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Isolation, Phenotypic Characterization, and Functional Potential of Indigenous Lactic Acid Bacteria from Fresh Duck Eggs

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ABSTRACT

Duck eggs are a highly nutritious source of animal protein; however, they are particularly susceptible to microbial contamination. Indigenous lactic acid bacteria (LAB) capable of colonizing fresh egg matrices may possess adaptive traits, making them effective natural biopreservation agents. The present study aimed to isolate, phenotypically characterize, and evaluate the antimicrobial activity of LAB derived from different compartments of fresh duck eggs, specifically the inner eggshell, egg membrane, and egg contents. Duck eggs were obtained from two intensive farms (I1 and I2) and two extensive farms (E1 and E2) located in Sidrap Regency, South Sulawesi, Indonesia, during 2024. The LAB isolates were obtained using a selective culture method employing De Man, Rogosa and Sharpe agar enriched with 1% $CaCO_2$. Twenty fresh duck eggs (five per location) were sampled, yielding 35 isolates, of which seven were confirmed as LAB based on morphological and biochemical analyses. Characterization included Gram, endospore, and acid-fast staining, catalase testing, and carbohydrate fermentation profiling. Antimicrobial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) was evaluated using agar well diffusion method at the original (acidic) and neutral pH levels to identify bacteriocin-like inhibitory substances (BLIS). Seven selected LAB isolates were obtained, including five from extensive farms (E1S1, E1S3, E1M1, E2S2, E2M2) and two from intensive farms (I1M2, I2M5). All isolates demonstrated complete glucose fermentation and a homofermentative pattern. Isolates E2S2, E2M2, and I1M2 fermented glucose, lactose, fructose, and mannitol, whereas E1S1, E1S3, E1M1, and I2M5 fermented glucose exclusively. Based on phenotypic data, the isolates were preliminarily classified as *Lactobacillus* and *Pediococcus* species. Antimicrobial activity testing demonstrated significant inhibitory potential, with maximum inhibition zones of 27.5 mm against *E. coli* and 17.0 mm against *S. aureus*. Although inhibition zone diameters decreased at neutral pH, the persistence of a clear zone against *E. coli* indicated BLIS production. The disappearance of inhibitory activity against *S. aureus* at neutral pH indicated that organic acids caused the pathogen's inhibition. The current findings indicated that fresh duck eggs contain a high abundance of homofermentative LAB, which could serve as effective natural biopreservation agents to improve food safety and prolong shelf life.

Keywords: Antimicrobial activity, Duck egg, Lactic acid bacteria, *Lactobacillus*, *Pediococcus*

INTRODUCTION

Duck eggs (*Anas platyrhynchos*) are a highly valuable local poultry product, known for their rich nutritional content and significant economic role within the national animal protein supply chain. In many regions across Indonesia, duck eggs have been served as a significant source of animal protein for local communities. Data from the Central Bureau of Statistics indicated that total duck egg production in Indonesia reached 358,000 tonnes in 2023, up from 349,000 tonnes in 2022 and 334,000 tonnes in 2021 (Statistics Indonesia, 2024). In Indonesia, South Sulawesi was ranked third among provinces in duck egg production in 2023. Several districts, including Sidrap and Wajo, have become prominent centers of duck farming, significantly contributing to local and regional egg supplies (Statistics Indonesia, 2024).

Duck eggs are widely consumed fresh and in processed forms, such as salted eggs, particularly in Asian countries, including Indonesia. Compared with chicken eggs, duck eggs are larger, have a deeper-colored yolk, and contain more protein and fat (Krismiyanto et al., 2020). In addition, duck eggs contain natural bioactive compounds, including avidin, ovotransferrin, and lysozyme, that have antimicrobial activity (Nian et al., 2022). Eggs play an important role in microbiological studies because they maintain internal sterility while serving as nutrient-rich substrates for microorganisms that penetrate their defenses (Wellman-Labadie et al., 2010). Similar to other avian eggs, duck eggs

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have a complex structure comprising an outer shell, membrane, and egg contents (albumen and yolk), with each compartment providing distinct nutrient profiles and inherent defense mechanisms (Guyot et al., 2016). Nevertheless, the eggshell and membrane are not entirely sterile and serve as primary biological barriers vulnerable to microflora from feed, feces, and the barn environment (Wierup, 2017). The nutrient-rich environment on and within the egg makes it a potential habitat for various microorganisms, including lactic acid bacteria (LAB; Saputra and Rahayu, 2013).

Lactic acid bacteria are a group of Gram-positive, non-spore-forming, facultatively anaerobic bacteria widely recognized for their important roles in food preservation and safety (Badis et al., 2022). The antimicrobial activity of LAB is primarily attributed to the production of lactic acid, which lowers the environmental pH and creates unfavorable conditions for the proliferation of spoilage microorganisms and pathogenic bacteria (Badis et al., 2022). Furthermore, LAB can produce additional antimicrobial substances, such as hydrogen peroxide, diacetyl, and bacteriocins, which enhance their inhibitory effects against foodborne pathogens (Dabiza et al., 2023). The potential of LAB in different livestock-derived food products has been demonstrated, with Murtari et al. (2020) successfully isolating *Lactobacillus plantarum* and *Lactobacillus fermentum*, and Sugata et al. (2024) identifying *Streptococcus* and *Lactobacillus* genera from fresh cow milk. These results verified the presence of active indigenous microorganisms, which could be further studied in other livestock products.

Although LAB have been found in several food products, investigations specifically targeting the different compartments of fresh duck eggs, including the inner shell, membrane, and egg contents, remain very limited. Previous studies mainly focused on fermented dairy products and processed egg products such as salted eggs. Saputra and Rahayu (2013) documented the presence of *Lactobacillus plantarum*, *Lactobacillus casei* subsp. *rhamnosus*, *Enterococcus gallinarum*, and *Pediococcus acidilactici* from salted eggs. Meanwhile, Surya and Nugroho (2025) reported isolating *Lactobacillus* and *Weissella* from egg white and egg yolk. The present study aimed to isolate and characterize indigenous LAB from fresh duck eggs to obtain locally adapted strains capable of colonizing the egg and demonstrating potential as natural biopreservation agents.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in accordance with the approved ethical procedures of the Hasanuddin University, Makassar, Indonesia, ethics committee. Since no live animals were used, handled, or subjected to any experimental interventions, formal institutional ethical approval was not required.

Study design

Fresh duck egg samples were collected directly from local commercial farmers after production. The study focused exclusively on isolating and characterizing LAB from these eggs. After isolating LAB from the eggshell, membrane, and interior, the culture was purified and characterized using different methods, including Gram, endospore, acid-fast, and cell-shape staining, as well as biochemical assays such as catalase activity and carbohydrate fermentation tests. The final step involved assessing antimicrobial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) by the agar well diffusion method. Isolations were performed in duplicate, and antimicrobial assays were repeated three times. The study was conducted at the Laboratory of Microbiology and Animal Health, Faculty of Animal Science, and the Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

Sample collection

Fresh duck eggs were collected directly from four farms in Sidrap Regency, South Sulawesi, Indonesia, during 2024, comprising two intensive and two extensive farming systems. The flock size at each location ranged from 500 to 1,000 ducks. A total of 20 fresh eggs (five per farm) were collected and labeled according to origin and management system. Sampling criteria included collection within 3 to 5 hours of laying, maintaining integrity without cracking, and remaining unwashed. Samples were transported to the laboratory in a cool box maintained at approximately 4°C and placed on sterile racks to prevent cross-contamination.

Isolation and preliminary selection of lactic acid bacteria

The LAB isolation procedure adhered to the modified methodology established by Sugata et al. (2024). Samples from each egg compartment, including the inner shell, membrane, and contents, were prepared under aseptic conditions and subsequently inoculated into De Man, Rogosa, and Sharpe (MRS) broth for pre-enrichment, using the swab method for the shell and membrane, and 1 g for the egg contents. After anaerobic incubation at 37°C for 24 hours, cultures were streaked onto MRS agar enriched with 1% calcium carbonate ($CaCO_3$). The addition of $CaCO_3$ served as a visual

indicator, with the formation of a clear zone around the colonies signifying acid production, a hallmark of LAB (Kurnia et al., 2020). Single colonies were identified by their morphological features, such as a white or yellowish color, a circular shape, a convex or flat elevation, and entire margins, as well as the presence of a clear zone. These colonies were purified through at least three rounds of streaking before being stored.

Microscopic identification and differential characterization of isolates

Cell morphology and Gram reaction were confirmed following the method of Sugata et al. (2019), with some modifications, using crystal violet, Lugol's iodine, 96% alcohol, and safranin. Gram-positive isolates with bacillus or coccus morphology under 1000x oil-immersion microscopy (Olympus, Japan) were identified as LAB candidates (Kurnia et al., 2020). Endospore staining verified that the organism did not form spores (Vasantha et al., 2021). Acid-fast staining was included as a supplementary step to exclude acid-fast genera such as *Mycobacterium*, although this test was not strictly essential for the identification of LAB (Tjayadi et al., 2017).

Catalase test

The catalase test was performed to determine whether the isolates produced catalase, following the method outlined by Hidayat et al. (2018). This test was critically important because LAB do not synthesize catalase and, as a result, were expected to yield a negative result. One loopful of the LAB isolate was smeared onto a clean glass slide, and 2-3 drops of 3% hydrogen peroxide (H_2O_2) were applied to the smear. The absence of bubble formation indicated a catalase-negative result, consistent with LAB identity.

Carbohydrate fermentation analysis

The metabolic pattern of isolates was determined through sugar fermentation tests covering glucose, lactose, sucrose, and mannitol, following the method of Liang et al. (2018). Phenol red carbohydrate broth (Oxoid, UK), supplemented with an inverted Durham tube, was used to detect gas production. The medium composition per liter of distilled water consisted of 10 g of peptone, 5 g of NaCl, 10 g of test sugar, 1 g of beef extract, and 180 mg of phenol red, serving as a pH indicator. After sterilization and inoculation, cultures were incubated microaerophilically at 37°C for 24 hours. Positive fermentation results were indicated by a color change from red to yellow (acid production), while gas bubbles in the Durham tube indicated heterofermentative LAB characteristics.

Antimicrobial activity testing

Antimicrobial potential was tested against *E. coli* and *S. aureus* using the agar well diffusion method (Rizal et al., 2019). Pathogen suspensions were adjusted to the 0.5 McFarland standard before inoculation onto Mueller-Hinton agar (Septian et al., 2020). Cell-free supernatant (CFS) was obtained by centrifugation at 6,000 × g for 15 minutes, followed by filtration through a sterile 0.2 µm membrane (Murtari et al., 2020). A volume of 100 µL of CFS was added to each well. The CFS was evaluated at its original acidic pH and at a neutral pH (6.5-7.0), adjusted with NaOH, to distinguish the activity of organic acids from that of bacteriocin-like inhibitory substances (BLIS). After incubation at 37°C for 24 hours, inhibition zone diameters (mm) were measured.

Data analysis

Data were analyzed descriptively using qualitative and quantitative approaches. Qualitative observations included colony morphology, Gram reaction, endospore staining, acid-fast staining, catalase activity, and carbohydrate fermentation profiles to characterize isolates. Quantitative data were obtained from inhibition zone diameter measurements against *E. coli* and *S. aureus* under original and neutral pH conditions. Tentative genus identification was conducted according to Bergey's Manual of Systematic Bacteriology (De Vos et al., 2009).

RESULTS AND DISCUSSION

Lactic acid bacteria isolation

Indigenous LAB isolates from different compartments of fresh duck eggs are presented in Table 1. The selected isolates demonstrated distinct acidogenic capabilities, as evidenced by clear zones around their colonies. The majority of isolates exhibited colony characteristics consistent with those of LAB, namely circular shape, white color, entire margin, convex/flat elevation, and glossy surface, with diameters of 0.1-0.4 mm (Table 1). However, selection analyses demonstrated that not all growing colonies produced a transparent zone. Isolates such as E1S4, E1M2, E1C2, and I1M1 did not create clear zones, suggesting they were non-acidogenic microbes that simply tolerated the MRSA medium.

Table 1. Colony morphology characteristics of isolates obtained from different compartments of fresh duck eggs in Sidrap Regency, South Sulawesi, Indonesia

Sample code	Shape	Color	Margin	Elevation	Surface	Size	Clear zone ($CaCO_3$)
E1S1	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E1S2	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E1S3	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E1S4	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
E1S5	Circular	White	Entire	Convex	Glossy	0.1 mm	Present
E1 M1	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E1M2	Circular	White	Entire	Convex	Non-glossy	0.2 mm	Absent
E1M3	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E1M4	Circular	White	Entire	Convex	Glossy	0.1 mm	Absent
E1M5	Circular	White	Entire	Convex	Glossy	0.3 mm	Present
E1C1	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E1C2	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
E1C3	Circular	White	Entire	Convex	Non-glossy	0.2 mm	Absent
E1C4	Circular	White	Entire	Convex	Glossy	0.4 mm	Absent
E1C5	Circular	White	Entire	Convex	Non-glossy	0.2 mm	Absent
E2S1	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
E2S2	Circular	White	Entire	Convex	Glossy	0.3 mm	Present
E2S3	Circular	White	Entire	Convex	Glossy	0.3 mm	Present
E2S4	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
E2S5	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E2M1	Circular	White	Entire	Convex	Glossy	0.4 mm	Absent
E2M2	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E2M3	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E2M4	Circular	White	Entire	Convex	Glossy	0.3 mm	Absent
E2M5	Circular	White	Entire	Convex	Glossy	0.3 mm	Absent
E2C1	Circular	White	Entire	Convex	Glossy	0.3 mm	Absent
E2C2	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E2C3	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
E2C4	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
E2C5	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
I1S1	Circular	White	Entire	Convex	Glossy	0.3 mm	Present
I1S2	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
I1S3	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I1S4	Circular	White	Entire	Convex	Glossy	0.3 mm	Present
I1S5	Circular	White	Entire	Convex	Non-glossy	0.2 mm	Absent
I1M1	Circular	White	Entire	Convex	Glossy	0.4 mm	Absent
I1M2	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I1M3	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I1M4	Circular	White	Entire	Convex	Glossy	0.3 mm	Absent
I1M5	Circular	White	Entire	Convex	Non-glossy	0.3 mm	Absent
I1C1	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
I1C2	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I1C3	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
I1C4	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I1C5	Circular	White	Entire	Convex	Non-glossy	0.2 mm	Absent
I2S1	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I2S2	Circular	White	Entire	Convex	Glossy	0.3 mm	Present
I2S3	Circular	White	Entire	Convex	Non-glossy	0.2 mm	Absent
I2S4	Circular	White	Entire	Convex	Glossy	0.3 mm	Absent
I2S5	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I2M1	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I2M2	Circular	White	Entire	Convex	Glossy	0.3 mm	Absent
I2M3	Circular	White	Entire	Convex	Glossy	0.2 mm	Present
I2M4	Circular	White	Entire	Convex	Non-glossy	0.2 mm	Absent
I2M5	Circular	White	Entire	Convex	Glossy	0.4 mm	Present
I2C1	Circular	White	Entire	Convex	Glossy	0.3 mm	Present
I2C2	Circular	White	Entire	Convex	Glossy	0.3 mm	Present
I2C3	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
I2C4	Circular	White	Entire	Convex	Glossy	0.2 mm	Absent
I2C5	Circular	White	Entire	Convex	Glossy	0.2 mm	Present

Source of data: Microbiology and Animal Health Laboratory, Faculty of Animal Science, Hasanuddin University, Indonesia, 2026

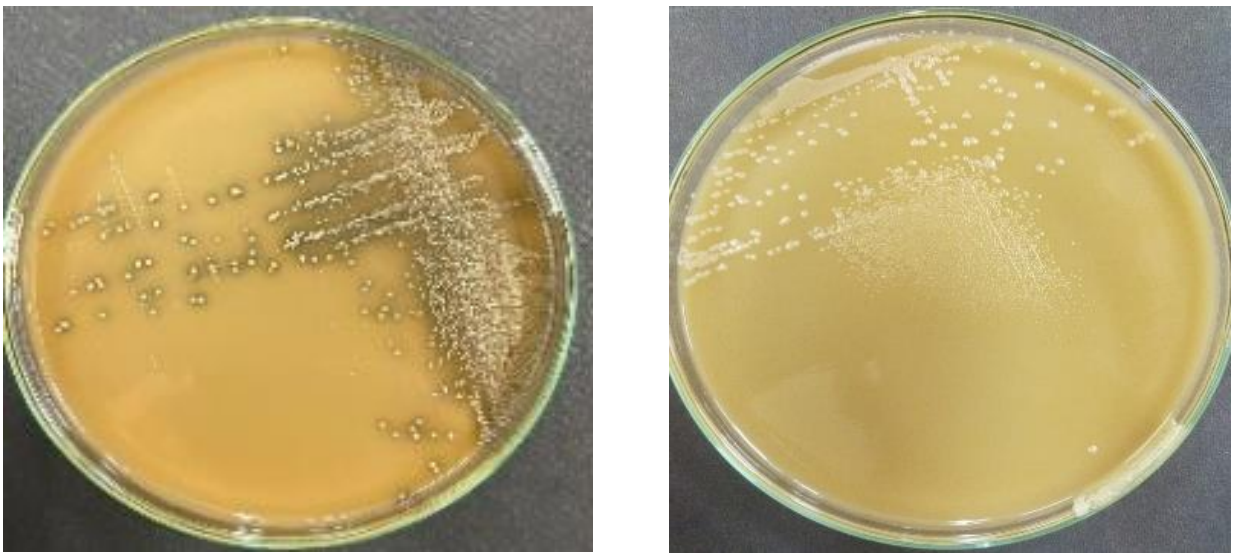
The majority of isolates exhibited colony characteristics consistent with those of LAB, namely a circular shape, white color, entire margin, convex/flat elevation, and glossy surface, with diameters of 0.1-0.4 mm (Table 1; Badis et al., 2022). However, selection analyses demonstrated that not all growing colonies formed a transparent zone. Isolates such

as E1S4, E1M2, E1C2, and I1M1 did not form clear zones, indicating that they were non-acidogenic microbes that merely tolerated the MRSA medium.

The distribution of suspected transparent zone-forming LAB exhibited a pattern correlated with management practices and ecological compartments in duck eggs. On extensive farms (E1 and E2), acidogenic isolates were randomly distributed across all egg compartments. This finding aligned with that of Wierup (2017), who reported that unconfined poultry environments facilitate intensive interactions between chickens and a diverse environmental microflora in rice-field or marsh ecosystems. On intensive farms (I1 and I2), transparent zone-forming isolates were more consistently identified in the outer compartments (eggshell and membrane). This pattern was consistent with the findings of Stepien-Pysniak et al. (2021), suggesting that regulated sanitation and biosecure feeding management limited arbitrary microbial infiltration while promoting the proliferation of specific surface-associated LAB groups.

The characteristics of each egg compartment serve as a primary differentiator. Consistent with the findings of Saputra and Rahayu (2013), who isolated acidogenic LAB from salted duck eggs, the present results indicated that the shell (S) and egg membrane (M) harbored significant acidogenic strains, such as E1S1, E1S5, E2M2, I1S4, and I2M1. This finding confirmed that the outer compartments functioned as a critical biological defense barrier. Organic acids produced by LAB on these surfaces lowered the local pH, thereby inhibiting the colonization of fecal- or soil-borne pathogenic bacteria prior to shell penetration (Stepien-Pysniak et al., 2021).

The successful isolation of bacterial colonies from the egg contents of E1C1, E2C2, E2C3, I1C2, I1C4, and I2C5 samples was particularly notable given the interior's strong antimicrobial environment. Albumen contains lysozyme, ovo transferrin, and avidin (Guyot et al., 2016) and maintains a highly alkaline pH of 9.0 to 9.6 (Wellman-Labadie et al., 2010). Microbes primarily penetrate via transovarian transmission during egg formation or horizontally through eggshell pores compromised by environmental moisture (Baron et al., 2016). A comparative visual representation of colony morphology between acidogenic and non-acidogenic isolates is illustrated in Figure 1.



(a) (b)

Figure 1. Colony morphology of isolates derived from fresh duck eggs in Sidrap Regency, South Sulawesi, Indonesia on De Man, Rogosa, and Sharpe agar supplemented with 1% $CaCO_3$. **a:** Sample E1S1 showing a wide transparent zone, **b:** Sample E1S4 showing no transparent zone.

Microscopic characteristics

Isolates demonstrating positive acidogenic activity underwent further characterization through Gram staining, endospore staining, acid-fast staining, and catalase testing to confirm their classification as LAB and to exclude non-LAB microorganisms or yeasts capable of dissolving $CaCO_3$ (Table 2; Badis et al., 2022). The cellular characteristics predominantly exhibited Gram-positive (+) cocci arranged in chains or pairs, as well as bacillus forms (Table 2). Isolates exhibiting Gram-negative (−) or catalase-positive (+) traits, including E1S5, E1M3, and E2M3, were discarded as environmental contaminants that merely tolerated the acidic medium. This rigorous elimination approach was consistent with the findings of Papadimitriou et al. (2015), who emphasized that catalase positivity and Gram-negativity are indicative of non-LAB taxa. Consequently, isolates E1S1, E1S3, E1M1, E2S2, E2M2, I1M2, and I2M5 were selected for subsequent analyses. Lactic acid bacteria status was confirmed by negative results for endospore staining, acid-fast staining, and catalase tests. In contrast to common egg-contaminating spore-formers such as *Bacillus* species (Liang et

al., 2018; Kurnia et al., 2020), the present isolated indigenous LAB were non-spore-forming. Furthermore, the catalase-negative property reflects an aerotolerant anaerobic metabolic pathway, a fundamental functional hallmark of true LAB.

Table 2. Microscopic characteristics, staining reactions, and biochemical results of lactic acid bacteria isolates derived from fresh duck eggs in Sidrap Regency, South Sulawesi, Indonesia

Isolate code	Gram	Endospore	Acid-fast	Cell shape	Cell arrangement	Catalase
E1S1	(+)	(−)	(−)	Coccus	Chain	(−)
E1S2	(+)	(−)	(−)	Coccus	Chain	(+)
E1S3	(+)	(−)	(−)	Coccus	Paired	(−)
E1S5	(−)	(−)	(−)	Coccus	Single	(+)
E1M1	(+)	(−)	(−)	Bacillus	Single	(−)
E1M3	(−)	(−)	(−)	Coccus	Single	(+)
E1M5	(−)	(−)	(−)	Coccus	Chain	(+)
E2C1	(−)	(−)	(−)	Coccus	Chain	(+)
E2S2	(+)	(−)	(−)	Bacillus	Single	(−)
E2S3	(+)	(−)	(−)	Bacillus	Chain	(+)
E2S5	(+)	(−)	(−)	Coccus	Chain	(+)
E2M2	(+)	(−)	(−)	Coccus	Paired	(−)
E2M3	(−)	(−)	(−)	Coccus	Chain	(+)
E2C2	(−)	(−)	(−)	Coccus	Single	(+)
E2C3	(−)	(−)	(−)	Coccus	Single	(+)
I1S1	(−)	(−)	(−)	Coccus	Chain	(+)
I1S3	(+)	(−)	(−)	Coccus	Single	(+)
I1S4	(+)	(−)	(−)	Coccus	Chain	(+)
I1M2	(+)	(−)	(−)	Coccus	Single	(−)
I1M3	(+)	(−)	(−)	Coccus	Single	(+)
I1C2	(+)	(−)	(−)	Coccus	Chain	(+)
I1C4	(+)	(−)	(−)	Bacillus	Single	(+)
I2S1	(+)	(−)	(−)	Coccus	Chain	(+)
I2S2	(+)	(−)	(−)	Coccus	Chain	(+)
I2S5	(+)	(−)	(−)	Bacillus	Single	(+)
I2M1	(+)	(−)	(−)	Coccus	Single	(+)
I2M2	(+)	(−)	(−)	Coccus	Single	(+)
I2M5	(+)	(−)	(−)	Coccus	Paired	(−)
I2C1	(+)	(−)	(−)	Coccus	Chain	(+)
I2C2	(−)	(−)	(−)	Coccus	Chain	(+)
I2C5	(−)	(−)	(−)	Coccus	Chain	(+)

Source of data: Microbiology and Animal Health Laboratory, Faculty of Animal Science, Hasanuddin University, Indonesia, 2026. E1: Extensive farm 1, E2: Extensive farm 2, I1: Intensive farm 1, I2: Intensive farm 2, S: Egg shell, M: Egg membrane, C: Egg contents, (+): Positive reaction, (−): Negative reaction

Biochemical characteristics and carbohydrate fermentation profiles

The metabolic capacity of the seven selected LAB isolates was evaluated through sugar fermentation analyses (Table 3). All seven selected isolates consistently fermented glucose, exhibiting a homofermentative pattern, as evidenced by a color change from red to yellow, with no CO_2 production (Table 3).

From a physiological perspective, this metabolic pattern confirmed that the isolates effectively convert sugars into lactic acid predominantly via the Embden-Meyerhof-Parnas (EMP) pathway, without generating gaseous byproducts that could potentially compromise or modify the texture of food products (Murtari et al., 2020; Badis et al., 2022). Isolates E2S2, E2M2, and I1M2 fermented glucose, lactose, fructose, and mannitol, while E1S1, E1S3, E1M1, and I2M5 fermented only glucose. The observed differences in sugar fermentation confirmed the diversity of indigenous LAB species, each with distinct competitive advantages in adapting to the fluctuating nutritional environment of the duck egg matrix (Saputra and Rahayu, 2013).

Table 3. Carbohydrate fermentation profiles and metabolic patterns of selected lactic acid bacteria isolates derived from fresh duck eggs in Sidrap Regency, South Sulawesi, Indonesia

Isolate code	Glucose	Lactose	Fructose	Mannitol	Fermentation pattern
E1S1	(+)	(−)	(−)	(−)	Homofermentative
E1S3	(+)	(−)	(−)	(−)	Homofermentative
E1M1	(+)	(−)	(−)	(−)	Homofermentative
E2S2	(+)	(+)	(+)	(+)	Homofermentative
E2M2	(+)	(+)	(+)	(+)	Homofermentative
I1M2	(+)	(+)	(+)	(+)	Homofermentative
I2M5	(+)	(−)	(−)	(−)	Homofermentative

Source of data: Microbiology Laboratory, Faculty of Medicine, Hasanuddin University, Indonesia, 2026. (+): Able to ferment, medium color changed to yellow, (−): Unable to ferment, E1: Extensive farm 1, E2: Extensive farm 2, I1: Intensive farm 1, I2: Intensive farm 2, S: Eggshell, M: Egg membrane

Genus identification of lactic acid bacteria

Cell morphology, metabolic profiles, and other phenotypic data were compiled and compared with Bergey's Manual of Determinative Bacteriology to indicate tentative taxonomic classifications of the selected isolates (Table 4).

Indigenous LAB isolates were classified into two genera, including *Lactobacillus* (E1M1 and E2S2) and *Pediococcus* (E1S1, E1S3, E2M2, I1M2, and I2M5). The prevalence of *Lactobacillus* and *Pediococcus* in fresh duck eggs aligned with the findings of Saputra and Rahayu (2013), thereby confirming that these two genera constituted stable and dominant indigenous microflora in avian egg environments. Furthermore, the capacity of E2S2, E2M2, and I1M2 isolates to ferment mannitol corresponded with the well-established taxonomic characteristics of *Pediococcus pentosaceus* or *Pediococcus acidilactici* (Garvie, 1986).

The presence of *Lactobacillus* and *Pediococcus* extending into the eggshell, membrane, and albumen was associated with physical penetration through shell pores that ranged from 10 to 65 µm in diameter. These pores are significantly larger than LAB cells, which measure between 0.5 and 1.2 µm for *Pediococcus* and 1 to 10 µm for *Lactobacillus*. The present study demonstrates that *Lactobacillus* strains could readily colonize the proteinaceous egg membrane. This finding was consistent with Saputra and Rahayu (2013), who emphasized the strong affinity of *Lactobacillus* cell walls for adhering to keratin fiber networks.

Pediococcus and *Lactobacillus* possess cell wall modifications, including high teichoic acid content and exocellular polysaccharides (Papadimitriou et al., 2015). In accordance with Wang et al. (2021), peptidoglycan modifications in *Lactobacillus* and *Pediococcus* enhance cell wall resistance against lysozyme-mediated degradation compared to other Gram-positive bacteria. Furthermore, their homofermentative EMP pathway facilitates rapid accumulation of lactic acid without the production of gases (Siren et al., 2023), thereby confirming that fresh duck eggs served as a natural reservoir for resilient biopreservative LAB strains.

Table 4. Genus identification of indigenous lactic acid bacteria isolates derived from fresh duck eggs in Sidrap Regency, South Sulawesi, Indonesia

Isolate code	Cell shape	Gram	Catalase	Fermentation pattern	Tentative genus
E1S1	Coccus	(+)	(−)	Homofermentative	*Pediococcus*
E1S3	Coccus	(+)	(−)	Homofermentative	*Pediococcus*
E1M1	Bacillus	(+)	(−)	Homofermentative	*Lactobacillus*
E2S2	Bacillus	(+)	(−)	Homofermentative	*Lactobacillus*
E2M2	Coccus	(+)	(−)	Homofermentative	*Pediococcus*
I1M2	Coccus	(+)	(−)	Homofermentative	*Pediococcus*
I2M5	Coccus	(+)	(−)	Homofermentative	*Pediococcus*

Source of data: Microbiology and Animal Health Laboratory, Faculty of Animal Science, Hasanuddin University, Indonesia, 2026. E1: Extensive farm 1, E2: Extensive farm 2, I1: Intensive farm 1, I2: Intensive farm 2, S: Eggshell, M: Egg membrane

Antimicrobial activity against pathogenic bacteria

Antimicrobial activity testing evaluated the ability of CFS derived from LAB isolates to inhibit the growth of *E. coli* and *S. aureus*. Results comparing inhibition zone diameters at original and neutral pH are presented in Table 5.

Table 5. Inhibition zone diameters (mm) of lactic acid bacteria isolates derived from fresh duck eggs in Sidrap Regency, South Sulawesi, Indonesia against *Escherichia coli* and *Staphylococcus aureus*

Isolate code	*Escherichia coli* (Original pH)	*Escherichia coli* (Neutral pH)	*Staphylococcus aureus* (Original pH)	*Staphylococcus aureus* (Neutral pH)
E1S1	16.0	15.5	16.0	13.0
E1S3	27.5	25.5	17.0	13.0
E1M1	21.5	15.0	19.0	13.0
E2S2	16.5	15.0	15.0	13.0
E2M2	17.0	14.5	15.5	13.0
I1M2	21.0	17.0	16.5	13.0
I2M5	19.0	14.0	15.0	13.0

Source of data: Microbiology and Animal Health Laboratory, Faculty of Animal Science, Hasanuddin University, Indonesia, 2026. The value of 13.0 mm was interpreted as the well diameter, indicating the absence of an inhibition zone against the indicator bacteria.

All seven LAB isolates demonstrated strong antagonistic activity under original acidic pH conditions (Table 5). Isolate E1S3 produced the highest inhibition zone against *E. coli* (27.5 mm), driven primarily by organic acid accumulation. Under original acidic conditions, all seven LAB isolates exhibited strong antagonistic activity against both pathogens (Table 5). This strong inhibition at original pH was consistent with findings of Septian et al. (2020) and Badis et al. (2022), demonstrating that organic acid accumulation, primarily lactic acid, served as the primary mechanism for LAB strains to suppress pathogens. Isolate E1S3 produced the largest zone against *E. coli* (27.5 mm) as visualized in Figure 2a.

Upon pH neutralization, inhibitory activity against *S. aureus* completely disappeared across all isolates, as indicated by a 13.0 mm well diameter. This finding contrasted with some broad-spectrum bacteriocin studies, but aligned with the findings of Septian et al. (2020), suggesting that the anti-*S. aureus* activity observed in these specific isolates was exclusively dependent on acidity. Conversely, under neutralized pH conditions, significant inhibition against Gram-negative *E. coli* was observed in several isolates, notably E1S3 (25.5 mm) and I1M2 (17.0 mm), which is depicted in Figure 2. This residual inhibition at neutral pH was consistent with the findings of Septian et al. (2020), indicating that non-acid antimicrobial metabolites such as BLIS maintain activity regardless of pH. The different sensitivity of *E. coli* and *S. aureus* at neutral pH implied that the BLIS or antimicrobial peptides produced have target-specific actions against the outer membranes of Gram-negative bacteria.

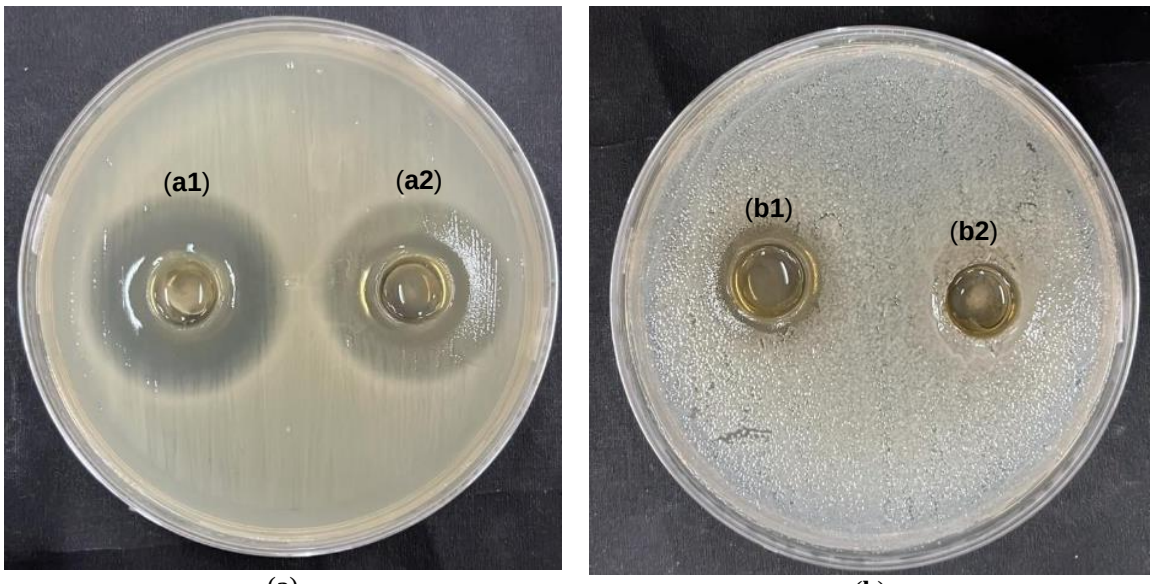


Figure 2. Visualization of antimicrobial activity of cell-free supernatants from lactic acid bacteria isolates. **a**: Isolate E1S3 in comparison to *E. coli*., **b**: Isolate E1S1 in comparison to *S. aureus*, **a1, b1**: Original pH, **a2, b2**: Neutral pH

CONCLUSION

The present study successfully isolated seven indigenous LAB isolates obtained from different compartments of fresh duck eggs in Sidrap Regency, South Sulawesi, Indonesia, each exhibiting strong acidogenic capabilities. These isolates

consisted of five extensive farms, as well as eggshell and egg membrane samples, and two isolates obtained from intensive farms, specifically from the egg membrane. All isolates were Gram-positive, catalase-negative, non-spore-forming, and homofermentative. Tentative phenotypic identification classified the isolates into the genera *Lactobacillus* and *Pediococcus*. At the original pH, all isolates demonstrated strong antimicrobial activity against *E. coli* and *S. aureus*; however, at neutral pH, inhibition persisted only against *E. coli*, thereby confirming BLIS production. Further purification and characterization are needed to verify the nature and spectrum of the putative BLIS produced by these isolates. Conversely, inhibition against *S. aureus* was exclusively attributable to organic acids. The current findings indicated that fresh duck eggs are a promising source of functional LAB for natural biopreservation applications. A limitation of the present study was that identification was based solely on phenotypic characteristics; molecular identification (*16S rRNA* gene sequencing) is recommended in future studies to confirm species-level classification and genetic diversity of the isolates.

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Authors' contributions

Muhammad Irham contributed to data collection, laboratory investigations, and manuscript preparation. Wahniyathi Hatta contributed to study design, manuscript drafting, and manuscript revision. Fatma Maruddin contributed as principal investigator, study design, data interpretation, and final manuscript approval. All authors have read and approved the final edition of the manuscript.

Availability of data and materials

Samples and data are available upon reasonable request from the corresponding author.

Competing interests

The authors declare no competing interests.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors used Gemini (Google) to improve the grammar and clarity of the text. After that, the authors revised the AI-edited text, reviewed it, and took full responsibility for using AI. The authors conducted all intellectual investigations, data collection, analysis, and scientific conclusions without using AI tools. No AI tools were used for data generation or scientific interpretation. All authors are solely responsible for the accuracy, originality, and integrity of the final content before submission, during the revisions, and final publication of the article.

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