



# Isolation and Antibacterial Activity Test of Lactic Acid Bacteria (LAB) from Pliek U and Pliek U Oil (Minyeuk Brok)

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**Abstract:** Lactic Acid Bacteria (LAB) are a group of microorganisms that play a crucial role in the fermentation of various traditional foods. The presence of LAB in fermented food results in the production of various types of metabolite compounds that can act as antimicrobials. The objective of this study is to isolate and characterise LAB from pliek u and pliek u oil (minyeuk brok), and to determine their potential antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The disc diffusion method was used to test the antibacterial activity. The results showed two LAB isolates from pliek u (IPU2 and IPU7) and one LAB isolate from pliek u oil (minyeuk brok) (IMP1). The colonies are cream-coloured, cocci in shape, with smooth edges and a raised elevation. The cells exhibit Gram-positive cocci and bacilli. Isolate IPU2 has the largest average inhibition zone diameter against *Staphylococcus aureus* (6.35 mm) but does not inhibit the growth of *Escherichia coli*.

**Keywords:** Antibacterial activity; Lactic acid bacteria; Pliek u; Pliek u oil (minyeuk brok)

## Introduction

Coconut (*Cocos nucifera* L.) has been used worldwide for centuries, including in Indonesia. Coconut meat and oil are primarily used as food and medicine. The fat content in coconut meat and oil is a functional component with physiological benefits, particularly as an antimicrobial (Nurliana, 2008). The people of Aceh Province traditionally use fermented coconut meat and oil processed in this way (Agustina et al., 2017). The coconut oil produced is known as simplah oil and pliek u oil (minyeuk brok), while the dregs produced after oil extraction are called pliek u (patarana). Pliek U oil is rich in fatty acids, primarily lauric, myristic, palmitic, and oleic acids, with lauric acid being the most abundant (Earlia et al. 2019)

Pliek u is a food originating from the Aceh region, produced by fermenting shredded coconut meat for 10-22 days. The process of making the product includes fermentation, squeezing, and sun drying. Pliek u is a popular seasoning in Aceh, used for cooking, salads, and

as a chilli sauce (Asril et al., 2019). The production of pliek u involves microbial fermentation of coconut meat. Sofyan et al. (2024) states that Lactic Acid Bacteria (LAB) play a crucial role in the fermentation process of pliek u, affecting its quality.

Khathir et al. (2021) reported that high-quality pliek u has low levels of free fatty acids and moisture, which help prevent rancidity and increase durability. Meanwhile, a low water content results in dry pliek u, which has a shelf life exceeding six months. Khathir et al. (2015) suggest that high water content can lead to fungal and mould infections and support oxidation and hydrolysis.

Lactic acid bacteria (LAB) are a group of microorganisms that play a crucial role in the fermentation of various traditional foods (Putri et al., 2012). LAB have been used by humans for a long time in food processing, resulting in products of higher quality and more favourable characteristics than the raw materials (Wikandari et al., 2012). These bacteria contribute to the distinct features of food products, such

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as aroma, flavour, texture, and longer shelf life. LAB can be isolated from various sources, including cheese, vegetables, fermented meat, and traditional foods, the digestive tract (Fachrial & Harmileni, 2018; Yulvizar et al. 2025; Buntin et al., 2008). Sofyan et al. (2024) research successfully isolated six LAB isolates from pliek u fermented food samples produced at home in Gp. Lambaro Sukon and Meunasah Blang, Aceh Besar.

LAB present in fermented foods produce various metabolites that can act as antimicrobials. Antimicrobials can inhibit or control the growth of unwanted microorganisms, such as pathogenic microbes and food destroyers (Santoso, 2008). LAB isolated from traditional foods has been reported to inhibit the growth of pathogenic microbes. Delvita (2021) research found that five LAB isolates from tempoyak can inhibit the growth of *Escherichia coli*. Kasi et al. (2017) also found that two LAB isolates from sago liquid waste can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. Adetunji and Oluwafemi (2011) isolated one LAB strain from Ogi, a fermented corn product, that inhibits the growth of *Candida albicans*. Based on the above, researchers are interested in isolating LAB from pliek u and pliek u oil (minyemuk brok) and testing their ability to inhibit the growth of pathogenic bacteria.

## Method

Solation was carried out by suspending 1 g of pliek u in 9 ml of sterile distilled water (dilution  $10^{-1}$ ). In addition, 1 ml of the  $10^{-1}$  dilution was taken and reconstituted in 9 ml of sterile distilled water ( $10^{-2}$  dilution). Dilutions were made up to dilution  $10^{-6}$ . Each dilution was taken to 1 ml and grown on MRSA media using the spread plate method. They were then incubated at  $37^{\circ}\text{C}$  for 24 hours. The bacteria that had grown were then transferred to fresh MRSA media using the quadrant scratch cup method to obtain a pure culture, and the culture was incubated again at  $37^{\circ}\text{C}$  for 48 hours. Isolation of pliek u oil (minyemuk brok) was performed by suspending 1 ml of pliek u oil sample in 9 ml of sterile distilled water ( $10^{-1}$  dilution). Subsequent steps were performed using the same method as for pliek u. Macroscopic characteristics were performed by observing the morphology of bacterial colonies, including shape, colour, margin and elevation.

Microscopic characteristics can be determined by Gram staining. Bacterial isolates are collected and placed on a glass slide using an Ose needle, then 0.9% NaCl is dripped onto the slide and dried on a Bunsen burner. The specimen glass was first treated with crystal violet stain for 1 minute, then rinsed with running water. Next, iodine was applied for 30 seconds, followed by a rinse with running water. Then, 96% alcohol was added and

allowed to stand for 15 seconds before being washed away. The final step involved adding safranin, allowing it to stand for 1 minute, washing it again with running water, and then drying the preparation. Once dry, the specimen can be observed under a microscope. If the results show red, the bacteria are Gram-negative. If they show blue or purple, the bacteria are Gram-positive.

### *Catalase Test*

To perform this test, add 2 drops of Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) to a glass slide containing LAB isolates. A positive test is indicated by the formation of gas bubbles, which shows that the LAB isolate produces a catalase enzyme that converts hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) into water ( $\text{H}_2\text{O}$ ) and oxygen ( $\text{O}_2$ ).

### *Growth at Different Temperatures*

A colony of LAB isolate was inoculated into MRSB media and incubated at  $15^{\circ}\text{C}$ ,  $30^{\circ}\text{C}$ , and  $45^{\circ}\text{C}$  for 7 days. Bacterial growth was identified by the formation of sediment on the media. Bacterial growth was identified by the formation of sediment on the media.

### *Growth at Different Salt Concentration*

Colony of LAB isolate was inoculated into MRSB media with 5% and 10% NaCl concentration and incubated at  $37^{\circ}\text{C}$  for 7 days.

### *Preparation of Test Bacterial Suspensions*

The bacteria used in the test were dissolved in 5 ml of 0.9% physiological NaCl and vortexed. The resulting turbidity was then compared to 0.5% McFarland solution ( $1.5 \times 10^8$  CFU/ml). The bacteria were then inoculated into Mueller Hinton Agar (MHA) media by dipping a sterile cotton bud into a test tube containing the bacteria, and then evenly scratching the media in a petri dish (Fachrial et al., 2020).

### *Antibacterial Activity Test*

The antibacterial activity test was conducted using the disc diffusion method (Kasi et al., 2017). The test microorganisms used in this study were *Escherichia coli* and *Staphylococcus aureus*. The positive control for the test bacteria was Ciprofloxacin, while sterile NaCl was used as the negative control. LAB isolates were taken at 3-4 Ose and then dissolved in 5 ml of MRSB media. Paper discs were soaked in the LAB solution for 25 minutes. The disc paper containing LAB isolate, positive control Ciprofloxacin, and negative control containing sterile NaCl was placed on the surface of the media that had been inoculated with test bacteria. The dish was then incubated at  $37^{\circ}\text{C}$  for 24 hours, and observations were made based on the inhibition zone formed. The zone of inhibition was measured with a vernier caliper. The presence of an inhibition zone indicates the absence

of test bacteria growth, and the antibacterial effect is declared positive (Arsy, 2022; Amezquita & Brashears, 2002).

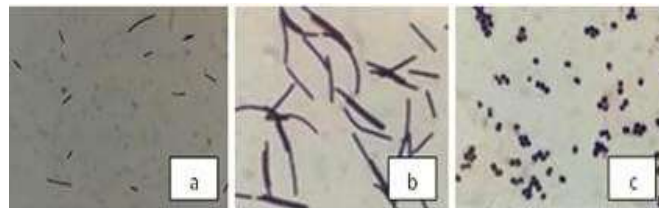
The research parameters included the number of LAB isolates produced, the characteristics of LAB colonies macroscopically and microscopically, and the classification of inhibition zones formed based on Morales et al. (2003) criteria: <5 mm (weak), 5-10 mm (medium), 11-20 mm (strong), and >20 mm (very strong).

## Result and Discussion

Three LAB isolates were obtained through isolation and purification, including two from pliek u (IPU2 and IPU7) and one from pliek u oil (minyeuk brok). All three LAB isolates had cocci-shaped (100%), cream-coloured (100%) colony morphology with smooth edges (100%) and convex elevation (100%). This is consistent with the findings of Sine et al. (2017), who obtained two LAB isolates from Gude seeds soaked for 24 hours, with round colony morphology, smooth edges, and convex surfaces. Giyatno et al. (2020) found that bacterial strains isolated from pliek u and pliek u oil (minyeuk brok) within the same species exhibited different cell morphologies due to genetic diversity. The presence of different LAB species in fermented products varies based on factors such as the type of substrate, processing technology, and even the breed of raw materials used (Comi et al., 2020). IPU2 and IPU7 were bacillus-shaped, while IMP1 was cocci-shaped and belonged to Gram-positive bacteria. LAB can be classified into two shapes: bacilli (rod-shaped) and cocci (spherical) (Ngasotter et al., 2020). This morphological variation reflects the taxonomic diversity of LAB commonly found in fermented food ecosystems. Mursalin et al. (2017) state that LAB are Gram-positive bacteria, in accordance with this the cell shape of IPU2 and IPU7, which is bacillus-shaped, while IMP1 is cocci-shaped. The results are consistent with the findings of Putriani et al. (2017), who isolated two rod-shaped bacteria and one cocci-shaped bacteria from fermented cocoa beans. Aisyah et al. (2014) also found three coccus-shaped and four rod-shaped LAB isolates in tempoyak, a fermented food. According to Rahmah et al. (2021), cocci-shaped LAB belong to the *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Enterococcus*, and *Pediococcus* genera, while the rod-shaped ones belong to the *Lactobacillus* genus.

The study results show that the three LAB isolates were catalase-negative, as evidenced by the absence of gas bubbles (Purwandani & Rahayu, 2003). LAB are indeed Gram-positive, catalase-negative microorganisms (Mokoena et al., 2021). This finding is consistent with the research conducted by Laily et al. (2013), who obtained 10 LAB isolates from salted

mustard greens, all of which were catalase-negative. Similarly, Ulfa et al. (2019) obtained six LAB isolates from the typical Batak traditional food 'Naniura' with negative catalase results. Choesri et al. (2013) found that LAB characteristics generally react negatively to catalase.



**Figure 1.** Gram staining results at 1000x magnification of Lactic Acid Bacteria (a) IPU2; (b) IPU7; (c) IMP1

The study found that the three LAB isolates grew at 15°C, 30°C, and 45°C. The purpose of this test was to determine the ability of LAB to grow at different temperatures. The best growth was observed at 30°C. These three LAB strains (IPU2, IPU7, and IMP1) belong to the mesophilic bacteria group. Surono (2004) classified LAB into two groups: mesophilic, with an optimum growth temperature of 25°C and a maximum temperature of 37°C-40°C, and thermophilic, with an optimum growth temperature of 37°C-45°C and a maximum temperature of 37°C-40°C.

The study results indicate that the three LAB isolates grew at 5% and 10% salt concentrations. LAB growth from pliek u and pliek u oil (minyeuk brok) was characterised by the cloudiness of the media and the presence of sediment at the bottom of the test tube. According to Efendi et al. (2017), bacterial growth in a liquid medium can be observed as sediment or as sediment and turbidity. Sediment refers to a collection of cells that gather at the bottom of the tube and disperse again when shaken. LAB demonstrate significant adaptation to their environments, as seen in studies of Indonesian fermented foods, where strains showed resistance to acidic and bile-salt conditions (Sihombing, 2025).

An antibacterial activity test was conducted to determine the effectiveness of LAB isolates in inhibiting the growth of pathogenic bacteria. *Escherichia coli* was used as a representative of Gram-negative bacteria, and *Staphylococcus aureus* was used as a representative of Gram-positive bacteria. The results of the antibacterial activity test indicate that isolate IPU2 does not inhibit the growth of *Escherichia coli*, but it does inhibit the growth of *Staphylococcus aureus*, with a moderate average inhibition zone diameter of 6.35 mm. Isolate IPU7 inhibits the growth of both *Escherichia coli* and *Staphylococcus aureus* bacteria with average inhibition zone diameters of 1.91 mm and 4.8 mm, respectively. Although IMP1 isolate is unable to inhibit the growth of

both *Escherichia coli* and *Staphylococcus aureus* bacteria. LAB produce antimicrobial compounds, such as organic acids, hydrogen peroxide, and bacteriocins (Okoye et al., 2025; Vuyst & Leroy, 2007). This antibacterial activity may be associated with metabolites produced during fermentation, including organic acids and fatty acids, which are commonly present in fermented coconut products such as pliek u oil (Earlia et al. 2019).

It is worth noting that Gram-positive bacteria, such as *Staphylococcus aureus*, are generally more susceptible to the bactericidal effect of LAB. Khairani (2013) and Muharni et al. (2017) state that the cell wall structure of *Staphylococcus aureus* bacteria is simpler than that of Gram-negative bacteria, making it easier for antibacterial compounds to enter the cell. Helmiyati et al. (2010) explain that the cell wall of Gram-positive bacteria is composed of polysaccharides, including peptidoglycan, teichoic acid, and teichuronic acid. Polysaccharide cell walls are more susceptible to denaturation. Compared with *Escherichia coli*, Gram-negative bacteria have a more complex, three-layered cell wall. The outer layer is lipoprotein, the middle layer is lipopolysaccharide, and the inner layer is peptidoglycan. This structural difference makes Gram-negative bacteria less susceptible to several antibacterial compounds. Furthermore, fatty acids such as lauric acid, myristic acid, palmitic acid, and oleic acid identified in pliek u oil have been reported as predominant components with potential biological activity, which may contribute to antimicrobial effects (Earlia et al. 2019).

## Conclusion

A total of three BAL isolates were successfully isolated, consisting of two isolates from pliek u (IPU2, IPU7) and one isolate from pliek u oil (minyeuk brok) (IMP1). The morphological characteristics of the IPU2, IPU7, and IMP1 colonies are round, cream and white in color, with smooth edges and a convex elevation. Meanwhile, the cell morphology of IPU2 and IPU7 is coccus-shaped, while IMP1 is bacillus-shaped. IPU2 is the isolate with the highest antibacterial activity based on the inhibition zone formed (6.35 mm) against the test bacterium *Staphylococcus aureus*. IPU2 has no antibacterial activity against the test bacterium *Escherichia coli*.

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## Author Contributions

Yulvizar, Ismail, and Amalina conceptualizing the study, collecting and analyzing data, and writing the manuscript. Mahzura and Zazila as the supervisor, conducting review,

validation, and ensuring the accuracy of the content. Haq and Fahrinnisa contributes in reviewing and improving the writing of the article.

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## Conflicts of Interest

The author declares no conflict of interest.

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