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Functional Characterization and Antagonistic Activity of Lactic Acid Bacteria Isolates from the Caecum of Broiler Chickens against *Staphylococcus aureus*

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Abstract: The use of Antibiotic Growth Promoters (AGP) in poultry farms increases the risk of antibiotic resistance so alternatives such as probiotics are needed. This study aims to determine the functional characterization and antagonistic activity of Lactic Acid Bacteria (LAB) from broiler chicken caecum against *Staphylococcus aureus*. The test included an antagonistic test using the agar well diffusion method, acid tolerance at pH 1.5, 2,5, and 3,5, amylolytic, proteolytic, and hemolytic activity. The results showed that LAB had antagonistic activity against *S. aureus* with an average inhibition zone diameter of 3.87 mm in the weak category. The LAB isolate showed good survival under acidic conditions with a survival rate of 97.2% at pH 3.5, 94.5% at pH 2.5, and 91.4% at pH 1.5. The amylolytic test showed a negative result, while the proteolytic test was positive and the hemolysis test showed a type of γ-hemolysis, which indicates non-hemolysis. It was concluded that LAB of broiler caecum origin has antagonistic activity against *S. aureus* and is likely to be a potential probiotic candidate. Further research is recommended to conduct genotype-based molecular identification as well as more comprehensive testing of other probiotic characteristics.

Keywords: lactic acid bacteria; chicken caecum; antagonistic; probiotic; *Staphylococcus aureus*

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Introduction

The digestive tract of chickens is a complex organ that houses a variety of microorganisms and plays an important role in the digestion process and absorption of nutrients to support the growth performance of chickens. Imbalances in the gut microbiota due to the use of uncontrolled Antibiotic Growth Promoters (AGP) in the long term can lead to antibiotic resistance (Untari et al., 2021). This makes the digestive tract more susceptible to the growth of pathogenic bacteria that can interfere with the health of poultry (Zhang et al., 2025). The presence of antibiotic-resistant pathogenic bacteria in animals, especially in chickens, can cause infections that are difficult to treat

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and potentially cause death. One of the pathogenic bacteria that is often associated with cases of various infections in humans and animals *is Staphylococcus aureus* (Manyi-Loh et al., 2018).

Staphylococcus aureus is known to be a bacterium that can cause *Staphylococcis* disease in chickens. It can cause infection when physical barriers, such as the skin and mucous membranes, are disrupted or when birds are immunosuppressed (Sato & El-Gazzar, 2025). Previous studies reported *S. aureus* isolation rates of 52.5% in healthy and diseased chickens and prevalence reaching 6.1% in broilers (Abd El-Ghany, 2021). The control of *S. aureus* is increasingly challenging due to the emergence of particularly *methicillin-resistant S. aureus* (MRSA), which have been detected in poultry, animals, and humans (Taylor et al., 2023). One potential control strategy is to utilize beneficial bacteria naturally present in the chicken digestive tract, particularly lactic acid bacteria (LAB) in the caecum, which can inhibit pathogenic bacteria (Zhang et al., 2025).

Lactic acid bacteria (LAB) are indigenous microorganisms that are predominantly found in chicken caecum and have the potential to be developed as probiotic candidates. LAB can inhibit the growth of pathogenic bacteria, including *Staphylococcus aureus*, through the production of antimicrobial compounds, such as organic acids and bacteriocins (Tian et al., 2024). The administration of LAB, particularly the genus Lactobacillus, in chicken feed has also been reported to improve the balance of the gut microbiota, nutrient utilization, immune response, and growth performance of poultry, which supports its potential as a probiotic (Sirisopapong et al., 2023).

Functional characterization is an important stage in the selection and development of lactic acid bacteria (LAB) as potential probiotic candidates. Basic criteria for selecting probiotic candidates include safety for their intended use, the ability to survive gastrointestinal conditions, particularly low pH and bile salts, antagonistic activity against pathogenic bacteria, and the ability to maintain viability during storage (Fachrial et al., 2018). These characteristics serve as preliminary screening parameters to assess the functional potential and safety of LAB isolates before further evaluation of their probiotic efficacy (Binda et al., 2020)

The initial characterization is carried out through several functional and safety parameters. Antagonistic activity was used to evaluate LAB's ability to inhibit the growth of pathogenic bacteria, while acid tolerance tests were used to assess LAB's ability to survive under acidic conditions in the gastrointestinal tract (Tian et al., 2024). Proteolytic and amylolytic assays were performed to evaluate the ability of LAB to produce protease and amylase enzymes that play a role in the hydrolysis of proteins and starches (Adnan et al., 2017), while hemolysis assays were used to assess hemolytic properties as one of the safety parameters of isolates (Yasmin et al., 2020).

Previous research by Rosyunita et al. (2024) has successfully isolated and identified LAB indigenous broiler caecum that is resistant to various types of antibiotics. However, it did not evaluate the antagonistic activity against *Staphylococcus aureus* nor the initial characterization of its probiotic potential. Therefore, this study was conducted to evaluate the antagonistic activity of LAB indigenous caecum broilers against *S. aureus* as well as its initial characterization of its potential as probiotic candidates through proteolytic, amylolytic, hemolysis and acid tolerance assays.

Materials and Methods

Antagonistic Activity Test

The antagonistic activity test used the agar diffusion method to inhibit the growth of pathogenic bacteria. S. *aureus* bacteria were inoculated into Tryptic Soy Broth (TSB) and cultured in an incubator shaker at 37°C at 60 rpm for 16 hours until it reached the logarithmic growth phase of 1×10^8 (CFU)/mL. The Nutrient Agar (NA) media was prepared and sterilized using an autoclave at a temperature of 121 °C for 15 minutes. *S. aureus* bacteria was inoculated into NA media at a concentration of 1% (v/v), mixed evenly, and poured into a petri dish. After the agar had solidified, as much as 200 µL of LAB inoculum was added to a well made on the agar surface and incubated at 37 °C for 48 hours. Once incubation is complete, the diameter of the bacterial growth inhibition zone is measured to assess the antimicrobial activity of LAB (Tian et al., 2024).

Acid Tolerance Test

The acid tolerance tests began with LAB inoculated into MRS broth then incubated at 37°C for 24

hours. The culture was then subcultured in MRS broth and re-incubated for 24 hours. The subculture was centrifuged at 4,000 x g for 5 minutes, the pellets were washed twice in PBS (Phosphate Buffered Saline), and re-suspended in PBS and homogenized in the vortex (Sirisopapong et al., 2023). MRS broth is prepared and the pH of the medium is adjusted 1.5, 2.5, and 3.5 respectively using 0.1 mol/L HCl with a pH meter then sterilized. LAB subculture inoculum (1×10^9 CFU/mL) was grown on MRS broth and incubated at 37°C for 24 hours. After incubation, 1 mL of the subculture was diluted with physiological NaCl 10^4 to 10^6 times. Then, 100 µL of each dilution was spread on a pH-adjusted MRSA media, followed by incubation at 37°C for 24 hours. Furthermore, the colony were expressed as log CFU/mL and the Survival rate (%) of the bacterial colony are calculated by the following formula (Tian et al., 2024)

$$\text{Survival rate (\%)} = \frac{\log \text{CFU/mL(F)} \times 100}{\log \text{CFU/mL(I)}}$$

Description:

Survival rate (%) : The percentage survival rate of bacterial colonies that grew after acid tolerance tests
log CFU/mL(I) : Number of initial bacterial colonies before acid tolerance test
log CFU/mL(F) : Number of final bacterial colonies after acid tolerance test

Amylolytic Test and Proteolytic Test

The amylolytic test was performed to see the ability to hydrolyze starch. Amylolytic activity was carried out by injecting one ose LAB in the *medium of Starch Agar* (SA). The bacterial isolates were incubated for 48 hours at 37°C. Then a few drops *of Gram's iodine* were then added to the bacterial culture, and the clear zone that formed was observed. The proteolytic test was performed to see the ability to hydrolyze casein. Protease activity was carried out with each LAB isolate inoculated into *the Skim Milk Agar* (SMA) media. The bacterial culture was inoculated into the medium and incubated at 37°C for 24 to 48 hours. (Adnan et al., 2017)

Hemolysis Test

The hemolysis test was performed to determine the hemolytic properties of lactic acid bacterial (LAB) isolate in lysing red blood cells. LAB isolate was inoculated on *Blood Agar* media, then incubated for 24 hours at 37°C. After that, observations were made of the clear zone around the colony. The pattern of hemolysis is classified based on α-hemolysis. (partial hemolysis) β-hemolysis (total hemolysis) and γ-hemolysis (non-hemolysis) (Yasmin et al., 2020).

Results and Discussion

Antagonistic Activity Test

Antagonistic activity test is a test that is carried out to find out if a bacterium inhibits the growth of other bacteria through the production of antimicrobial compounds it has. Antagonistic activity was tested using the well diffusion method. Antagonistic activity is shown by the formation of an inhibition zone around the wells due to the diffusion of antimicrobial compounds produced by LAB into agar media so as to inhibit the growth of indicator bacteria (Sarkar et al., 2024).

The results showed that lactic acid bacteria (LAB) of MRSA 10 isolates were isolated from chicken caecum had antagonistic activity against *Staphylococcus aureus*, which was characterized by the formation of clear zones (inhibition zones) around the wells in all triple replication groups (triplo). The mean diameter inhibition zone can be seen in **Table 1**.

Table 1. Antagonistic Test Results

Isolation Code	Average diameter of the barrier zone (mm)	Category
LAB isolate 10	2.08 mm	Weak
	4.73 mm	Weak
	4.81 mm	Weak
Average ± SD (mm)	3.87 ± 1.55 mm	Weak

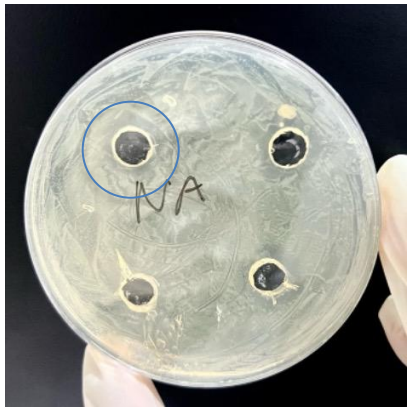


Figure 1. Antagonistic test result of LAB isolate 10 againts *Staphylococcus aureus*

According to Datta et al. (2019) the activity of the inhibition zone is grouped into four categories ≥ 20–30 mm is stated as very strong, 10–20 mm as strong, and 5–10 mm as the medium category. Diameters that reach ≤ 5 mm are considered to have weak inhibiting activity. Based on the measurement results, the diameter of the inhibition zone formed in three repeats was 2.08 mm each; 4.73 mm; and 4.81 mm with an average diameter of the inhibition zone and a standard deviation of 3.87 ± 1.55 mm. From this data, antagonistic activity is classified as a weak inhibition category.

Based on these data, LAB isolate 10 is able to inhibit the growth of *S. aureus*. This ability is influenced by the production of antimicrobial compounds, such as organic acids, bacteriocins, and hydrogen peroxide. Bacteriocins play a role through bactericidal mechanisms and inhibit the synthesis of peptidoglycan which leads to cell wall integrity and leads to cell lysis (Tian et al., 2024). Organic acids in the form of lactic acid play a role in lowering intracellular pH and hydrogen peroxide form oxidative stress that can damage membranes, proteins, and other important components in bacterial cells (Hou et al., 2025)

The mean inhibition zone diameter of 3.87 mm indicates weak antagonistic activity based on Datta et al. (2019). The low antagonistic activity is thought to be related to the low production of antimicrobial metabolite concentrations in inhibiting pathogenic bacteria and the level of metabolite concentrations that diffuse into the media so that they tend to be low (Gajic et al., 2022). Variations in the diameter of the inhibition zone can also be affected by culture conditions, such as pH, temperature, inoculum homogeneity, and incubation length (Balouiri et al., 2016). In addition, the structural characteristics and ability of *S. aureus* as a target bacteria have a defense ability of pH homeostasis that can affect the response to LAB antimicrobial compounds (Zhou et al., 2020).

The results of this study are in line with Tian et al. (2024) which reported that as many as 79 LAB isolates isolated from chicken caecum, 7 of which were from the *Lactiplantibacillus pentosus* R26 and *Lactiplantibacillus plantarum* R32 strains were able to inhibit the growth of *S. aureus* with the diameter of the inhibition zone respectively of 21.11 mm and 22.80 mm which are classified as strong. When compared to Tian et al. (2024), the antagonistic activity of LAB isolate 10 in this study is relatively lower. These differences can be influenced by the genetic characteristics of each LAB strain that determine the metabolic pathway as well as the type and concentration of antimicrobial metabolites produced. In addition, fermentation conditions, such as pH, temperature, and substrate type, can affect gene expression and antimicrobial metabolite production resulting in different antagonistic activity between strains (Dwijastuti et al., 2025).

Antagonistic activity is one of the important characteristics in the selection of probiotics. According to FAO (2002), one of the criteria for microorganisms that can be categorized as probiotics is that they have antimicrobial activity against pathogenic bacteria (Chotiah et al., 2018). LAB possesses this ability through the production of various metabolites, such as bacteriocins, organic acids, and exopolysaccharides, which play a role in inhibiting the colonization of pathogenic bacteria in the intestines. Thus, LAB isolate 10 has the potential to be developed as a probiotic candidate because it is able to inhibit the growth of S. *aureus*. Although, the resulting inhibition was still relatively weak (Tian et al., 2024).

Acid Tolerance Test

The acid tolerance test is a test carried out to determine the ability of bacteria to survive in low pH conditions that resemble the stomach environment, so that its viability can be evaluated during the gastrointestinal tract (Wendel, 2022). This ability is important because LAB must be able to withstand a gastric pH that ranges from 1.5–3.5 due to the secretion of hydrochloric acid (HCl), so that it can reach the

intestine in a living state and provide a probiotic effect for the host (Bawole et al., 2018).

The results showed that the LAB isolate 10 from chicken caecum had good survival ability under acidic pH conditions. The description of the acid tolerance test results is presented in the form of percentage survival rate (%) at various pH levels which can be seen in **Table 2**.

Table 2. Acid Tolerance Test Results

pH	Number of Early Colonies	Log CFU/ mL (l)	Number of Final Colonies	Log CFU/ mL (F)	Survival Rate (%)
7	300	7,47	300	7,47	100
1,5	300	7,26	182	7,26	97,2
2,5	300	7,07	115	7,07	94,5
3,5	300	6,86	73	6,86	91,4

Based on the results of acid tolerance tests, LAB isolate 10 showed good survival under various acidic pH conditions. At normal pH (control), the number of bacteria was 3×10^7 CFU/mL with a log value of CFU/mL of 7.47 and a survival rate of 100%. After treatment at pH 3.5, the number of bacterial colonies decreased to 1.82×10^7 CFU/mL with a log value of CFU/mL of 7.26 and a survival rate of 97.2%. At pH 2.5, the number of bacterial colonies decreased to 1.15×10^7 CFU/mL with a log CFU/mL of 7.06 and a survival rate of 94.5%, while at pH 1.5 the number of bacterial colonies decreased by 0.63×10^7 CFU/mL with a log CFU/mL of 6.86 and a survival rate of 91.4%.

Acid resistance tests are carried out based on viability values or survival rate after exposure to acidic conditions, strain with a viability of ≥80% is categorized as very tolerant to acid, while a viability of ≥50% is still considered resistant to low pH (Zommara et al., 2023). Based on the results of the study, LAB isolate 10 showed good tolerance to acidic environments with a value of survival rates at pH 3.5 97,2%. and pH 2.5 of 94,5%, and pH 1.5 of 91,4% which indicates that LAB isolate 10 are classified as highly tolerant to acidic conditions (Dwijastuti et al., 2025)

The ability of LAB isolate 10 to survive in acidic conditions is related to their ability to maintain pH homeostasis, which is the ability to maintain intracellular pH balance even in an acidic environment. pH homeostasis is important for maintaining metabolic activity, nutrient absorption, and protein and nucleic acid synthesis for cells to function properly. In acidic environmental conditions such as gastric conditions containing HCl, a drastic drop in external pH occurs. LAB maintains pH homeostasis through a proton pumping mechanism by removing H^+ ions from inside the cell so that intracellular pH remains stable (Guan & Liu, 2020).

The results of this study are in line with Tian et al. (2024) who reported that 79 LAB isolates, 2 leading isolates, namely *Lactiplantibacillus pentosus* R26 and *Lactiplantibacillus plantarum* R32, showed survival rates at pH 3.5 of 94.72% and 92.23% then decreased to 88.69% and 89.33% at pH 2.5 and remained at pH 1.5 with survival rates of 86.29% and 87.99%. The difference in survival rates compared with the study by Tian et al. (2024) may suspected to be influenced by the characteristics of LAB which are strain-specific, so that each strain has different physiological adaptability to acidic conditions. In addition, culture conditions, growth phases, inoculum concentration, and testing conditions can affect the survival rate obtained (Mendonça et al., 2023).

A drastic drop in environmental pH increases the concentration of extracellular H^+ ions that encourage the entry of protons into the cell. This condition makes the energy needed to maintain pH homeostasis also greater. When the cell is no longer able to compensate for the entry of H^+ ions, the intracellular pH decreases so that proteins and DNA are damaged which has an impact on the decreased viability of the cell. Exposure to acidic conditions can also damage bacterial cell membranes, resulting in the loss of intracellular components, cell death, so that the overall bacterial population decreases (Yanti et al., 2024)

Tolerance to acidic conditions is one of the important characteristics of probiotic candidates because microorganisms must be able to survive gastric conditions that have a pH of around 1.5 during fasting to 3.0–5.0 after eating in order to reach the intestines in a live state (Unnikrishnan & V, 2025; Fasha et al., 2025). This resistance is necessary because highly acidic gastric fluid can reduce the viability of bacteria, so only microorganisms are able to withstand exposure to acids that have the potential to colonize in the intestines and carry out their biological functions as probiotics (Tian et al., 2024). Thus, LAB isolate 10 shows good acid

tolerance and has the potential to be developed as a probiotic candidate for indigenous poultry.

Amylolytic Test and Proteolytic Test.

The amylolytic test is a test carried out to determine the ability of bacteria to produce the enzyme amylase which can hydrolyze starch into simpler molecules. The test was observed using a medium containing starch and iodine solution (Adnan et al., 2017). Amylolytic activity is indicated by the formation of clear zones around the colony due to the loss of the blue-colored complex between the starch and iodine reactions (Gaenssle et al., 2020).

The proteolytic test is a test to determine the ability of bacteria to produce protease enzymes that can hydrolyze proteins. The test generally uses skim milk media so that the casein contained in it acts as a substrate for protease enzyme activity. Proteolytic activity is indicated by the formation of clear zones around the colony, which suggests that bacterial isolates can secrete protease enzymes (Irsyadah & Santosa, 2024)

The results showed that LAB isolate 10 from chicken caecum did not exhibit amylolytic (negative) activity, but had consistent proteolytic activity (positive) in all three replicates groups (triplo). The results of the amylolytic and proteolytic assays are presented in the form of the enzymatic activity of LAB isolate which can be seen in **Table 3**.

Table 3. Amylolytic and Proteolytic Tests Result

Isolation Code	Amylolytic Activity	Proteolytic Activity
LAB isolate 10	Negative	Positive

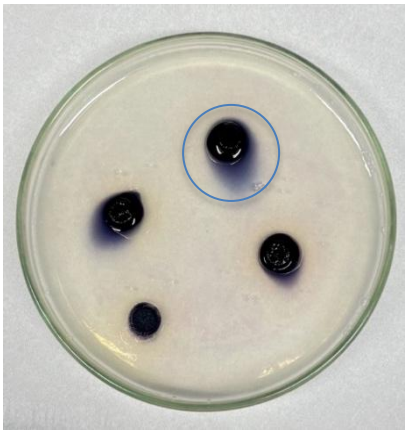


Figure 2. Amylolytic Test Results of LAB isolate 10

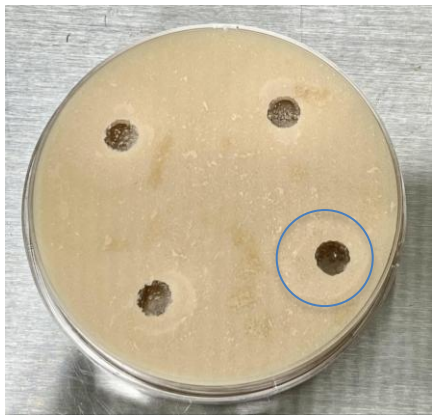


Figure 3. Proteolytic Test Results of LAB isolate 10

Amylolytic activity shows negative results characterized by the absence of clear zones in the media after the addition of iodine solution. The results showed that LAB isolate 10 was unable to produce extracellular amylase enzymes. This enzyme plays a role in hydrolyzing starch into simple sugars, such as maltose, dextrin, and glucose. In LAB, extracellular amylase enzymes belong to the endoamylase group which is able to break the bonds of α-(1-4) and α-(1-6) in starch. The hydrolysis products are then used as a source of carbon to be fermented into lactic acid. However, this ability is strain-dependent, so it is not possessed by all LAB isolates (Auliyah et al., 2023).

The absence of amylolytic activity in LAB isolate 10 is thought to be related to physiological characteristics and differences in gene expression that play a role in the synthesis of amylase enzymes. The variation in gene expression causes each strain of LAB to have a different ability to produce extracellular amylase enzymes. As a result, not all isolates are able to hydrolyze starch into simple sugars that can be fermented into lactic acid (Velikova et al., 2016).

The results of this study are supported by the research of Suciati et al (2016) who reported that LAB WK 28 (*Pediococcus* sp.) and WK 33 (*Lactobacillus* sp.) isolates were isolated from the gastrointestinal tract of mangrove crabs (*Scylla* spp.) did not show amylolytic activity characterized by the absence of clear zones around the colony on starch agar media after the addition of iodine solution. This condition occurs due to the inability of LAB isolate to synthesize extracellular enzymes inside the cell to hydrolyze starch into simpler sugars. The difference in the ability to form clear zones in each bacterial isolate may be influenced by differences

in their ability to utilize starch as carbon source (Suciati et al., 2016).

Proteolytic activity showed positive results characterized by the formation of clear zones formed in each repeat (triplo) with diameters of 1.35 mm, 3.27 mm, and 5.15 mm, respectively, with an average of 3.26 ± 1.90 mm. The results suggest that LAB isolate 10 can produce extracellular proteolytic enzymes that play a role in hydrolyzing casein into simple peptides. The activity of bacterial extracellular enzymes plays an important role in breaking down complex macromolecular compounds, including proteins, into simpler components so that they can be utilized for bacterial growth and metabolism (Adnan et al., 2017).

The proteolytic activity in LAB is also related to its ability to utilize casein as a source of amino acids to support growth and metabolism. This mechanism involves proteinase enzymes, peptidases, and peptide transport systems. Extracellular proteinases bound to the cell wall hydrolyze casein into oligopeptides, then transported into the cell. Furthermore, oligopeptides are hydrolyzed by various peptidases into free amino acids that are utilized as a source of nutrients for LAB (Kieliszek et al., 2021).

The results of this study are consistent with the findings reported by Adnan et al. (2017) who found LAB were isolated from fermentation of brown rice laundry water showed proteolytic activity characterized by the formation of clear zones around the colony in the skim milk agar media. Of the LAB isolate 10 obtained, as many as seven showed positive results on the casein hydrolysis test. The formation of clear zones indicates that the isolate produces protease enzymes that hydrolyze casein into soluble peptides and amino acids. The enzymes are secreted to the surrounding environment so that the proteins in the media are degraded and the appearance of the media changes from cloudy to clear around the colony (Adnan et al., 2017).

The difference in the diameter of the clear zone in each repeat can be influenced by various factors, such as the number of bacterial inoculums, cell metabolic activity, incubation conditions, media composition, and the diffusion ability of protease enzymes in agar media. Environmental factors such as temperature and incubation pH also affect the enzymatic activity of LAB. Optimal incubation temperature will increase the metabolic activity of bacteria so that the production of protease enzymes is maximized (Fachrial & Harmileni, 2022).

One characteristic of probiotics is their ability to produce digestive enzymes that break down complex compounds into simpler molecules thereby increasing nutrient absorption (Auliyah et al., 2023). Although the LAB isolate 10 did not exhibit amylolytic activity, this finding does not preclude its potential as a probiotic candidate because probiotic potential can be demonstrated through various other functional characteristics, namely the presence of proteolytic activity. Thus, the absence of amylolytic activity does not reduce the potential of LAB isolate 10 as probiotic candidates, since the isolates still have proteolytic activity that supports nutrient utilization and is one of the expected functional characteristics in probiotic bacteria (Dwijastuti et al., 2025).

Hemolysis Test

The hemolysis test is a test to identify the hemolytic properties of a bacterium based on its ability to lyze red blood cells (erythrocytes). Hemolytic activity is one of the indicators of safety aspect of an isolate because it is related to the virulence factor of pathogenic bacteria. Based on the pattern of hemolysis, bacteria can be divided into three types, namely alpha hemolysis (α) indicates the occurrence of partial lysis of red blood cells so that hemoglobin turns into methemoglobin which is characterized by the formation of a greenish zone around bacterial growth, beta hemolysis (β) indicates complete lysis of red blood cells accompanied by the degradation of hemoglobin by bacteria which is characterized by the formation of clear zones around the colony, and gamma hemolysis (γ) is characterized by the absence of red blood cell damage or changes in the media around the colony (Harmoko et al., 2025).

The results showed that the lactic acid bacteria (LAB) isolate 10 from chicken caecum in hemolysis testing produced γ-hemolytic activity. This is characterized by the absence of clear zones or discoloration around the colony, which indicates that there is no red blood cell lysis. The hemolysis test results are presented in the form of the type of hemolysis of LAB isolate which can be seen in **Table 4**.

Table 4. Hemolysis Test Results

Isolation Code	Hemolysis Area	Category	Remarks
LAB isolate 10	No zones	γ-hemolysis	Non-hemolysis



Figure 6. Hemolysis Test Results of LAB isolate 10

The results showed that LAB isolate 10 fall into the category of γ-hemolysis, which is characterized by the absence of erythrocyte lysis in the blood medium. This condition indicates that the isolates do not produce hemolysin, which can cause erythrocyte lysis. The absence of hemolysis activity causes erythrocytes in the media to remain intact, so that no clear zones or greenish zones are formed around the colony. Hemolysis activity reflects the presence of virulence factors that are generally possessed by pathogenic bacteria, especially the ability to lyze red blood cells which can negatively impact the host. So that LAB isolate 10 can support the safety aspect of the isolate because it does not have hemolysis activity related to pathogenic bacterial virulence factors (Yasmin et al., 2020).

In the context of probiotic selection, LAB isolate are expected to show non-hemolytic properties as an indicator that the isolate does not have the potential for pathogenicity or cytotoxic activity on red blood cells, so that their safety is more guaranteed for food or health applications (Dwijastuti et al., 2025). Safety is one of the important aspects in the selection of probiotic candidates. According to the Food and Agriculture Organization (FAO) it is recommended that the probiotic strains used must be safe for the host, one of these findings was indicated by the absence of hemolytic properties in LAB isolate. The absence of hemolytic activity supports the safety assessment of the isolate. However, additional safety tests are required to confirm its suitability as a probiotic candidate (Khushboo et al., 2023).

The results of this study are consistent with those of Yasmin et al. (2020) who reported that 8 LAB isolates grown in Columbia Blood Agar media all showed γ-hemolysis type without the presence of α- or β-hemolysis, which indicates that the isolates do not have the ability to lyse red blood cells. This condition indicates that the isolates do not produce hemolysin so they are not pathogenic and have a good level of safety to be developed as a probiotic candidate. Thus, the LAB isolate 10 in this study has show a favorable hemolysis safety profile because it does not exhibit hemolytic activity, thus meeting one of the basic requirements as a probiotic candidate.

Conclusion

Based on the results of the study, LAB isolate 10 from broiler chicken caecum showed antagonistic activity against *Staphylococcus aureus* with a weak inhibition category, as indicated by an average inhibition zone diameter of 3.87 ± 1.55 mm. The isolate exhibited proteolytic activity but did not show amylolytic activity. It was also non-hemolytic (γ-hemolysis) and demonstrated good acid tolerance, with survival rates of 97.2% at pH 3.5, 94.5% at pH 2.5, and 91.4% at pH 1.5. Overall, these functional and safety characteristics indicate that LAB isolate 10 has preliminary potential as a probiotic candidate, although it does not exhibit amylolytic activity. However, further characterization is required, particularly through genotype-based molecular identification, bile salt tolerance testing, and adhesion assays on intestinal epithelial cells to evaluate the suitability of LAB isolate 10 for further development as an indigenous poultry probiotic candidate.

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