



Antibacterial Activity of Lactic Acid Bacteria From Chao Pangkep Against The Growth of Pathogenic Bacteria Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

Chao is a traditional fermented food that comes from the Pangkep area. The microorganism that dominates Chao is the lactic acid bacteria (LAB). LAB bacteria belong to a group of probiotic bacteria that have many health benefits. Methicillin-Resistant Staphylococcus aureus (MRSA) bacteria are bacteria that cause infections whose prevalence is increasing. The purpose of this study is to explore LAB from Chao Pangkep as a new source to find LAB that can be used to treat MRSA infection. The research methods carried out were LAB isolate rejuvenation, lactic acid bacterial selection through hemolytic tests and antibacterial activity tests using the agar diffusion method (Kirby and Bauer disc diffusion). The test results showed that three LAB isolates, namely C2, C10, and C11, were γ -hemolytic (non-hemolytic) so they were not pathogenic. The three non-pathogenic isolates were subsequently used as LAB candidates for testing antibacterial activity. Antibacterial activity is shown by the presence of clear zones formed around bacterial colonies. The existence of LAB's ability as an antibacterial is due to the production of metabolite compounds. Therefore, the lactic acid (LAB) bacterial isolate with code C11 has antibacterial activity, so it can be used as a potential candidate to control pathogenic bacteria.

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INTRODUCTION

Indonesia is a country rich in natural resources. This potential has led to the development of a variety of traditional foods. Griana & Kinasih, (2020) stated that almost every island in Indonesia has its own traditions and dishes, each with its own distinct flavors. Fermented food products from various regions in Indonesia include tempeh, oncom, dadih, tape, tauco, tempoyak, brem, minas, and dangke.

One traditional fermented product originating from Pangkajene and the Islands (Pangkep) in South Sulawesi is Chao. This traditional food is known as a fermented fish product. Chao is a fermented product made from fish and rice, then fermented for one week

(Langkong *et al.*, 2019). The chao fermentation process involves adding rice that has been fermented with tape yeast for 24 hours. This distinguishes chao from other traditional fish fermentations (Matti *et al.*, 2019). Matti & Kumalasari, (2017) stated that the microorganisms that dominate chao fermentation are lactic acid bacteria (LAB).

Lactic acid bacteria (LAB) are a type of microorganism or microbe widely distributed in nature and can produce various metabolite compounds. In general, LAB are considered safe and do not cause negative health effects. Bacteria LAB do not produce toxins, therefore they are known as food-grade microorganisms (Afriani *et al.*, 2017). Lactic acid bacteria are probiotic bacteria with numerous benefits, including lowering blood pressure, boosting immunity, and lowering cholesterol (Beltrán-Barrientos *et al.*, 2016). In addition, LAB can produce metabolite compounds with antibacterial properties.

Antibacterial is a compound that controls the growth of bacteria. These compounds kill or suppress the growth of microorganisms. The existence of these properties makes antibacterial compounds very important to be used in treating infectious diseases caused by bacteria. An example of bacteria that can cause infection is *Staphylococcus aureus*. *S. aureus* bacteria are part of the normal flora of the human body but under certain conditions can cause infectious diseases. The most common and potentially fatal *S. aureus* infection is Methicillin-Resistant *Staphylococcus aureus* (MRSA).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a pathogenic bacteria that are resistant to antibiotics, especially the β -lactam antibiotic group. Pristianingrum *et al.*, (2021) explain that there are gene changes in *S. aureus* so that infections caused by MRSA bacteria are difficult to treat. In addition, Hasanpour *et al.*, (2023) stated that methicillin-resistant *Staphylococcus aureus* is a difficult infection to treat. The prevalence of deaths due to MRSA in Indonesia has shown a significant increase since 2017. The prevalence of MRSA in 2015 was 7.69%, decreased to 5.63% in 2016, rose sharply to 10.81% in 2017, and reached a peak of 12.94% in 2018 (Nuryah *et al.*, 2019).

Treatment for bacterial infections is carried out using antibiotics. However, inappropriate (irrational) antibiotic use triggers bacterial resistance. Resistance is a condition that occurs when a drug, such as an antibiotic, is ineffective in controlling or killing pathogenic bacteria in the body. Bacterial resistance is triggered by using antibiotics for too short a period, using too low a dose, misdiagnosing the initial infection, and using antibiotics without a prescription Putra & Kusmiati, (2019) stated that developing new antibacterial agents to control MRSA bacteria is a top priority in the health sector. This situation necessitates effective solutions to address MRSA infections.

Research on the use of lactic acid bacteria (LAB) has been widely explored, but the potential of LAB from the traditional Chao Pangkep food as an antibacterial against MRSA has not been studied, even though this traditional food has the potential to be a new source for discovering LAB with significant potential for health. Several previous studies have shown that LAB isolated from traditional fermented products have antimicrobial effects, which is the basis for this research.

METHODE

This study used a quantitative research design with a Laboratory experimental method to examine the potential of lactic acid bacteria from Chao Pangkep as an antibacterial alternative for Methicillin-Resistant *Staphylococcus aureus* (MRSA). The materials used in this study were De Man, Ragosa and Sharpe (MRS) agar and broth, blood agar, Mueller-Hinton Agar (MHA), distilled water, sheep blood, and disc paper. There were



7 lactic acid bacteria (LAB) isolates used obtained from previous research (Ardeni *et al.*, 2025).

The research procedure began with the rejuvenation of bacterial isolates. The rejuvenation stage of LAB isolates was carried out by culturing LAB on MRS agar media using the streak plate method and growing at 24 °C for 24 hours. Next, LAB selection was carried out through a hemolytic activity test. Seven bacterial isolates had LAB characteristics. The LAB isolates were streaked on blood agar media and incubated at 37 °C for 24 hours (Irma *et al.*, 2018). Selection was carried out on the pathogenic properties of LAB through the hemolytic activity formed by the LAB isolates. The final stage carried out was the antibacterial activity test against the pathogenic bacteria MRSA. The antibacterial activity test method used in this study was the agar diffusion method (Kirby and Bauer disc diffusion). The diffusion method often used for antibacterial analysis (Farhat *et al.*, 2025). The paper discs used were 6 mm in size. The first step was to make MHA (Mueller Hinton Agar) media.

Then the test bacteria or pathogenic bacteria, Methicillin-Resistant *Staphylococcus aureus* (MRSA) were diluted according to the McFarland standard. The McFarland standard is used in antibacterial testing to standardize the turbidity of bacterial suspensions, ensuring the concentration of bacteria used. The McFarland standard value is 0.5 or equivalent to 1.5×10^8 CFU/mL of bacterial suspension (Wardaniati & Gusmawarni, 2021). The standard is prepared by mixing a solution of barium chloride (BaCl₂) and sulfuric acid (H₂SO₄). After that, the test bacteria are taken and mixed with MHA media or by the pour plate method. After solidifying, paper discs soaked in LAB isolate samples are placed on the surface of the MHA media. The positive control used is ciprofloxacin, while the negative control is sterile distilled water.

RESULT

Seven lactic acid bacteria (LAB) isolates were used in the study, coded C2, C4, C9, C10, C11, C14, and C15. These isolates were rejuvenated on MRS agar. Furthermore, the pathogenic activity of the seven LAB isolates was analyzed using a hemolysis test using blood agar with 5% sheep blood added.

Table 1. Results of Pathogenicity Tests On Lactic Acid Bacteria (LAB) Isolates

No	Isolat Code LAB	Hemolysis Test
1	C2	Non Pathogenic (No Hemolysis)
2	C4	Pathogenic (Partial Hemolysis)
3	C9	Pathogenic (Complete Hemolysis)
4	C10	Non-Pathogenic (No Hemolysis)
5	C11	Non-Pathogenic (No Hemolysis)
6	C14	Pathogenic (Partial Hemolysis)
7	C15	Pathogenic (Complete Hemolysis)

The results of the hemolysis activity test showed that the seven BAL isolates showed different hemolytic activity. Lactic acid (LAB) bacterial isolates C2, C10, and C11 are non-hemolysis or cannot lyse blood cells so they can be categorized as non-pathogenic. Hemolysis activity is often used as a rapid method for the initial assessment of the pathogenic properties of bacteria (Greco *et al.*, 2020). The isolates C4 and C14 showed partial hemolysis samples while C9 and C15 included total hemolysis.

An antibacterial activity test was conducted to evaluate the ability of the three LAB isolates (C2, C10, and C11) to inhibit the growth of pathogenic microorganisms, particularly Methicillin-Resistant *Staphylococcus aureus* (MRSA), using the disc diffusion method on Mueller-Hinton Agar (MHA) media. The formation of an inhibition zone is an indicator of LAB antibacterial activity against MRSA pathogenic bacteria.

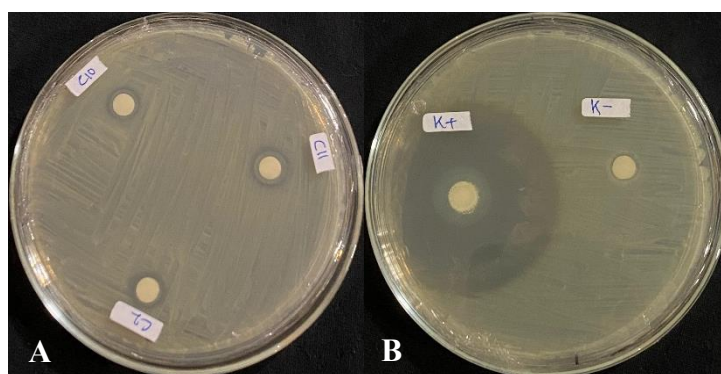


Figure 1. Clear zones formed around the paper discs (A) Lactic Acid Bacteria Isolates and (B) Positive and Negative Control Treatments

Table 2. Measurement Results of the Inhibition Zone Diameter of Pathogenic Bacteria: Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Sample Treatment (LAB isolates & control)	Clear Zone Diameter		Average (mm)	Strength Criteria
	Repetition of the 1	2		
C2 Isolate	2.30	2.70	2.50	Weak
C10 Isolate	3.20	3.50	3.35	Weak
C11 Isolate	5.20	4.90	5.05	Moderate
Positive Control (Ciprofloxacin)	46.40	45.10	45.75	Very Strong
Negative Control (Aquadest)	0.00	0.00	0.00	-

The results showed that all three lactic acid bacterial isolates (C2, C10, and C11) have potential to fight MRSA pathogenic bacteria. This can be seen from the clear zone formed around the disc paper. The size of the clear zone in C2 and C10 isolates was relatively weak, while C11 isolate showed a moderate category of inhibition. The diameter of the inhibition zone for positive control of ciprofloxacin is very strong.



DISCUSSION

Lactic acid bacteria (LAB) play a crucial role in the food fermentation process. LAB are also considered probiotics because they are non-pathogenic and safe for human health. They are also categorized as generally recognized as safe (GRAS), meaning they are safe for consumption (Arrioja-Bretón *et al.*, 2020). The LAB isolates used in this study were C2, C4, C9, C10, C11, C14, and C15. These bacteria were isolated from fermented Chao food from Pangkep (Ardeni *et al.*, 2025).

The seven isolates were selected to determine which LAB isolates had the potential to cause adverse health effects. Pathogenicity testing was performed using the hemolytic activity of the bacteria. Of the seven isolates, three LAB isolates, C2, C10, and C11, showed no hemolytic zone around the bacterial colonies on blood agar media supplemented with 5% sheep blood. This indicates that the three bacterial isolates are γ -hemolytic or non-hemolytic, meaning they are unable to lyse red blood cells. Lactic acid bacteria generally lack hemolytic activity because they do not produce hemolysins (Suryani & Gaffar, 2024). Therefore, these three LAB isolates do not produce toxins like hemolysins, which can break down red blood cells (erythrocytes). Reuben *et al.*, (2020) explain that non-pathogenic lactic acid bacteria can be used as probiotic candidates and can also be applied in industry.

Preliminary analysis of the pathogenicity of bacteria can be found through the results of the hemolysis activity test. The hemolysis test serves as a rapid early indicator to determine the pathogenicity of bacteria in destroying erythrocyte cells (Greco *et al.*, 2020). BAL isolates with codes C4, C9, C14, and C15 indicate the presence of hemolytic activity. The presence of bacterial hemolytic activity is related to bacterial virulence factors (Tao *et al.*, 2022). Total hemolysis (β -hemolysis) indicates the ability of bacteria to produce hemolysin toxins that can destroy the erythrocyte cell membrane, forming a clear zone in the media. Meanwhile, partial hemolysis (α -hemolysis) indicates partial or partial destruction of the erythrocyte membrane, resulting in a green zone that appears around the colony in the media. The differences in hemolytic activity of lactic acid bacteria are influenced by biological and environmental factors (Yumnam *et al.*, 2025).

The selection results of three lactic acid bacteria (LAB) isolates, namely C2, C10, and C11, which showed non-pathogenic characteristics, were then analyzed for their antibacterial activity against the growth of the pathogenic bacteria Methicillin-Resistant *Staphylococcus aureus* (MRSA). Antibacterials are substances that can control the growth of pathogenic bacteria. The three bacterial isolates tested showed the ability of LAB to inhibit pathogenic bacteria. The ability as an antibacterial is seen from the presence of an inhibition zone or clear zone formed around the paper disc (blank disc). The ability as an antibacterial is caused by the production of metabolite compounds produced by LAB. Fatimah *et al.*, (2020) explained that lactic acid bacteria are able to produce antibacterial compounds in the form of lactic acid, hydrogen peroxide, and bacteriocins.

Based on the inhibition zone measurements in Table 2, it shows that lactic acid bacteria isolates have varying inhibitory effects on the growth of the pathogenic MRSA bacteria. The LAB isolate coded C11 has an average inhibition diameter of 5.05 mm, categorized as moderate inhibition. The other two isolates, C2 and C10, have average diameters of 2.50 mm and 3.35 mm, respectively, categorized as weak inhibition. Davis & Stout, (1971) state that the minimum inhibitory concentration criteria for antibacterial power are as follows: an inhibition zone diameter of 5 mm is categorized as weak, an inhibition zone of 5-10 mm is categorized as moderate, an inhibition zone diameter of 10-20 mm is categorized as strong, and an inhibition zone value greater than 20 mm is categorized as very strong. The presence of inhibition shown by lactic acid bacterial isolate with code C11 proves that the isolate is a potential candidate in inhibiting pathogenic bacteria. The ability of lactic acid bacteria as a natural antibacterial agent is shown by the presence of an inhibitory or clear zone formed around the blank disk (Riadi *et al.*, 2020).

The antibacterial activity of LAB isolates has a variety of inhibitions. The results obtained are in accordance with research conducted by Anindita, (2021) that the inhibitory ability of lactic acid bacteria (LAB) varies for each type of LAB isolate and type of pathogenic bacteria. The antibacterial mechanism of lactic acid bacteria is the production of natural metabolite compounds such as lactic acid that can damage bacterial cells and the production of bacteriocins which are antimicrobial proteins that are bactericidal. In addition, lactic acid bacteria are able to inhibit the formation of biofilms from pathogenic bacteria. Bacteriocin activity is able to disrupt cell membrane permeability and pore formation, inhibit bacterial cell wall biosynthesis, and inhibit the main metabolic pathway or essential metabolism of pathogens (El Ahmadi *et al.*, 2025). The results of research by Mgomi *et al.*, (2023) showed that LAB can prevent colonization (initial attachment) of pathogens through the activity of inhibiting the formation of pathogenic bacterial biofilms. Pathogenic biofilms can cause antibiotic-resistant infections. Therefore, lactic acid bacteria (C11) could be a potential candidate for controlling antibiotic-resistant pathogenic bacteria.

CONCLUSION

Lactic acid bacteria (LAB) isolated from the traditional fermented food Chao Pangkep, South Sulawesi, have antibacterial activity. The results of the hemolytic test showed that the three bacterial isolates of lactic acid C2, C10, and C11 were gamma hemolysis (γ -hemolysis) which means that they did not lyse blood cells. Antagonistic activity testing against the pathogenic bacteria Methicillin-Resistant *Staphylococcus aureus* (MRSA) showed that the three selected isolates (C2, C10, and C11) were able to inhibit the growth of the pathogenic bacteria, with isolate C11 producing the largest inhibition zone. This antibacterial activity is thought to originate from metabolite compounds produced by LAB isolates, such as organic acids, bacteriocins, and hydrogen peroxide. Although the inhibitory power shown was still smaller than the positive control (ciprofloxacin), these results indicate that LAB isolates from Chao Pangkep have potential as natural antibacterial agents against resistant pathogenic bacteria. The suggestion in this study is that it is necessary to vary the inhibition concentration and molecular identification of potential isolates of lactic acid bacteria.



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