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Probiotic potential of indigenous lactic acid bacteria isolated from spiny lobster (*Panulirus* sp.)

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Abstract. *Lingga R, Fabiani VA, Zulfarina, Yurnaliza, Dalimunthe NP, Kurniawan B, Angrahini T, Kustaria S, Gusdur. 2026. Probiotic potential of indigenous lactic acid bacteria isolated from spiny lobster* (Panulirus *sp.*)*. Biodiversitas 27 (5): d270525. https://doi.org/10.13057/biodiv/d270525*. Feed management represents a fundamental factor influencing the efficiency and sustainability of lobster aquaculture operations. The nutritional requirements of lobsters encompass essential macronutrients, including proteins, carbohydrates, and lipids, traditionally supplied through fresh or formulated feeds. This study investigated the probiotic potential of indigenous Lactic Acid Bacteria (LAB) isolated from the intestines of spiny lobsters (*Panulirus* sp.) to address feed-related challenges in lobster aquaculture. Five marine lobster intestines were used as samples for LAB isolation using the spread plate method onto de Man, Rogosa, and Sharpe Agar (MRSA) medium supplemented with 1% $CaCO_3$. From 39 initial isolates, eight distinct taxa belonging to *Lactobacillus* and *Enterococcus* genera were characterized through morphological, biochemical, and physiological analyses. The isolates exhibited LAB properties and were Gram-positive, with diverse morphologies (cocci, bacilli, and coccobacilli). Eight isolates namely LM1, LM4, LP2, LP3, LP4, LP12, LP40, and LP33 were selected for further probiotic potential testing. Physiological characterization revealed robust adaptability across varied environmental conditions, with growth sustained at temperatures between 27-45°C and pH levels ranging from 2.5 to 8.0. Antimicrobial assays demonstrated significant pathogenic inhibition, with LM04 showing the largest inhibition zone against *Staphylococcus aureus* (maximum zone 25.94±1.58 mm) and *Escherichia coli* (maximum zone 15.51±1.06 mm). Isolates LP40 and LP12 showed effectiveness against both *Pseudomonas aeruginosa* and *Vibrio* sp. Most isolates showed antibiotic resistance to chloramphenicol while maintaining intermediate sensitivity to tetracycline and ampicillin. Isolate LM1 showed the highest growth rate, with populations reaching 23.5×10^8 CFU/mL, LP2 showed the highest adhesion capabilities (9.9%±0.09). Identification based on 16S rRNA gene analysis showed that isolates LP3 and LP12 were *Enterococcus faecalis*, whereas the other isolates were identified only based on phenotypic characteristics. These findings established the indigenous LAB from *Panulirus* sp. as a promising probiotic candidate, particularly for enhancing disease resistance in lobster aquaculture systems, while highlighting areas for future optimization.

Keywords: Gut microbiota, lactic acid bacteria, lobster, *Panulirus* sp., probiotics

INTRODUCTION

Spiny lobsters (*Panulirus* sp.) represent large crustaceans inhabiting tropical and subtropical marine ecosystems worldwide. These decapod crustaceans have emerged as premium seafood commodities, commanding high market values due to their exceptional palatability and superior nutritional profile, making them integral to the hospitality, tourism, and culinary sectors (Amali and Sari 2020). Indonesia's ranking as the 17th-largest global lobster exporter underscores its economic significance (Sudarwati 2020). However, the Indonesian lobster aquaculture sector faces significant challenges, with survival rates ranging from 20 to 50%. The challenges are primarily attributed to feed-related constraints, insufficient settlement habitat, and fish predation (Jones et al. 2019).

Feed management constitutes a critical determinant in successful lobster aquaculture. The nutritional requirements of lobsters include essential macronutrients, which are traditionally supplied through fresh or formulated feeds. Trash fish remains the predominant feed source, accounting for approximately 70% of feed inputs in lobster aquaculture systems (Muthu Rathinam et al. 2014). In Vietnam, the nursing process for lobsters involves raising juvenile lobsters in small cages, submerged or suspended and feeding them a diet of fresh seafood. These juvenile lobsters are

cultivated until they reach an advanced stage, then sold to grow-out farmers. These farmers transfer them to larger floating cages suspended from basic floating structures for further growth (Jones et al. 2019). Commercial feed, like pellets, was also used in lobster aquaculture. The concern in pellet application is that consumption may decline when their size and shape do not suit the species' natural feeding habits. Developing feed in a suitable form is essential to address certain limitations in artificial feeding. Lobster feed must be appropriately sized to match the lobster's mouth, as its length and diameter influence its palatability (Syafrizal et al. 2018; Adiputra et al. 2020).

The other concerns in lobster culture are health and disease, which cause substantial yearly losses to lobster production. In Vietnam, substantial losses due to milky disease occurred in 2007 and 2008, when production declines ranged from 31% to 71% across all provinces. On average, the industry experienced a 50% drop in production during that period, leading to an estimated loss of US$50 million and affecting over 5,000 households. Before 2002, the survival rate during the grow-out phase was 70%, but by 2008, it had fallen below 50% due to disease outbreaks. However, by 2011, survival rates had recovered to 70% following the implementation of disease prevention measures. Despite a Milky disease outbreak in 2012, its impact was significantly lower compared to that of 2007-2008. In recent years, milky disease has remained the most critical issue in lobster farming, though its effects have been mitigated by improved surveillance and health management practices (Jones et al. 2019).

While commercial pelleted feeds present an alternative, recent research suggests that probiotic supplementation in moist pellet formulations could enhance the growth and survival of fish (Baños et al. 2019; Soltani et al. 2019; Nakhei et al. 2023) and increase digestive activity (Afrilasari et al. 2016). Probiotics are defined as beneficial living microorganisms that promote host health when administered in adequate amounts. The action mode of probiotics involves modifying the microbial populations associated with the host and has been demonstrated to improve nutrition, enhance feed utilization, and enhance immunity, ultimately increasing disease resistance (Krausova et al. 2019). These beneficial microorganisms exhibit competitive exclusion of pathogens through colonization of attachment sites and competition for nutrients within the digestive tract, thereby promoting resistance to pathogens (Safari et al. 2016). Lactic Acid Bacteria (LAB) have garnered particular attention among probiotic candidates. LAB are characterized as Gram-positive, non-spore-forming, microaerophilic bacteria that produce lactic acid as their primary fermentation end-product (Ibrahim and Uowehand 2019; Ayivi et al. 2020). Their probiotic efficacy is attributed to their ability to ferment carbohydrates, producing substantial amounts of lactic acid that enhance nutrient absorption and promote gastrointestinal health (Vinoj et al. 2015; Meidong et al. 2021).

Despite the potential applications of probiotics in lobster aquaculture, there remains a significant knowledge gap regarding the characterization of indigenous bacteria in the digestive tract of *Panulirus* sp. This study aimed to isolate and characterize indigenous lactic acid bacteria from lobster intestines and to evaluate their potential as probiotic candidates to support sustainable lobster aquaculture.

MATERIALS AND METHODS

Sample collection and preparation

The research used five adult lobster intestinal samples from 4-to 8-inch-long specimens. According to Marsela et al. (2022), this size range corresponds to mature or consumption-grade lobsters, typically measuring 4.5-8 in. We used five naturally captured samples from seawater without prior rearing. The specimens were collected using a traditional net from Tikus Emas Beach, Sungailiat, Bangka District, Bangka Belitung Islands, Indonesia. The samples were transported to the Biology Laboratory in a cooler box for isolation procedures.

Species identification was conducted before further analysis. Visual identification of lobster species was performed by examining color patterns on body segments, following the taxonomic keys in "Marine Lobsters of the World" (Holthuis 1991). The extraction of digestive tract contents as inoculum sources was performed through dissection. Sample preparation involved surface sterilization of the lobsters using 70% alcohol, followed by a sterile incision from the dorsal region to the anus using a sterilized scalpel. The intestines were then carefully extracted using sterile forceps. The collected intestinal samples from all five specimens were subsequently subjected to isolation procedures (Nurhasanah and Fatturrahman 2019).

Isolation and selection of lactic acid bacteria

The five prepared marine lobster intestinal samples were individually transferred into test tubes containing 5 mL of selective MRSB (de Man, Rogosa, and Sharpe Broth) medium supplemented with 0.9% NaCl. The samples were then incubated at 37°C for 48 hours. Subsequently, 1 mL of each lobster intestinal isolation solution was transferred to sterile test tubes and subjected to serial dilution up to 10^{-9} using 9 mL NaCl solution. The solutions were homogenized using a vortex mixer.

Selected dilutions (10^{-3}, 10^{-5}, 10^{-7}, and 10^{-9}) were aseptically inoculated using the spread plate method onto de Man, Rogosa, and Sharpe Agar (MRSA) medium supplemented with 1% $CaCO_3$. All cultures were incubated at 37°C for 48 hours. Isolates that produced clear zones were purified on MRSA medium (Shafakatullah and Chandra 2014).

Morphological characterization and purification

The bacterial isolates were identified by observing their colony morphological characteristics, such as shape, edge, elevation, margin, color, optical character, and surface. Following a comprehensive colony morphological characterization, the bacterial isolates were inoculated onto fresh MRS agar medium to ensure purification and preparation for subsequent analytical procedures.

Bacterial cell morphology and Gram staining tests were conducted using standard microbiological techniques to

characterize cellular structure and taxonomic classification. Following the protocol by O'Toole (2016), bacterial isolates were prepared on microscope slides and subjected to a differential staining procedure. The method involves sequential application of crystal violet, Gram's iodine, decolorizing agent, and safranin to distinguish bacterial cell wall characteristics.

Microscopic examination using a binocular microscope CX21 revealed cellular morphology, allowing differentiation between Gram-positive (purple-stained) and Gram-negative (red-stained) bacteria. Morphological observations focused on cell shapes, including spherical (cocci), rod-shaped (bacilli), and spiral forms, as described by Hardiansyah et al. (2020). This comprehensive approach enabled the precise identification and characterization of bacterial isolates, providing fundamental insights into their structural properties.

Biochemical properties and hemolysis test

Biochemical and metabolic characterization of the isolates was performed through a series of standardized tests (Nadia et al. 2020; Nofiani et al. 2022). Catalase activity was assessed by placing a pure bacterial isolate on a microscope slide using a sterile inoculation loop, then adding 3% hydrogen peroxide (H_2O_2). The formation of oxygen bubbles indicated a positive catalase reaction, while their absence denoted a negative result. Bacterial motility was evaluated using a Semi-solid Indole Motility (SIM) medium. Pure isolates were inoculated through straight-line stab inoculation into tubes containing SIM medium and incubated at 37°C for 24 hours. Diffuse growth from the stab line indicated motile organisms, while growth restricted to the stab line represented non-motile bacteria. For the Methyl Red (MR) test, pure cultures were inoculated into tubes containing Methyl Red-Voges Proskauer (MRVP) broth, homogenized, and incubated at 37°C for 72 hours. After incubation, 3-4 drops of methyl red indicator were added, and color changes were recorded to assess mixed-acid fermentation activity. Carbohydrate utilization patterns were investigated using Triple Sugar Iron Agar (TSIA). Pure isolates were both stabbed into the butt and streaked on the slant surface of the TSIA medium, followed by incubation at 37°C for 72 hours.

Hemolysis test followed the procedure by Buxton (2005), using the method: a loopful of each bacterial isolate from pure culture was streaked onto blood agar supplemented with 5% (v/v) fresh goat or sheep blood. Following incubation at 28°C for 24-48 hours, the appearance of a clear zone or color change surrounding the colonies was interpreted as a positive hemolytic reaction.

Identification of 16S rRNA gene from bacterial isolate

The LAB isolate DNA was isolated and processed using a modified method from Sambrook and Russell (2001). The isolated DNA was processed using the Polymerase Chain Reaction (PCR) technique to amplify the 16S rRNA gene with forward primer 63F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and reverse primer 1387R (5′-GGG CGG WGT GTA CAA GGC-3′). PCR amplification was performed with pre-denaturation, denaturation, annealing, extension, and post-amplification steps, for a total of 30 cycles. The amplified DNA products were visualized by mini-gel electrophoresis on 1% agarose gels, stained with ethidium bromide and observed under a UV transilluminator. The 16S rRNA gene amplicons were subsequently sent to 1st BASE DNA Sequencing Service (Malaysia) for sequencing. Raw sequence data were trimmed and assembled using ChromasPro version 1.5, and the resulting sequences were compared with sequences available in the NCBI database using BLAST. Phylogenetic analysis was performed in MEGA version 7.0 to determine relationships among LAB isolates and other bacterial species using the Neighbor-Joining method with 1,000 bootstrap replications.

Evaluation of probiotic potential

Selected isolates that underwent characterization and identification were evaluated for probiotic potential through multiple analyses. These included bacterial population enumeration, antibiotic sensitivity testing against 10 mg ampicillin, 30 mg tetracycline, and 30 mg chloramphenicol. Antimicrobial activity against pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Vibrio* sp. was assessed using the disc diffusion test (Verawaty et al. 2020). Additionally, physiological tolerance tests were conducted to examine temperature resistance and pH tolerance (Gotcheva et al. 2002).

Population of lactic acid bacteria

The assessment of probiotic potential was conducted in liquid MRSB (de Man, Rogosa, and Sharpe Broth) medium, which was preliminarily sterilized in an autoclave. The preparation of the inoculum (active culture) of lactic acid bacterial isolates in MRSB and prebiotic media was performed according to a modified method by Kurnia et al. (2020). The modification involved transferring two loopfuls of bacterial culture into 25 mL of MRSB and prebiotic media, then incubating at 37°C for 48 hours. The bacterial cultures from both MRSB and prebiotic media were subsequently analyzed for enumeration of the lactic acid bacterial population.

The Total Plate Count (TPC) method was used to perform enumeration of lactic acid bacteria. About 1 mL of culture was serially diluted in NaCl 0.85% solution in the test tubes up to 10^{-7}. From 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions, 1 mL of the samples was cultured on MRS Agar using the pour plate method and incubated for 24 hours at 37°C. The enumeration of total lactic acid bacteria was performed using plates containing between 30 and 300 colonies, following standard microbiological practices. The bacterial count was expressed in colony-forming units per milliliter (CFU/mL). The bacterial concentration per milliliter was calculated using the following formula (Arya et al. 2020).

$$\frac{\text{CFU}}{\text{mL}} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{volume of sample plated (mL)}}$$

Antibiotic susceptibility test

Antibiotic susceptibility was tested using 10 mg ampicillin, 30 mg chloramphenicol, and 30 mg tetracycline.

LAB isolates were inoculated into MRS Agar medium using the swab method. Sterile paper discs were placed on the agar surface. Each disc was then loaded with approximately ±20 µL of the test antibiotic solution. After 24 hours of incubation at 37°C, inhibition zones were measured (Sukarya et al. 2021). Results were categorized as sensitive (S: ≥21 mm), intermediate (I: 16-20 mm), or resistant (R: ≤15 mm) according to Vlková et al. (2006).

Antimicrobial test

Antimicrobial activity was assessed using the McFarland 0.5 standard (equivalent to 1.5×10^8 CFU/mL) as a reference for bacterial suspension density. The test organisms, *S. aureus*, and *E. coli*, were cultured on Nutrient Agar (NA) at 37°C for 24 hours and standardized in 0.9% NaCl to match the McFarland standard.

The well-diffusion method was employed, in which 100 µL of test bacteria were inoculated into NA using the pour plate technique. After 15 minutes of absorption, 20 µL of a 24-hour lactic acid bacterial culture (in MRS Broth) was added to wells with sterile MRSB as a negative control. Plates were incubated at 37°C for 24 hours. Inhibition zones were measured and classified as weak (≤5 mm), moderate (6-10 mm), strong (11-20 mm), or very strong (≥21 mm) (Nigam et al. 2012; Verawaty et al. 2020).

Physiological properties test

Physiological properties include acid and temperature resistance of the isolates. This test aimed to evaluate the growth of bacterial isolates under varying temperature and pH conditions. In examining temperature tolerance, 500 µL of LAB (Lactic Acid Bacteria) culture was added to test tubes containing 4.5 mL of MRSB (de Man, Rogosa, and Sharpe Broth) medium. The samples were incubated for 48 hours at four distinct temperatures: 4°C (low), 27°C (ambient), 37°C (optimal incubation), and 45°C (elevated) (Todorov et al. 2004).

The MRSB medium was adjusted to pH 2.5, 4, 6, and 8 for acid-resistance assessment using 0.1% HCl. A 1 mL aliquot of each bacterial isolate from stock culture was inoculated into test tubes containing 9 mL of pH-adjusted MRSB medium. These cultures were then incubated at 37°C for 48 hours to monitor bacterial growth in response to acidic conditions (Mannan et al. 2017).

LAB adhesion test

Bacterial adhesion was evaluated by immersing sterile stainless steel plates (2×2 cm) in bacterial cultures for 48 hours at room temperature under constant agitation (150 rpm). Following incubation, the SS plates were washed with PBS and aseptically swabbed. The samples were transferred to 0.85% NaCl, and serial dilutions were performed to 10^{-5}, followed by incubation at 37°C for 48 hours. For initial cell enumeration, 1 mL of culture medium was subjected to serial dilution (10^{-5}), plated using the pour plate method, and incubated at 37°C for 48 hours (Alp and Kulea 2020). At the end of the adhesion test, the adhesion rate was calculated using the equation below (Lau and Chye 2018).

$$\% \text{ Adhesion: } \frac{\text{CFU of adhered cell on plates}}{\text{CFU of initial inoculated cell}} \times 100$$

Data analysis

Data on antimicrobial activity, antibiotic resistance and adhesion ability were analyzed descriptively, including average values and standard deviations. Data is presented in tables and graphs.

RESULTS AND DISCUSSION

Morphological characteristics of bacteria

The bacterial population was cultured on a MRSA selective medium, supplemented with 1% $CaCO_3$. $CaCO_3$ is commonly used to select lactic acid bacteria that form clear zones, as it reacts with lactic acid to form calcium lactate. The formation of clear zones serves as an initial characterization criterion for the selection of lactic acid bacteria (Haro et al. 2020), indicating that $CaCO_3$ dissolution can help identify LAB on MRS agar medium supplemented with $CaCO_3$ (Paswan et al. 2023). Supplementation with $CaCO_3$ can increase lactic acid production, though the optimal $CaCO_3$ concentration for lactic acid production was 0.5% (Yang et al. 2015). A total of 39 lactic acid bacterial colonies were successfully isolated from lobster intestines, characterized by clear zones surrounding each colony.

Morphological examination revealed consistent colony characteristics across all isolates: circular form, convex elevation, entire margins, opaque optical character, and white coloration (Table 1). All of lactic acid bacteria isolates were Gram-positive. Gram-positive bacteria were identified through purple cell coloration, while Gram-negative bacteria displayed red coloration under the microscope. These characteristics suggested that the isolates were lactic acid bacteria (Ghanbari et al. 2009; Vijayaram et al. 2016; Nasri et al. 2021; Lingga et al. 2023).

Cell morphological analysis revealed three distinct bacterial shapes: bacilli, coccobacilli, and cocci. One isolate showed bacilli morphology, 29 isolates exhibited coccobacilli morphology, and nine showed cocci morphology. The coccus-shaped isolates were identified as LP2, LM4, LP15, LP40, LP49, LP30, LP31, LP32, and LP33. Generally, lactic acid bacteria have a coccoid or rod-shaped cell morphology (Wang et al. 2021). LAB constitutes more than 60 genera, including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Weissella*, *Streptococcus*, *Enterococcus*, etc. (Mokoena 2017). Lactic acid bacteria (LAB) are Gram-positive, non-spore-forming microorganisms that use carbohydrates as their only or primary carbon source (George et al. 2018).

Biochemical properties

Biochemical characterization included catalase, motility, Methyl-Red (MR), and Triple Sugar Iron Agar (TSIA) tests. Results revealed 30 catalase-negative and 9 catalase-positive isolates; the latter were excluded from further analysis (Table 1). These results are consistent with previous studies showing that LAB is catalase-negative (Yelnetty et al. 2014; Ramadhanti et al. 2021). The catalase test identifies bacteria capable of producing the catalase

enzyme, which decomposes hydrogen peroxide (H_2O_2) formed during aerobic respiration into water (H_2O) and oxygen (O_2) (Khatoon et al. 2022). As facultative anaerobes, Lactic Acid Bacteria (LAB) produce peroxidase enzymes that degrade H_2O_2 into organic compounds and water without generating bubbles (Saryono et al. 2023).

The motility test evaluates bacterial movement in the growth medium. Positive motility is observed when bacterial growth radiates outward from the stab line. Motility testing demonstrated that 28 isolates were non-motile, showing no diffuse growth around the SIM medium stab. In contrast, isolate LM02 displayed motile behavior with distinct growth patterns along the stab line.

The MR test determines the production of acidic metabolites during glucose fermentation. The MR test yielded positive results for 28 isolates, as evidenced by a red color change upon addition of the methyl red reagent. In contrast, isolate LM02 showed negative results and was subsequently excluded from further testing. This result indicates acid production, lowering the medium's pH due to the accumulation of lactic, acetic, succinic, and formic acids during glucose fermentation. These findings align with the study by Kursia et al. (2020), which reported similar positive MR test results in LAB isolates from vegetable waste.

TSIA testing demonstrated glucose fermentation by all isolates, as indicated by a yellow color change in the butt portion of the medium. However, differential responses were observed in sucrose and lactose fermentation: isolates LM1, LP3, and LP4 were unable to ferment these sugars, as evidenced by the red color retained in the slant portion. Meanwhile, the other isolates showed a yellow coloration on both the slant and the butt, indicating a presumed ability to ferment all three sugars (glucose, lactose, and sucrose). Similar results were reported by Suhartono et al. (2021), who demonstrated that LAB isolates from Aceh buffalo meat fermented glucose, sucrose, and lactose, leading to yellow coloration of the medium.

Lactic acid bacterial population

Probiotic potential evaluation was conducted after Multi-Variate Statistical Package (MVSP) analysis of the isolates. MVSP analysis was employed for bacterial isolate clustering. Eight distinct taxa were identified based on Simple Matching Coefficient analysis, represented by isolates LM1, LM4, LP2, LP3, LP4, LP12, LP40, and LP33. The identification results revealed that the lactic acid bacteria isolated from lobster intestines exhibited characteristics consistent with those of the genera *Lactobacillus* and *Enterococcus*.

Lactic acid bacterial population enumeration was done using the The Total Plate Count (TPC) technique. TPC method is a commonly employed technique in microbiology to estimate bacterial populations in a sample by incubating the sample on solid media until colonies form. Total Plate Count (TPC) analysis of the LAB isolates revealed that isolate M01 exhibited the highest bacterial population, with an average count of 23.5×10^8 CFU/mL, indicating superior adaptability and growth performance under the experimental conditions. Isolate LM04 demonstrated the second-highest population density, with an average count of 12.4×10^8 CFU/mL (Table 2).

Variations in population among the isolates may be attributed to several factors, including differences in metabolic capabilities, nutrient availability, and resilience to environmental conditions during incubation. Lactic acid bacteria often exhibit varying adaptability due to genetic and metabolic differences, enabling better growth under specific conditions (Patel et al. 2019). External factors such as media composition and environmental pH can also influence LAB viability and growth rates in culture, as highlighted in previous studies (Tomás et al. 2002).

Both LM1 and LM4 showed a higher population than the isolate-coded LP. LM was isolated from pearl lobsters, while LP was isolated from sand lobsters. The significant differences in LAB populations between pearl lobsters (*Panulirus ornatus*) and sand lobsters (*Panulirus homarus*) may stem from habitat disparities. Pearl lobsters typically inhabit coral reef ecosystems with high diversity in food sources, fostering gut conditions that support more substantial LAB colonization. In contrast, sand lobsters reside in sandy or muddy habitats with lower food diversity than coral reefs (Setyanto et al. 2019). These dietary variations between the two habitats likely affect the LAB populations thriving in their respective lobster hosts.

Table 1. Morphological characteristics and biochemical properties of LAB isolates

Characteristics	Isolate code							
	LP2	LP3	LP4	LP12	LP40	LP33	LM1	LM4
Morphological								
Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colony color	White	White	White	White	White	White	White	White
Cell shape-arrangement	Cocci	Cocco-bacilli	Cocco-bacilli	Cocco-bacilli	Cocci	Cocci	Bacilli	Cocci
Gram staining	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Biochemical								
Catalase	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
motility	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
TSIA	Yellow	Red	Red	Yellow	Yellow	Yellow	Red	Yellow
MR	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Haemolytic activity	Gamma	Gamma	Gamma	Gamma	Gamma	Gamma	Gamma	Gamma

Table 2. LAB populations growing on MRS medium

Isolate code	LAB populations (CFU/mL)
LP2	4.2×10^8
LP3	1.3×10^8
LP4	2.9×10^8
LP12	2.8×10^8
LP40	2.7×10^8
LP33	2.8×10^8
LM1	23.5×10^8
LM4	12.4×10^8
NC (MRS broth)	0

Table 3. Temperature and pH tolerances of selected LAB isolates

Isolate	LP2	LP3	LP4	LP12	LP40	LP33	LM1	LM4
Temperature (°C)								
4	-	-	-	-	-	-	-	-
27	+	+	+	+	+	+	+	+
37	+	+	+	+	+	+	+	+
45	+	+	+	+	+	+	+	+
pH								
2.5	+	+	+	+	-	-	+	+
4	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+

Note: +: Visible growth, -: No visible growth

Physiological properties

Physiological tests were conducted on Lactic Acid Bacteria (LAB) isolates from the gut of *Panulirus* sp., focusing on pH and temperature tolerances. All isolates were unable to grow at 4°C and remained stable at temperatures up to 45°C (Table 3).

The pH tolerance test was performed under varying conditions (pH 2.5, 4, 6, and 8). Results demonstrated that all isolates could survive at all pH levels, except isolates LP40 and LP33, which could not grow on pH 2.5 (Table 3). Lactic acid bacteria resistance to acid involves several mechanisms, including central metabolic pathways, proton pumps, changes in cell membrane composition and cell density, nucleic acid and protein damage repair, and neutralization processes (Liu et al. 2015; Guan and Liu 2020). Additionally, these mechanisms include the generation of alkaline compounds via the arginine dihydrolase system to counteract acidity, proton consumption through carbon dioxide formation during malolactic fermentation, and active proton export via membrane-associated pumps such as F1-F0-ATPase (Wang et al. 2018).

Temperature tolerances were evaluated at 27°C, 37°C, and 45°C. Observations revealed that all isolates grew under all tested temperatures, indicated by increased turbidity in the media and sediment formation at the bottom of the test tubes. The isolates remained viable at room temperature (27°C), incubation temperature (37°C), and shipping temperature (45°C) (Table 3). These results categorize the LAB isolates as mesophilic bacteria, with optimal growth temperatures ranging from 30 to 45°C (Terpou et al. 2019). Information on LAB stress response and adaptation mechanisms is of great practical significance for selecting appropriate LAB strains as well as for artisanal or large-scale industrial production (Sionek et al. 2024). These findings highlight the robustness of LAB isolates from *Panulirus* sp., further supporting their potential use as probiotics under diverse environmental conditions.

Antimicrobial activity

The antimicrobial activity of selected LAB isolates was tested against *E. coli*, *S. aureus*, *P. aeruginosa*, and *Vibrio* sp. The results indicated significant inhibitory effects on all pathogens (Table 4). Isolates LP2, LM1, and LM4 showed potent inhibitory effects against *E. coli* and strong effects against *S. aureus*. Isolate LM04 showed the highest inhibition zones, with 25.94±11.58 mm against *S. aureus* and 15.51±1.06 mm against *E. coli*. Meanwhile, isolates LP4, LP3, LP12, LP40, and LP33 demonstrated moderate to strong inhibitory activity. Isolate LP40 exhibited the largest inhibition zone against *P. aeruginosa* (12,22±0.21 mm), while isolate LP12 showed maximum inhibition against *Vibrio* sp. (11.00±0.02mm). The study conducted by Zainuddin et al. (2024) showed significant inhibition zone and bacteriocin activity of lactic acid bacteria isolated from shrimp against *Vibrio harveyi* and *V. parahaemolitycus*. These results suggest that LAB isolates can effectively inhibit pathogenic bacteria, likely due to their production of organic acids and other antimicrobial metabolites. LAB inhibits pathogens primarily by producing organic acids (e.g., lactic acid, acetic acid, and propionic acid), diacetyl, hydrogen peroxide, and bacteriocins.

Lactic acid, the primary metabolite of LAB glucose metabolism, is crucial for lowering environmental pH and creating unfavorable conditions for many pathogens (Bangar et al. 2022). The inhibition zone in Gram-positive bacteria was larger than that in Gram-negative bacteria. The ability of LAB to secrete lactic acid and antimicrobial peptides is likely the reason why lowering the environment's pH disrupts the cell structure of Gram-positive bacteria. In Gram-negative bacteria, the situation is different because of the outer membrane, which provides a physical barrier that mitigates the effects of LAB metabolic products (Hou et al. 2025).

Lactic acid can rapidly diffuse into microbial cells, causing cytoplasmic acidification and membrane preturbation, affecting protein folding, disruption of substrate transport, and inhibition of macromolecule synthesis, which collectively suppress bacterial growth (Mira et al. 2010; Peetermans et al. 2021). Additionally, bacteriocins produced by LAB inhibit peptidoglycan synthesis in the cell walls of Gram-positive and Gram-negative bacteria, resulting in weakened cell structures and eventual cell lysis (Hamidah et al. 2019). These findings reinforce the potential of LAB isolates as effective antimicrobial agents, highlighting their suitability for probiotic applications in controlling pathogenic bacteria.

Table 4. Antimicrobial activities of selected isolates of lactic acid bacteria

Isolate code	Inhibition zones (mm)±st dev			
	E. coli	***S. aureus***	***P. aeruginosa***	***Vibrio* sp.**
LP2	12.20±0.87	24.90±10.85	NT	NT
LM1	14.41±0.91	24.27±8.99	NT	NT
LM4	15.51±1.06	25.94±11.58	NT	NT
LP4	NT	NT	10.28±0.20	9.65±0.07
LP3	NT	NT	11.05±0.33	10.52±0.04
LP12	NT	NT	10.05±0.28	11.00±0.02
LP40	NT	NT	12.22±0.21	9.69±0.21
LP33	NT	NT	11.67±0.21	9.40±0.21
NC (MRS medium)	-	-	-	-

Note: ≤5 mm: Weak, 6-10 mm: Intermediate, 11-20 mm: Strong, ≥21 mm: Very strong, NT: Not Tested (Wally et al. 2022)

Table 5. Antibiotic sensitivity of selected isolates of lactic acid bacteria

Isolate code	Inhibition zone (mm)±st dev		
	Ampicillin	**Tetracycline**	**Chloramphenicol**
LP2	12.86±0.67	21.34±4.61	11.35±0.85
LP4	12.07±0.98	19.79±0.30	11.16±0.68
LP3	12.89±0.97	19.04±0.99	11.49±0.27
LP12	13.43±0.57	18.61±0.99	10.65±0.14
LP40	16.36±0.71	18.96±0.58	11.92±0.57
LP33	11.84±0.99	17.68±0.43	9.78±0.78
LM1	12.75±0.30	9.87±0.33	0
LM4	17.29±0.39	0	0

Note: Sensitive ≥21mm, Intermediate = 16-20 mm, Resistent ≤15 mm (Vlková et al. 2006)

Antibiotic sensitivity

LAB isolates' antibiotic sensitivity profiles were evaluated with paper disc assays for tetracycline, chloramphenicol, and ampicillin (Table 5). The sensitivity test for ampicillin showed that most LAB isolates were resistant, with inhibition zones <15 mm. However, isolates LP40 and LM04 showed intermediate sensitivity to ampicillin, with zone diameters of 16.36±0.71 mm and 17.29±3.94 mm, respectively. The enzymatic activity of β-lactamase primarily confers resistance to ampicillin (Gross et al. 2024), which cleaves the β-lactam ring of the antibiotic, thereby reducing its effectiveness. Additionally, the thick peptidoglycan layer in the cell wall of LAB provides structural integrity and shape, which can hinder the penetration of antibiotics by reducing cell wall permeability, thereby limiting antibiotic access to the cytoplasm (Sujadmiko and Wikandari 2017).

For tetracycline sensitivity, almost all isolates coded LP showed intermediate sensitivity, with inhibition zones ranging from 16 to 20 mm, while isolate LP2 showed sensitive results (21.34±4.61 mm). Meanwhile, isolates LM01 (9.87±0.33 mm) and LM04 (0 mm) are resistant to tetracycline. The LAB's resistance to tetracycline is due to genetic factors that enable the bacteria to survive antibiotic exposure. The main mechanisms of tetracycline resistance were efflux pumps, ribosomal protection, and enzymatic inactivation (Blake et al. 2025). Anisimova and Yarullina (2019) found that *Lactobacillus fermentum* showed tetracycline resistance due to genes such as tet(K), tet(M), tet(W), tet(S), and tet(L). The tet(K) gene encodes an efflux pump, while tet(M) protects the bacterial ribosome and contributes to resistance.

Regarding chloramphenicol, all LAB isolates showed resistance, with inhibition zones of less than 15 mm. Resistance to antimicrobials such as chloramphenicol is commonly found in *Lactobacillus casei* strains compared with other lactobacilli (Başbülbül et al. 2015). *Lactobacillus* spp. are generally susceptible to chloramphenicol, erythromycin and tetracycline (D'Aimmo et al. 2007). The ability of bacteria to resist chloramphenicol is linked to the presence of the cat gene, which encodes chloramphenicol acetyltransferase (Schwarz et al. 2004). Hummel et al. (2007) noted that while *Lactobacillus* species typically carry the cat gene, mutations in the bacterial genome can sometimes prevent its expression, thereby contributing to chloramphenicol resistance or reduced sensitivity. Generally, probiotic bacteria are tolerant of antibiotics, allowing them to continue living in the digestive tract, which may be exposed to antibiotics (Gueimonde et al. 2013). These findings underscore the variability in antibiotic resistance among LAB isolates and provide insight into the genetic mechanisms behind their resistance to specific antibiotics. Intrinsic antibiotic resistance in LAB can confer a survival advantage to the gut microbiota when these bacteria are exposed to antibiotics used to treat certain diseases (Refaat et al. 2025). LAB are increasingly being identified as potential reservoirs of Antibiotic Resistance (AR) genes, which may hinder effective treatment of infections caused by pathogenic bacteria (Nunziata et al. 2022). Understanding these mechanisms is crucial for evaluating the potential of LAB as probiotics and ensuring their safety in various applications.

LAB adhesion

A critical criterion for probiotic potential assessment is the ability to adhere to intestinal mucosal surfaces. Adhesion testing of LAB isolates revealed relatively low attachment capabilities across all eight isolates (Table 6). Isolate LP2 demonstrated the highest adhesion rate at 9.9±0.09%, while isolate LP40 showed the lowest at 3.5±0.25%. The present study used stainless steel as a testing surface. The results showed a pattern similar to that of Leska et al. (2022), in which LAB showed weaker adhesion to abiotic surfaces (glass and polystyrene) than to biotic surfaces. Bacteria enhance adhesion to substrates with similar surface charge (An and Friedman 1998). This adhesion demonstrates hydrophobic interactions between bacterial cell membranes and host cell surfaces (Krausova et al. 2019). Although the LAB isolate demonstrated the ability to adhere to stainless steel, further research is required to evaluate various LAB strains for their ability to adhere to Caco-2 intestinal epithelial cells and to confirm their probiotic potential in vivo.

Bacterial adhesion can be facilitated by adhesion-promoting factors such as lectins. Additionally, bacteria's production of exopolysaccharides (EPS) plays a significant role in supporting attachment mechanisms (García-Ruiz et al. 2014). The ability of LAB to adhere is crucial in blocking

the invasion and colonization of gut pathogens, shaping their competitive advantage and determines the competitive abilities of these bacteria (Du et al. 2022). Lactic acid bacteria can form biofilm on different surface (biotic and abiotic) to function as antagonistic effectors (Tatsaporn and Kornkanok 2020). LAB has abilty to produces metabolites Exopolysaccaride (EPS) that can inhibits the formation of the biofilms of pathogens such as *E. coli*, *S. aureus*, and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (Lee et al. 2021; Werning et al. 2022).

LAB identification using 16S rRNA gene

The identification of lactic acid bacteria as potential probiotic strains was performed through analysis of the 16S rRNA gene. Based on lactic acid production, tolerance to various temperature and pH conditions, hemolytic activity, antimicrobial activity and antibiotics resistance assay, two promising isolates (LP3 and LP12) were selected for molecular characterization. The 16S rRNA gene was amplified by PCR yielding an amplicon of approximately 1300 bp (Figure 1). Following sequencing, the edited nucleotide sequences were compared with those available in the GenBank database using the BLAST tool on the NCBI platform.

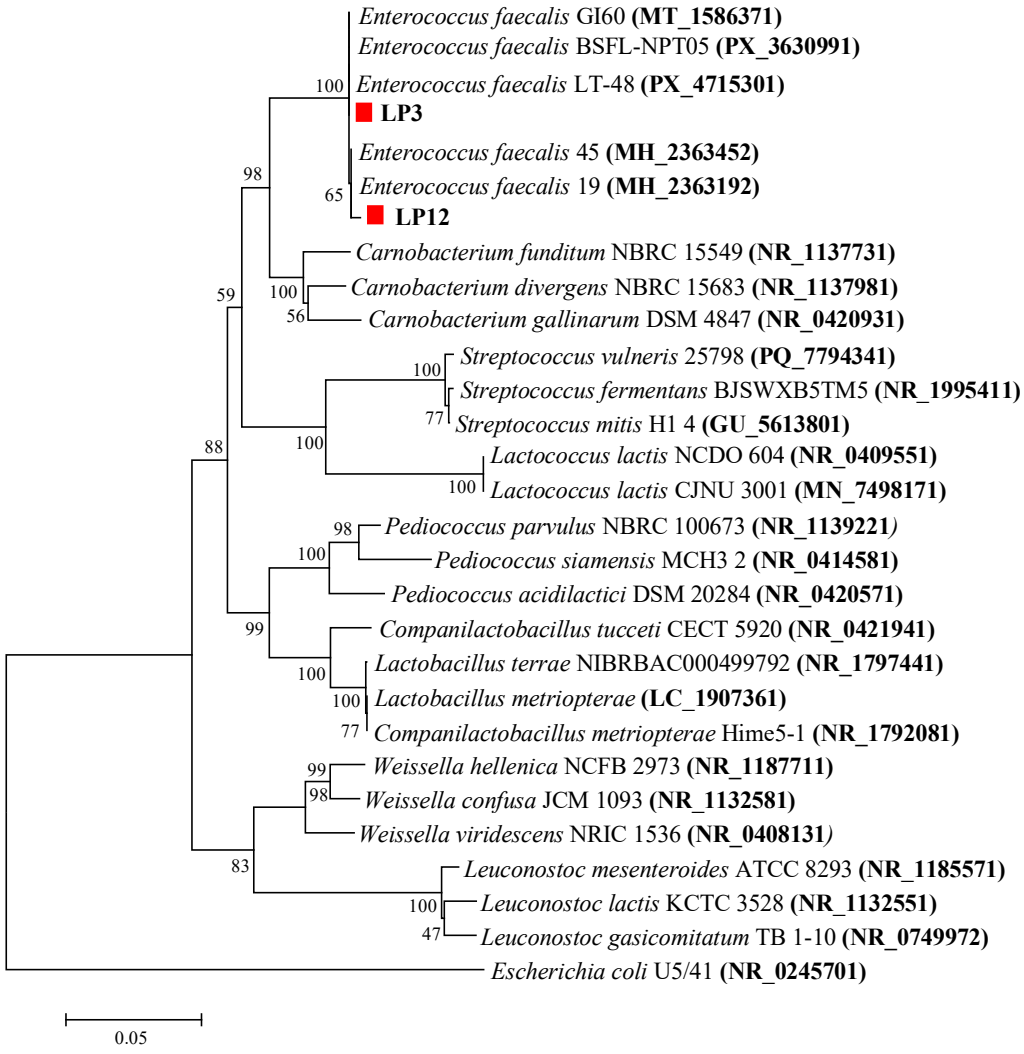

Figure 1. Phyllogenetic analysis of lactic acid bacteria isolated from spiny lobster using Maximum Composite Likelihood model

Table 6. Adhesion capability of LAB isolates on stainless steel surface

Isolate	LP2	LP4	LP3	LP12	LP40	LP33	LM1	LM4
Adhesion capability (%) ± st dev	9.9±0.09	4.1±0.08	7.3±0.15	4.1±0.05	3.5±0.25	4.7±0.12	9.7±0.37	9.6±0.05

The BLASTn results for 16S rRNA gene sequences revealed that isolates LP3 and LP12 were identified as *Enterococcus faecalis*, with minimum nucleotide sequence similarity to the GenBank database of 99,27% and 99,05%, respectively. *E. faecalis* can produce organic acids, including lactic and acetic acids, which lower the pH of the surrounding microenvironment. It also releases antimicrobial compounds, such as bacteriocins, that disrupt the cell membranes of pathogenic bacteria (Yin et al. 2024; Ladjouzi et al. 2025), and it can competitively limit pathogen colonization in the host intestine (Baccouri et al. 2019). In addition, *E. faecalis* may strengthen both local and systemic immune responses, potentially by activating gut-associated lymphoid tissue and/or producing immunomodulatory metabolites (Lin et al. 2018; Boeder et al. 2024).

In conclusion, this study demonstrated the potential of indigenous lactic acid bacteria isolated from the intestines of spiny lobsters (*Panulirus* sp.) as a probiotic agent. Eight distinct bacterial taxa were successfully isolated and characterized, belonging to the genera *Lactobacillus* and *Enterococcus*. These isolates exhibited remarkable physiological adaptability, maintaining viability across a wide range of pH (2.5-8.0) and temperatures (27-45°C). Notably, isolate LM4 demonstrated superior antimicrobial activity against pathogenic bacteria, producing inhibition zones of 25.94±11.58 mm against *S. aureus* and 15.51 15.51±1.06 mm against *E. coli*. Isolates LP40 and LP12 showed effectiveness against both *P. aeruginosa* and *Vibrio* sp. The highest bacterial population was observed in isolate LM1 (23.5×10^8 CFU/mL), LP2 showed the highest adhesion capabilities (9.9%±0.09). Meanwhile, further research is needed to optimize adhesion properties. These findings suggest that LAB isolates from *Panulirus* sp. possess potential for development as probiotics in sustainable lobster aquaculture, particularly for improving host health and disease resistance. Our research focused on exploring potential isolates by testing its capabilities in vitro with a limited replication rate. Future studies should focus on in vivo trials to validate these promising in vitro results and explore methods to enhance bacterial adhesion capabilities.

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REFERENCES

Adiputra YT, Zairin Jr. M, Suprayudi MA, Manalu W, Widanarni, Brite M. 2020. The effects of thyroxine hormone on gonadal maturation and growth of male spiny lobster (*Panulirus homarus*). Malays J Sci 39 (1): 30-40. https://doi.org/10.22452/mjs.vol39no1.3

Afrilasari W, Widanarni, Meryandini A. 2016. Effect of probiotic *Bacillus megaterium* PTB 1.4 on the population of intestinal microflora, digestive enzyme activity and the growth of catfish (*Clarias* sp.). Hayati J Biosci 23 (4): 168-172. https://doi.org/10.1016/j.hjb.2016.12.005.

Alp D, Kuleaşan H. 2020. Determination of competition and adhesion abilities of lactic acid bacteria against gut pathogens in a whole-tissue model. Biosci Microbiota Food Health 39 (4): 250-258. https://doi.org/10.12938/bmfh.2020-033.

Amali I, Sari PDW. 2020. Growth performance of cultivated spiny lobster (*Panulirus homarus*, Linnaeus 1758) in Tuban, East Java, Indonesia. Egypt J Aquat Biol Fish 24 (3): 381-388. https://doi.org/10.21608/ejabf.2020.92321.

An YH, Friedman RJ. 1998. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res 43 (3): 338-348. https://doi.org/10.1002/(sici)1097-4636(199823)43:3<338::aid-jbm16>3.0.co;2-b.

Anisimova EA, Yarullina DR. 2019. Antibiotic resistance of *Lactobacillus* strains. Curr Microbiol 76: 1407-1416. https://doi.org/10.1007/s00284-019-01769-7.

Arya RK, Singh J, Garg A. 2020. Detection and enumeration of lactic acid bacteria from human colostrum using traditional microbiology techniques. Adv Biores 11 (4): 84-88. https://doi.org/10.15515/abr.0976-4585.11.4.8488.

Ayivi RD, Gyawali R, Krastanov A, Aljaloud SO, Worku M, Tahergorabi R, da Silva RC, Ibrahim SA. 2020. Lactic acid bacteria: Food safety and human health applications. Dairy 1 (3): 202-232. https://doi.org/10.3390/dairy1030015.

Baccouri O, Boukerb AM, Farhat LB, Zébré A, Zimmermann K, Domann E, Cambronel M, Barreau M, Maillot O, Rincé I, Muller C, Marzouki MN, Feuilloley M, Abidi F, Connil N. 2019. Probiotic potential and safety evaluation of *Enterococcus faecalis* OB14 and OB15, isolated from traditional Tunisian Testouri cheese and Rigouta, using physiological and genomic analysis. Front Microbiol 10: 881. https://doi.org/10.3389/fmicb.2019.00881.

Bangar SP, Suri S, Trif M, Ozogul F. 2022. Organic acids production from lactic acid bacteria: A preservation approach. Food Biosci 46: 101615. https://doi.org/10.1016/j.fbio.2022.101615.

Baños A, Ariza JJ, Nuñez C, Gil-Martínez L, García-López JD, Martínez-Bueno M, Valdivia E. 2019. Effects of *Enterococcus faecalis* UGRA10 and the enterocin AS-48 against the fish pathogen *Lactococcus garvieae*: Studies in vitro and in vivo. Food Microbiol 77: 69-77. https://doi.org/10.1016/j.fm.2018.08.002.

Başbülbül G, Özteber M, Biyik HH. 2015. Antibiotic resistance in lactic acid bacteria isolated from fermented dairy products and boza. J Microbiol Biotechnol Food Sci 4 (6): 513-517. https://doi.org/10.15414/jmbfs.2015.4.6.513-517.

Blake KS, Xue Y-P, Gillespie VJ, Fishbein SRS, Tolia NH, Wencewicz TA, Dantas G. 2025. The tetracycline resistome is shaped by selection for specific resistance mechanisms by each antibiotic generation. Nat Commun 16: 1452. https://doi.org/10.1038/s41467-025-56425-5.

Boeder AM, Spiller F, Carlstrom M, Izídio GS. 2024. *Enterococcus faecalis*: Implications for host health. World J Microbiol Biotechnol 40: 190. https://doi.org/10.1007/s11274-024-04007-w.

Buxton R. 2005. Blood Agar Plates and Hemolysis Protocols. ASM Microbe Library. American Society for Microbiology, Washington, DC.

D'Aimmo MR, Modesto M, Biavati B. 2007. Antibiotic resistance of lactic acid bacteria and *Bifidobacterium* spp. isolated from dairy and pharmaceutical products. Intl J Food Microbiol 115 (1): 35-42. https://doi.org/10.1016/j.ijfoodmicro.2006.10.003.

Du Y, Li H, Shao J, Wu T, Xu WL, Hu X, Chen J. 2022. Adhesion and colonization of the probiotic *Lactobacillus plantarum* HC-2 in the intestine of *Litopenaeus vannamei* are associated with bacterial surface proteins. Front Microbiol 13: 878874. https://doi.org/10.3389/fmicb.2022.878874.

García-Ruiz A, de Llano DG, Esteban-Fernández A, Requena T, Bartolomé B, Moreno-Arribas MV. 2014. Assessment of probiotic properties in lactic acid bacteria isolated from wine. Food Microbiol 44: 220-225. https://doi.org/10.1016/j.fm.2014.06.015.

George F, Daniel C, Thomas M, Singer E, Guilbaud A, Tessier FJ, Revol-Junelles A-M, Borges F, Foligné B. 2018. Occurrence and dynamism of lactic acid bacteria in distinct ecological niches: A multifaceted functional health perspective. Front Microbiol 9: 2899. https://doi.org/10.3389/fmicb.2018.02899.

Ghanbari M, Rezaei M, Jami M, Nazari RM. 2009. Isolation and characterization of *Lactobacillus* species from intestinal contents of beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*). Iran J Vet Res 10 (2): 152-157. https://doi.org/10.22099/ijvr.2009.1668.

Gotcheva V, Hristozova E, Hristozova T, Guo M, Roshkova Z, Angelov A. 2002. Assessment of potential probiotic properties of lactic acid

bacteria and yeast strains. Food Biotechnol 16 (3): 211-225. https://doi.org10.1081/fbt-120016668.

Gross R, Yelin I, Lázár V, Datta MS, Kishony R. 2024. Beta-lactamase dependent and independent evolutionary paths to high-level ampicillin resistance. Nat Commun 15: 5383. https://doi.org/10.1038/s41467-024-49621-2.

Guan N, Liu L. 2020. Microbial response to acid stress: Mechanisms and applications. Appl Microbiol Biotechnol 104: 51-65. https://doi.org/10.1007/s00253-019-10226-1.

Gueimonde M, Sánchez B, de los Reyes-Gavilán CG, Margolles A. 2013. Antibiotic resistance in probiotic bacteria. Front Microbiol 4: 202. https://doi.org/10.3389/fmicb.2013.00202.

Hamidah MN, Rianingsih L, Romadhon. 2019. Aktivitas antibakteri isolat bakteri asam laktat dari papeda dengan jenis ikan berbeda terhadap *E. coli* dan *S. aureus*. J Ilmu dan Teknologi Perikanan 1 (2): 11-21. https://doi.org/10.14710/jitpi.2019.6742.

Hardiansyah MY, Musa Y, Jaya AM. 2020. Identifikasi plant growth promoting rhizobacteria pada rizosfer bambu duri dengan gram KOH 3%. Agrotech Res J 4 (1): 1-5. https://doi.org/10.20961/agrotechresj.v4i1.40875. [Indonesian]

Haro G, Iksen I, Nasri N. 2020. Identification, characterization and antibacterial potential of probiotic lactic acid bacteria isolated from *naniura* (a traditional Batak fermented food from carp) against *Salmonella typhi*. Rasayan J Chem 13 (1): 464-468. https://doi.org/10.31788/rjc.2020.1315530.

Holthuis LB. 1991. FAO Species Catalogue. Marine Lobsters of the World. An Annotated and Illustrated Catalogue of Species of Interest to Fisheries Known to Date. FAO Fisheries Synopsis. FAO, Rome.

Hou Y, Duan Y, Wu G, Zhang J, Luo X, Zhang M, Pang H, Hao Y, Wang Y, Cai Y, Wang L, Tan Z. 2025. Antibacterial activity, probiotic potential, and biocontrol efficacy of two lactic acid bacteria against *Penicillium expansum* on fresh grapes. Foods 14 (3): 493. https://doi.org/10.3390/foods14030493.

Hummel AS, Hertel C, Holzapfel WH, Franz CMAP. 2007. Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. Appl Environ Microbiol 73 (3): 730-739. https://doi.org/10.1128/aem.02105-06.

Ibrahim F, Ouwehand AC. 2019. The genus *Lactobacillus*. In: Lactic Acid Bacteria 5th Edition. CRC Press, Boca Raton.

Jones CM, Anh TL, Priyambodo B. 2019. Lobster aquaculture development in Vietnam and Indonesia. In: Radhakrishnan E, Phillips B, Achamveetil G (eds). Lobsters: Biology, Fisheries and Aquaculture. Springer, Singapore. https://doi.org/10.1007/978-981-32-9094-5_12.

Khatoon H, Anokhe A, Kalia V. 2022. Catalase test: A biochemical protocol for bacterial identification. AgriCos e-Newsletter 3 (1): 18.

Krausova G, Hyrslova I, Hynstova I. 2019. In vitro evaluation of adhesion capacity, hydrophobicity, and auto-aggregation of newly isolated potential probiotic strains. Fermentation 5 (4): 100. https://doi.org/10.3390/fermentation5040100.

Kurnia M, Amir H, Handayani D. 2020. Isolasi dan identifikasi bakteri asam laktat dari makanan tradisional Suku Rejang di Provinsi Bengkulu: "Lemea". Alotrop 4 (1): 25-32. https://doi.org/10.33369/atp.v4i1.13705. [Indonesian]

Kursia S, Imrawati, Ismail, Halim A, Ramadhani N, Ramadhani F, Priska F, Hanifah F. 2020. Identifikasi biokimia dan aktivitas antibakteri isolat bakteri asam laktat limbah sayur bayam. Media Farmasi 16 (1): 21-32. https://doi.org/10.32382/mf.v16i1.1369. [Indonesian]

Ladjouzi R, Taminiau B, Daube G, Lucau-Danila A, Drider D. 2025. The efficacy of the bacteriocinogenic *Enterococcus faecalis* 14 in the control of induced necrotic enteritis in broilers. Microbes Infect 27 (3): 105477. https://doi.org/10.1016/j.micinf.2025.105477.

Lau LYJ, Chye FY. 2018. Antagonistic effects of *Lactobacillus plantarum* 0612 on the adhesion of selected foodborne enteropathogens in various colonic environments. Food Control 91: 237-247. https://doi.org/10.1016/j.foodcont.2018.04.001.

Lee S-J, Jeon H-S, Yoo J-Y, Kim J-H. 2021. Some important metabolites produced by lactic acid bacteria originated from kimchi. Foods 10 (9): 2148. https://doi.org/10.3390/foods10092148.

Leska A, Nowak A, Szulc J, Motyl I, Czarnecka-Chrebelska KH. 2022. Antagonistic activity of potentially probiotic lactic acid bacteria against honeybee (*Apis mellifera* L.) pathogens. Pathogens 11 (11): 1367. https://doi.org/10.3390/pathogens11111367.

Lin D, Gao Y, Zhao L, Chen Y, An S, Peng Z. 2018. *Enterococcus faecalis* lipoteichoic acid regulates macrophages autophagy via PI3K/Akt/mTOR pathway. Biochem Biophys Res Commun 498 (4): 1028-1036. https://doi.org/10.1016/j.bbrc.2018.03.109.

Lingga R, Adibrata S, Roanisca O, Sipriyadi, Wibowo H, Arsyadi A. 2023. Probiotics potential of lactic acid bacteria isolated from slender walking catfish (*Clarias nieuhofii*). Biodiversitas 24 (8): 4572-4580. https://doi.org/10.13057/biodiv/d240839.

Liu Y, Tang H, Lin Z, Xu P. 2015. Mechanisms of acid tolerance in bacteria and prospects in biotechnology and bioremediation. Biotechnol Adv 33 (7): 1484-1492. https://doi.org/10.1016/j.biotechadv.2015.06.001.

Mannan SJ, Rezwan R, Rahman MS, Begum K. 2017. Isolation and biochemical characterization of *Lactobacillus* species from yogurt and cheese samples in Dhaka metropolitan area. Bangladesh Pharm J 20 (1): 27-33. https://doi.org/10.3329/bpj.v20i1.32090.

Marsela, Kautsari N, Ahdiansyah Y, Mardhia D, Bachri S, Latuconsina H. 2022. Metode penangkapan, jenis dan ukuran lobster yang tertangkap di perairan Suka Mulya, Labangka, Sumbawa. Albacore: J Penelitian Laut 6 (3): 293-304. https://doi.org/10.29244/core.6.3.293-304. [Indonesian]

Meidong, Nakao M, Sakai K, Tongpim S. 2021. *Lactobacillus paraplantarum* L34b-2 derived from fermented food improves the growth, disease resistance and innate immunity in *Pangasius bocourti*. Aquaculture 531: 735878. https://doi.org/10.1016/j.aquaculture.2020.735878.

Mira NP, Teixeira MC, Sá-Correia I. 2010. Adaptive response and tolerance to weak acids in *Saccharomyces cerevisiae*: A genome-wide view. OMICS: J Integr Biol 14 (5): 525-540. https://doi.org/10.1089/omi.2010.0072.

Mokoena MP. 2017. Lactic acid bacteria and their bacteriocins: Classification, biosynthesis and applications against uropathogens: A mini-review. Molecules 22 (8): 1255. https://doi.org/10.3390/molecules22081255.

Muthu Rathinam M, Kizhakudan JK, Vijayagopal P, Jayasankar V, Leslie VA, Sundar R. 2014. Effect of dietary protein levels in the formulated diets on growth and survival of juvenile spiny lobster *Panulirus homarus* (Linnaeus). Indian J Fish 61 (2): 67-72.

Nadia AB, Jannah SN, Purwantisari S. 2020. Isolation and characterization of lactic acid bacteria from *Apis mellifera* stomach and their potential as antibacterial using in vitro test against growth of *Staphylococcus aureus* and *Salmonella typhimurium*. Niche J Trop Biol 3 (1): 35-44. https://doi.org/10.14710/niche.3.1.35-44.

Nakhei RMH, Haghighi Khiabania AA, Azari TG, Razavillar V, Tehrani SM. 2023. The effects of probiotics and prebiotics on growth, stress responses, blood and immune parameters of rainbow trout. Iran J Fish Sci 22 (2): 278-302.

Nasri, Harahap U, Silalahi J, Satria D. 2021. Antibacterial activity of lactic acid bacteria isolated from dengke naniura of carp (*Cyprinus carpio*) against diarrhea-causing pathogenic bacteria. Biodiversitas 22 (8): 3098-3104. https://doi.org/10.13057/biodiv/d220802.

Nigam A, Kumar A, Madhusudan HV, Bhola N. 2012. In-vitro screening of antibacterial activity of lactic acid bacteria against common enteric pathogens. J Biomed Sci 1: 1-6.

Nofiani R, Sarwiyati, Ardiningsih P, Adhitiyawarman. 2022. Characteristics of lactic acid bacteria isolated from traditional fermented fish. Biodiversitas 23 (11): 5662-5669. https://doi.org/10.13057/biodiv/d231116.

Nunziata L, Brasca M, Morandi S, Silvetti T. 2022. Antibiotic resistance in wild and commercial non-enterococcal lactic acid bacteria and bifidobacteria strains of dairy origin: An update. Food Microbiol 104: 103999. https://doi.org/10.1016/j.fm.2022.103999.

Nurhasanah AK, Faturrahman F. 2019. Komunitas bakteri pada saluran pencernaan lobster mutiara (*Panulirus ornatus*). J Sains Teknologi Lingkungan 5 (1): 1-9. https://doi.org/10.29303/jstl.v5i1.100. [Indonesian]

O'Toole GA. 2016. Classic spotlight: How the Gram stain works. J Bacteriol 198 (23): 3128. https://doi.org/10.1128/jb.00726-16.

Paswan M, Adhikary S, Salama HH, Rusu AV, Zuorro A, Dholakiya BZ, Trif M, Bhattacharya S. 2023. Microbial synthesis of lactic acid from cotton stalk for polylactic acid production. Microorganisms 11 (8): 1931. https://doi.org/10.3390/microorganisms11081931.

Patel M, Ray B, Chen S. 2019. Environmental adaptation of lactic acid bacteria: A review of physiological factors affecting growth. Lactic Acid Bacteria Res 53 (4): 298-306.

Peetermans A, Foulquié-Moreno MR, Thevelein JM. 2021. Mechanisms underlying lactic acid tolerance and its influence on lactic acid production in *Saccharomyces cerevisiae*. Microb Cell 8 (6): 111-130. https://doi.org/10.15698/mic2021.06.751.

Ramadhanti N, Melia S, Hellyward J, Purwati E. 2021. Characteristics of lactic acid bacteria isolated from palm sugar from West Sumatra,

Indonesia and their potential as a probiotic. Biodiversitas 22 (5): 2610-2616. https://doi.org/10.13057/biodiv/d220520.

Refaat SS, Erdem ZA, Kasapoğlu MZ, Ortakcı F, Dertli E. 2025. Assessing the antibiotic resistance in food lactic acid bacteria: Risks in the era of widespread probiotic use. Food Sci Nutr 13 (8): e70740. https://doi.org/10.1002/fsn3.70740.

Safari R, Adel M, Lazado CC, Caipang CMA, Dadar M. 2016. Host-derived probiotic *Enterococcus casseliflavus* improves resistance against *Streptococcus iniae* infection in rainbow trout (*Oncorhynchus mykiss*) via immunomodulation. Fish Shellfish Immunol 52: 198-205. https://doi.org/10.1016/j.fsi.2016.03.020.

Sambrook J, Russell DW. 2001. Molecular Cloning: A Laboratory Manual. 3rd eds. Cold Spring Harbor Laboratory Press, New York.

Saryono, Ismawati, Pratiwi NW, Devi S, Sipayung MY, Suraya N. 2023. Isolation and identification of lactic acid bacteria from traditional food sarobuong of Kuantan Singingi District, Riau, Indonesia. Biodiversitas 24 (4): 2201-2206. https://doi.org/10.13057/biodiv/d240432.

Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev 28 (5): 519-542. https://doi.org/10.1016/j.femsre.2004.04.001.

Setyanto A, Rachman NA, Yulianto ES. 2019. Distribution and composition of lobster species caught in Java Sea of East Java, Indonesia. J Perikanan Universitas Gadjah Mada 20 (2): 49-55. https://doi.org/10.22146/jfs.36151.

Shafakatullah N, Chandra M. 2014. Screening of raw buffalo's milk from Karnataka for potential probiotic strains. Res J Recent Sci 3 (9): 73-78.

Sionek B, Szydłowska A, Trząskowska M, Kołożyn-Krajewska D. 2024. The impact of physicochemical conditions on lactic acid bacteria survival in food products. Fermentation 10 (6): 298. https://doi.org/10.3390/fermentation10060298.

Soltani M, Kane A, Taheri-Mirghaed A, Pakzad K, Hosseini-Shekarabi P. 2019. Effect of the probiotic, *Lactobacillus plantarum* on growth performance and haematological indices of rainbow trout (*Oncorhynchus mykiss*) immunized with bivalent streptococcosis/lactococcosis vaccine. Iran J Fish Sci 18 (2): 283-295. https://doi.org/10.22092/ijfs.2018.117757.

Sudarwati. 2020. Metodologi Penelitian Bisnis. PT Gramedia, Jakarta. [Indonesian]

Suhartono S, Misrahanum M, Yulvizar C, Khairami IR. 2021. *Lactococcus lactis* and *Lactococcus garvieae* identified from Aceh buffalo (*Bubalus bubalis*) associated lactic acid bacterial isolates. IOP Conf Ser Earth Environ Sci 711: 012005. https://doi.org/10.1088/1755-1315/711/1/012005.

Sujadmiko WKKY, Wikandari PR. 2017. Resistensi antibiotik amoksisilin pada strain *Lactobacillus plantarum* B1765 sebagai kandidat kultur probiotik. Unesa J Chem 6 (1): 54-58. https://doi.org/10.26740/ujc.v6n1.p%25p. [Indonesian]

Sukarya IBJ, Pratiwi IDPK, Arihantana NMIH, Puspawati NN. 2021. Ketahanan isolat bakteri asam laktat indigenus kombucha dan dadih terhadap antibiotik. J Ilmu Teknologi Pangan 10 (4): 734-745. https://doi.org/10.24843/itepa.2021.v10.i04.p18. [Indonesian]

Syafrizal, Jones CM, Permana IG, Utomo NBP. 2018. Effect of feeding frequency on survival and growth of juvenile spiny lobster *Panulirus versicolor* in Indonesia. AACL Bioflux 11 (5): 1427-1434.

Terpou A, Papadaki A, Lappa IK, Kachrimanidou V, Bosnea LA, Kopsahelis N. 2019. Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. Nutrients 11 (7): 1591. https://doi.org/10.3390/nu11071591.

Todhanakasem T, Ketbumrung K. 2020. Using potential lactic acid bacteria biofilms and their compounds to control biofilms of foodborne pathogens. Biotechnol Rep 26: e00477. https://doi.org/10.1016/j.btre.2020.e00477.

Todorov SD, Vaz-Velho M, Gibbs P. 2004. Comparison of two methods for purification of plantaricin ST31, a bacteriocin produced by *Lactobacillus plantarum* ST31. Braz J Microbiol 35 (1-2): 157-160. https://doi.org/10.1590/S1517-83822004000100026.

Tomás MSJ, Bru E, Wiese B, De Ruiz Holgado AAP, Nader-Macías ME. 2002. Influence of pH, temperature and culture media on the growth and bacteriocin production by vaginal *Lactobacillus salivarius* CRL 1328. J Appl Microbiol 93 (4): 714-724. https://doi.org/10.1046/j.1365-2672.2002.01753.x.

Verawaty M, Apriani N, Tarigan LR, Aprian ET, Laurenta WC, Muharni. 2020. Antibiotics resistant *Escherichia coli* isolated from aquatic ecosystems in Palembang, South Sumatra, Indonesia. Biodiversitas 21 (1): 86-97. https://doi.org/10.13057/biodiv/d210113.

Vijayaram S, Kannan S, Muthukumar S. 2016. Isolation and characterization of probiotic bacteria isolated from diverse fish fauna of the trodden Vaigai river at Theni district. J Chem Pharm Res 8 (7): 883-889.

Vlková E, Rada V, Popelářová P, Trojanová I, Killer J. 2006. Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. Livestock Sci 105 (1-3): 253-259. https://doi.org/10.1016/j.livsci.2006.04.011.

Vinoj G, Jayakumar R, Chen J-C, Withyachumnarnkul B, Shanthi S, Vaseeharan B. 2015. N-hexanoyl-L-homoserine lactone-degrading *Pseudomonas aeruginosa* PsDAHP1 protects zebrafish against *Vibrio parahaemolyticus* infection. Fish Shellfish Immunol 42: 204-212. https://doi.org/10.1016/j.fsi.2014.10.033.

Wally P, Marwah AS, Warang AF. 2022. Efektivitas ekstrak *Myristica fragrans* Houtt terhadap bakteri patogen *Pseudomonas aeruginosa* dan methicilin resistensi *Staphylococcus aureus*. J Biotek 10 (2): 224-239. https://doi.org/10.24252/jb.v10i2.31930. [Indonesian]

Wang C, Cui Y, Qu X. 2018. Mechanisms and improvement of acid resistance in lactic acid bacteria. Arch Microbiol 200: 195-201. https://doi.org/10.1007/s00203-017-1446-2.

Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, Bai X, Xie J, Wang Y, Geng W. 2021. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. Front Bioeng Biotechnol 9: 612285. https://doi.org/10.3389/fbioe.2021.612285.

Werning ML, Hernández-Alcántara AM, Ruiz MJ, Soto LP, Dueñas MT, López P, Frizzo LS. 2022. Biological functions of exopolysaccharides from lactic acid bacteria and their potential benefits for humans and farmed animals. Foods 11 (9): 1284. https://doi.org/10.3390/foods11091284.

Yang P-B, Tian Y, Wang Q, Cong W. 2015. Effect of different types of calcium carbonate on the lactic acid fermentation performance of *Lactobacillus lactis*. Biochem Eng J 98: 38-46. https://doi.org/10.1016/j.bej.2015.02.023.

Yelnetty A, Purnomo H, Purwadi, Mirah A. 2014. Biochemical characteristics of lactic acid bacteria with proteolytic activity and capability as starter culture isolated from spontaneous fermented local goat milk. J Nat Sci Res 4 (10): 137-146.

Yin H, Zhang X, Jiang X, Liu D. 2024. Characterization of the probiotic and functional properties of *Enterococcus faecalis* AQ10 isolated from chicken cecum. Lett Appl Microbiol 77 (12): ovae116. https://doi.org/10.1093/lambio/ovae116.

Zainuddin M, Pringgenies D, Brotosudarmo THP. 2024. Bacteriocin-producing lactic acid bacteria in shrimp intestine as a potential probiotic consortium. AACL Bioflux 17 (1): 80-90.