

# Screening Probiotics from Genetically Unselected Indonesian Local Chicken to Enhance Meat Quality in Broiler Chicken

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## ABSTRAK

Wardhani R, Afidah U, Syamsurya D, Lee J, Fufa AJ, Gani F, Husain DR. 2025. Skrining probiotik pada ayam lokal Indonesia yang belum terseleksi secara genetik untuk meningkatkan kualitas daging ayam pedaging. JITV 30(3):150-158. DOI:<http://dx.doi.org/jitv.v30i3.3456>.

Penelitian ini bertujuan untuk mengisolasi dan mengkarakterisasi bakteri probiotik dari usus ayam lokal Indonesia (*Gallus gallus domesticus*) yang secara genetik belum terseleksi, untuk meningkatkan kualitas daging ayam pedaging. Ayam kampung berperan sebagai sumber bakteri probiotik yang menjanjikan dikarenakan ketahanan dan kemampuan dalam beradaptasi. Probiotik dipilih menggunakan media MRSA (de Man Rogosa Sharpe Agar) dengan suplementasi 1% CaCO<sub>3</sub>. Potensi probiotik isolat bakteri dievaluasi berdasarkan ketahanannya terhadap keasaman lambung (pH) dan garam empedu. Karakterisasi meliputi morfologi koloni, uji antibiotik menggunakan *Escherichia coli* dan *Salmonella typhi*, dan uji biokimia seperti MR-VP, TSIA, motilitas, dan katalase. Empat isolat bakteri probiotik diperoleh, dengan tiga diantaranya bersifat Gram-positif (J2, J3, and J4) dan satu bersifat Gram-negatif (J1). Semua isolat berbentuk batang (basil), katalase-negatif, tidak motil, dan menunjukkan efek penghambatan terhadap *E. coli* dan *S. typhi*. Isolat J2 menunjukkan aktivitas penghambatan terkuat, dengan zona penghambatan berukuran 15,5 mm terhadap *S. typhi* dan 11,5 mm terhadap *E. coli*. Ketika digunakan sebagai aditif pakan, isolat J2 meningkatkan kualitas daging ayam pedaging dengan mempertahankan pH optimal, meningkatkan kapasitas menahan air, dan mengurangi kadar kolesterol dan lemak, meskipun meningkatkan kehilangan pemasakan. Hasil ini menunjukkan bahwa isolat J2 memiliki potensi signifikan sebagai agen probiotik dalam nutrisi unggas, dengan implikasi yang menjanjikan untuk meningkatkan kualitas daging dan keamanan pangan. Penelitian di masa depan harus fokus pada pengoptimalan formulasi probiotik dan mengeksplorasi efek jangka panjang pada kesehatan dan produktivitas unggas.

**Kata Kunci:** Ayam Lokal, Kualitas Daging, Sumber Probiotics

## ABSTRACT

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This study investigated the isolation and characterization of probiotic bacteria from the intestines of genetically unselected Indonesian local chickens (*Gallus gallus domesticus*) to improve the quality of broiler chicken meat. Due to their resilience and adaptability, Indonesian local chickens serve as a valuable source of probiotic bacteria. Probiotics were selected using MRSA (de Man, Rogosa, and Sharpe Agar) medium supplemented with 1% CaCO<sub>3</sub>. The probiotic potential of bacterial isolates was assessed based on their resistance to gastric acidity (pH) and bile salts. Characterization included colony morphology, pathogenic inhibition assays using *Escherichia coli* and *Salmonella typhi*, and biochemical tests such as MR-VP, TSIA, motility, and catalase. Among the four probiotic bacterial isolates obtained, three were Gram-positive (J2, J3, and J4) and one was Gram-negative (J1). All isolates were rod-shaped (bacilli), catalase-negative, non-motile, and inhibited *E. coli* and *S. typhi*. Isolate J2 exhibited the most potent inhibitory activity, with inhibition zones measuring 15.5 mm against *S. typhi* and 11.5 mm against *E. coli*. When used as a feed additive, the J2 isolate improved broiler meat quality by maintaining optimal pH, enhancing water-holding capacity, and reducing cholesterol and fat content, although it increased cooking losses. These results indicate that the J2 isolate has significant potential as a probiotic agent in poultry nutrition, with promising implications for improving meat quality and food safety. Future studies should focus on optimizing probiotic formulations and exploring the long-term effects on poultry health and productivity.

**Key Words:** Local Chicken, Meat Quality, Probiotics Source

## INTRODUCTION

Genetically unselected Indonesian local chicken (*Gallus gallus domesticus*), commonly known as free-range chicken, possesses several advantages, including its ability to adapt to diverse environments and greater disease resistance compared to modern broiler chickens. Free-range chickens are allowed to roam freely and consume natural food sources available in their surroundings. This natural diet, combined with their robust adaptability, influences the intestinal microflora, leading to the development of a bacterial community that is well-adapted and resistant to the chickens' environment (Husain et al. 2023).

Probiotics derived from free-range chickens have been the subject of extensive research due to their significant potential in enhancing poultry health and productivity. These probiotics, primarily comprising lactic acid bacteria (LAB), have shown remarkable efficacy in improving gut health and growth performance in poultry (Shi et al. 2022; Johnson et al. 2024). Research has demonstrated that LAB isolated from the gastrointestinal tracts of chickens can inhibit the colonization of harmful pathogens, thereby promoting a healthy gut environment (Husain et al., 2023).

Kobierecka et al. (2017) highlighted that certain *Lactobacillus* strains from chicken digestive tracts effectively inhibited the growth of *Campylobacter jejuni*, a common poultry pathogen, thereby enhancing gut health and reducing infection risks (Kobierecka et al., 2017). Additionally, Wang et al. (2023) explored the survival and antibacterial properties of LAB from Tibetan chickens, demonstrating their potential as a natural alternative to antibiotics in broiler chicken diets (Wang et al. 2023). Probiotics play a crucial role in optimizing digestive tract function by fostering the growth of beneficial bacteria and enhancing the activity of digestive enzymes, thereby improving feed digestibility and nutrient absorption (Zhang et al. 2012; Ghodrati et al. 2021). These findings underscore the importance of chicken-derived probiotics in promoting sustainable, health-oriented poultry farming practices.

Probiotics have been shown to positively influence various physiological parameters in broiler chickens, including blood parameters, immune responses, and meat quality (Kim et al. 2017; Bohatko 2023; Purba et al. 2023; Yang et al. 2023). These beneficial microorganisms promote growth and enhance antioxidant enzyme activities, contributing to overall health improvements (Aluwong et al. 2013). Additionally, probiotics are known to boost immune responses, which are critical for broiler immune development, as well as to improve feed conversion efficiency, water-holding capacity, and the oxidative stability of meat, thereby enhancing carcass quality (Khan et al. 2023). Furthermore, probiotics have been reported to alleviate the effects of heat stress in broilers

by increasing antioxidant enzyme activity and reducing oxidative stress (Ogbuagu et al. 2018). They also improve intestinal morphology, which facilitates better nutrient absorption and overall health (Aluwong et al. 2017). In this study, we focus on isolating and characterizing probiotic bacteria from the intestines of genetically unselected Indonesian local chicken (*Gallus gallus domesticus*) to enhance the quality of modern broiler chicken meat.

## MATERIALS AND METHODS

### Isolation of probiotic bacteria

Probiotic bacteria from the intestines of genetically unselected Indonesian local chickens were collected in Luwu Timur Regency, South Sulawesi, Indonesia. The samples were collected from the inner walls of the chicken intestines and placed in sterile physiological saline. This suspension was subjected to a series of serial dilutions. One milliliter of the diluted sample was inoculated onto De Man, Rogosa, and Sharpe (MRS) agar supplemented with 1% calcium carbonate (CaCO<sub>3</sub>). The inoculated plates were incubated at 37°C for 24–48 hours.

### Purification, morphological characterization, and stock culture preparation of probiotic bacteria

Purification was performed by selecting single colonies that exhibited clear zones on MRS agar, indicative of potential probiotic activity. These colonies were incubated at 37°C for 48 hours. The purification process was repeated 2–3 times to ensure the purity of the colonies. The morphology of each purified colony was examined for characteristics such as shape, margin, color, surface elevation, and odor. Purified colonies were then cultured on slant MRS agar to prepare stock cultures for further analysis.

### Acid resistance testing

The resistance of isolates to gastric acidity was assessed using MRS broth (MRSB) adjusted to pH 2.5–3.0 with 0.1 N HCl, simulating stomach conditions. The ability of the bacteria to grow in this acidic MRSB environment indicated positive acid resistance, while the absence of growth indicated a lack of resistance.

### Biochemical characterization and bile salt resistance

Biochemical characteristics were assessed using the Methyl Red Voges-Proskauer (MR-VP) test, catalase test, and Triple Sugar Iron Agar (TSIA) motility test. To

evaluate bile salt resistance, MRSB was supplemented with synthetic bile salts (ox bile) at concentrations of 1% and 5%. Each bacterial isolate from the stock culture was inoculated into the MRSB-bile salts medium and incubated at 37°C for 2–3 hours. Colony counts were conducted before and after incubation to assess bile salt tolerance.

### Inhibitory activity against pathogenic bacteria

The inhibitory potential of the probiotic isolates against pathogenic bacteria, specifically *Salmonella typhi* and *Escherichia coli*, was evaluated using the agar diffusion method on Mueller-Hinton agar (MHA) (Husain et al., 2023). Paper disks were soaked in bacterial supernatant for 15 minutes. One mL suspension of each test pathogen was added to sterile petri dishes, followed by the addition of MHA medium at 45°C, which was then allowed to solidify. The soaked disks were placed on the solidified MHA, and the plates were incubated at 37°C for 24 and 48 hours. The diameters of the inhibition zones were measured to assess the antibacterial activity. Ciprofloxacin was used as a control because of its broad-spectrum antibiotic activity against gram-positive and gram-negative bacteria.

### Broiler chicken feed and the addition of probiotics

The probiotic isolate that performed best in the initial screening was then evaluated for its effect on meat quality in broiler chickens. The starter probiotics were prepared by dilution. Initially, 9 mL of 0.9% NaCl physiological saline was transferred into a test tube and homogenized. Subsequently, a 0.5 mL aliquot of the bacterial stock was drawn using a syringe and added to the dilution tube. The concentration of probiotic bacteria was determined using the Standard Plate Count (SPC) method, achieving a final concentration of  $10^7$  cells/mL. The artificial feed was composed of 30 grams of rice bran, 40 grams of ground corn, and 30 grams of fish meal, a composition sourced from a local farmer. Every

100 grams of the artificial feed received a spray of the probiotic starter. A commercial feed, BP-11, was used as the control.

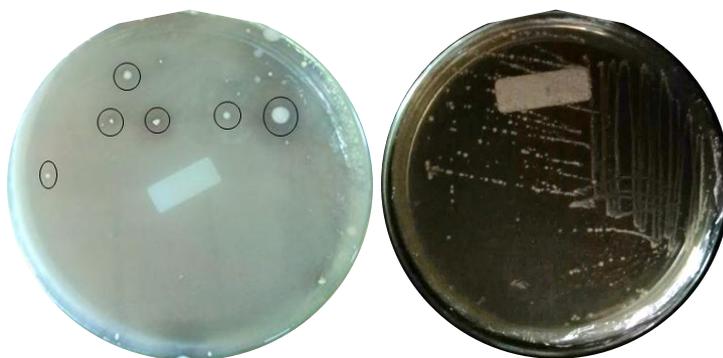
The groups were divided into two: a control group that received feed without probiotic supplementation and a treatment group that received feed supplemented with probiotics. Six broiler chickens per treatment group were fed ad libitum for 6 weeks. The chickens were raised following standard broiler husbandry practices. Meat quality parameters, including color ( $L^*$  for lightness,  $a^*$  for redness, and  $b^*$  for yellowness), pH, water-holding capacity (WHC), cooking losses (%), cholesterol (mmol/L), and fat content (%), were subsequently analyzed.

## RESULTS AND DISCUSSION

### Isolation and purification of probiotic bacteria

Six isolates of lactic acid bacteria (LAB) were isolated. The presence of clear zones around the colonies, as shown in Figure 1, indicates the growth of LAB. These six LAB isolates were further purified to obtain pure bacterial colonies. After three rounds of purification, four distinct bacterial isolates were successfully obtained. The morphology of these four LAB isolates was then observed and documented.

Observations of bacterial colony morphology revealed that all four isolates exhibited distinct colony morphologies. The isolates, labeled as J1, J2, J3, and J4, each displayed a circular shape. J1 and J2 isolates showed smooth colony edges and a convex surface, but J1 was milky white while J2 was clear white. Additionally, both J3 and J4 exhibited smooth colony edges. However, J3 had a clear white colony with flat elevation, whereas J4 had a milky white colony with raised elevation. Furthermore, the results of the bacterial cell morphology, as determined by Gram staining, are presented in Table 1.



**Figure 1.** Morphology of probiotic isolates (left) and purification results of probiotic isolates (right)

**Table 1.** Microscopic observation of probiotic bacteria isolates of genetically unselected Indonesian local chicken after Gram staining

Bacterial Isolate Code	Cell Shape	Gram Type
J1	Bacilli	Negative
J2	Bacilli	Positive
J3	Bacilli	Positive
J4	Bacilli	Positive

Gram staining was performed to identify the Gram type of bacterial isolates, based on differences in cell wall structure. Crystal violet initially stains all bacteria purple, but during the alcohol decolorization step, Gram-positive bacteria retain the stain due to their thicker peptidoglycan layer, while Gram-negative bacteria lose it. Safranin is then applied as a counterstain, which colors Gram-negative bacteria pink, while Gram-positive bacteria remain purple (Becerra et al. 2016). As shown in Table 1, three bacterial isolates were Gram-positive, and one was Gram-negative. The J1 isolate was a Gram-negative bacillus, while the J2, J3, and J4 isolates were Gram-positive bacilli.

#### Probiotic test

Probiotics are typically administered orally, so they must survive the digestive tract, particularly the acidic environment of the stomach, and reach the small intestine alive (Shehata et al. 2016). To assess this, resistance tests were conducted at pH 3 for gastric acidity and with 5% bile salts. The results are presented in Table 2 below. Based on the observations in Table 2, the LAB isolates grew in a medium with a pH of 3 and in a medium containing 5% synthetic bile salts; this is evident from the turbidity and growth of bacterial colonies in the test tubes, indicating that these LAB isolates can survive the acidic conditions of the stomach and reach the small intestine, making them suitable as probiotic bacteria.

According to Jannah et al. (2018), lactic acid bacteria can regulate their cytoplasmic or intracellular pH to maintain near-neutral conditions even when exposed to low extracellular pH (Jannah et al. 2018). The ability of lactic acid bacteria to tolerate bile salts is linked to the activity of the bile salt hydrolase (BSH), which hydrolyzes conjugated bile acids, thereby reducing their bactericidal effect (Cho et al. 2006). Additionally, other studies suggest that the resistance of lactic acid bacteria to low pH and bile salts in the digestive tract may also be due to the role of extracellular polysaccharides (EPSs) (Boke et al. 2010; Jurášková et al. 2022).

#### Biochemical characterization test

Based on the biochemical characterization results shown in Table 3, isolates J1, J2, and J3 tested positive

for the Methyl Red (MR) test, indicating their ability to produce stable acid end-products from glucose fermentation. However, only J1 and J3 tested positive for the Voges-Proskauer (VP) test, while J2 showed a negative result, suggesting that J2 did not produce the fermentation byproduct. All three isolates were negative in the catalase and motility tests, indicating they did not produce catalase and were non-motile. Further analysis using the Triple Sugar Iron Agar (TSIA) test revealed differences in sugar fermentation capabilities among the isolates. Isolates J3 and J4 could ferment all three sugars—glucose, sucrose, and lactose—whereas isolated J2 was only able to ferment glucose. This variability in sugar fermentation profiles among the isolates could affect their metabolic versatility and potential as probiotics.

#### Inhibitory activity of probiotic bacteria against pathogenic bacteria

The ability of LAB isolates to inhibit the growth of pathogenic bacteria can be determined through an antimicrobial susceptibility test. The presence of clear or inhibitory zones indicates the inhibitory effect on pathogenic bacteria. Inhibitory test results of probiotic bacteria isolated against pathogenic bacteria are also presented in Table 4.

The observations in Table 4 indicate that all four bacterial isolates inhibited the pathogenic bacteria *E. coli* and *S. typhi*, as evidenced by clear inhibitory zones around the paper disks. This inhibitory activity is a key characteristic of probiotic bacteria, which are known to produce extracellular antimicrobial compounds such as organic acids (e.g., lactic acid, acetate, propionate, and formate), hydrogen peroxide, diacetyl, and bacteriocins, which collectively inhibit the growth of pathogenic bacteria (Shehata et al. 2016; Haghshenas et al. 2023; Husain et al. 2023).

The specific mechanism by which bacteriocins exert their inhibitory effects involves direct interaction with the bacterial cell membrane. Bacteriocin molecules disrupt the proton motive force (PMF) by creating pores in the bacterial cell membrane, leading to impaired cell growth and eventual cell death (Farha et al. 2013; Arfani et al. 2017). This pore formation is particularly effective

**Table 2.** The resistance of probiotic bacteria isolates to gastric acidity at pH 3 and resistance to bile salt at a concentration of 5%

Bacterial Isolates Code	pH Test	Bile Salt Test
J1	++	++
J2	++	+++
J3	++	+++
J4	+	++

+ = Slight sediment, less turbid; ++ = Moderate sediment, slightly turbid; +++ = Significant sediment, turbid

**Table 3.** Biochemical characterization test of probiotic bacteria isolates

Biochemical Characterization Test	Probiotic Bacteria Isolates			
	J1	J2	J3	J4
MR Test	+	+	+	+
VP Test	-	+	+	+
TSIA Test	<i>Slant</i>	Red	Yellow	Yellow
	<i>Butt</i>	Yellow	Yellow	Yellow
	H <sub>2</sub> S	-	-	-
	Gas	-	-	-
Catalase Test	-	-	-	-
Motility Test	-	-	-	-

(-) = Negative result; (+) = Positive result

**Table 4.** The result of the inhibitory activity of probiotic bacteria

Bacterial Isolates Code	Diameter of Inhibiting Zone (mm)			
	<i>Escherichia coli</i>		<i>Salmonella typhi</i>	
	1x24 hour	2x24 hour	1x24 hour	2x24 hour
J1	4	4	6	6
J2	11.5	11.5	15.5	15.5
J3	12	12	13,5	13,5
J4	6	6	11	11
Ciprofloxacin	18	18	22	22

in preventing the proliferation of harmful bacteria. The results in Table 4 also highlight variability in the size of the inhibitory zones formed by each probiotic isolate. Among the isolates, J3 exhibited the largest inhibition zone against *E. coli* (12 mm), while J2 showed the most significant inhibition against *S. typhi* (15.5 mm). For comparison, the positive control, which utilized tetracycline, produced inhibition zones of 18 mm for *E.*

*coli* and 22 mm for *S. typhi*. The size of the inhibition zone is a direct measure of the isolate's ability to inhibit pathogenic bacteria. According to Li et al. (2016), inhibition zones between 20-25 mm are considered very strong, 15-20 mm as strong, 10-15 mm as moderate, and 5-10 mm as weak (Li et al. 2016).

Overall, the findings suggest that J2, isolated from the intestines of probiotic bacteria obtained from

genetically unselected Indonesian local chickens, was the most effective probiotic in inhibiting the growth of both *E. coli* and *S. typhi*. Although the inhibition zones produced by the J2 and J3 isolates were classified as moderate, their ability to inhibit these pathogenic bacteria demonstrates their potential as effective probiotic agents. The J2 isolate showed the most significant overall inhibitory effect, indicating its promise for further development and application in probiotic formulations.

### Meat quality

Given that the J2 isolate demonstrated strong inhibitory effects, we conducted a further evaluation to assess its impact as a feed additive on meat quality, as seen in Table 5. The result was aligned with Mohammed et al. (2021), who found that probiotic-supplemented broilers have a lower value in lightness, resulting in less pale meat, redness, and yellowness compared to control ( $P < 0.05$ ) (Mohammed et al. 2021). The color quality of the samples remained strong and had not degraded, and the presence of probiotics maintained the pigment's quality. Previous studies have reported that the ultimate pH range in broiler meat (breast) is 5.9 to 6.2 at 15 min after slaughter, while values at  $\leq 5.8$  are considered pate, soft, and exudative meat, and  $\geq 6.3$  is for dark, firm, and dry meat (Ristic & Damme 2013). In this study, the meat pH values of broilers that were given probiotics were higher, around ultimate pH 5.9, while the meat pH values of broilers that were given BP-11 (control) were lower than 5.7, which was defined as PSE (pate, soft, and exudative) meat. Similarly, Cramer et al. (2018) also showed that dietary supplementation with *B. subtilis* increased the  $\text{pH}_{24\text{h}}$  of breast muscle (Cramer et

al. 2018). The probiotics' roles in maintaining meat pH and color could account for the findings in the current studies.

The ionic strength and pH of meat significantly influenced the WHC value of probiotic-supplemented broilers, which was higher than that of BP-11-supplemented broilers. Mohammed et al. (2021) conducted a previous study that yielded a similar result: the probiotic diet increases WHC%, thereby improving meat moisture retention (Mohammed et al. 2021). However, the impact of probiotic supplementation on meat WHC is not always consistent. Zhang et al. (2012) found in another study that supplementing broilers with *Bacillus subtilis* did not affect their water-holding capacity (Zhang et al. 2012).

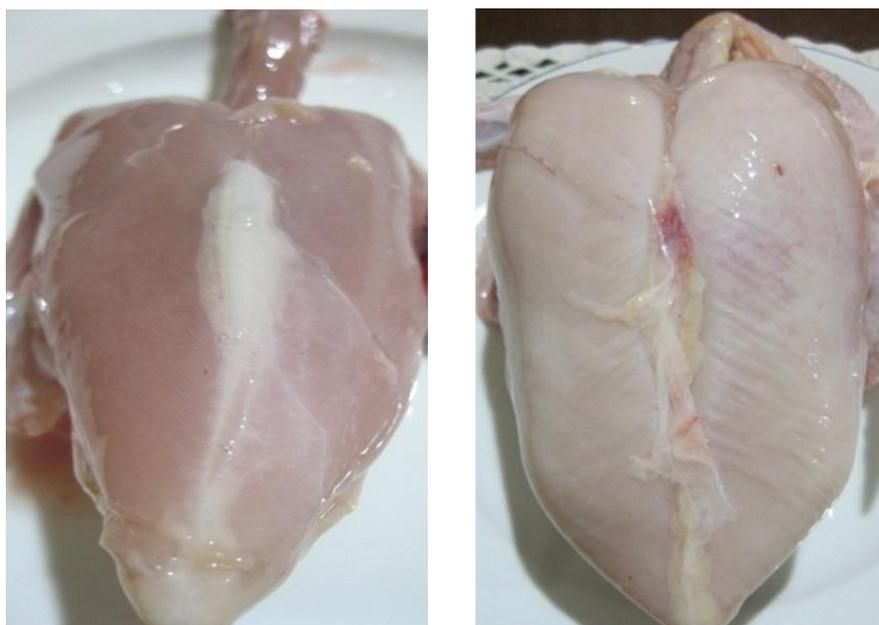
pH and water-holding capacity correlate with cooking loss. Higher pH and WHC values result in reduced meat cooking loss. However, the current findings showed that probiotic-supplemented broilers had higher cooking losses than BP-11-supplemented broilers. Factors that influence meat cooking loss include pH, relative humidity, time, and temperature (Devi et al. 2019). Other factors that affect cooking loss include genetics and the feeding system (Barbanti & Pasquini 2005).

Cholesterol and fat values in probiotic-supplemented broilers were lower than those in BP-11-supplemented broilers. Abdurrahman et al. (2016) conducted a previous study that revealed that the addition of probiotics to broilers' feed significantly reduced the cholesterol value of meat (Abdurrahman et al. 2016). Bacterial growth cells can achieve this phenomenon through assimilation or incorporation into the probiotic microorganism's cell surface, thereby inhibiting the body's absorption of cholesterol (Aluwong et al. 2013).

**Table 5.** Meat quality result of modern broiler chicken, fed a diet supplemented with J2 probiotic bacteria

Meat Quality	Treatment	Control
Color (L*) lightness	44.24±0.34	45.24±0.20
Color (a*) redness	4.46±0.04	4.56±0.04
Color (b*) yellowness	4.14±0.10	4.49±0.08
pH	5.96±0.01	5.55±0.05
Water holding capacity (WHC)	54.12±0.27	52.35±0.84
Cooking losses (%)	25.25±0.39	21.75±1.02
Cholesterol (mmol/L)	3.07±0.06	3.20±0.01
Fat Rate (%)	1.65±0.01	1.79±0.03

Data represented as value±STD; J2= Bacilli shape – gram-positive type of probiotic bacteria, which had the most significant inhibitory effect on *E. coli* and *S. typhi*



**Figure 3.** Broiler chicken meat of the chicken fed diet, A) with probiotic (Treatment), B) without probiotic (control)

### CONCLUSION

The study conducted on the isolation of probiotic bacteria from genetically unselected Indonesian local male chickens in Malakaji Village, Gowa Regency, has yielded four distinct isolates, labeled J1, J2, J3, and J4, each demonstrating notable probiotic characteristics. Among these, the J2 isolate emerged as particularly promising, showing the most significant inhibitory effects against *S. typhi* and *E. coli*, with inhibition zones measuring 15.5 mm and 11.5 mm, respectively. All isolates demonstrated resilience in gastrointestinal conditions, including resistance to gastric acidity at pH 3 and tolerance to 5% bile salts, characteristics essential for probiotic efficacy. The isolates were identified as Gram-positive (J2, J3, J4) and Gram-negative (J1), all of which were rod-shaped, catalase-negative, and non-motile. Moreover, the application of J2 as a probiotic feed additive was found to enhance meat quality by maintaining optimal pH, improving water-holding capacity, and reducing cholesterol and fat content. However, it also led to increased cooking losses, underscoring the intricate balance required in dietary supplementation. These findings highlight the potential of probiotics in enhancing meat quality and underscore the need for further research to refine their application in poultry nutrition.

### CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported. All authors have agreed with the contents of the manuscript.

### AUTHOR'S CONTRIBUTION

The authors confirm their contribution to the paper as follows: study conception and design: DRH; data collection: DS, RW, FG; analysis and interpretation of results: DRH, DS, RW, UA; draft manuscript preparation: RW, UA, AJF, FG, JL.

### ETHICS APPROVAL

This research was conducted at the Microbiology Laboratory, Universitas Hasanuddin, Indonesia. The procedure in this study followed the guidelines for the use of animals (Buchanan et al., 2012).

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