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JOURNAL TEMPLATE

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.* ***Aims:*** *This study aims to isolate and identify LAB from 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 similar to Lactococcus lactis, Lactobacillus plantarum, and Lactobacillus fermentum.* ***Conclusion:*** *The LAB isolated from gambut tape show potential as antibacterial agents for disease prevention. Further research is needed to evaluate their antibacterial activity and characterize bacteriocins produced by these LAB.*

Keywords*: Lactic Acid Bacteria, Gambut Tape*

1. Introduction

Fermentation is the oldest biotechnology technique used to extend the shelf life of food products without the use of 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 have a positive impact on 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 the popular traditional fermented products in Indonesia 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 tape Gambut 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 LAB present in Gambut tape.

2. Research Methods

Materials

This study used Gambut tape sold in the traditional market on Jln. Ahmad Yani, Central Kalimantan, Palangka Raya City, Man Rogosa Sharpe (MRS) agar medium, and Man Rogosa Sharpe (MRS) broth medium, 3% H_2O_2 solution, Gram staining reagents, Triple Iron Sugar Agar (TSIA) medium, citrate medium, Sulfur Indole Motility (SIM) medium, and MR-VP media.

Equipment

The equipment used in this study includes jars, knives, an analytical balance, measuring cylinders, Erlenmeyer flasks, Petri dishes, incubators, laminar air flow,

autoclaves, microscope slides, hot plates, tweezers, Bunsen burners, test tube racks, and light microscopes.

Research Activity

1. **Isolation and Purification of LAB**

   The isolation and purification method of LAB refers to Martani et al. (2025) with modifications. One gram of tape ketan 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 clear 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 again for 24 hours at 37°C (12).

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, SIM test, citrate test, and MR-VP test.

3. **Fermentation Type Test**

   The fermentation type test refers to Martani et al. (2025). LAB isolates were inoculated into tubes containing MRS broth medium and Durham tubes. The inoculated tubes were then incubated at 37°C for 24-48 hours. After the incubation period, gas formation in the Durham tubes was observed (12).

3. Results and Discussions

Isolation of Lactic Acid Bacteria (LAB) from Gambut Tape

A total of three LAB isolates were successfully isolated from Gambut tape (Figure 1) and were 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, while isolate T2 had a very small (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.

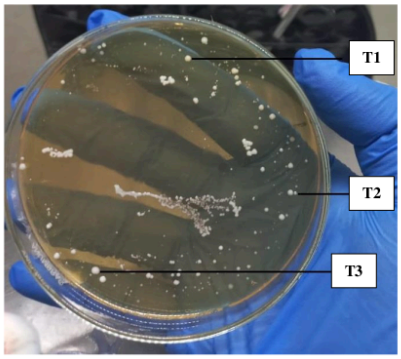

Figure 1. Macroscopic morphological characteristics of LAB from Gambut tape

Table 1. Colony morphological characteristics of bacterial isolates from 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	-	-	-

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

The results of Gram staining 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 (13–17), where isolate T1 suggests characteristics of *Lactococcus lactis*, isolate T2 suggests *Lactobacillus fermentum*, and isolate T3 suggests *Lactobacillus plantarum*. The genera *Lactococcus* and *Lactobacillus* have been widely reported as dominant LAB groups in various fermented products, such as tapes. These results support the theory that, although only one microorganism species may be used in the

fermentation process, other microorganisms can emerge and contribute to enriching the flavor and aroma of the fermented product (18).

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 (13,16). 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 (19–21).

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 lantibiotic class I (22). Meanwhile, *L. plantarum* is a Gram-positive, rod-shaped bacterium, non-motile, non-sporulating, homofermentative, and a facultative anaerobe (23). *L. plantarum* produces antimicrobial compounds known as plantaricins (24). *L. fermentum* is a Gram-positive, rod-shaped bacterium that can be found as single or paired cells, non-sporulating, and without flagella. This bacterium also produces various types of bacteriocins, including Fermentcin B (19). 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* (19–21).

Further confirmation tests are needed to obtain more accurate results, such as using the API 20 method and VITEK 2 Compact, which focus on the biochemical characteristics of the bacteria. Additionally, MALDI-TOF MS techniques, based on bacterial protein profiles, and 16S rRNA sequencing, which relies on genetic analysis, can provide deeper identification. Future research could focus on antibacterial activity tests and bacteriocin characterization to evaluate the probiotic potential of bacteria isolated from Gambut tape.

4. 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 similar to the species *L. lactis*, *L. plantarum*, and *L. fermentum*.

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