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Isolatıon, Characterızatıon, and Antıfungal Assay of Lactıc Acıd Bacterıa Isolated from Green Glutınous Rıce Tape Agaınst *Aspergillus flavus*

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Abstract: Green glutinous rice tape is a fermented food made from glutinous rice, katuk leaves (*Sauropus androgynus*), and yeast, originating from Tembilahan, Riau Province. This product can serve as a source of lactic acid bacteria (LAB) capable of producing antimicrobial compounds to inhibit pathogenic microorganisms, including *Aspergillus flavus*, a well-known spoilage fungus in food products. However, to date, no reports have explored the ability of LAB from green glutinous rice tape to inhibit the growth of *Aspergillus flavus*. This study aimed to isolate LAB from green glutinous rice tape, conduct macroscopic, microscopic, and biochemical characterizations, and evaluate the antifungal activity of the LAB isolates against *Aspergillus flavus* using the good diffusion method. The isolation process yielded four LAB isolates from Tembilahan green tape (TKHT-2, TKHT-3, TKHT-5, and TKHT-7), which were identified as members of the genus *Lactobacillus* sp., with antifungal activities of 25.50 ± 5.78 mm, 24.33 ± 0.62 mm, 22.16 ± 6.56 mm, and 18.66 ± 4.28 mm, respectively. The corresponding cell-free supernatants (CFS) from these isolates (TKHT-S2, TKHT-S3, TKHT-S5, and TKHT-S7) also demonstrated antifungal activity with inhibition zones of 19.83 ± 3.47 mm, 19.83 ± 4.24 mm, and 14.50 ± 3.26 mm, respectively. LAB cells and their cell-free supernatants partially inhibited *Aspergillus flavus*, indicating a fungistatic effect. These findings suggest the potential application of LAB from green glutinous rice tape as a natural preservative or antifungal agent in food products.
Keywords: Anti-mold; isolation; lactic acid bacteria; Tembilahan green tape.

INTRODUCTION

Indonesian traditional foods are an essential part of the nation's cultural heritage and are closely tied to the characteristics of its society. Nearly every island in Indonesia has unique culinary traditions and distinctive flavors in each region (Griana & Kinasih, 2020). One of the conventional biotechnological methods used in traditional food processing is fermentation (Faridah & Sari, 2019). Fermentation is a process that produces new products different from the original raw materials with the help of microorganisms, including bacteria, molds, and yeasts (Stefanny & Pamungkaningtyas, 2023). These groups of bacteria are considered safe for food applications as they do not produce toxins and pose no health risks (Rahmah et al., 2021).

Riau Province, particularly Indragiri Hilir Regency, with its capital Tembilahan, is known as a producer of traditional fermented foods. One of the region's distinctive products is green glutinous rice tape. As the name suggests, this fermented food is made from glutinous rice and naturally colored using katuk leaves (*Sauropus androgynus*) (Rikizaputra et al., 2022). Katuk leaves offer various health benefits, including enhancing breast milk production, preventing osteoporosis, and aiding in

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weight loss. In addition, katuk leaves exhibit antimicrobial properties (Herawati et al., 2024) and possess antioxidant, antidiabetic, and anti-inflammatory activities. They are also rich in bioactive compounds such as flavonoids and polyphenols (Zhang et al., 2020).

Glutinous rice tape is a yeast-fermented food and a potential source for isolating lactic acid bacteria (LAB). According to Koriasih et al. (2019), LAB from glutinous rice tape has the potential to be used in controlling pathogenic microbes, particularly as an anti-mold agent to inhibit *Aspergillus flavus*. Rahmiati and Mumpuni (2017) further support this, stating that lactic acid bacteria (LAB) can function as an anti-mold agent. LAB is a heterogeneous group of bacteria capable of fermenting sugars into lactic acid (Wedajo, 2015). According to Septianti (2019), LAB produces lactic acid, acetic acid, phenylacetic acid (PLA), dipeptide compounds, fatty acids, and hydroxyl compounds, which have anti-mold properties and inhibit protein-based fungal growth. These effects are attributed to bacteriocins and other low-molecular-weight antimicrobial compounds. Additionally, LAB secrete antimicrobial compounds such as ethanol, hydrogen peroxide, and fatty acids as part of their pathogen inhibition mechanism (Chen et al., 2019).

Aspergillus flavus is commonly found in legumes (especially peanuts), spices, oil-rich seeds, and sometimes in dried fruits (Gandjar et al., 2000). This mold is a significant cause of food poisoning due to its production of aflatoxins, a group of mycotoxins known for their carcinogenic, mutagenic, and teratogenic effects. There are four primary aflatoxins: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). Among these, AFB1 is the most toxic and highly carcinogenic (Wisnu, 2018). Research by Ningrum et al. (2023) identified a LAB isolate from fermented glutinous rice tape sold at Pasar Citra Niaga, Jombang, labeled as TR354, with an irregular shape, curled edges, medium size, shiny and translucent appearance, textured surface, and a cream color. Nisa et al. (2020) isolated five LAB strains (BTP isolates) from plastic-packaged glutinous rice tape, which exhibited bacillus or coccus shapes, yellow to cream coloration, were Gram-positive, and catalase-negative, with the ability to inhibit *Fusarium sp.* growth, showing an average inhibition diameter of 12.00 mm. Meanwhile, Koriasih et al. (2019) isolated seven LAB strains (BALTD isolates) from glutinous rice tape, characterized by round shape, white/cream color, smooth and shiny surfaces, and an average inhibition diameter of 6.80 mm against *Aspergillus flavus*.

Several studies have successfully isolated and characterized LAB from glutinous rice tape. However, research focusing on LAB isolation and characterization from green glutinous rice tape, a traditional food of Tembilahan, has not yet been conducted. Due to the limited information available, this study aims to isolate, characterize, and evaluate the anti-mold activity of LAB isolates from green glutinous rice tape against *Aspergillus flavus*. Based on this background, this study seeks to obtain and identify LAB isolates from green glutinous rice tape collected from Tembilahan through macroscopic and microscopic characterization. These isolates can potentially be used in pathogenic microbial control, particularly as anti-mold agents to inhibit *Aspergillus flavus*. In the future, these isolates could be applied to reduce *Aspergillus flavus* contamination in food products.

MATERIALS AND METHODS

Sample Collection

Green glutinous rice tape was obtained from producers who make and sell green glutinous rice tape in Tembilahan City, Indragiri Hilir Regency.

Isolation and Purification of Lactic Acid Bacteria

Isolation was carried out using a serial dilution method followed by spread plate inoculation. A total of 10 grams of green glutinous rice tape was diluted in 90 mL of sterile distilled water up to a 10^{-9} dilution. From the 10^{-7} to 10^{-9} dilutions, 100 µL of each was spread onto *de Man Rogosa Sharpe Agar* (MRSA) medium supplemented with 1% $CaCO_3$ and incubated at 37°C for 48 hours. Colonies that formed clear zones were re-purified on the same medium (Koriasih et al., 2019). LAB colonies grown on an MRSA medium were purified using the quadrant streak method until single colonies were obtained. The purification process was repeated if purity was not achieved (Darma, 2023).

Subculturing of Lactic Acid Bacteria

Subculturing of lactic acid bacteria for preparing stock and working cultures was carried out by inoculating 1–2 loops of bacteria from pure stock into MRSA medium on slant test tubes and Petri dishes. The media were then incubated at 37℃ for 24 hours (Darma, 2023; Ayen et al., 2017).

Lactic Acid Bacteria Characterization

The macroscopic characterization of lactic acid bacteria (LAB) colonies involved observing their shape, edges, elevation, and color. Pure colonies were identified by uniform morphology, such as round, single, paired, rod-shaped, or chained forms (Joni et al., 2018). Microscopically, 24-hour-old LAB isolates were Gram-stained to differentiate Gram-positive bacteria, which appeared purple, from Gram-negative bacteria, which stained pink (Idroes et al., 2019). Endospore staining was also performed, where endospores appeared green and vegetative cells appeared red after staining with malachite green and safranin (Amaliah et al., 2018).

Biochemical tests included the catalase test, where bubble formation after adding 3% hydrogen peroxide indicated a positive result. Carbohydrate fermentation was evaluated using phenol red as an indicator in sugar-containing media; a color change to yellow or orange signified a positive reaction. The Triple Sugar Iron Agar (TSIA) test detected acid (yellow), alkaline reactions (red), hydrogen sulfide (black precipitate), and gas production (medium lifting). The Sulfide Indole Motility (SIM) test showed motility through medium turbidity and spread of growth, while a red ring after adding Kovacs reagent indicated indole production. Citrate utilization on Simmon Citrate Agar (SCA) was confirmed by a blue color change. In the urease test, a shift from orange-red to purple in urea broth indicated urea hydrolysis. The oxidase test used oxidase strips, with a purple color within 15 seconds indicating a positive result, while pink indicated a negative one (Tang et al., 2006).

Antifungal Activity Test

Fungal Identification

The Microbiology Laboratory, Faculty of Medicine, University of Riau, identified *Aspergillus flavus* based on macroscopic (colony color and texture) and microscopic (conidia, hyphae, and vesicle) characteristics on PDA medium (Sukmawati et al., 2018).

Fungal Revitalization

Fungal revitalization was performed by inoculating 1–2 streaks of fungi from pure stock onto PDA medium in slanted test tubes, then incubated at 25°C for 120 hours (Hakim, 2009).

Preparation of Fungal Suspension

The revitalized fungi were streaked 3–4 times into test tubes containing physiological NaCl solution and homogenized using a vortex. The suspension's

concentration was measured using a UV-Vis spectrophotometer until it achieved 90% transmittance at a wavelength of 530 nm.

Inoculation of Fungal Inoculum

0.1 mL of *Aspergillus flavus* suspension was inoculated into a Petri dish, followed by the addition of 25 mL MRSA medium. The mixture was homogenized by gently rotating the Petri dish and left to solidify.

Antifungal Activity Test by LAB Cells

Antifungal activity was tested using the good diffusion method on an MRSA medium inoculated with fungi. Wells with a diameter of 6.5 mm were made using a cork borer. LAB isolates incubated for 24 hours were adjusted to 25% transmittance (580 nm), and 40 µL of each isolate was added to the wells. Ketoconazole was used as the positive control, and de Man, Rogosa, and Sharpe Broth (MRSB) as the negative control. Incubation was conducted at room temperature and 37°C for 48 hours. The clear zone around the wells was measured using a caliper (Koriasih et al., 2019).

Antifungal Activity Test of Cell-Free Supernatant (CFS) of LAB

Cell-free supernatant was obtained by centrifuging LAB cultures incubated for 24 hours at 10,000 rpm for 15 minutes. This is just an explanation: the following procedure is the same as the Antifungal Activity Test by LAB Cells.

Data Analysis

The data collected included descriptive data, such as microscopic and macroscopic characteristics and biochemical test results of the LAB isolates, as well as quantitative data in the form of the average inhibition zone diameter against *Aspergillus flavus*. The variation in results was measured using standard deviation to indicate the consistency of inhibition. Although no inferential statistical tests were performed, the standard deviation helped assess the reliability and biological variability among the isolates.

RESULTS AND DISCUSSION

The lactic acid bacteria isolates were obtained from green glutinous rice tape at dilution levels of 10^{-7} and 10^{-8}. These isolates exhibited clear zones around the colonies after 24 hours of incubation on MRSA medium supplemented with 1% $CaCO_3$. This phenomenon occurs because the LAB produces lactic acid, which reacts with $CaCO_3$ to form soluble calcium lactate, causing the dissolution of $CaCO_3$ around the growing colonies.

The purification of bacterial isolates from green glutinous rice tape resulted in seven pure isolates labeled TKHT (Tape Ketan Hijau Tembilahan), namely TKHT-1, TKHT-2, TKHT-3, TKHT-4, TKHT-5, TKHT-6, and TKHT-7 (Figure 1). The macroscopic characterization results of the seven isolates—TKHT-1, TKHT-2, TKHT-3, TKHT-4, TKHT-5, TKHT-6, and TKHT-7—showed that all isolates had round shapes, smooth edges, convex elevations, and milky white coloration (Figure 1, Table 1). Based on these observations, it can be concluded that all isolates exhibit morphological characteristics typical of lactic acid bacteria (LAB). Sulmiyati et al. (2018) stated that LAB colonies generally have diameters of 0.5–2 mm, a round shape, smooth edges, convex surfaces, and a milky white or cream color. According to Joni et al. (2018), the color of bacterial colonies arises from the pigments produced by the bacteria, such as melanin, anthocyanin, carotenoids, phenazines, and tripyrilmethene. In this study, the colonies exhibited a milky white color. Rather et al. (2025) also noted that bacteria containing carotenoid pigments could result in cell coloration ranging from dark orange, orange, yellow, and cream white to milky white.

Table 1. Macroscopic Identification of Lactic Acid Bacteria Isolates from Green Glutinous Rice Tape

Isolate Code	Colony Shape	Colony Margin	Colony Elevation	Colony Color
TKHT-1	Circular	Entire	Convex	Milky White
TKHT-2	Circular	Entire	Convex	Milky White
TKHT-3	Circular	Entire	Convex	Milky White
TKHT-4	Circular	Entire	Convex	Milky White
TKHT-5	Circular	Entire	Convex	Milky White
TKHT-6	Circular	Entire	Convex	Milky White
TKHT-7	Circular	Entire	Convex	Milky White

Table 2. Microscopic Identification of Lactic Acid Bacteria Isolates from Green Glutinous Rice Tape Based on Gram Staining and Endospore Staining

Isolate Code	Cell Shape	Cell Color	Gram Reaction	Endospore Staining
TKHT-1	Bacillus	Purple	Positive	With Endospore
TKHT-2	Bacillus	Purple	Positive	Without Endospore
TKHT-3	Bacillus	Purple	Positive	Without Endospore
TKHT-4	Coccus	Purple	Positive	Without Endospore
TKHT-5	Bacillus	Purple	Positive	Without Endospore
TKHT-6	Coccus	Purple	Positive	Without Endospore
TKHT-7	Bacillus	Purple	Positive	Without Endospore

Table 3. Biochemical Test Results of Lactic Acid Bacteria Isolates from Green Glutinous Rice Tape

Isolate Code	Biochemical Test								Genus
	Cat	Oxy	TSIA	SIM	Urease	SCA	Carbohydrate Fermentation		
							Glucose	Lactose	
TKHT-1	+	-	K/A	+	+	+	+	+	*Bacillus* sp
TKHT-2	-	-	A/A	-	-	-	+	+	*Lactobacillus* sp
TKHT-3	-	-	A/A	-	-	-	+	+	*Lactobacillus* sp
TKHT-4	+	-	K/K	+	+	+	+	-	*Staphylococcus aureus*
TKHT-5	-	-	A/A	-	-	-	+	+	*Lactobacillus* sp
TKHT-6	+	-	K/K	+	+	+	+	-	*Staphylococcus epidermidis*
TKHT-7	-	-	A/A	-	-	-	+	+	*Lactobacillus* sp

Note:
TSIA (Triple Sugar Iron Agar) Test:

- A/A = Fermentation of glucose and one or more disaccharides (lactose and/or sucrose)
- K/A = Fermentation of glucose only
- K/K = No sugar fermentation (alkaline reaction)

SIM: Sulfide, Indole, Motility test
SCA: Simmons Citrate Agar
Cat: Catalase Test
Oxy: Oxidase test

Microscopic examination of the seven isolates—TKHT-1, TKHT-2, TKHT-3, TKHT-5, and TKHT-7—showed rod-shaped (bacillus) cells, purple coloration, and Gram-positive characteristics. Meanwhile, isolates TKHT-4 and TKHT-6 exhibited spherical (coccus) cell shapes and purple coloration and were also Gram-positive (Figure 1, Table 2). Lactic acid bacteria are classified as Gram-positive bacteria. According to Mustaqim et al. (2014), during Gram staining, bacteria undergo dehydration when treated with 96% alcohol, causing their pores to shrink. As a result, the primary stain (crystal violet) cannot escape because Gram-positive bacteria possess thick cell walls and a single-layered cell membrane.

Based on the endospore staining, it was found that isolate TKHT-1 contained spores, while isolates TKHT-2, TKHT-3, TKHT-4, TKHT-5, TKHT-6, and TKHT-7 did not contain spores in their vegetative cells (Figure 2, Table 2). These six isolates are categorized as lactic acid bacteria (LAB), characterized by negative endospore staining due to the absence of spore formation. The staining was performed using the Schaeffer–Fulton method, which utilizes malachite green as the primary stain and safranin as the counterstain. In this method, malachite green penetrates and stains the highly resistant endospores, turning them green. In contrast, the less resistant vegetative cells are decolorized and then counterstained red or pink by safranin. Since the LAB isolates did not produce spores, their cells did not retain the green stain. Instead, they appeared red under the microscope due to the uptake of safranin, confirming the absence of endospore structures (Oktari et al., 2017).

The biochemical test results of seven bacterial isolates from green glutinous rice tape revealed diverse physiological and metabolic characteristics supporting bacterial genera identification (Table 3). Isolate TKHT-1 showed positive reactions for catalase, SIM, urease, and SCA and was capable of fermenting both glucose and lactose, indicating its classification as *Bacillus* sp., consistent with the findings of Elshaghabee et al. (2017), who reported that several *Bacillus* strains are catalase-positive and possess probiotic potential. Four other isolates (TKHT-2, TKHT-3, TKHT-5, TKHT-7) displayed traits typical of *Lactobacillus* sp., including negative reactions for catalase and oxidase and the ability to ferment glucose and lactose, aligning with the characteristics of lactic acid bacteria described by Axelsson (2004). Meanwhile, isolates TKHT-4 and TKHT-6 were identified as *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively, based on their positive reactions for catalase, SIM, urease, and SCA, but negative lactose fermentation, by Becker et al. (2014), who noted that *Staphylococcus* species are facultative anaerobes and catalase-positive. These findings indicate that the fermentation of green glutinous rice tape involves a variety of bacteria, with *Lactobacillus* sp. predominating as the lactic acid bacteria with probiotic potential.

The classification of bacterial isolates into genus and species was based on colony morphology, cell morphology, and biochemical tests, referring to Bergey's Manual of Determinative Bacteriology. The identification results showed that isolate

TKHT-1 belongs to the genus *Bacillus sp*., isolates TKHT-2, TKHT-3, and TKHT-5 belong to the genus Lactobacillus sp., isolate TKHT-4 belongs to the species *Staphylococcus aureus*, isolate TKHT-6 belongs to the species *Staphylococcus epidermidis*, and isolate TKHT-7 belongs to the genus *Lactobacillus sp*. Thus, the green glutinous rice tape isolation results revealed four bacterial genera/species: *Bacillus sp., Lactobacillus sp., Staphylococcus* aureus, and *Staphylococcus epidermidis.*

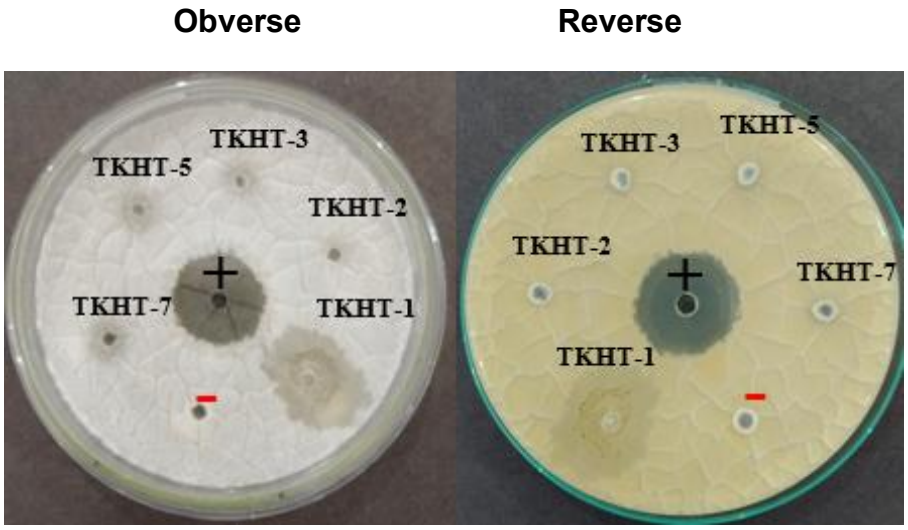

Figure 1. Results of Antifungal Activity Test by LAB Cells on Green Glutinous Rice Tape Against Aspergillus flavus: (a) At Room Temperature for 48 Hours, (b) At 37℃ for 48 Hours.

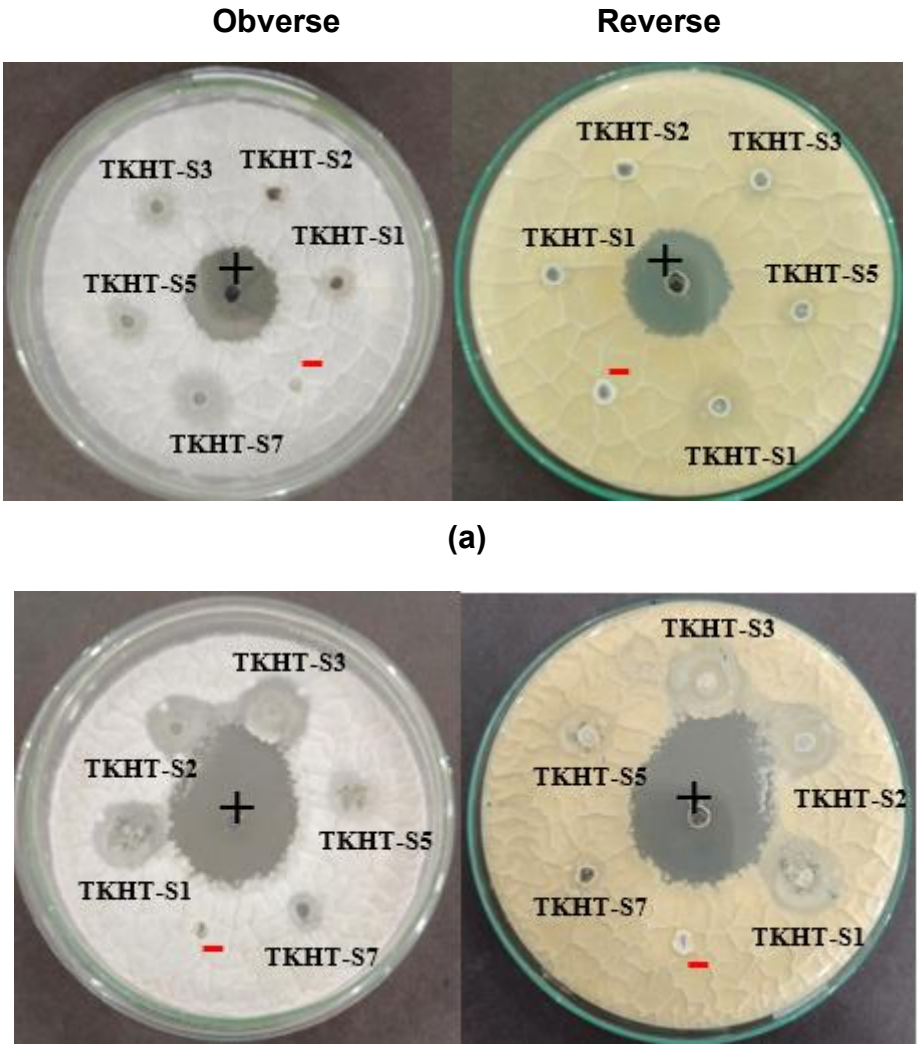

Figure 2. Results of Antifungal Activity Test of Cell-Free Supernatant (CFS) from Green Glutinous Rice Tape Against *Aspergillus flavus*: (a) At Room Temperature for 48 Hours, (b) At 37℃ for 48 Hours

Table 5. Measurement of Inhibition Zone Diameter in Antifungal Activity Test by LAB Cells at 37℃ for 48 Hours

Treatment	Inhibition Zone Diameter (mm)			Average ± SD	Remarks
	I	II	III		
K (+)	37,00	42,00	45,00	41,33 ± 3,29	Total Inhibition
K (-)	6,50	6,50	6,50	6,50 ± 0,00	No Activity
TKHT-1	13,00	23,50	26,50	21,00 ± 5,78	Partial Inhibition
TKHT-2	17,50	28,00	31,00	25,50 ± 5,78	Partial Inhibition
TKHT-3	25,00	23,50	24,50	24,33 ± 0,62	Partial Inhibition
TKHT-5	13,00	28,00	25,50	22,16 ± 6,56	Partial Inhibition
TKHT-7	13,50	24,00	18,50	18,66 ± 4,28	Partial Inhibition

Description:

- Positive Control: Ketoconazole 1%
- Negative Control: MRSB

Table 6. Measurement of Inhibition Zone Diameter in Antifungal Activity Test of Cell-Free Supernatant (CFS) from LAB at Room Temperature for 48 Hours

Treatment	Inhibition Zone Diameter (mm)			Average ± SD	Remarks
	I	II	III		
K (+)	29,50	29,00	29,50	29,33 ± 0,23	Total Inhibition
K (-)	6,50	6,50	6,50	6,50 ± 0,00	No Activity
TKHT-1	10,00	6,50	6,50	7,66 ± 1,64	Partial Inhibition
TKHT-2	6,50	11,00	14,00	10,50 ± 3,08	Partial Inhibition
TKHT-3	10,50	9,00	18,00	12,50 ± 3,93	Partial Inhibition
TKHT-5	9,00	8,50	18,50	12,00 ± 4,60	Partial Inhibition
TKHT-7	21,50	11,50	6,50	13,16 ± 6,23	Partial Inhibition

Description:
- Positive Control: Ketoconazole 1%
- Negative Control: MRSB

Table 7. Measurement of Inhibition Zone Diameter in Antifungal Activity Test of Cell-Free Supernatant (CFS) from LAB at 37℃ for 48 Hours

Treatment	Inhibition Zone Diameter (mm)			Average ± SD	Remarks
	I	II	III		
K (+)	39,00	44,50	40,50	41,33 ± 2,32	Total Inhibition
K (-)	6,50	6,50	6,50	6,50 ± 0,00	No Activity
TKHT-1	22,00	26,00	16,50	21,50 ± 3,89	Partial Inhibition
TKHT-2	20,00	24,00	15,50	19,83 ± 3,47	Partial Inhibition
TKHT-3	21,50	24,00	14,00	19,83 ± 4,24	Partial Inhibition
TKHT-5	17,00	28,50	12,00	19,16 ± 6,90	Partial Inhibition
TKHT-7	10,50	18,50	14,50	14,50 ± 3,26	Partial Inhibition

Description:
- Positive Control: Ketoconazole 1%
- Negative Control: MRSB

The measurements were carried out at two temperatures, namely room temperature and 37°C, to determine the effect of temperature on the antifungal activity of lactic acid bacteria (LAB) and their cell-free supernatant (CFS), as well as its relation to the growth conditions of *Aspergillus flavus*. Room temperature represents common environmental conditions, while 37°C reflects human body temperature and approximates the optimal growth temperature of *Aspergillus flavus* (30–37°C). Therefore, testing at both temperatures aims to evaluate the effectiveness of LAB under different conditions, including when *Aspergillus flavus* is growing optimally, and to identify the most favorable temperature for LAB to produce active antifungal compounds.

Based on the data from Tables 4 to 7, incubation temperature significantly affects the antifungal activity of lactic acid bacteria (LAB) cells and their cell-free supernatant (CFS). At 37°C, the inhibition zone diameters were generally larger than those observed at room temperature for both LAB cells and CFS. For example, isolate TKHT-3 produced an inhibition zone of 24.33 ± 0.62 mm at 37°C using LAB cells, whereas it only reached 10.33 ± 2.89 mm at room temperature. Similarly, for TKHT-3 CFS, the inhibition zone increased from 12.50 ± 3.93 mm (room temperature) to 19.83 ± 4.24 mm (37°C). This indicates that higher incubation temperatures can enhance

the production and/or activity of antifungal compounds by LAB, both those released into the medium (in the CFS) and those retained within the cells (Mani-López., et al., 2022). The elevated temperature likely stimulates the metabolism of LAB, producing greater quantities or more potent bioactive compounds, such as organic acids, antimicrobial peptides, or hydrogen peroxide, which contribute to antifungal activity (Mishra et al., 2021).

The antifungal activity of LAB isolates from green glutinous rice tape tested against *Aspergillus flavus* includes LAB cells and cell-free supernatant (CFS) from LAB, with 1% ketoconazole as a positive control. The antifungal activity test by LAB cells aims to determine the inhibitory ability of LAB cells along with their metabolites against fungal growth. In contrast, the antifungal activity test of cell-free supernatant (CFS) aims to determine the antifungal activity of metabolites produced by LAB, such as organic acids (lactic acid, acetic acid, propionic acid, and phenylacetic acid) and their antagonistic compounds (diacetyl, hydrogen peroxide, and bacteriocins) (Koriasih et al., 2019).

The antifungal activity test by LAB cells and cell-free supernatant (CFS) on green glutinous rice tape against *Aspergillus flavus* showed partial inhibition, indicating that the antifungal compounds produced are fungistatic. Partial inhibition or fungistatic activity is characterized by thinning fungal growth around the well, but no clear zone is observed. Meanwhile, the positive control ketoconazole showed total inhibition or fungicidal activity, as evidenced by the clear zone around the well (Natasia et al., 2020).

Poeloengan (2009) states that a total inhibition zone is a clear area around the well, indicating the presence of bioactive compounds capable of killing the test fungus, while a partial inhibition zone is an area with reduced or thin fungal growth around the well, indicating that bioactive compounds inhibit microbial growth.

The results of the antifungal activity test by LAB cells on green glutinous rice tape against *Aspergillus flavus* showed that isolate TKHT-2 had the highest inhibitory activity among the other isolates, with an average inhibition zone diameter of 25.50 mm. In comparison, isolate TKHT-7 showed the smallest inhibitory activity, with an average inhibition zone diameter of 18.66 mm.

The ability of LAB cells to inhibit the growth of *Aspergillus flavus* can occur due to competition for nutrients in the medium, as well as the organic acids and metabolites produced by LAB. The ability of bacteria to suppress and control the growth of pathogenic microbes is due to competition for nutrients and space. LAB with the potential to inhibit *Aspergillus flavus* are heterofermentative LAB, as these bacteria not only produce lactic acid but also generate other organic acids and metabolites such as lactic acid, CO2 gas, diacetyl, ethanol, and bacteriocins (Ahuja et al., 2024).

The bacteriocin compound suspected of inhibiting the growth of *Aspergillus flavus* is reuterin. Reuterin is produced by *Lactobacillus reuteri*, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus collinoides*, and *Lactobacillus coryniformis*. Reuterin is made during the stationary phase of growth of *Lactobacillus reuteri* under anaerobic conditions. Reuterin has a broad antimicrobial spectrum and can inhibit Gram-negative and Gram-positive bacteria, yeast, fungi, and protozoa. The mechanism of action of reuterin occurs through the inhibition of ribonucleotide reductase, which interferes with DNA synthesis. Antifungal compounds from other LAB species include cyclo (L-Phe-L-Pro), cyclo (L-Phe-trans-4-OH-L-Pro), and phenylacetic acid. Phenylacetic acid can inhibit the formation of chitin and β-glucan in fungal cell wall synthesis (Kadyan et al., 2020).

Although isolates TKHT-1, TKHT-4, and TKHT-6 were identified as Bacillus and Staphylococcus species rather than Lactobacillus, they were included in antifungal testing because Bacillus and Staphylococcus species are well-known producers of various antimicrobial compounds, including antifungal agents. Bacillus species, in particular, are recognized for synthesizing lipopeptides, enzymes, and other bioactive metabolites that inhibit fungal and pathogen growth (Nakkeeran., et al., 20019). Similarly, some Staphylococcus strains produce antimicrobial substances that can contribute to food safety and preservation. Some *Staphylococcus* species, particularly coagulase-negative strains such as *Staphylococcus carnosus*, *S. xylosus*, and *S. piscifermentans*, are known to produce bacteriocins—protein-based antimicrobial compounds that are effective against pathogenic and spoilage bacteria. These bacteriocins, often called staphylococcins, have great potential for microbial control in food products (Milshteyn et al., 2018). Therefore, testing these isolates broadens the scope of potential biocontrol agents beyond lactic acid bacteria, emphasizing the diverse microbial community in fermented foods like tape ketan hijau that enhances product safety and quality.

The antifungal activity observed in isolates from green glutinous rice tape showed varying inhibition zones against *Aspergillus flavus*, with the highest average inhibition zone recorded for isolate TKHT-S1 (21.50 ± 3.89 mm) and the lowest for TKHT-S7 (14.50 ± 3.26 mm). These results are consistent with the findings of Taheur et al. (2019), which demonstrated that *Lactobacillus* strains have inhibitory effects against *Aspergillus flavus*. Similarly, the study by Zhang et al. (2008) reported that *Bacillus* strains produce organic acids and bioactive compounds that suppress fungal growth, which corresponds with the partial inhibition observed in this study. Although the inhibition zones are smaller than chemical fungicides used as positive controls, such microbial antagonism presents a promising natural biocontrol method for enhancing food safety in fermented products.

The antifungal activity of the cell-free supernatant (CFS) of LAB is attributed to lactic acid and other acids, which lower the environmental pH, thereby inhibiting the growth of acid-sensitive fungi. Additionally, metabolites with antifungal properties, such as diacetyl, hydrogen peroxide, and bacteriocins, contribute to this effect. Guan et al. (2020) state that the inhibition of fungal growth caused by organic acids such as lactic acid, acetic acid, propionic acid, and phenylacetic acid results in the release of H+ into the cytoplasm, leading to a rapid drop in pH within the cell membrane (Guan et al., 2020).

This study has limitations that may affect the interpretation of the antifungal activity of LAB isolates. Factors such as temperature, pH, incubation time, bacterial density, and cell age can influence the inhibition zone (Sutrisna et al., 2015). Additionally, the effectiveness of antimicrobial substances depends on compound concentration, microbial sensitivity, diffusion in the medium, and environmental interactions (Erlyn, 2016). Therefore, in vitro, results may differ from real-world conditions. Future research should optimize fermentation parameters, test antifungal activity under practical conditions, and identify specific bioactive compounds. Exploring synergistic effects between LAB isolates or other agents could also enhance antifungal potential.

CONCLUSION

Based on the conducted research, four isolates identified as lactic acid bacteria (LAB) from green glutinous rice tape in Tembilahan—namely TKHT-2, TKHT-3, TKHT-5, and TKHT-7—are confirmed to belong to the genus *Lactobacillus* sp. Other isolates

were identified as *Bacillus* sp. (TKHT-1), *Staphylococcus aureus* (TKHT-4), and *Staphylococcus epidermidis* (TKHT-6). The antifungal activity tests of LAB cells and cell-free supernatant (CFS) against *Aspergillus flavus* showed partial inhibition, indicating fungistatic effects. Further research is recommended to isolate and characterize the specific antifungal compounds involved, optimize conditions for maximum inhibitory activity, and evaluate the effectiveness of these isolates in real food preservation applications.

CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest to disclose.

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