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ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM GAMBUT TAPE

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Abstract

Background: Lactic acid bacteria (LAB) are Gram-positive bacteria with probiotic properties, contributing to digestive health by balancing gut microflora. These bacteria are commonly found in fermented products, including gambut tape, a traditional Indonesian fermented food from Kalimantan. Gambut tape is known for its distinctive green color derived from katuk and pandan leaves.
Objective: This study aims to isolate and identify LAB from the gambut tape.
Methods: LAB were isolated using Man, Rogosa, and Sharpe (MRS) agar and incubated at 37°C for 24 hours. Identification was performed through biochemical tests, including Gram staining, catalase, citrate, H2S, motility, indole, carbohydrate fermentation, methyl red, and Voges-Proskauer tests.
Results: Three isolates were obtained, exhibiting similar morphological features: milky white color, round shape, intact edges, convex elevation, and smooth surface. Biochemical results suggested that the isolates are identical to *Lactococcus lactis, Lactobacillus plantarum,* and *Lactobacillus fermentum*.
Conclusions: The LAB isolated from the gambut tape shows potential as an antibacterial agent for disease prevention. Further research is needed to evaluate their antibacterial activity and characterize bacteriocins produced by these LAB.

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INTRODUCTION

Fermentation is the oldest biotechnology technique used to extend the shelf life of food products without chemical preservatives. This process also enhances the nutritional value of food and provides health benefits due to the presence of probiotic microorganisms (1). Probiotic microorganisms consist of various species, including several lactic acid bacteria (LAB) and yeasts. LAB are a group of Gram-positive bacteria (2); they do not form spores, are either cocci or rod-shaped, and produce lactic acid as the main product during carbohydrate fermentation (3). LAB are taxonomically divided into two main phyla: Firmicutes and Actinobacteria. The Firmicutes phylum includes various genera such as *Lactobacillus, Lactococcus, Streptococcus, Enterococcus, Aerococcus,* and *Weissella,* while the Actinobacteria phylum includes *Bifidobacterium* (4).

LAB do not produce toxins, making them safe to be added to food, and they produce bacteriocins that positively impact health (5). In a review by Das et al. (2022), the bioactivity of LAB provides health benefits through various biological mechanisms, such as protecting the body from pathogen invasion; regulating gut microbiota balance and strengthening gut defenses; inhibiting pathogen colonization; producing antimicrobial compounds like lactic acid and bacteriocins that inhibit pathogen growth; immunomodulatory activity; interacting with Toll-like Receptors (TLRs) in the gut; influencing Nucleotide Oligomerization Domain-like NLRs in immune response regulation; enhancing lactose digestion in individuals with lactose intolerance; preventing diarrhea; preventing Irritable Bowel Syndrome (IBS); preventing urogenital infections; accelerating ulcer healing; reducing food allergy symptoms; reducing body fat; lowering blood cholesterol levels; regulating blood sugar levels and improving insulin sensitivity; maintaining liver health; binding carcinogens to reduce cancer risk; inhibiting tumor growth; increasing Short-Chain Fatty Acids (SCFAs) production; maintaining oral health; improving oral microbiota balance; and boosting the body's immune response to viruses.

LAB can be found in various fermented products such as kimchi, natto, tempeh, miso, yogurt, kefir, tepache, cheese, anchovy fish, wine, and kombucha (7). One of Indonesia's popular traditional fermented products is tape ketan, which is made from glutinous rice fermented using the yeast *Saccharomyces cerevisiae*. Tape ketan has a sweet-sour taste, a slight alcoholic content, and a distinct fresh aroma (8). One variation of tape ketan commonly found in the Kalimantan region is Gambut tape, which has a green color and is shaped like a ping pong ball (9). The green color of Gambut tape is obtained from the use of katuk leaves (*Sauropus androgynus*) (10) and pandan leaves (*Pandanus amaryllifolius*) (11) in the manufacturing process.

Research on LAB in black glutinous rice tape has been widely conducted, but studies on Gambut tape are still limited. Therefore, this study aims to isolate and identify the LAB in Gambut tape.

MATERIALS AND METHODS

Materials

The Gambut tape used in this study was sourced from a single vendor at a traditional market located on Jalan Ahmad Yani, Palangka Raya City, Central Kalimantan. We chose to take samples from only one vendor because all the Gambut tape vendors in the area are from the same producer. The media and reagents used for the isolation of LAB are Man Rogosa Sharpe (MRS) agar medium (Himedia), Man Rogosa Sharpe (MRS) broth medium (Himedia), 3% H2O2 solution, Gram staining reagents (Indo Reagen), Triple Iron Sugar Agar (TSIA) medium (Himedia), citrate medium (Himedia), Sulfur Indole Motility (SIM) medium (Himedia), and MR-VP media (Himedia).

Equipment

The equipment used in this study includes jars, knives, an analytical balance, measuring cylinders, Erlenmeyer flasks, Petri dishes, incubators, laminar air flow Biobase, China), autoclaves (GEA Medical LS-75LJ, China), microscope slides, hot plates, tweezers, Bunsen burners, test tube racks, and light microscopes.

Research Activity

1. Isolation and Purification of LAB

LAB's isolation and purification method refers to Martani et al. (15) with modifications. One gram of Gambut tape was diluted with 9 mL of MRS broth solution to obtain an initial suspension (10^{-1}), which was then incubated for 24 hours at 37°C. After the initial incubation, the suspension at the 10^{-1} dilution was transferred to a test tube containing 9 mL of MRS broth and then serially diluted until reaching a 10^{-10} dilution. A 1000 µL sample from the 10^{-10} dilution suspension was plated on a Petri dish containing MRS agar medium and then incubated for 48 hours at 37°C. After incubation, single colonies suspected to be LAB, exhibiting white color, round shape, and precise edges, were transferred to MRS agar medium for further purification. These colonies were purified using the quadrant streak method with an inoculation needle and incubated for 24 hours at 37°C.

2. Biochemical Tests

To confirm all LAB isolates, we looked at the shape of the bacteria, used Gram staining, and performed several tests, including the catalase test, glucose gas production test to see how fermentation occurs, Sulfide Indole Motility (SIM) test, Triple Sugar Iron agar (TSIA) test, citrate test, and Methyl Red (MR) and Voges-Proskauer (VP) tests.

3. Fermentation Type Test

The fermentation type test refers to Martani et al. (15). LAB isolates were inoculated into tubes containing MRS broth medium and Durham tubes. The inoculated tubes were incubated at 37°C for 24-48 hours. After the incubation period, gas formation in the Durham tubes was observed.

RESULTS AND DISCUSSIONS

Isolation of Lactic Acid Bacteria (LAB) from Gambut Tape

Three LAB isolates were successfully isolated from Gambut tape (Figure 1) and coded as isolates T1, T2, and T3. Based on observations, these three isolates exhibited similar colony morphological characteristics, except for their size (Table 1). Isolate T1 had a small colony size, isolate T2 had a tiny (punctiform) colony size, and isolate T3 had a moderate size with flat elevation. All three isolates shared similarities such as milky white color, round shape, intact edges, convex elevation, and smooth surface.

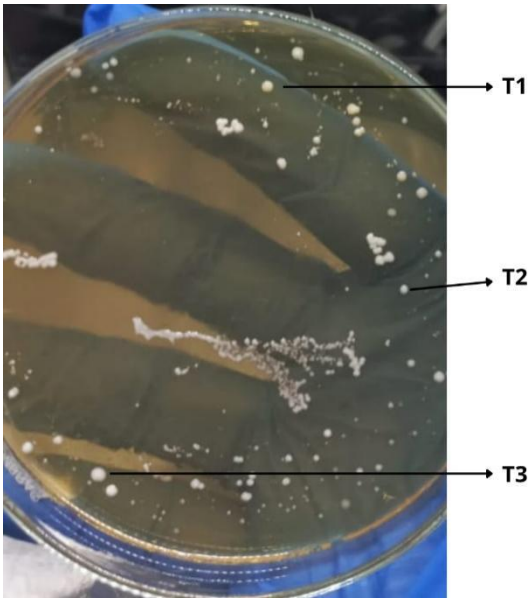


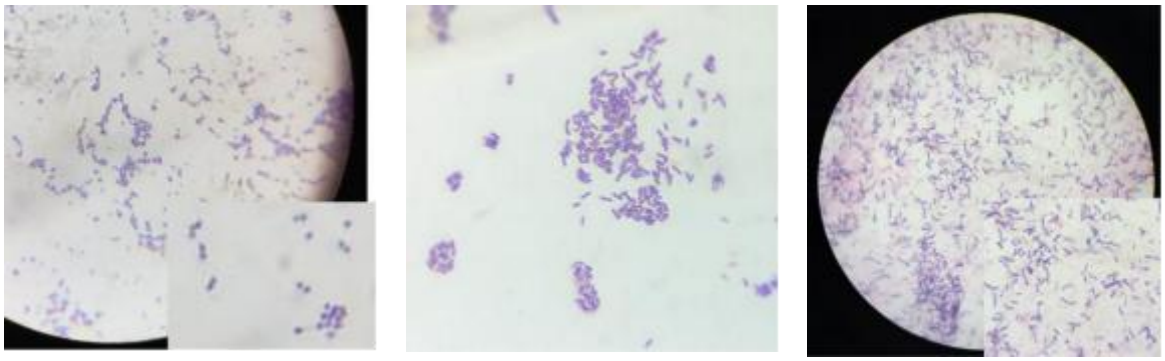
Figure 1. Macroscopic morphological characteristics of LAB from Gambut tape

Table 1. Colony morphological characteristics of bacterial isolates from the Gambut tape

Isolate Code	Color	Size	Form	Elevation	Margin	Surface
T1	White	Small	Circular	Convex	Entire	Smooth
T2	White	Punctiform	Circular	Convex	Entire	Smooth
T3	White	Moderate	Circular	Convex	Entire	Smooth

Table 2. Results of biochemical tests

Characteristic	Isolate T1	Isolate T2	Isolate T3
Gram Staining	+	+	+
Cell Shape	Coccus	Basil	Basil
Catalase	-	-	-
SIM Test	Sulfide (-), Indole (-), Motility (-)	Sulfide (-), Indole (-), Motility (-)	Sulfide (-), Indole (-), Motility (-)
TSIA Test	K/A; Gas (-); H_2S (-)	A/A; Gas (-); H_2S (-)	A/A; Gas (-); H_2S (-)
Fermentation Type	Homofermentative	Heterofermentative	Homofermentative
Citrate	-	+	-
Methyl red	-	+	+
Voges-Proskauer	+	-	-



a b c

Figure 2. Gram staining results of Isolate T1, Isolate T2, and Isolate T3

Identification of Lactic Acid Bacteria (LAB) Based on Biochemical Characteristics

The Gram staining results indicated that all three isolates were Gram-positive, with T1 being cocci-shaped, while T2 and T3 were rod-shaped. Based on the biochemical test results listed in Table 2, we draw preliminary conclusions regarding the species of bacteria isolated from Gambut tape. We summarize the findings from (16–20), where isolate T1 suggests characteristics of *Lactococcus lactis*, isolate T2 suggests *Lactobacillus fermentum*, and isolate T3 suggests *Lactobacillus plantarum*. However, the results are only presumptive because this study relies solely on standard biochemical tests.

Based on the morphological characteristics of the colony in Table 2, microscopically, the identified bacteria are suspected to be *Lactococcus lactis* from Gambut tape, which appear as cocci, Gram-positive, as shown in Figure 2a. Table 2 lists the results of several biochemical tests used to identify the bacterial isolate. The results are in line with the study conducted by Chaurasi et al. (20), who also identified *L. lactis* with similar characteristics, including negative results on catalase, indole, citrate, and Voges-Proskauer tests, as well as positive results on the Methyl Red test and the ability to ferment glucose. These findings support the conclusion that *L. lactis* has a consistent biochemical profile across different isolates from various sources, such as Gambut tape. Based on the results of the biochemical characteristic tests, isolates T2 and T3 are Gram-positive, rod-shaped,

and non-motile bacteria. Both isolates showed positive results for citrate and methyl red tests, and negative results for Voges-Proskauer and catalase tests. The TSIA test results indicate that both isolates can produce gas and ferment glucose, sucrose, and lactose. This identification is supported by the similarity in biochemical and morphological characteristics to *L. fermentum* and *L. plantarum*, which have been isolated by Ahmad et al. (16) and Abid et al. (17).

Lactococcus and *Lactobacillus* genera have been widely reported as dominant LAB groups in fermented products, such as cheeses. These results support the theory that, although only one microorganism species may be used in the fermentation process, other microorganisms can emerge and enrich the fermented product's flavor and aroma (21). *L. lactis, L. plantarum,* and *L. fermentum* have been granted GRAS ("generally recognized as safe") status through scientific procedures, with evidence that these bacteria do not contain genes encoding antibiotic resistance or virulence factors (16,19). These three bacteria have been isolated from various fermented food products, including pickles, kimchi, sourdough bread, cheese, and dried fish, as well as from the oral cavity, gastrointestinal tract, and vagina (22–24).

L. lactis is a Gram-positive, cocci-shaped, non-sporulating bacterium without flagella and is homofermentative. This bacterium produces nisin, a bacteriocin that belongs to the lantibiotic class I (25). Meanwhile, *L. plantarum* is a Gram-positive, rod-shaped bacterium, non-motile, non-sporulating, homofermentative, and a facultative anaerobe (26). *L. plantarum* produces antimicrobial compounds known as plantaricins (27). *L. fermentum* is a Gram-positive, rod-shaped bacterium found as single or paired cells, non-sporulating, and without flagella. This bacterium also produces various bacteriocins, including Fermentcin B (22). Several studies have shown that L. *lactis, L. plantarum,* and *L. fermentum* exhibit probiotic activity that effectively inhibits the growth of pathogenic bacteria such as *Listeria monocytogenes, Staphylococcus aureus,* and *Clostridium botulinum* (22–24).

The results of this study indicate that Gambut tape, a local product from Kalimantan, contains lactic acid bacteria. Further exploration with a larger sample size is needed to identify the probiotic properties of the LAB isolates obtained from Gambut tape.

CLINICAL IMPLICATION

The LAB species isolated from Gambut tape are *L. lactis, L. plantarum,* and *L. fermentum.* This finding suggests that Gambut tape has potential in the health field, particularly in supporting digestive health.

LIMITATIONS

This study has several limitations that need to be considered. The analysis relies on basic biochemical tests to identify bacterial species, which only provide preliminary identification and cannot be confirmed accurately. Therefore, further confirmation methods such as API 20, VITEK 2 Compact, MALDI-TOF MS, or 16S rRNA sequencing are needed to obtain more precise identification. In addition to gaining a clearer understanding of the diversity of lactic acid bacteria (LAB) from Gambut tape, a larger sample size is required.

CONCLUSIONS

Based on the results of lactic acid bacteria isolation from Gambut tape, three bacterial isolates were found to have similar colony morphology, including milky white color, round shape, intact edges, convex elevation, and smooth surface. The biochemical test results showed characteristics identical to *L. lactis, L. plantarum,* and *L. fermentum*.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTOR CONTRIBUTIONS

All authors are responsible for the research planning, data collection, and analysis.

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