakened by the fermentation process. Due to fermentation, the cempedak mesocarp has a large amount of air, is hard, and degraded. Due to fermentation, the cempedak mesocarp has a large amount of air, is hard, and degraded. After several days of fermentation, the texture of the cempedak mesocarp becomes soft, its color becomes more brown or dark, and its skin becomes slimy. After several days of fermentation, the texture of the cempedak mesocarp becomes soft, its color becomes browner or darker, and its skin becomes slimy. This phenomenon is caused by the cells in the mesocarp of the cempedak being immersed in a large amount of salt solution (hypertonic). This creates turgor and air resistance, which will make the cell structure more substantial. In hypertonic conditions, air and turgor pressure are affected, making the cell structure more robust. It becomes more substantial. Plasmolysis occurs when there is a significant decrease in air quality in hypertonic conditions. When there is a significant decrease.

Able to counteract the decrease in cell pressure to the point where the protoplasm separates from the cell wall, which will counteract the space between the two tissues. Experiences softening and shrinkage. One hundred grams of cempedak mesocarp contains about 2.5 percent protein, 0.4 percent fat, 25 percent carbohydrates, 3.4 percent fiber, and cempedak mesocarp contains about 2.5 percent protein, 0.4 percent fat, 25 percent carbohydrates, 3.4 percent fiber, and 67.9% minerals (Sunaryono, 2005). % minerals (Sunaryono, 2005). Before the salt was replaced, the cempedak mesocarp had a neutral pH of 6.8, but after the salt was replaced, the pH became 5.3.

A significant decrease in pH occurred during the 14-day fermentation process, with a final pH of 3.83. This low pH level makes the cempedak mesocarp an ideal environment for the growth of indigenous bacteria in the natural fermentation process. The bacteria then decompose the substrate and ferment it, producing acid and changing the texture of the cempedak mesocarp during the fermentation process.

1. Bacterial Isolation During the Mandai Fermentation Process

A total of 17 bacterial isolates were obtained throughout the 14-day fermentation process. Nine Gram-negative isolates were detected during days 0–4, whereas eight Gram-positive isolates dominated from days 6–14. Log-transformed microbial counts increased significantly from 4.2 log CFU/mL on day 0 to 7.1 log CFU/mL on day 14 ($p < 0.05$, ANOVA). This shift indicates microbial succession, where Gram-negative bacteria initially grew but were gradually replaced by LAB, consistent with their ability to thrive in acidic and high-salt environments. Similar microbial succession has been reported in other traditional fermented foods such as pekasam (Rahman et al., 2023) and dadih (Gupta et al., 2024).

Table. 1. Bacterial isolates at 14 days of mandai fermentation.

Hari Ke-	Kode Isolat	Gram
0	M_1	-
	M_2	-
2	M_3	-
	M_4	-
4	M_5	-
	M_6	-
	M_7	-
	M_8	-
	M_9	-
6	M_{10}	+
8	M_{11}	+
	M_{12}	+
10	M_{13}	+
	M_{14}	+
12	M_{15}	+
	M_{16}	+
14	M_{17}	+

Table 4.1 shows the number of bacterial isolates successfully obtained during the mandai fermentation process, namely nine Gram-negative isolates found on days 0 to 4, and eight Gram-positive isolates that appeared on days 6 to 14. The growth of Gram-negative and Gram-positive bacteria on MRS Agar medium (OXOID - CMO0359) is likely due to the high nutrient content in the medium. The abundant nutrients in MRS Agar allow some Gram-negative bacteria to continue to grow by utilizing the available nutrients, even though this medium is basically designed for the selection of Gram-positive bacteria.

This is in line with the opinions of Hasrul (2009) and Muchtadi (2010), who stated that it is possible for Gram-negative bacteria to grow in selective media intended for the isolation of Gram-positive bacteria due to their ability to adapt to the available nutrients. Meanwhile, lactic acid bacteria generally belong to the Gram-positive group. A total of eight Gram-positive isolates (codes M10 to M17) suspected of being lactic acid bacteria were then further analyzed through colony morphology observation, cell characteristics, and biochemical tests to confirm their identity. The results of morphological characterization and biochemical testing are presented in Figure 4.2 and Table 4.2, with testing procedures referring to Cowan (1995), Cowan et al. (1974), and Salminen (1993).

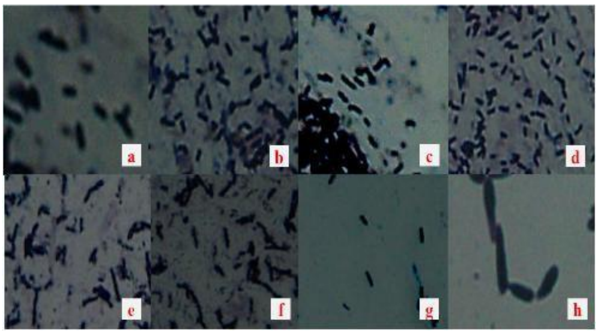


Figure 2. Morphology of mandai fermentation bacterial isolates at 1000X magnification*.
*Description:
a. Isolate M10 e. Isolate M14
b. Isolate M11 f. Isolate M15
c. Isolate M12 g. Isolate M16
d. Isolate M13 h. Isolate M17

2. Morphological Characteristics and Biochemical Tests of Bacterial Isolates

The eight Gram-positive isolates (M10–M17) exhibited typical LAB characteristics: Gram-positive rods or cocci, catalase negative, non-motile, and facultative anaerobes. Biochemical tests confirmed their metabolic versatility: All isolates produced acid and gas from glucose, reducing medium pH to below 4.0 within 48 h. Growth occurred at 37 °C and 48 °C, but not at 16 °C.

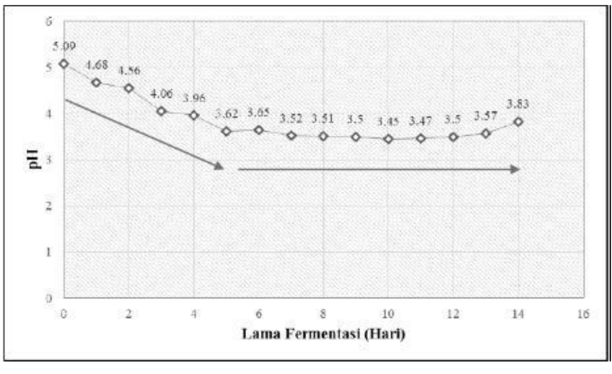
Clear hydrolysis zones were observed on starch, skim milk, and fat agar, confirming amylase, protease, and lipase activities. The enzymatic capabilities suggest that mandai-associated LAB may contribute to the soft texture and sour flavor by degrading complex substrates into fermentable sugars, peptides, and fatty acids. This agrees with studies on LAB in Sui Wu'u and dairy products, where amylolytic and proteolytic activities were linked to flavor development (Rubak et al., 2023; Khan et al., 2024).

3. pH Changes During Fermentation

The initial pH of the mandai substrate was 6.8. A significant decline was observed during fermentation, reaching 3.83 on day 14 ($p < 0.05$, ANOVA). The steepest decrease occurred between days 0–5, after which the pH stabilized (Figure 3). The correlation between decreasing pH and LAB dominance supports their role as the main acid producers. Low pH values (3–4) are optimal for LAB survival but inhibitory to spoilage and pathogenic bacteria, thereby improving product safety (Marco et al., 2021).

4. Proximate Composition of Fermentation Mandai

Proximate analysis revealed that protein and crude fiber contents were relatively stable throughout fermentation, while fat decreased significantly after 14 days ($p < 0.05$). Ash content increased slightly, suggesting mineral concentration due to water loss. Total lactic acid content rose from 0.2% to 1.8% (w/w), confirming active acid production. Compared with tempeh and dadih, mandai showed a higher lactic acid yield, which may explain its intense sour flavor (Sari et al., 2025).



5. Scientific and Practical Implications

This study provides the first detailed isolation and characterization of LAB from mandai. The eight dominant LAB isolates demonstrated acid tolerance, enzymatic activity, and growth at elevated temperatures, traits that are valuable for industrial fermentation. Their ability to hydrolyze starch, fat, and casein suggests potential applications in food biotechnology, including as starter cultures to standardize mandai production, enhance flavor consistency, and improve food safety. Moreover, these isolates may serve as candidates for probiotic development or sources of industrial enzymes, highlighting the global significance of exploring LAB from local traditional foods.

CONCLUSION AND SUGGESTION

This study successfully isolated and characterized lactic acid bacteria (LAB) from mandai, a traditional fermented food of East Kalimantan made from cempedak (Artocarpus champeden) peel. A total of 17 bacterial isolates were obtained during the 14-day fermentation, with eight Gram-positive isolates identified as dominant LAB. These isolates exhibited key characteristics including acid and gas production from glucose, catalase-negative activity, non-motility, tolerance to high temperatures (growth at 48 °C), and enzymatic capabilities such as starch, fat, and casein hydrolysis. Such properties demonstrate the functional role of LAB in producing acidity, flavor, and texture during mandai fermentation. This research represents one of the first detailed studies to document LAB from mandai, highlighting its potential as a novel reservoir of indigenous microorganisms with unique traits. The findings emphasize that mandai is not only of cultural and nutritional value but also a promising source of LAB that may serve as starter cultures to improve the safety and consistency of mandai production, and as candidates for probiotic or industrial enzyme applications. Future research should include: Molecular identification (16S rRNA sequencing or whole-genome analysis) to confirm species-level taxonomy of LAB isolates. In vitro probiotic assessment (acid and bile tolerance, adhesion ability, antimicrobial activity) to evaluate their suitability as probiotics. Pilot-scale application of the isolates as starter cultures to test their effectiveness in improving the quality, safety, and sensory properties of mandai. Comparative studies with LAB from other Indonesian fermented foods (e.g., tempeh, dadih, tape, pekasam) to map microbial diversity and functional potential. Industrial exploration of enzymatic properties (amylase, protease, lipase) for possible applications in food biotechnology.

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