

## Isolation and antimicrobial activity of lactic acid bacteria from the traditional fermented food *cangkuok semaung* in Riau, Indonesia

### Isolasi dan aktivitas antimikroba bakteri asam laktat dari pangan fermentasi tradisional *cangkuok semaung* di Riau, Indonesia

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#### ARTICLE INFO

##### Article History

Received: Jun 17, 2025

Accepted: Jul 23, 2025

Available Online: Jul 30, 2025

##### Keywords:

functional food,  
cangkuok semaung,  
fermentation,  
kepayang fruit,  
indigenous microbiota

##### Cite this:

J. Ilm. Pertan., 2025, 22 (2) 105-116

##### DOI:

<https://doi.org/10.31849/jip.v22i2.27537>

#### ABSTRACT

Fermentation can be an effective step in addressing postharvest handling challenges and enhancing nutritional value through the conversion of perishable crops into more stable products. One of the traditional fermented foods originating from Kuantan Singingi Regency, Riau Province, Indonesia, is *cangkuok semaung* (CS). This food is prepared by fermenting all fruit components of the *semaung* (*Pangium edule*). This research aimed to isolate and identify lactic acid bacteria (LAB) from CS and investigate their antimicrobial properties. The bacteria were isolated from the food, purified using the quadrant streak method on the De Man–Rogosa–Sharpe agar, and continued with morphological analysis and antimicrobial activities evaluation. The findings of this study revealed that 45 isolates were gram-positive bacteria identified as *Lactobacillus* sp. Among the LAB isolates, the highest antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* were shown by isolates of S12, S14, S27, and S24, respectively. These results suggest that LAB from CS, especially isolates S12 and S14, have promising antimicrobial properties and show potential as probiotic candidates that merit further investigation into possible applications in food and health sectors.

#### ABSTRAK

Fermentasi dapat menjadi langkah efektif dalam mengatasi tantangan penanganan pascapanen dan meningkatkan nilai gizi melalui konversi hasil panen menjadi produk yang lebih stabil. Salah satu makanan fermentasi tradisional yang berasal dari Kabupaten Kuantan Singingi, Provinsi Riau, Indonesia adalah *cangkuok semaung* (CS). Makanan ini dibuat dengan cara memfermentasikan seluruh komponen buah *semaung* (*Pangium edule*). Penelitian ini bertujuan untuk mengisolasi dan mengidentifikasi bakteri asam laktat (*lactic acid bacteria*, LAB) dari CS dan mengetahui sifat antimikrobanya. Bakteri diisolasi dari makanan, lalu dimurnikan menggunakan metode kuadran guratan pada agar De Man–Rogosa–Sharpe, dan dilanjutkan dengan analisis morfologi dan evaluasi aktivitas antimikroba. Hasil penelitian ini menunjukkan bahwa 45 isolat merupakan bakteri gram positif yang diidentifikasi sebagai *Lactobacillus* sp. Isolat LAB yang menunjukkan aktivitas tertinggi terhadap *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, dan *Klebsiella pneumoniae* masing-masing adalah S12, S14, S27, dan S24. Hasil penelitian ini menunjukkan bahwa LAB dari CS, khususnya isolat S12 dan S14, memiliki sifat antimikroba yang menjanjikan dan menunjukkan potensi sebagai kandidat probiotik yang layak untuk diselidiki lebih lanjut untuk kemungkinan aplikasi di sektor pangan dan kesehatan.

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## INTRODUCTION

*Cangkuok semaung* (CS) is a fermented dish from Kuantan Singingi Regency, Riau Province, Indonesia, that is made by fermenting the fruit of the *semaung*, *kepayang*, or *keluak* (*Pangium edule*). The *semaung* tree is prevalent in various tropical regions, including Micronesia, Melanesia, and Southeast Asia, with Indonesia being one of its habitats (Bintsis, 2018). The flesh of the *semaung* seed primarily comprises carbohydrates, water, fat, protein, and a limited quantity of minerals and vitamins. *Semaung* is also reported to have the ability to prevent oxidative stress that causes senescence due to its antioxidant activity from phenolic groups and conjugated double bonds. Therefore, *semaung* has the potential to be developed as an anti-senescence agent in the form of cosmetic preparations that have local effects, such as hydrogels that are easy and safe to use in the body (Wardani et al., 2021).

The CS is currently becoming scarce due to the younger generation's diminished interest in its tangy flavor; however, it is noteworthy for its abundance of lactic acid bacteria (LAB) and is a potential source of probiotics. When consumed in sufficient amounts, probiotics can help the host's health by balancing the microbes in the body (Somashékaraiah et al., 2019). LAB are bacteria commonly used as probiotics, which convert carbohydrates into lactic acid. These bacteria are gram-positive, non-spore-forming, and exhibit either a spherical (coccus) or rod-shaped morphology (Bintsis, 2018). LAB encompass various genera such as *Lactobacillus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, and *Sporolactobacillus*. *Lactobacillus* is a member of the LAB and occurs naturally in raw foods and feeds, and it is also a part of the digestive tract of most mammals (Huys et al., 2013). The characterization of LAB with probiotic potential involves being innocuous, capable of surviving during processing and storage, exhibiting antagonistic effects against pathogenic bacteria, tolerating gastric acid, pancreatic juice, and bile, and having the ability to safeguard the host epithelium.

Fermented food refers to foods or beverages created through regulated microbial growth and alterations in food components facilitated by enzymatic activity (Dimidi et al., 2019). In the production of CS, fermentation plays a key role in reducing the natural toxicity of *Pangium edule* seeds, while also enhancing the sensory properties such as flavor and texture. Generally, fermented products have three main pathways in the manufacture and development of flavors: glycolysis, lipolysis, and proteolysis (Bintsis, 2018). The natural lactic acid fermentation that happens when indigenous LAB are present lowers the pH, making it harder for microorganisms and pathogens to grow. Adding turmeric and lemongrass leaves not only gives CS its unique smell and taste, but it may also have antimicrobial and antioxidant effects because of the bioactive compounds they contain. These herbal ingredients may work together to help good LAB grow while keeping bad microbes from growing during fermentation.

In previous studies, isolation and investigation of LAB from various foods have been conducted. Cho et al. (2013) successfully isolated LAB from the traditional Korean dish, Kimchi, obtaining 106 LAB isolates from multiple regions in Korea. Hwanhlem et al. (2011) conducted a study on LAB isolation from the traditional Thai fermented fish, *Plasom*, across different fermentation periods, identifying 133 LAB isolates out of 138. On the other hand, Indonesia has numerous fermented foods; however, many still need to be explored regarding LAB, such as CS. This food is rich in nutrients and probiotics, but the lack of attention from the public and the next generation has led this food to close to disappearing. Despite the growing interest in traditional fermented foods as sources of functional microbes, CS remains poorly studied in scientific literature. Moreover, the use of turmeric and lemongrass during fermentation may create a unique microenvironment that influences LAB composition and activity.

Therefore, this study aimed to isolate and identify LAB from traditional fermented food CS and investigate their antimicrobial ability against *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. The isolation and characterization of LAB from this unique indigenous food are expected to contribute to the discovery of novel probiotic candidates with potential applications in the development of functional foods and natural antimicrobial agents for food preservation.

## MATERIALS & METHODS

### *Materials*

Samples of CS were collected from a traditional household producer in Kuantan Singingi Regency, Riau Province, Indonesia. A total of 5 samples were obtained, each weighing approximately 200 g. Samples were aseptically transferred into sterile, tightly sealed polyethylene bags and stored in a cooler box with ice packs ( $4\pm 2^{\circ}\text{C}$ ) to maintain their microbiological integrity during transport. All samples were processed within 6 h after collection in the microbiology laboratory under aseptic conditions.

De Man-Rogosa-Sharpe (MRS) and Nutrient Broth (NB) media,  $\text{H}_2\text{O}_2$  30%, and ethanol 96% EMSURE® Reag. Ph Eur were purchased from Merck (Darmstadt, Germany); while NaCl for molecular biology and Lugol's iodine were obtained from Himedia (Pennsylvania, USA). D-(+)-glucose, D-lactose monohydrate, D-(-)-fructose, and sucrose (all from Sigma-Aldrich, USA) were used as carbon sources in fermentation assays.

### *Isolation and screening of LAB from CS*

The isolation and screening of LAB from CS followed the procedure from Saryono et al. (2023). The sample was weighed as much as 1 g and placed in a test tube, and 9 mL of physiological NaCl solution ( $10^{-1}$ ) was added. Subsequently, the culture was then diluted until  $10^{-5}$ . From this dilution range ( $10^{-3}$  to  $10^{-5}$ ), 100  $\mu\text{L}$  of the culture solution was extracted and spread onto a petri dish containing MRS media. LAB isolates were incubated at room temperature ( $28\pm 2^{\circ}\text{C}$ ) for 24–48 h under aerobic conditions.

### *Purification of LAB isolates*

LAB colonies cultivated on MRS agar were subjected to purification by the streak plate method until a single colony or separate colonies were obtained (Saryono et al., 2023). Next, the colonies were examined for the shape and movement of the cells under a microscope. If the characteristics of the colonies examined were the same, then the colony was considered pure. In cases where impurities persisted, a re-streaking process was performed on MRS media until a singular colony was attained.

### *Macroscopic and microscopic observation*

Macroscopic assessments involved the examination of colony characteristics such as shape, edge, elevation, and surface (Saryono et al., 2023). A light microscope Olympus CX23 (Tokyo, Japan) was utilized for morphological evaluations of LAB form. Pure colonies exhibited consistent morphological features, appearing either round, single, in pairs, stems, or chains. The purified isolates were also characterized by a Gram test. Initially, 18–20 h-aged bacterial cultures were smeared onto a glass slide with one loop of isolate. The slide was then fixed on the glass by passing it over a Bunsen burner for 5 seconds, followed by a 30-s rinsing with distilled water. After adding 1–2 drops of iodine, the preparations were left for 30 s and rinsed with distilled water. Afterward, the preparations were washed with 90% ethanol and rinsed with running water. LAB was then subjected to 1–2 drops of safranin, left to stand for 1 min, and rinsed with distilled water. The bacteria-containing preparations were dried and examined under a light microscope. A purple color indicated a Gram-positive bacterial cell, while a pink color indicated a Gram-negative.

### *Catalase test*

The LAB isolate from MRS agar was taken as much as 1 loop, then smeared on a glass preparation that had been cleaned with 70% alcohol. The addition of 1–2 drops of 3%  $\text{H}_2\text{O}_2$  was carried to check the catalase activity. If air bubbles formed, then the BAL was positive for the catalase test (Nofiani et al. 2022).

### *Carbohydrate fermentation test*

For the carbohydrate fermentation test, glucose, lactose, fructose, and sucrose were employed. Each carbohydrate source, amounting to 7.5 g, was dissolved in 1000 mL of NB media, and 3–4 drops of phenol red were incorporated. Subsequently, 8 mL of each solution was dispensed into a test tube, and the Durham tube was carefully introduced to

prevent the formation of air spaces. The medium was then sterilized through autoclaving at 121°C and 15 lbs pressure for 15 min. Following sterilization, one loop of a 24-h bacterial culture was inoculated into each test tube containing the medium, and the mixture was incubated for 48 hours. A positive reaction was indicated by a change in the medium color to yellow and/or the presence of gas in the Durham tube. The type of fermentation was determined by the formation of bubbles in the Durham tube. The fermentation was categorized as homofermentative if no air bubbles were observed in the Durham tube (Goa et al. 2022).

#### *Salt resistance test*

A test tube containing 8 mL of MRS Broth (MRSB) media was inoculated with the 24-h bacterial culture was inoculated into. This media was then dripped with 3-4 drops of 6.5% NaCl and incubated at 37°C for 48 h. A positive result was identified by observing a change in the media's clarity, contrasting it with a negative control (Goa et al. 2022).

#### *Temperature resistance test*

Temperature tolerance of LAB isolates was assessed following Reuben et al. (2019) with slight adjustments, by inoculating each isolate into 5 mL of MRS broth, followed by incubation at two different temperatures: 15°C and 45°C for 48 h. The presence of growth was determined based on visual turbidity in the broth, with visible cloudiness indicating a positive result for temperature tolerance at the tested condition.

#### *Antimicrobial activity of LAB*

The LAB antibacterial test was carried out using the disc diffusion method. The LAB isolate was taken and dissolved in 5 mL of sterile distilled water; then, the paper discs were soaked for 15 min with the LAB isolate solution. As a control, another paper disc was soaked in 5 mL of sterile distilled water, while chloramphenicol was used as a positive control. The pathogenic bacteria *E. coli*, *S. aureus*, *A. baumannii*, and *K. pneumoniae* (0.1 mL) were each inoculated on the surface of the NA media using a spread plate and left for 5 min. Next, a paper disc soaked in LAB isolate was placed on the surface of the medium and incubated for 48 h at 37°C. Measurement of the inhibition zone of LAB isolates against pathogenic bacteria followed equation (Verawaty et al. 2020):

$$D_z = \frac{(D_v - D_c) + (D_h - D_c)}{2} \quad (1)$$

where,

$D_z$  = diameter of inhibition zone (mm)

$D_v$  = vertical diameter of the inhibition zone around the paper disc (mm)

$D_h$  = horizontal diameter of the inhibition zone around the paper disc (mm)

$D_c$  = diameter of disc paper (6 mm)

Due to the exploratory nature of this study, no statistical analysis was performed.

## RESULTS & DISCUSSIONS

Besides improving protein digestibility and the nutritional value of plant-based commodities, fermentation plays a broader role in facilitating the transition toward sustainable agricultural systems (Abbaspor, 2024). The environmental advantages of applying fermentation in plant-based food production include significantly reduced greenhouse gas emissions compared to traditional livestock farming, as well as decreased land and water demands (Agrawal et al., 2023). In this context, exploring microbial diversity in traditional fermented foods can support the development of functional foods and natural bio-preservatives, aligning with sustainability goals in agriculture, as reported by Mutamima et al. (2023).

The spontaneous fermentation of CS creates a selective environment that promotes the growth of native lactic acid bacteria (LAB) naturally present on raw materials such as lemongrass, turmeric leaves, and *Pangium edule*. The fruit's initial

carbohydrate content provides a fermentable substrate for LAB metabolism, resulting in lactic acid production and a gradual decrease in pH. This acidification inhibits the proliferation of acid-intolerant bacteria. The predominance of facultative anaerobic LAB is supported by the ambient temperature and the mildly anaerobic conditions during natural fermentation. Additionally, lemongrass and turmeric leaves may enhance microbial selectivity due to their mild antimicrobial and antioxidant properties (Parham et al., 2020). Curcumin and citral, two bioactive compounds found in these herbs, may inhibit spoilage or pathogenic bacteria while encouraging the growth of acid-tolerant LAB. Therefore, the microbial ecology of CS is shaped not only by the substrate composition but also by the presence of local herbs that modulate selective pressure toward LAB enrichment.

*Isolation and identification of lactic acid bacteria*

Following the isolation of bacteria from CS on the MRS media, a total of 45 isolates were obtained. The identification outcomes are presented in Table 1.

**Table 1.** Morphological characteristics of LAB isolates (S1–S45) from CS.

Morphological characterization	Observation
Colony shape	Globular
Colony color	Milky white
Colony edge	Entire
Colony elevation	Convex
Cell morphology	Bacilli
Gram staining	+



**Figure 1.** Gram staining images of selected bacterial isolates from CS: (a) Isolate S3, (b) Isolate S8, and (c) Isolate S9. All images were captured at 40× magnification.

The macroscopic observations revealed that all 45 isolates shared similar features, presenting a round shape, milky white color, flat edges, and convex elevation. Microscopic identification results indicated that all isolates exhibited a bacilli form and showed a purple color and, thus, were gram-positive (Figure 1). Thus, based on macroscopic and microscopic morphology, all the isolates were identified as *Lactobacillus* sp. One limitation of the present study is the reliance on morphological and biochemical methods for LAB identification. Even though these tests provide helpful initial information, they are insufficient for conclusive species-level classification, especially in the diverse *Lactobacillus* genus, where many species share phenotypic characteristics. Future work should incorporate molecular techniques such as 16S rRNA gene sequencing to confirm and extend the identification of the isolates.

One of the most important and well-known genera in the LAB group is *Lactobacillus*. (Heczko et al., 2024). *Lactobacillus* genera are Gram-positive, non-spore-forming, catalase-negative, and generally rod-shaped bacteria widely distributed in

nature and play a crucial role in various industrial and biotechnological applications (Mokoena, 2017). These bacteria are known for their ability to produce lactic acid as the primary end-product of carbohydrate fermentation, which contributes to the food industry, particularly in the dairy sector. The metabolic activity of lactic acid bacteria, particularly *Lactobacillus* spp., which are essential to fermentation, finds a suitable substrate in the high carbohydrate content of *semaung* by breaking down available sugars, which helps to acidify the product, prevent spoiling microbes, and give CS its distinctively sour flavor. The metabolic activity of *Lactobacillus* during the fermentation of CS not only ensures microbial safety but also plays a crucial role in shaping the product's sensory characteristics. Its distinctive tangy flavor is attributed to lactic acid, while the overall aroma and flavor complexity are enhanced by other fermentation byproducts, such as acetic acid and diacetyl (Kouakou-Kouamé et al., 2023). Additionally, by softening the plant matrix, acidification may affect texture and improve the product's palatability (Gustaw et al., 2021).

#### *Biochemical and physiological characterization*

Biochemical and physiological tests of the isolates became necessary characterization steps for LAB claims, which in this study were carried out by testing catalase, salt resistance, and carbohydrate fermentation. The catalase test helps differentiate LAB by checking for the presence of the enzyme catalase produced. Additionally, assessing salt resistance and carbohydrate fermentation profiles aids in understanding the adaptability and metabolic capabilities of the isolates, ensuring accurate identification and functional potential. The results of biochemical and physiological tests of LAB isolates can be seen in Table 2.

The catalase test results confirmed that all isolates are catalase-negative, evidenced by the absence of gas bubbles upon the addition of 10% H<sub>2</sub>O<sub>2</sub> to the medium. This negative catalase result aligned with LAB characteristics (Khushboo et al. 2023), signifying the absence of this enzyme in LAB that prevented the breakdown of H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O. Salt testing at a concentration of 6.5% indicated that all isolates could thrive in high osmotic NaCl concentrations. Bacterial cells will experience a loss of turgor pressure when cultivated in a high salt concentration, which affects the cells' physiology, enzyme activity, water activity and metabolism. Some cells address this condition by regulating the osmotic pressure between the inside and outside of the cell. Previous studies report that strains of lactococci (Uguen et al., 1999) and lactobacilli (Glaasker et al., 1998) performed decreased growth rate with increasing medium osmolarity. Uguen et al. (1999) also added that an increased amount of osmolyte in the form of glycine betaine in lactococci cells grown in high NaCl concentration might become an adaptive effort to deal with increasing external osmotic pressure. The same mechanism could also apply in this study, where all the lactococci isolates grew at higher NaCl concentrations. In commercial fermentation, LAB with high osmo-tolerance could resist the number of free acids converted into salt after alkali neutralization to prevent the dramatic pH decrease (Menconi et al. 2014). Stress-tolerant LAB strains provide practical advantages, including improved fermentation efficiency, greater product stability, and enhanced overall quality (He et al., 2025).

The results revealed that all the bacterial isolates fermented lactose, sucrose, glucose, and fructose homofermentatively. The carbohydrate fermentation test revealed that all isolates were homofermentative, evidenced by the change in medium color from red to yellow, confirming the occurrence of glucose fermentation to produce lactic acid, especially under anaerobic conditions. Homofermentative bacteria metabolize carbohydrates via the hexose diphosphate pathway. During the dehydrogenation process of glyceraldehyde phosphate, hydrogen is split and transferred to pyruvic acid, resulting in 90-95% lactic acid and small amounts of succinic acid, malic or other organic acids, alcohols, and aromatic substances can be formed (Gunkova et al. 2021). These findings aligned with the research of Nursyirwani et al. (2018), which reported a homofermentative activity of LAB isolates of the *Lactobacillus* genus. Subsequently, as many as 39 of the total 45 LAB isolates obtained could endure a temperature of 15°C, while 38 isolates showed their growth at 45°C. The LAB ability to grow at low temperatures indicates that the bacteria can still survive during cold storage. On the contrary, resistance to high temperatures means an increase in growth rate and lactic acid production, as well as reducing contamination by other microorganisms (Menconi et al., 2014).

**Table 2.** Biochemical and physiological properties of LAB isolates from CS.

Isolate code	Biochemical and physiological tests							
	Catalase	Salt	Carbohydrate fermentation				Temperature resistancy	
			Lactose	Sucrose	Glucose	Fructose	15 °C	45 °C
S1	-	+	Ho	Ho	Ho	Ho	+	+
S2	-	+	Ho	Ho	Ho	Ho	-	-
S3	-	+	Ho	Ho	Ho	Ho	+	-
S4	-	+	Ho	Ho	Ho	Ho	+	+
S5	-	+	Ho	Ho	Ho	Ho	+	+
S6	-	+	Ho	Ho	Ho	Ho	+	+
S7	-	+	Ho	Ho	Ho	Ho	+	+
S8	-	+	Ho	Ho	Ho	Ho	-	-
S9	-	+	Ho	Ho	Ho	Ho	+	+
S10	-	+	Ho	Ho	Ho	Ho	+	+
S11	-	+	Ho	Ho	Ho	Ho	+	+
S12	-	+	Ho	Ho	Ho	Ho	-	-
S13	-	+	Ho	Ho	Ho	Ho	+	+
S14	-	+	Ho	Ho	Ho	Ho	+	+
S15	-	+	Ho	Ho	Ho	Ho	+	+
S16	-	+	Ho	Ho	Ho	Ho	+	+
S17	-	+	Ho	Ho	Ho	Ho	+	+
S18	-	+	Ho	Ho	Ho	Ho	+	+
S19	-	+	Ho	Ho	Ho	Ho	+	+
S20	-	+	Ho	Ho	Ho	Ho	+	+
S21	-	+	Ho	Ho	Ho	Ho	-	+
S22	-	+	Ho	Ho	Ho	Ho	+	+
S23	-	+	Ho	Ho	Ho	Ho	+	+
S24	-	+	Ho	Ho	Ho	Ho	+	+
S25	-	+	Ho	Ho	Ho	Ho	+	+
S26	-	+	Ho	Ho	Ho	Ho	+	+
S27	-	+	Ho	Ho	Ho	Ho	+	+
S28	-	+	Ho	Ho	Ho	Ho	+	+
S29	-	+	Ho	Ho	Ho	Ho	+	+
S30	-	+	Ho	Ho	Ho	Ho	+	-
S31	-	+	Ho	Ho	Ho	Ho	+	+
S32	-	+	Ho	Ho	Ho	Ho	+	+
S33	-	+	Ho	Ho	Ho	Ho	+	+
S34	-	+	Ho	Ho	Ho	Ho	+	+
S35	-	+	Ho	Ho	Ho	Ho	+	+
S36	-	+	Ho	Ho	Ho	Ho	+	+
S37	-	+	Ho	Ho	Ho	Ho	+	+
S38	-	+	Ho	Ho	Ho	Ho	+	+
S39	-	+	Ho	Ho	Ho	Ho	+	+

## Biochemical and physiological tests

Isolate code			Carbohydrate fermentation				Temperature resistancy	
	Catalase	Salt	Lactose	Sucrose	Glucose	Fructose	15 °C	45 °C
S40	-	+	Ho	Ho	Ho	Ho	+	+
S41	-	+	Ho	Ho	Ho	Ho	+	+
S42	-	+	Ho	Ho	Ho	Ho	+	+
S43	-	+	Ho	Ho	Ho	Ho	-	-
S44	-	+	Ho	Ho	Ho	Ho	+	+
S45	-	+	Ho	Ho	Ho	Ho	-	-

Note: +: growth; -: no growth; Ho: homofermentative

Overall, biochemical characterization results supported the results of macroscopic and microscopic morphological identification, where all obtained bacterial isolates were in line with the classification of LAB. The isolates specifically exhibited characteristics of *Lactobacillus*, such as being gram-positive, catalase-negative, oxidase-negative, and salt-resistant (Mokoena, 2017). These findings correlated with the research of Rezac et al. (2018), which emphasized that LAB present in fermented foods commonly included bacteria from the genera *Lactobacillus*, *Streptococcus*, and *Leuconostoc* (Rezac et al. 2018). Goa et al. (2011) found 12 LAB isolates in fermented milk (Ergo) from southwest Ethiopia, which belonged to the genera *Lactococcus*, *Lactobacillus*, and *Leuconostoc*. Nofiani et al. (2022) isolated LAB from 3 traditional foods derived from fish, i.e., *buduk*, *jasam*, and *jasam ale-ale*. A total of 12 isolates were identified as *Pediococcus* sp, *Enterococcus* sp, *Streptococcus* sp, and *Lactobacillus* sp.

#### Inhibitory effects of LAB on pathogenic bacteria

Based on the data obtained in Table 3, LAB isolates from CS were potential as probiotics because they could hinder the growth of pathogenic bacteria *E. coli*, *S. aureus*, *A. baumannii*, and *K. pneumoniae*. These findings indicated LAB's release of antibacterial compounds into the extracellular environment, as evidenced by a clear zone formed. The LAB isolates the highest antibacterial activity against *E. coli*, *S. aureus*, *A. baumannii*, and *K. pneumoniae* were S12 (11.5 mm), S14 (10.3 mm), S27 (7.1 mm), and S24 (5.6 mm), respectively. The inhibitory abilities of LAB isolates against *E. coli* and *S. aureus* were classified as strong since it was in range (>10-20 mm), while for *A. baumannii* and *K. pneumoniae* it was classified as moderate, falling within the medium range (5-10 mm) (Goa et al. 2022). It should be noted that the current study did not include statistical analysis to assess the significance of differences in antimicrobial activity between LAB isolates. As this was an exploratory study, the results are presented in a descriptive manner based on inhibition zone diameters. Although the findings provide valuable preliminary insights, future studies should include biological replicates and appropriate statistical tests to determine if the differences between isolates are statistically significant.

This difference may be attributed to the varying susceptibility of these pathogens to LAB-derived antimicrobial compounds. *A. baumannii* and *K. pneumoniae* are known for their intrinsic resistance mechanisms, including efflux pumps, outer membrane impermeability, and biofilm formation, which reduce the effectiveness of antimicrobial peptides and acidic stress (Kyriakidis et al., 2021; Huy, 2024). Also, LAB metabolites like bacteriocins work better against Gram-positive bacteria like *S. aureus* because they can get to the peptidoglycan cell wall more easily. On the other hand, Gram-negative bacteria like *K. pneumoniae* and *A. baumannii* have an extra outer membrane that can act as a barrier (Simons et al., 2020). This study, however, found that *E. coli* had a large inhibition zone. This suggests that some LAB isolates may also make antimicrobial metabolites other than bacteriocins, like organic acids or hydrogen peroxide, that can get through Gram-negative barriers when conditions are favorable.

Differences in the amount of lactic acid produced probably caused the difference in antimicrobial activity of these six isolates. LAB exert antimicrobial effects through the production of organic acids, particularly lactic and acetic acid, which lower the pH and create an inhospitable environment for many pathogens. These acids disrupt bacterial membrane potential and interfere with nutrient transport, especially in Gram-negative bacteria whose outer membrane is sensitive to acid-induced permeability changes. Bacteriocins—ribosomally synthesized antimicrobial peptides—are more effective against Gram-positive bacteria, as they can directly penetrate the peptidoglycan-rich cell wall and form pores in the cytoplasmic membrane, leading to cell death. Nofiani et al. (2022) found that LAB typically releases antimicrobial compounds such as organic acids, particularly diacetyl lactic acid, hydrogen peroxide, or protein bacteriocins. These results align with several prior studies reporting the inhibitory effect of LAB on pathogenic bacteria. Saryono et al. (2023) reported that LAB isolated from *Sarobuong*, another traditional food from Kuantan Singingi prepared from fermented bamboo shoots, inhibited the growth of *E. coli* and *S. aureus* bacteria.

**Table 3.** Antibacterial test of LAB isolates from CS

Isolate code	Clear zone formed against pathogens (mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>A. baumannii</i>	<i>K. pneumoniae</i>
S1	6.4	4.3	-	3.1
S2	6.0	5.5	-	-
S3	3.5	6.2	-	5.3
S4	2.6	3.6	2.4	-
S5	4.7	5.8	1.2	-
S6	6.6	2.4	0.4	-
S7	3.8	5.1	-	-
S8	5.3	6.2	3.8	0.3
S9	4.7	3.8	4.6	-
S10	7.1	5.7	-	-
S11	5.4	5.8	3.2	-
S12	11.5	3.3	6.1	-
S13	5.6	7.0	4.0	1.1
S14	2.4	10.3	2.4	0.2
S15	4.6	2.4	0.5	2.0
S16	-	-	-	-
S17	0.5	0.6	-	-
S18	2.6	2.8	0.7	5.0
S19	4.6	1.2	0.8	-
S20	6.3	4.7	0.3	-
S21	4.1	6.4	-	4.6
S22	4.0	5.2	-	-
S23	5.1	4.5	1.0	0.4
S24	3.7	5.6	0.1	5.6
S25	5.0	6.2	4.1	-
S26	2.7	2.2	-	3.2
S27	4.5	6.3	7.1	0.3
S28	2.4	3.2	-	0.3
S29	3.4	4.8	0.9	-
S30	2.2	5.0	-	-
S31	3.4	3.7	-	-

Isolate code	Clear zone formed against pathogens (mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>A. baumannii</i>	<i>K. pneumoniae</i>
S32	9.7	8.0	-	-
S33	5.2	3.2	0.5	-
S34	5.9	3.0	1.8	2.2
S35	4.2	4.5	-	0.1
S36	1.9	3.2	-	-
S37	6.8	4.6	3.1	3.4
S38	4.9	3.8	2.4	-
S39	6.7	4.7	0.3	-
S40	4.2	6.6	-	-
S41	3.8	4.6	1.3	-
S42	4.5	4.5	-	0.5
S43	1.7	3.5	2.2	-
S44	5.2	2.5	0.6	-
S45	4.2	4.2	-	-

The obtained results indicated weak inhibitory activity against *E. coli* (<5 mm) and categorized *S. aureus* inhibition as weak to moderate (5-10 mm). Yap et al. (2021) conducted a LAB antagonist test against clinical strains of *A. baumannii*, a pathogenic bacterium associated with nosocomial infections and antibiotic resistance. The results revealed that 50% of the high-LAB strains exhibited substantial inhibitory activity, reaching 66%. Savinova et al. (2021) reported the efficient inhibition of *K. pneumoniae* bacterial growth and biofilm formation by LAB. The results show that LAB is thought to produce proteins, especially metabolic enzymes, that control the spread of *K. pneumoniae*.

## CONCLUSIONS

There were 45 bacterial isolates obtained from CS, a traditional fermented dish from Kuantan Singingi Regency, Riau Province, Indonesia. The morphological identification, biochemical and physiological characterization collectively confirmed that all isolates belonged to the LAB group, specifically from the *Lactobacillus* genus. All isolates exhibited the ability to inhibit *E. coli* and *S. aureus* growth with strong efficacy (>10-20 mm), as observed in isolate S12 for *E. coli* inhibition and S14 for *S. aureus* inhibition. In contrast, for *A. baumannii* and *K. pneumoniae*, the majority displayed a moderate inhibitory capacity (5-10 mm). These findings suggest that selected LAB strains, particularly S12 and S14, hold potential applications including food biopreservation or antimicrobial agents and development as probiotic candidates. Further research is recommended to characterize their functional properties, survivability under gastrointestinal conditions, and their efficacy in food systems.

## ACKNOWLEDGEMENTS

This research was funded by the Ministry of Education, Culture, Research, and Technology of Indonesia under the research grant awarded to SS (Contract No. 1636/UN19.5.1.3/PT.01.03/2022).

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