Research Article

Phenotypic Characterization and *In vitro* Screening of Potential Probiotic Lactic Acid Bacteria (LAB) Isolated from the Intestinal Tract of Indonesian Native Chickens

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Abstract | Lactic acid bacteria (LAB) isolated from the gastrointestinal tract of indigenous poultry are considered promising probiotic candidates because of their adaptation to the host intestinal environment. However, information regarding the probiotic characteristics of LAB isolated from Indonesian native chickens remains limited. This study aimed to isolate, phenotypically characterize, and evaluate the *in vitro* probiotic properties of lactic acid bacteria (LAB) isolated from the intestinal tract of Indonesian native chickens. Nine bacterial isolates obtained from the jejunum, duodenum, and ileum were characterized based on colony morphology, Gram staining, catalase activity, and carbohydrate fermentation patterns. The isolates were further evaluated for acid tolerance (pH 2, 3, and 4), bile salt tolerance (1% and 5%), temperature tolerance (20°C, 30°C, and 50°C), and antibacterial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 using the agar well diffusion method. The results indicate that isolates D1 and I1 exhibit promising preliminary probiotic characteristics, as evidenced by phenotypic evaluation and *in vitro* functional assays. Nevertheless, molecular identification, safety assessment, and in vivo evaluation are required before these isolates can be considered for practical application as poultry probiotics.

Keywords | Lactic acid bacteria (LAB), Indigenous chicken, Probiotic, Phenotypic characterization, Antimicrobial activity, Poultry

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INTRODUCTION

The poultry industry plays a crucial role in supplying high-quality animal protein to meet the increasing global demand for food. However, sustainable poultry production continues to face major challenges, including the increasing cost of feed ingredients and the high incidence of infectious diseases, both of which negatively affect production efficiency and profitability (Jha and Berrocoso, 2020; Denli and Demirel, 2018). For decades, Antibiotic growth promoters (AGPs) have been widely incorporated into poultry diets to improve growth performance and feed efficiency. Nevertheless, the prolonged use of AGPs has contributed to the emergence of antimicrobial-resistant bacteria and the accumulation of antibiotic residues in animal products, raising concerns regarding food safety and

public health (Anadón, 2006; Denli and Demirel, 2018). Consequently, many countries have restricted or prohibited the use of AGPs, creating an urgent need for effective and sustainable alternatives for maintaining poultry health and productivity (Jha and Berrocoso, 2020).

Among the available alternatives, probiotics have received considerable attention because of their ability to improve intestinal microbial balance, enhance nutrient utilization, stimulate immune responses, and inhibit the proliferation of pathogenic microorganisms (FAO and WHO, 2002; Jha and Berrocoso, 2020). LAB are among the most extensively studied probiotic microorganisms due to their Generally Recognized as Safe (GRAS) status and their ability to produce antimicrobial metabolites, including organic acids, bacteriocins, and hydrogen peroxide, which contribute to pathogen inhibition and intestinal homeostasis (Papadimitriou *et al*., 2016). However, probiotic properties are strain-dependent; therefore, each LAB isolate must be individually evaluated before being considered a potential probiotic candidate (Ayyash *et al*., 2021).

Host-derived LAB have attracted increasing interest because microorganisms originating from the gastrointestinal tract of the target host generally exhibit better adaptation, colonization potential, and persistence in the intestinal environment than isolates from unrelated hosts or environmental sources (Dunne *et al*., 2001; Ayyash *et al*., 2021). Indonesian native chickens constitute valuable indigenous poultry genetic resources that have adapted to tropical environmental conditions and diverse feeding systems (Balitbangtan, 2018). These unique characteristics may influence the composition and functional diversity of their intestinal microbiota, making them a promising source of indigenous LAB with potential probiotic properties for poultry production.

Although numerous studies have reported the isolation and characterization of LAB from poultry gastrointestinal tracts, information regarding the phenotypic characteristics and probiotic potential of LAB isolated from Indonesian native chickens remains limited (Nguyen *et al*., 2023). Most previous investigations have focused on commercial broiler chickens or evaluated only a limited number of probiotic characteristics. Moreover, comprehensive preliminary screening involving acid tolerance, bile salt tolerance, temperature tolerance, and antibacterial activity has rarely been performed using LAB isolated from Indonesian native chickens. This lack of information limits the development of host-adapted probiotic candidates for sustainable poultry production in Indonesia.

Therefore, the present study aimed to isolate, phenotypically characterize, and evaluate the *in vitro* probiotic properties of lactic acid bacteria (LAB) isolated from the intestinal tract of Indonesian native chickens. Phenotypic characterization was performed based on colony morphology, Gram staining, catalase reaction, and carbohydrate fermentation profiles, whereas probiotic potential was evaluated through acid tolerance, bile salt tolerance, temperature tolerance, and antibacterial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The findings of this study provide preliminary scientific evidence supporting the selection of indigenous LAB as potential probiotic candidates for poultry. Nevertheless, molecular identification and in vivo evaluation are required to further validate the probiotic potential of the selected isolates.

To our knowledge, limited information is available on the isolation and phenotypic characterization of presumptive lactic acid bacteria (LAB) from the gastrointestinal tract of local Indonesian chickens, as well as on the evaluation of their probiotic-related properties. Therefore, this study provides baseline information for the development of indigenous poultry-derived probiotic candidates and serves as a foundation for future molecular identification and in vivo validation.

MATERIALS AND METHODS

Sample collection and preparation

Presumptive lactic acid bacteria (LAB) were isolated from the gastrointestinal tract of Indonesian Local chickens. The isolates obtained were subsequently characterized by phenotypic properties and evaluated for their *in vitro* probiotic potential.

Isolation and purification of lactic acid bacteria (LAB)

Intestinal samples were collected aseptically from the duodenum, jejunum, and ileum of healthy three-day-old Indonesian local chickens that had not received antibiotic supplementation. Each intestinal segment was homogenized in sterile physiological saline (0.9% NaCl). Subsequently, 1 mL of the homogenate was transferred into 9 mL of sterile 10% (w/v) reconstituted skim milk (RSM) and incubated anaerobically at 37°C for 24 h to enrich for LAB growth, following the method described by Malaka *et al*. (2013) with slight modifications. Reconstituted skim milk was used as an enrichment medium because it provides lactose and milk proteins that support the recovery and growth of lactic acid bacteria (Pham and Shah, 2008).

Following enrichment, the cultures were serially diluted in sterile physiological saline (0.9% NaCl). Dilutions of 10^{-7} and 10^{-8} were prepared, and 0.1 mL aliquots from each dilution were spread onto the surface of de Man, Rogosa and Sharpe agar (MRS agar; Oxoid, UK) supplemented with 1% (w/v) calcium carbonate ($CaCO_3$) using a sterile

L-shaped spreader. The inoculated plates were incubated under anaerobic conditions at 37°C for 48 h.

Colonies surrounded by clear halo zones were regarded as presumptive LAB because the organic acids produced during growth dissolved the calcium carbonate incorporated into the medium. Representative colonies with distinct morphological characteristics were selected and purified by repeated streaking on fresh MRS agar until pure cultures were obtained. The purity of the isolates was confirmed by Gram staining, and only Gram-positive isolates with uniform cellular morphology were selected for further phenotypic characterization. Pure cultures were maintained on MRS agar slants at 4°C as working cultures and preserved in 20–30% glycerol at −20°C as stock cultures for subsequent probiotic evaluation.

The purified isolates were further characterized by colony morphology, Gram reaction, catalase activity, motility, and carbohydrate fermentation patterns, following standard microbiological procedures. Only isolates exhibiting characteristics typical of lactic acid bacteria (LAB), including Gram-positive reaction, catalase-negative reaction, non-motility, and the ability to ferment carbohydrates, were selected for subsequent evaluation of probiotic properties.

Phenotypic and biochemical characterization of lactic acid bacteria (LAB)

Purified isolates were subjected to phenotypic and biochemical characterization following standard microbiological procedures (Cappuccino and Welsh, 2017). Colony morphology, including colony shape, elevation, margin, surface appearance, and pigmentation, was observed after incubation on de Man, Rogosa, and Sharpe agar (MRS agar). Gram staining was performed to determine cell morphology and Gram reaction using a light microscope under oil immersion (1000× magnification). Catalase activity was determined by adding one drop of 3% hydrogen peroxide (H_2O_2) to a fresh bacterial colony. The absence of bubble formation was interpreted as a negative catalase reaction, which is a characteristic feature of lactic acid bacteria (LAB).

Motility was evaluated using semi-solid motility medium after incubation at 37°C for 24–48 h. Isolates exhibiting growth only along the stab line were considered non-motile, whereas those showing diffuse growth away from the inoculation line were classified as motile.

Biochemical characterization was further performed using Triple Sugar Iron Agar (TSIA) and Methyl Red–Voges Proskauer (MR–VP) tests following standard microbiological methods (MacFaddin, 2000). TSIA reactions were interpreted based on changes in slant and butt color, gas production, and hydrogen sulfide (H_2S) formation after incubation at 37°C for 24 h. The MR test was carried out by adding methyl red reagent to the incubated culture, whereas the VP test was performed by adding Barritt's reagents A and B. Color development was interpreted according to standard microbiological criteria.

Carbohydrate fermentation profiles were determined using fermentation media containing individual carbohydrates. Fermentation ability was assessed based on color changes of the indicator medium following incubation at 37°C for 24–48 h. Isolates exhibiting Gram-positive reaction, catalase-negative reaction, non-motility, and biochemical characteristics consistent with lactic acid bacteria (LAB) were selected for subsequent evaluation of probiotic properties.

Acid tolerance

The acid tolerance of the isolates was evaluated using MRS broth adjusted to pH 2.0, 3.0, 4.0, and 7.0 using sterile hydrochloric acid (HCl). Fresh cultures of each isolate were inoculated into the respective media and incubated anaerobically at 37°C for 48 h. Bacterial growth was assessed qualitatively based on the presence or absence of turbidity in the culture medium. Isolates exhibiting visible turbidity were considered capable of surviving under the corresponding acidic condition, whereas the absence of turbidity indicated inhibition of bacterial growth. The acid tolerance assay was performed in duplicate.

Bile salt tolerance

Bile salt tolerance was determined by inoculating each isolate into MRS broth supplemented with 1% and 5% (w/v) synthetic bile salts. Cultures were incubated anaerobically at 37°C for 48 h. Bacterial growth was evaluated qualitatively based on the presence or absence of turbidity in the culture medium. The appearance of visible turbidity indicated bacterial growth, whereas a clear medium indicated the inability of the isolate to tolerate the tested bile salt concentration. The bile salt tolerance assay was performed in duplicate.

Temperature tolerance

The temperature tolerance of the isolates was evaluated by inoculating fresh cultures into MRS broth, followed by incubation at 20°C, 30°C, and 50°C for 48 h. Bacterial growth was determined qualitatively based on the presence or absence of turbidity in the culture medium. Isolates showing visible turbidity were considered capable of growing at the respective incubation temperature. The temperature tolerance assay was performed in duplicate.

Antimicrobial activity test

The antibacterial activity of the selected lactic acid bacteria

(LAB) was evaluated using the agar well diffusion method against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Mueller–Hinton agar plates were inoculated with the respective indicator bacteria, and wells of 8 mm in diameter were prepared using a sterile cork borer. Subsequently, 100 µL of fresh LAB culture was introduced into each well. Tetracycline was used as the positive control. The plates were incubated at 37°C for 24 h, after which the inhibition zones were measured using a digital caliper. The antibacterial activity assay was performed in triplicate, and the mean inhibition zone diameter was used for statistical analysis.

Data analysis

Data obtained from the antibacterial activity assay were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) to determine significant differences among treatments at $P < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 19.0 (IBM Corp., Armonk, NY, USA).

This investigation received approval from the Ethics Committee of Hasanuddin University (Protocol No. 052/UN4.12/EC/VI/2026, Approval Date: June 2026). All procedures involving live birds adhered strictly to institutional guidelines governing the care and utilization of laboratory animals, and every reasonable effort was undertaken to minimize potential distress or suffering.

RESULTS

Isolation of lactic acid bacteria (LAB)

Nine bacterial isolates were successfully recovered from the duodenum, jejunum, and ileum of Indonesian local chickens following enrichment in 10% reconstituted skim milk (RSM) and subsequent cultivation on MRS agar supplemented with 1% $CaCO_3$ under anaerobic conditions. Colonies presumed to be lactic acid bacteria (LAB) were selected based on the presence of clear halos surrounding the colonies. Repeated streaking on fresh MRS agar produced morphologically uniform colonies, indicating that pure cultures had been successfully obtained.

The successful isolation of nine presumptive LAB from the gastrointestinal tract of Indonesian local chickens indicates that the intestinal environment provides a suitable ecological niche for the growth of beneficial microorganisms. Enrichment in 10% reconstituted skim milk (RSM) followed by cultivation on MRS agar supplemented with 1% $CaCO_3$ effectively promoted the recovery of acid-producing bacteria. The appearance of clear halos surrounding the colonies resulted from the production of organic acids, primarily lactic acid (LAB), which dissolved calcium carbonate in the medium. This characteristic has been widely used as a preliminary indicator for the isolation of presumptive LAB, although further phenotypic and biochemical characterization is required to confirm their identity (de Man *et al.*, 1960; Cappuccino and Welsh, 2017).

The successful purification of all isolates provided morphologically homogeneous cultures suitable for subsequent probiotic characterization. Indigenous LAB isolated from the intestinal tract are considered promising probiotic candidates because they are naturally adapted to the gastrointestinal environment of the host, which may enhance their survival and functional performance after administration. Previous studies have also demonstrated that host-derived LAB possess considerable potential as alternatives to antibiotic growth promoters in poultry production owing to their beneficial effects on intestinal health and microbial balance (Vieco-Saiz *et al.*, 2019; Papadimitriou *et al.*, 2016).

Phenotypic and biochemical characterization of lactic acid bacteria

The phenotypic and biochemical characteristics of the isolates were consistent with those commonly reported for LAB. The colony morphology characteristics of the presumptive LAB isolates are presented in Table 1 and Figure 1. All isolates were Gram-positive, rod-shaped, catalase-negative, non-motile, and exhibited positive Methyl Red (MR) but negative Voges–Proskauer (VP) reactions as shown in Figure 2. These characteristics indicate that the isolates primarily metabolize carbohydrates through fermentative pathways, producing stable organic acids, particularly lactic acid, as the major end products. The absence of catalase activity further supports their classification as LAB because these bacteria generally lack catalase enzymes and rely on fermentative metabolism for energy production (Papadimitriou *et al.*, 2016; Cappuccino and Welsh, 2017).

Variations in carbohydrate fermentation profiles were observed among the isolates, with most isolates fermenting glucose, while lactose fermentation was detected only in isolate I2, and no isolates fermented sucrose or mannitol. Such differences in carbohydrate utilization are commonly observed among indigenous LAB and reflect strain-dependent metabolic diversity and adaptation to different ecological niches within the gastrointestinal tract. These phenotypic and biochemical properties provide strong preliminary evidence that the isolates belong to the LAB group and represent promising candidates for further probiotic evaluation. However, molecular identification is still required in future studies to determine the isolates at the species level (Papadimitriou *et al.*, 2016; Surono, 2015).

Acid tolerance (pH)

The acid tolerance of the nine presumptive LAB isolates was evaluated by observing bacterial growth in MRS broth adjusted to pH 2, 3, 4, and 7 (Table 2) all isolates maintained detectable growth. Moderate growth (+) was observed at pH 2 and pH 3, whereas good growth (++) was recorded at pH 4. Abundant growth (+++) was observed at pH 7 for all isolates. No variation in growth response was observed among the isolates, indicating that all isolates tolerated acidic conditions within the tested pH range.

Table 1: Colonial morphology features of LAB isolates obtained from the gastrointestinal tract of Indonesian local chickens.

Isolate	Colony morphology			
	Shape	Elevation	Margin	Color
J1	Round	Convex	Entire	White
J2	Round	Convex	Entire	White
J3	Round	Convex	Entire	White
D1	Round	Convex	Entire	White
D2	Round	Convex	Entire	White
D3	Round	Convex	Entire	White to Yellowish
I1	Round	Convex	Entire	White to Yellowish
I2	Round	Convex	Entire	White
I3	Round	Convex	Entire	White to Yellowish

The ability of all presumptive LAB isolates to grow at pH 2, 3, 4, and 7 demonstrates their capacity to tolerate acidic environments, which is an important prerequisite for probiotic microorganisms because they must survive gastric acidity before reaching the intestine. Although bacterial growth was reduced at pH 2 and 3 compared with pH 7, all isolates remained viable under the tested acidic conditions, suggesting the presence of adaptive mechanisms that maintain cellular integrity and physiological functions during acid stress. Similar observations have been reported for poultry-derived LAB, where acid tolerance is considered one of the principal selection criteria for probiotic candidates due to its role in enhancing survival during gastrointestinal transit (Papadimitriou *et al*., 2016; Corcoran *et al*., 2005; Neveling *et al*., 2020). Therefore, the acid tolerance exhibited by the isolates in the present study indicates their potential for further evaluation as probiotic candidates for poultry.

Bile salt tolerance

The bile salt tolerance of the presumptive LAB isolates was evaluated in MRS broth supplemented with 1% and 5% synthetic bile salts (Table 2). All isolates exhibited good growth (++) in medium containing 1% bile salts, whereas no visible growth (–) was observed at 5% bile salts. These findings indicate that the isolates were able to tolerate 1% bile salts but were inhibited by the higher bile salt concentration.

Table 2: Phenotypic characteristics and qualitative growth responses of presumptive lactic acid bacteria (LAB) isolated from the gastrointestinal tract of Indonesian local chickens under different pH values, bile salt concentrations, and incubation temperatures.

Test bio-chemical	Isolate code								
	J1	J2	J3	D1	D2	D3	I1	I2	I3
Gram stain	+	+	+	+	+	+	+	+	+
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Catalase	-	-	-	-	-	-	-	-	-
MR	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-
Gas production	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	-	-	+
Lactose	-	-	-	-	-	-	-	+	-
Mannitol	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-
Temperature									
20 °C	+++	+++	+++	+++	+++	+++	+++	+++	+++
30 °C	+++	+++	+++	+++	+++	+++	+++	+++	+++
50 °C	+	+	+	+	+	+	+	+	+
pH									
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	++	++	++	++	++	++	++	++	++
7	+++	+++	+++	+++	+++	+++	+++	+++	+++
Bile salt									
1%	++	++	++	++	++	++	++	++	++
5%	-	-	-	-	-	-	-	-	-

(+): positive; (-): negative; MR: Methyl Red; VP: Voges Proskauer; Growth intensity was assessed qualitatively based on visual observation of turbidity after incubation, where (+++) = abundant growth, (++) = good growth, (+) = moderate growth, and (–) = no visiblegrowth.

The ability of all presumptive LAB isolates to exhibit good growth in the presence of 1% bile salts indicates a relatively high tolerance to bile stress, which is an important prerequisite for probiotic microorganisms because they must survive exposure to bile salts in the small intestine. In contrast, no visible growth was observed at 5% bile salts, suggesting that this concentration exceeded the tolerance limit of the isolates. Bile salts are known to damage bacterial cell membranes, alter membrane permeability, and disrupt cellular homeostasis, thereby inhibiting bacterial growth at elevated concentrations. Similar observations have been reported in poultry-derived LAB, where isolates generally tolerate lower bile salt concentrations but exhibit reduced growth or complete inhibition as bile salt levels increase (Begley *et al*., 2005; Papadimitriou *et al*., 2016; Neveling

et al., 2020).

Temperature tolerance

The temperature tolerance of the presumptive LAB isolates was evaluated by observing bacterial growth in MRS broth incubated at 20°C, 30°C, and 50°C (Table 2). All isolates exhibited abundant growth (+++) at both 20°C and 30°C, whereas only moderate growth (+) was observed at 50°C. These results indicate that all isolates exhibited growth over a wide temperature range, although bacterial growth decreased at the highest incubation temperature.

The ability of all presumptive LAB isolates to exhibit detectable growth at 20°C, 30°C, and 50°C demonstrates their adaptability to a relatively wide range of incubation temperatures. Abundant growth observed at 20°C and 30°C indicates that these temperatures provide favorable conditions for bacterial metabolism and cell proliferation, whereas only moderate growth at 50°C suggests that elevated temperatures impose physiological stress that limits bacterial growth. High temperatures are known to affect membrane fluidity, enzyme activity, protein stability, and other cellular processes essential for bacterial metabolism. Nevertheless, the ability of all isolates to maintain visible growth at 50°C indicates a certain degree of thermotolerance, which may enhance their stability during processing and storage of probiotic preparations. Similar observations have been reported for poultry-derived LAB, in which growth is generally optimal at mesophilic temperatures but decreases as incubation temperature approaches the upper tolerance limit (Papadimitriou *et al*., 2016; Holzapfel and Wood, 2014; Neveling *et al*., 2020).

Antimicrobial activity

The antimicrobial activity of the presumptive LAB isolates against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 was evaluated using the agar well diffusion method (Table 3).

Significant differences ($p < 0.05$) were observed among isolates against both test bacteria. Against *S. aureus*, isolate D1 produced the largest inhibition zone (21.50 ± 0.50 mm), comparable to the tetracycline control (21.67 ± 0.58 mm), followed by isolates I1 (20.00 ± 3.91 mm), J3 (14.50 ± 1.80 mm), and D2 (14.17 ± 0.58 mm). In contrast, inhibition zones against *E. coli* were relatively small, ranging from 2.33 ± 0.58 to 6.33 ± 1.53 mm, with isolate J2 showing the greatest inhibitory activity. Overall, the isolates exhibited stronger antibacterial activity against the Gram-positive bacterium *S. aureus* than against the Gram-negative bacterium *E. coli*.

Table 3: Antibacterial activity of presumptive lactic acid bacteria isolated from Indonesian local chickens against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

Isolate	Inhibition zone (mm)	
	***S. aureus* ATCC 25923**	***E.coli* ATCC 25922**
Tetracycline	21.67 ± 0.58a	6.50 ± 1.00a
J1	5.33 ± 0.76d	5.83 ± 0.76abc
J2	8.17 ± 0.76c	6.33 ± 1.53ab
J3	14.5 ± 1.80b	3.67 ± 0.58d
D1	21.5 ± 0.50a	5.17 ± 0.29abc
D2	14.17 ± 0.58b	6.17 ± 0.29ab
D3	2.33 ± 0.29e	2.33 ± 0.58e
I1	20 ± 3.91a	5.33 ± 0.58abc
I2	6.67 ± 0.58cd	5.00 ± 0.50bcd
I3	5.33 ± 0.58d	4.67 ± 0.58cd
F- hitung	74,186	8,895
p-value	0.001	0.001

*Note: Values are expressed as mean ± standard deviation (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$) based on one-way ANOVA followed by Duncan's Multiple Range Test (DMRT).

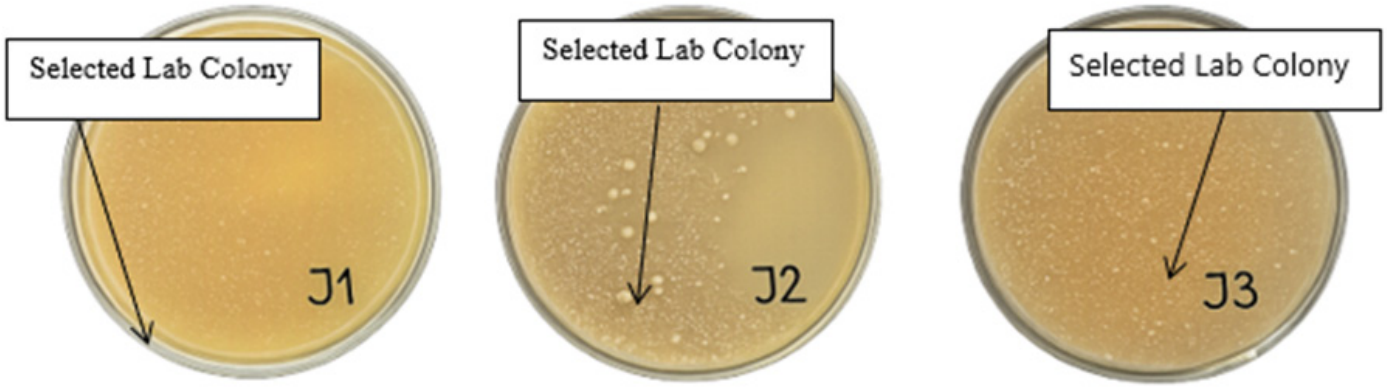

Figure 1: Colony morphology of presumptive actic acid bacteria (LAB) isolated from the gastrointestinal tract of Indonesian local chickens on de Man, Rogosa and Sharpe agar (MRSA) supplemented with 1% $CaCO_3$. Clear halos surrounding the colonies indicate acid production.

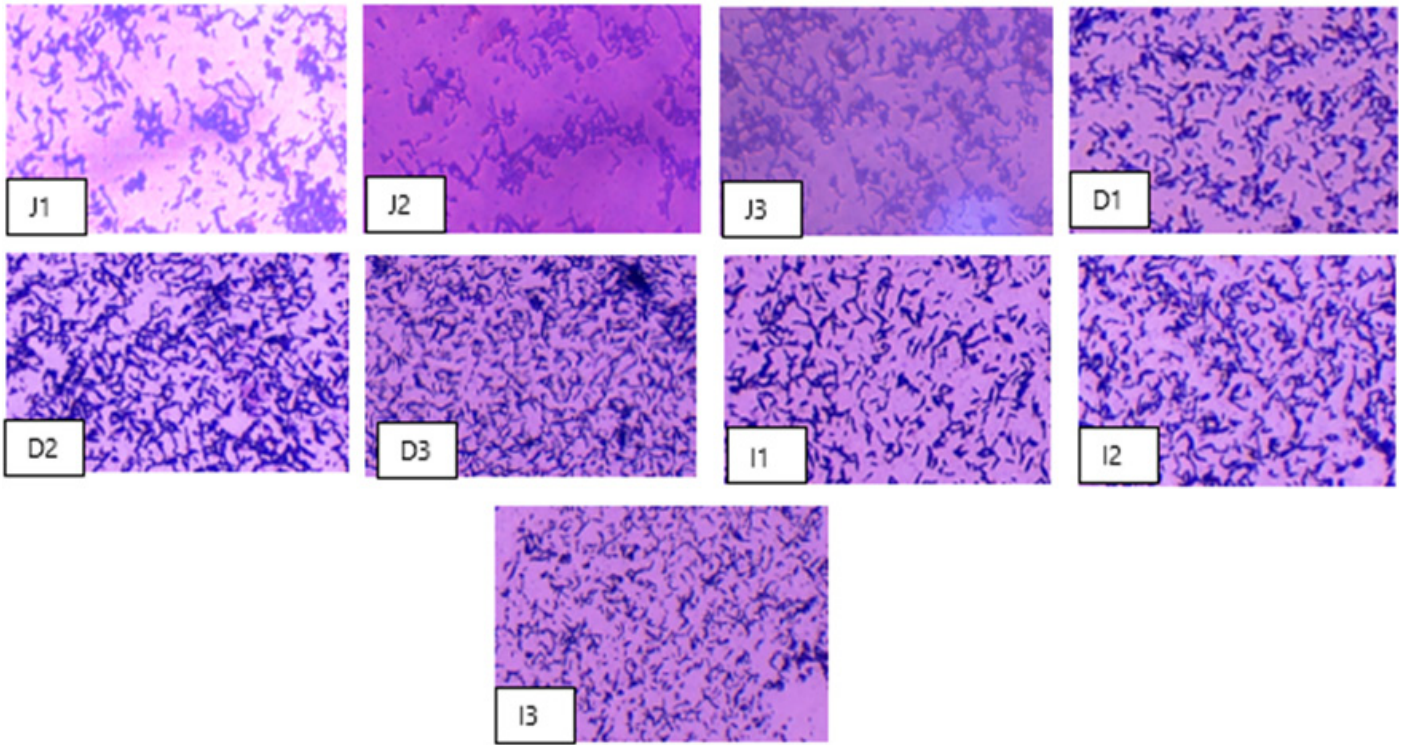

Figure 2: Gram staining of presumptive lactic acid bacteria (LAB) isolated from the gastrointestinal tract of Indonesia local chickens (1000× magnification). All isolates exhibited Gram-positive, rod-shaped morphology.

The antimicrobial activity of the presumptive LAB isolates varied significantly among isolates and between the two indicator bacteria. Overall, the isolates exhibited stronger inhibitory activity against *Staphylococcus aureus* than against *Escherichia coli*. Isolate D1 produced the largest inhibition zone against *S. aureus*, followed by isolates I1, J3, and D2, whereas isolate J2 exhibited the greatest inhibitory activity against *E. coli*. The observed differences among isolates indicate strain-dependent variation in antimicrobial activity, suggesting that each isolate produces different types or amounts of antimicrobial metabolites. Similar variability among indigenous poultry-derived LAB has been reported previously, highlighting that antimicrobial activity is a strain-specific characteristic rather than a species-specific trait (Papadimitriou *et al*., 2016; Vieco-Saiz *et al*., 2019).

The greater susceptibility of *S. aureus* compared with *E. coli* may be explained by differences in cell wall structure between Gram-positive and Gram-negative bacteria. Gram-negative bacteria possess an outer membrane composed of lipopolysaccharides that restricts the diffusion of antimicrobial compounds, whereas Gram-positive bacteria lack this protective barrier and are therefore generally more susceptible to inhibitory metabolites produced by LAB, including organic acids, hydrogen peroxide, and bacteriocins. The antibacterial activity demonstrated by the isolates in the present study indicates their potential as probiotic candidates for poultry, particularly for improving intestinal microbial balance and suppressing pathogenic bacteria. Nevertheless, further studies are required to characterize the specific antimicrobial compounds produced by the most active isolates and to evaluate their efficacy under in vivo conditions (Begley *et al*., 2005; Neveling *et al*., 2020; Papadimitriou *et al*., 2016).

CONCLUSION

Nine presumptive LAB isolates were successfully isolated from the gastrointestinal tract of Indonesian local chickens and exhibited phenotypic and biochemical characteristics typical of LAB, including Gram-positive, rod-shaped, catalase-negative, non-motile, Methyl Red-positive, and Voges–Proskauer-negative reactions. All isolates tolerated acidic conditions (pH 2–7), exhibited good growth in the presence of 1% bile salts, and were able to grow at incubation temperatures of 20°C, 30°C, and 50°C, although growth was reduced at the highest temperature. In addition, all isolates demonstrated antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with generally stronger inhibition against *S. aureus*. These findings indicate that indigenous LAB isolated from the gastrointestinal tract of Indonesian local chickens possess desirable probiotic characteristics and represent promising candidates for further investigation and development as probiotic cultures for poultry production. Future studies should focus on molecular identification and *in vivo* evaluation to confirm their safety, efficacy, and functional performance.

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NOVELTY STATEMENT

This study provides baseline information on the phenotypic characteristics and preliminary in vitro probiotic potential of lactic acid bacteria isolated from the gastrointestinal tract of Indonesian native chickens. The novelty of this study lies in the preliminary screening of indigenous poultry-derived LAB based on acid tolerance, bile salt tolerance, temperature tolerance, and antibacterial activity, providing a basis for the further selection and development of host-adapted probiotic candidates for Indonesian poultry production.

AUTHORS CONTRIBUTION

MJM: Conceptualization, methodology, investigation, writing original draft. RM: Conceptualization, supervision, writing review and editing. SP and A: Formal analysis, data curation, validation. All authors approved the final version.

Ethical approval

Ethics Committee on the Use of Research and Learning Animals, Faculty of Animal Science, Hasanuddin University, No. 052/UN4.12/EC/VI/2026.

Data availability

Data are available from the corresponding author upon reasonable request.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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