BIODIVERSITAS
Volume 26, Number 4, April 2025
Pages: 1827-1835

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260432

Lactic acid bacteria isolated from the digestive tract of spiny lobsters (*Panulirus homarus*) and their potency as probiotics

FATURRAHMAN[1,2,♥], KIKI RIZKI AULIYAH[1], LARAS ALISHIA JUANDA[1], TRI WAHYU SETYANINGRUM[1], SARKONO[1,2], BAMBANG FAJAR SURYADI[1,2], FARIQ AZHAR[3], BAYU PRIYAMBODO[4], WANDA QORIASMADILLAH[5]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Mataram. Jl. Majapahit No. 62, Mataram 83115, West Nusa Tenggara, Indonesia. Tel.: +62-370-646-506, ♥email: fatur@unram.ac.id
[2]Microbial Technology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Mataram. Jl. Majapahit No. 62, Mataram 83115, West Nusa Tenggara, Indonesia
[3]Department of Aquaculture, Faculty of Agriculture, Universitas Mataram. Jl. Pendidikan No. 37, Mataram 83115, West Nusa Tenggara, Indonesia
[4]Lombok Marine Aquaculture Center, The Ministry of Marine Affairs and Fisheries. Jl. Raya Sekotong, Lombok Barat 83365, West Nusa Tenggara, Indonesia
[5]Biodiversity Warriors, The KEHATI Foundation. Jl. Benda Alam I No. 73 Cilandak Timur, Jakarta Selatan 12560, Jakarta, Indonesia

Manuscript received: 10 October 2024. Revision accepted: 13 April 2025.

Abstract. *Faturrahman, Auliyah KR, Juanda LA, Setyaningrum TW, Sarkono, Suryadi BF, Azhar F, Priyambodo B, Qoriasmadillah W. 2025. Lactic acid bacteria isolated from the digestive tract of spiny lobsters (*Panulirus homarus*) and their potency as probiotics. Biodiversitas 26: 1827-1835.* Spiny lobsters (*Panulirus homarus*) are an on-demand marine fishery commodity with significant economic value. However, collected data indicate that the lobsters exhibit low growth rates and high mortality due to disease infection caused by *Vibrio* spp. The provision of probiotics in feed represents a potential solution to this issue. Lactic acid bacteria (LAB) have been identified as probiotics due to their ability to counteract pathogenic bacteria and synthesize enzymes capable of hydrolyzing complex molecules, such as proteins, carbohydrates, and lipids, thereby promoting organismal growth. The objective of this study was to isolate LAB from the digestive tract of spiny lobsters as probiotic candidates. The selection of LAB isolates from the digestive tract was based on their ability to inhibit the growth of *Vibrio harveyi* bacteria and their capacity to produce a range of extracellular enzymes, including proteases, lipases, and amylases. The results demonstrated the isolation of seven LAB isolates, classified *as Pediococcus*, *Enterococcus*, and *Lactobacillus* genera, which exhibited the capacity to impede the proliferation of *V. harveyi*. The majority of the isolated LAB were found to possess the ability to hydrolyze protein, starch, and fat, in addition to exerting inhibitory effects against the growth of *V. harveyi*. Among the isolates, SP6 exhibited the most promising characteristics as a probiotic candidate by demonstrating robust protein and fat hydrolysis abilities, along with a significant capacity to inhibit the growth of *V. harveyi*.

Keywords: Enzymes, spiny lobsters, LAB, probiotic, pathogenic bacteria, *Vibrio harveyi*

INTRODUCTION

Spiny lobsters (*Panulirus homarus*) are highly valuable marine commodities in Indonesia due to their significant economic worth and global market demand, leading to a substantial increase in production through aquaculture (Erlania et al. 2014). However, lobster farming faces several challenges, including long rearing periods due to slow growth rates and high mortality. The slow growth of these animals is thought to be linked to deficiencies in key nutrients, possibly caused by inefficient feed absorption during digestion (Agustin et al. 2023; Maharani et al. 2023). In addition, infectious diseases such as vibriosis caused by *Vibrio* spp. are commonly reported in lobster farming, contributing to high mortality rates (Valente and Wan 2021).

Probiotics have shown promise in regulating the microbial balance in the digestive tract, inhibiting the growth of harmful bacteria, and secreting extracellular enzymes that aid in digestion (Sumarsih et al. 2012). Bacteriocins or proteins that reduce harmful bacteria proliferation can be derived from beneficial bacteria like *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, and *Bifidobacteria*. These probiotics produce antimicrobial substances that prevent bacterial adhesion and translocation, as well as decrease the presence of harmful microorganisms in the gastrointestinal tract (Anee et al. 2021). A study by Daniels et al. (2013) found that *Bacillus* sp. probiotics, when added to the diet of *Homarus gammarus* lobsters, promoted growth and improved stress tolerance.

Lactic acid bacteria (LAB), a group commonly used as probiotics, produce bioactive compounds such as lactic acid, bacteriocins, hydrogen peroxide, ethanol, free fatty acids, organic acids, benzoic acid, and enzymes. These compounds possess antimicrobial properties that inhibit the growth of pathogenic bacteria (Chizhayeva et al. 2022). Additionally, LAB can secrete extracellular enzymes like protease, amylase, and lipase, which enhance feed absorption efficiency (Chizhayeva et al. 2022). LAB are also natural microflora in the digestive tract of aquatic animals, which suggests that using LAB isolates from spiny lobsters or similar organisms could improve compatibility and effectiveness (Muñoz-Atienza et al. 2013).

Research on the digestive tract microbiota of lobsters has identified various bacteria, including *Vibrio*, *Bacillus*, *Pseudomonas*, *Micrococcus*, and *Flavobacterium*, which vary in abundance depending on environmental conditions (Lein et al. 2022). These microorganisms produce digestive enzymes (lipase, protease, cellulase) that aid in food digestion, nutrient absorption, and vitamin synthesis while also protecting the host from pathogenic infections by activating immune responses in the digestive tract (Lein et al. 2022). Additionally, studies have shown that bacteria symbiotic with lobsters produce bacteriocins antibacterial peptides that may replace antibiotics, reducing the risks of bacterial resistance and drug residue in the organism (Nguyen et al. 2014).

Although previous studies have reported the antibacterial activity of symbiotic bacteria isolated from *Panulirus ornatus*, there remains a significant knowledge gap regarding the antibacterial potential of symbiotic microorganisms, particularly lactic acid bacteria (LAB), associated with *Panulirus homarus*. Notably, no scientific investigations have been conducted to evaluate the ability of LAB isolated from *P. homarus* to inhibit the growth of *V. harveyi*, a pathogenic bacterium known to cause vibriosis and high mortality rates in aquaculture species, including lobsters. Considering the increasing interest in the development of environmentally friendly alternatives to antibiotics, LAB have gained recognition for their potential as effective probiotics due to their capacity to produce antimicrobial compounds, compete with pathogens, and modulate host immune responses.

In this context, the present study aims to isolate and characterize LAB from the digestive tract of *P. homarus* and to assess their antibacterial activity specifically against *V. harveyi*. The findings of this research are expected to contribute to the foundational knowledge necessary for the future formulation of probiotic candidates with multistrain, multiaction, and site-specific colonization potential. Such probiotics may possess the ability to survive and function in distinct compartments of the lobster digestive system including the pyloric, cardiac, and intestinal regions thereby enhancing digestive efficiency and providing localized protection against pathogenic bacteria. This study represents an innovative approach to probiotic development in marine invertebrates and is expected to lay the groundwork for further research into tailored probiotic strategies for sustainable lobster aquaculture.

MATERIALS AND METHODS

Sampling

Spiny lobsters were collected from local fishermen in Telong Elong Village (-8.8093, 116.4951), East Lombok District, West Nusa Tenggara Province, Indonesia. To maintain their viability, the lobsters were wrapped in newspapers and placed in ice boxes until laboratory processing.

Isolation of LAB from the digestive tract of spiny lobsters

The digestive contents of spiny lobsters were obtained by dissecting from the cephalothorax to the abdomen. The digestive organs were then separated into cardiac, pyloric, and intestinal segments. Each segment (1 g) was transferred into 9 mL of physiological saline (NaCl), followed by serial dilutions up to 10^{-6} CFU/mL. Samples (1 mL) from the 10^{-4}, 10^{-5}, and 10^{-6} CFU/mL dilutions were plated on de Man Rogosa Sharpe (MRS) agar supplemented with 0.5% calcium carbonate ($CaCO_3$) using the pour-plate method. The plates were incubated at 30°C for 48 hours (Faturrahman et al. 2019).

Morphological characterization

Colony morphology was assessed based on shape, elevation, color, margin, and surface appearance of colonies grown on MRS agar. Cell morphology was further examined using Gram staining to determine bacterial shape and Gram reaction.

Biochemical characterization

For biochemical characterization, the following tests were conducted: (i) Catalase Test: Two drops of 3% hydrogen peroxide (H_2O_2) were added to a bacterial smear on a glass slide. The formation of gas bubbles indicated a positive result, signifying catalase enzyme production (Cappucino and Sherman 2002). (ii) Motility Test: Bacteria were stabbed into a semi-solid Sulfite Indole Motility (SIM) medium and incubated at 30°C for 24 hours. Motile bacteria exhibited spreading growth, whereas non-motile bacteria showed confined growth (Cappucino and Sherman 2002). (iii) Fermentation Type Test: LAB isolates were grown in MRS broth at 30°C for 48 hours to differentiate between homofermentative and heterofermentative bacteria. Gas bubble formation indicated heterofermentative LAB, whereas its absence suggested homofermentative LAB (Cappucino and Sherman 2002).

Physiological characterization

The physiological characterization of LAB was assessed by evaluating their growth under varying conditions. Temperature tolerance was determined by incubating LAB isolates in MRS broth at 10°C and 45°C for 48 hours. Salt tolerance was tested by culturing the isolates in MRS broth containing of 6.5% and 18% NaCl concentrations. Additionally, pH tolerance was examined by incubating the isolates in MRS broth at pH 4.4 and pH 9.6 to observe their adaptability to acidic and alkaline environments (Axelsson 2004).

Enzyme activity test

Casein hydrolysis test

LAB isolates were inoculated into MRS agar supplemented with 1% skim milk and incubated at 30°C for 48 hours. The presence of clear zones around colonies indicated protein hydrolysis (Khushboo et al. 2023).

Starch hydrolysis test

Bacteria were inoculated into MRS agar enriched with 1% starch and incubated at 30°C for 48 hours. Clear zones around colonies signified starch hydrolysis (Dida 2018).

Lipid hydrolysis test

Bacteria were inoculated into MRS agar supplemented with 1% olive oil and Tween 80. After incubation at 30°C for 48 hours, clear zones around colonies indicated lipid hydrolysis (Furini et al. 2018).

Hydrolysis index measurement

The hydrolysis index for protein, starch, and lipids was determined by comparing the diameter of the clear zone formed with the bacterial colony diameter (Silitonga et al. 2020).

$$\text{Hydrolysis Index} = \frac{\text{Diameter of Clear Zone (mm)}}{\text{Diameter of Colony (mm)}}$$

Cell-free supernatant hydrolysis test

The initial stage of this test included the production of extracellular enzymes through bacterial culture in MRS broth incubated at 30°C for 48 hours, then centrifugation was performed at 3200 rpm for 20 minutes to obtain cell-free supernatant. Approximately 100 µL of the cell-free supernatant containing crude enzyme extract was injected into wells on the proteolytic, amylolytic, and lipolytic test media. Another incubation was conducted at 30°C for 48 hours, while clear zones formed on the test media were observed and measured (Wihartati et al. 2022).

Antibacterial test

Preparation of LAB cell-free supernatant

The LAB cell-free supernatant was prepared following a modified method by Wihartati et al. (2022). LAB cultures were incubated in MRS broth at 30°C for 48 hours, then transferred into Falcon tubes. The suspension was centrifuged at 3200 rpm for 20 minutes, followed by a second centrifugation for 10 minutes. The supernatant was carefully collected from the top layer.

Preparation of test bacteria suspension

A bacterial suspension of *Vibrio harveyi* was prepared by transferring a single colony into a test tube containing 9 mL of sterile 0.9% NaCl solution. The suspension was vortexed for homogenization, and its turbidity was adjusted to match a 0.5 McFarland standard (~1.5×10^8 CFU/mL).

Antivibrio activity test

The antibacterial activity of LAB isolates was assessed using the well diffusion method. *V. harveyi* was inoculated onto Thiosulfate Citrate Bile Sucrose (TCBS) agar in Petri dishes. Once the medium solidified, 100 µL of LAB cell-free supernatant, a positive control (chloramphenicol), and a negative control (MRS broth) were added into 8 mm diameter wells (Truc et al. 2019). The plates were incubated at 30°C for 24 hours, and the diameters of the inhibition zones were measured using the following formula (Rumampuk et al. 2017):

$$\text{Inhibition Zone Diameter} = \frac{Dv + Dh}{2} - Ds$$

Where:
Dv: Vertical diameter
Dh: Horizontal diameter
Ds: Well diameter

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria from the digestive tract of spiny lobsters

A total of 14 LAB strains were isolated from the cardiac, pyloric, and intestinal segments of the digestive tract. As shown in Figure 1, multiple colonies formed on MRS agar supplemented with CaCO, exhibiting clear zones around them. The addition of $CaCO_3$ facilitated the differentiation of LAB from non-acid-producing bacteria by reacting with lactic acid to form calcium lactate ($C_6H_{10}CaO_6$). This reaction resulted in the formation of clear zones in the medium, indicating the presence of acid-producing LAB (Sari 2018).

A total of one, six, and seven LAB isolates were obtained from the cardiac, pyloric, and intestinal segments, respectively, with the highest abundance found in the intestinal segment. This variation in bacterial quantity is likely due to differences in pH levels along the digestive tract. The stomach's highly acidic environment (pH 2) may inhibit bacterial growth, whereas the intestine, with its neutral pH of 6-7, provides more favorable conditions for LAB proliferation (Ruiz Rodríguez et al. 2019). According to Derunets et al. (2024), most LAB exhibit slower growth in highly acidic environments and may experience cellular damage or loss of viability when exposed to low pH conditions.

Figure 1. LAB colonies growing on MRS agar enriched with 0.5% $CaCO_3$, showing clear zones

Morphological appearance of isolates

The morphological analysis of the 14 LAB isolates revealed that all colonies had a round shape with smooth margins. Most exhibited convex elevations, while some appeared flat. The colonies were predominantly white in color, with a few showing a slight yellowish tint. These morphological characteristics are in line with the findings of Rahayu and Setiadi (2023), who reported nine isolates capable of producing acid and exhibiting typical features of lactic acid bacteria, such as round cell shape, Gram-positive staining, non-endospore-forming, and non-motile behavior.

Cell morphology observations confirmed that all isolates were Gram-positive bacteria. The majority were round-shaped, while a few were rod-shaped. Under the microscope, Gram-positive bacteria appeared purple due to the presence of peptidoglycan in their cell walls, which retained the crystal violet stain (O'Toole 2016). Table 1 summarizes the isolation results.

Biochemical and physiological characterization

The biochemical and physiological characterization of the obtained isolates revealed that seven were catalase-negative, while five were catalase-positive (Table 2). All isolates were non-motile, homofermentative (Ho), and exhibited optimal growth at pH 4.4. According to Finanda et al. (2021), LAB are typically catalase-negative because they are facultative anaerobes that utilize peroxidase enzymes to break down H_2O_2 into organic compounds and water without generating air bubbles.

Hydrolysis of casein, starch, and lipid

Eight of the 14 LAB isolates obtained from the digestive tract of spiny lobsters exhibited enzymatic activity, indicated by the formation of clear zones around the colonies on each test medium. Table 3 presents the enzymatic activity of these isolates, along with data from the cardiac and pyloric segments, which form the upper and lower parts of the stomach.

Table 3. Enzymatic activity screening of LAB isolates from the spiny lobster digestive tract

Segment of origin	Number of isolate	Screening		
		Proteolytic	Amylolytic	Lipolytic
Cardiac	1	0	1	0
Pyloric	6	3	3	3
Intestine	7	3	2	3
Total	14	6	6	6

Table 1. The appearance of colony morphology of LAB isolates from the digestive tract of spiny lobsters

Isolate	Color	Formed	Edges	Elevation	Cell form	Gram
SK1	White	Round	Smooth	Convex	Round	Positive
SP1	White	Round	Smooth	Convex	Round	Positive
SP2	White	Round	Smooth	Convex	Rod	Positive
SP3	Yellow	Round	Smooth	Flat	Round	Positive
SP4	White	Round	Smooth	Convex	Round	Positive
SP5	Yellow	Round	Smooth	Convex	Rod	Positive
SP6	White	Round	Smooth	Flat	Round	Positive
SU1	White	Round	Smooth	Flat	Round	Positive
SU2	Yellow	Round	Smooth	Convex	Round	Positive
SU3	White	Round	Smooth	Convex	Rod	Positive
SU4	White	Round	Smooth	Convex	Round	Positive
SU5	Yellow	Round	Smooth	Convex	Round	Positive
SU6	White	Round	Smooth	Flat	Round	Positive
SU7	White	Round	Smooth	Convex	Rod	Positive

Table 2. Biochemical and physiological characterization of LAB isolates

Characteristics	Isolate code														
	SK1	SP1	SP2	SP3	SP4	SP5	SP6	SU3	SU4	SU5	SU6	SU7	Pc	Ec	Lb
Catalase	+	-	+	-	-	+	-	-	-	+	+	-	-	-	-
Motiliy		-		-	-		-	-	-			-	-	-	-
Fermentation Type		Ho		Ho	Ho		Ho	Ho	Ho			Ho	Ho	Ho	Ho/Hf
Temperature:															
10°C		+		-	+		-	+	+			+	±	+	±
45°C		-		+	+		+	-	+			+	±	+	±
pH:															
4.4		+		+	+		+	+	+			+	+	+	±
9.6		-		-	+		-	-	-			-	-	+	-
NaCl:															
6.5%		+		-	+		-	+	+			+	±	+	±
18%		-		-	-		-	-	-			-	-	-	-

Note: "+" indicates the presence of growth, "-" indicates the absence of growth, "±" indicates variability in growth, where some species show growth while others do not

Amylolytic bacteria were primarily found in the cardiac segment, likely because lobsters initially digest carbohydrates as an energy source. The pyloric segment contained a higher number of bacteria with proteolytic, amylolytic, and lipolytic activities, as this part of the digestive tract undergoes more intensive chemical digestion. According to Perera and Simon (2015), protease enzymes in the lobster digestive tract function optimally at neutral to slightly alkaline pH, whereas amylase is more active in acidic conditions. The neutral pH of the intestine facilitates the digestion of both carbohydrates and proteins, leading to the presence of proteolytic and amylolytic bacteria, along with lipolytic bacteria, which indicate lipid digestion in the pyloric and intestinal segments.

Proteolytic, amylolytic, and lipolytic activity tests were performed using both cell cultures and cell-free supernatants containing crude enzyme extracts. The formation of clear zones in these tests indicated enzymatic activity, with variations in zone size reflecting different hydrolysis efficiencies for proteins, starch, and fats. The highest protein and fat hydrolysis index was observed in isolate SP6, while the greatest starch hydrolysis index was recorded for isolate SU4. Table 4 presents the calculated values for these enzymatic activities.

Based on Table 4, six bacterial isolates from the digestive tract of spiny lobsters exhibited proteolytic activity, as indicated by the formation of clear zones on protein-containing (casein) media. The highest proteolytic hydrolysis index was recorded for isolate SP6, with a value of 3.32. The formation of clear zones around the colonies confirmed that casein was hydrolyzed by extracellular enzymes produced by the bacteria (Suciati et al. 2016).

Amylolytic activity tests (Table 3) demonstrated that starch was hydrolyzed by isolates SK1, SP4, SP5, SP6, SU3, and SU4. Among them, SU4 exhibited the highest amylolytic activity, with a hydrolysis index of 3.67. Similarly, lipolytic activity tests (Table 3) showed that six isolates (SP3, SP4, SP6, SU3, SU4, and SU5) demonstrated fat hydrolysis, as evidenced by clear zones around the colonies. The highest lipolytic activity was observed in SP6, with a fat hydrolysis index of 2.62.

Complex macromolecules such as proteins, fats, and starch are broken down into simpler compounds by extracellular enzymes produced by bacteria, facilitating their growth and development. LAB carry out proteolytic activity through a proteolytic system comprising proteinases, specific protein transporters, and peptidases, which help convert casein into free amino acids necessary for growth. Proteinases attached to the LAB cell wall hydrolyze casein into dipeptides, tripeptides, and oligopeptides (Kieliszek et al. 2021). These peptides are transported into the cell and further degraded by endopeptidases, proline-specific peptidases, dipeptidases, and aminopeptidases, releasing free amino acids (Wang et al. 2021). The movement of amino acids and peptides across the cytoplasmic membrane is facilitated by specific protein transporters such as Opp, DtpP, and DtpT in LAB (Kieliszek et al. 2021), with the resulting free amino acids supporting bacterial growth (Kieliszek et al. 2021).

Amylolytic bacteria hydrolyze starch through the secretion of amylase enzymes, which break down starch into simpler compounds such as glucose, maltose, and dextrin (Nangin and Sutrisno 2015). Starch degradation is carried out by four classes of enzymes: exoamylases, transferases, endoamylases, and debranching enzymes (Phieter et al. 2020). Starch consists of amylose, a linear chain of α-glucose linked by α-(1-4) glycosidic bonds, and amylopectin, a branched structure with α-(1-4) and α-(1-6) bonds. The extracellular amylase enzyme produced by *Lactobacillus* sp. is an endoamylase that hydrolyzes both α-(1-6) and α-(1-4) glycosidic bonds found in pullulan, amylopectin, and amylose (Veronica et al. 2022). However, certain LAB species cannot degrade starch, as this activity depends on their ability to directly utilize starch as a carbon source for lactic acid production (Maryati et al. 2021).

Table 4. Hydrolysis index of protein, starch, and fat by LAB isolates from the digestive tract of spiny lobsters

Hydrolysis test	Isolate	Diameter of clear zone (mm)	Colony diameter (mm)	Clear zone with cell-free supernatant (mm)	Hydrolysis index
Proteolytic	SP3	6.67	4.00	12.20	1.67
	SP4	16.67	6.34	18.67	2.63
	SP6	11.01	3.34	18.71	3.32
	SU3	11.67	4.67	29.67	2.50
	SU4	13.00	6.00	28.67	2.16
	SU5	6.67	5.00	12.17	1.33
Amylolytic	SK1	6.97	5.43	11.50	1.28
	SP4	1.20	7.00	19.70	1.71
	SP5	3.67	2.67	11.37	1.38
	SP6	10.34	3.34	22.40	3.10
	SU3	7.00	4.00	16.00	1.75
	SU4	22.00	6.00	24.33	3.67
Lipolytic	SP3	5.00	3.67	10.83	1.36
	SP4	12.33	6.67	15.00	1.85
	SP6	11.33	4.33	17.33	2.62
	SU3	9.67	5.33	13.00	1.81
	SU4	11.07	5.54	12.67	1.98
	SU5	9.00	4.00	16.00	2.25

Lipolytic activity reflects the ability of bacteria to produce lipases, which catalyze the hydrolysis of ester bonds in triacylglycerols, breaking them down into glycerol and fatty acids at the hydrophobic substrate-water interface (Chandra et al. 2020). Lipases can be intracellular or extracellular, with LAB primarily producing intracellular lipases that require autolysis to interact with substrates. Previous studies have shown that higher substrate concentrations result in greater lipolytic activity (Thierry et al. 2017). In this study, olive oil was used as the lipid substrate, as it is rich in unsaturated fatty acids, particularly oleic acid, which enhances lipase activity (Kivanc and Acu 2022).

Probiotic potential can be assessed based on the ability to produce multiple extracellular enzymes. In this study, isolates SP6 and SU4 exhibited the highest enzymatic activity, placing them in the strong category, as extracellular enzyme activity is classified as strong when the hydrolysis index is >2. A hydrolysis index of 1-2 is considered moderate, while 0-1 is classified as weak (Elida et al. 2022). Given their strong enzymatic activity, SP6 and SU4 show potential as probiotic bacteria for use as feed additives for spiny lobsters. The supplementation of spiny lobster feed with a consortium of these two LAB isolates could promote growth and serve as an alternative strategy for sustainable lobster aquaculture.

As isolates derived directly from the gastrointestinal tract of spiny lobsters (*P. homarus*), SP6 and SU4 exhibit specific adaptations to their host and environment, enabling them to function more effectively within the lobster's gastrointestinal system. These adaptations enhance the digestibility of the feed and optimize the bioavailability of nutrients, which are essential for the growth and health of the lobsters. Their exceptional multienzymatic capabilities, which are rarely observed in common probiotic strains, enable the efficient degradation of proteins, starch, and fats, thus providing comprehensive support for digestive processes. This advantage is further amplified by their potential to act synergistically as a probiotic consortium, with SP6 excelling in proteolytic and lipolytic activities, while SU4 demonstrates superior amylolytic activity. This combination yields a synergistic effect that not only facilitates the breakdown of complex nutrients but also optimizes nutrient metabolism efficiency, thus positioning SP6 and SU4 as superior candidates in comparison to generic or single-strain probiotics.

Inhibition test of LAB isolates against *V. harveyi*

The selection of probiotic candidates in this study was based on several essential criteria, including antibacterial activity. Chloramphenicol was used as a positive control, while MRS broth served as a negative control. The results of the antibacterial activity tests are presented in Table 5. Based on the table, seven LAB isolates were found to inhibit the growth of *V. harveyi*, as evidenced by the formation of inhibition zones around the wells. Three isolates (SP1, SP6, and SU7) demonstrated strong inhibition, while the remaining four isolates (SP3, SP4, SU3, and SU4) showed moderate inhibition. According to Prastiyanto et al. (2020), inhibition strength is categorized as very strong (>20 mm), strong (11-20 mm), moderate (6-10 mm), and weak (<5 mm).

The inhibition zones around the wells were caused by *V. harveyi* growth being restricted by the supernatant containing LAB, which produced compounds such as lactic acid, bacteriocins, (H_2O_2) and carbon dioxide (CO_2) capable of inhibiting the pathogenic bacteria. LAB inhibits pathogens through various mechanisms, including cell wall damage, suppression of cell wall synthesis, changes in membrane permeability leading to plasmolysis, and interference with protein and nucleic acid synthesis, as well as enzyme activity (Riadi et al. 2020).

Clear and turbid zones were observed when LAB inhibited *V. harveyi*, with clear zones indicating bactericidal activity from antimicrobial compounds like bacteriocins and organic acids. Turbid zones, often referred to as subinhibitory inhibition, are thought to result from fewer metabolites with bacteriostatic properties (Zhang et al. 2022). According to Maťátková et al. (2019), subinhibitory concentrations are those below the minimum inhibitory concentration but still capable of inhibiting bacterial growth. In this study, isolates SP1 and SU7 produced clear inhibition zones, while SP3, SP4, SP6, SU3, and SU4 displayed subinhibitory or turbid inhibition zones.

LAB produces metabolites, such as lactic acid, that inhibit *V. harveyi*, primarily through the release of organic acids during fermentation (Sandi and Subagiyo 2022). Lactic acid, in particular, exhibits bactericidal properties at a pH of 4.5 and concentrations greater than 0.2%. During LAB fermentation, lactic acid lowers the pH, disrupting enzyme activity and preventing metabolic functions in pathogenic bacteria (Fauziah et al. 2015).

Bacteriocins, which are proteinaceous toxins produced by LAB, also play a significant role in inhibiting pathogenic bacteria by disrupting protein synthesis and energy metabolism (Hamidah et al. 2019). Bacteriocins have bactericidal properties and can eliminate harmful bacteria even at low concentrations (Manoharan and Balasubramanian 2022). For example, the Gram-positive bacterium *Pediococcus pentosaceus* produces pediocin, a bacteriocin that strongly inhibits *V. harveyi* by disrupting its cell membrane (Haliman et al. 2023). Similarly, *Lactobacillus rhamnosus* produces lactocin, which significantly inhibits *V. harveyi* growth (Wu et al. 2022).

Table 5. Inhibition capacity of LAB supernatants against *V. harveyi* on TCBS agar incubated for 24 hours at 30°C

Isolate	Inhibition zone diameter (mm)	Inhibition strength	Categorization of inhibition (mm)
SP1	18	Strong	11-20
SP3	7.5	Moderate	6-10
SP4	6.83	Moderate	6-10
SP6	11.83	Strong	11-20
SU3	9.33	Moderate	6-10
SU4	7	Moderate	6-10
SU7	11	Strong	11-20
Control (+)	16	Strong	11-20
Control (-)	0	-	-

Note: Control (+): Chloramphenicol: Positive control using chloramphenicol (antibiotic standard) and control (-): MRS broth: Negative control using MRS broth without bacterial filtrate

Among the seven LAB isolates tested, SP1, SP6, and SU7 exhibited strong antivibrio activity, with inhibition zones measuring 18 mm and 11.83 mm for isolates from the genus *Pediococcus*, and 11 mm for an isolate from the genus *Lactobacillus*. Based on these results, LAB isolates from the genera *Pediococcus* and *Lactobacillus* have potential as probiotics to suppress *V. harveyi* growth and can be developed as additives for functional feeds. These probiotic candidates could promote digestive tract health in spiny lobsters and provide a safer alternative to antibiotics, which are known to contribute to bacterial resistance.

Comparative efficacy of LAB probiotics in aquaculture species

The application of lactic acid bacteria (LAB) in aquaculture has been the subject of extensive study across a variety of species, with findings demonstrating broad-spectrum benefits in improving growth performance, disease resistance, and overall aquaculture sustainability. For example, a study on the administration of the probiotic *Lactobacillus plantarum* to shrimp demonstrated significant improvements in growth performance, increased serological immunity indicators and hepatopancreatic immunity gene expression levels, and a reduction in mortality in shrimp exposed to *V. parahaemolyticus* (Wei et al. 2022). Furthermore, lactic acid bacteria have been demonstrated to enhance feed utilization, promote growth, and bolster disease resistance in shellfish. LAB have been demonstrated to enhance water quality and augment stress resistance. The administration of LAB to shellfish resulted in enhanced innate immune responses and elevated survival rates against pathogens. The immunomodulatory effects of mixed LAB strains have been demonstrated to be more pronounced than those of single strains (Ringø et al. 2020).

LAB have been demonstrated to stimulate gastrointestinal development, improve digestive function and enhance immune responses in finfish. LAB are capable of producing antibacterial substances that are effective against fish and human pathogens. Fish that have been fed LAB have exhibited increased innate immune activity, including neutrophil activity and cytokine production, which has resulted in increased resistance to disease. However, it should be noted that the immunological effects of LAB may vary depending on the species and specific LAB strain used (Ringø et al. 2018).

Challenges in probiotic development and application

Lobster aquaculture could greatly benefit from the use of probiotics, specifically LAB, but several challenges must be addressed in the development and commercialization process. One major challenge is the variability in probiotic efficacy, which can be influenced by factors such as dosage, mode of administration, and strain specificity. In this study, in vitro trials, including enzyme activity assays and inhibition tests, were used to assess the potential of probiotics. However, to apply these results effectively in real-world aquaculture, in vivo trials are essential for assessing the actual impact of probiotics on lobster growth and health.

Another significant challenge is ensuring the stability and viability of probiotics in commercial feed formulations. Probiotic bacteria are often sensitive to heat, moisture, and oxygen, which can reduce their efficacy during storage and feeding (El-Haroun et al. 2006). To address this, developing encapsulation or other protective delivery methods is crucial to maintaining the stability of LAB during feed processing and storage, ensuring their effectiveness in aquaculture.

Regulatory challenges surrounding the use of probiotics in aquaculture must also be considered. The safety of probiotic strains needs to be thoroughly evaluated to prevent adverse effects on both the aquaculture organisms and human consumers. Regulatory frameworks vary across countries, and the approval process for probiotic products can be lengthy and complex (Torres-Maravilla et al. 2024).

The LAB isolates from the digestive tract of spiny lobsters examined in this study show promising probiotic potential, particularly in their ability to produce beneficial enzymes and inhibit pathogenic bacteria. However, to fully harness the benefits of probiotics in lobster aquaculture, further research is necessary to address challenges related to probiotic stability, environmental impact, and regulatory approval (Fachri et al. 2024).

Future studies should focus on large-scale in vivo trials to validate the efficacy of LAB probiotics in aquaculture settings. Additionally, exploring new methods for effectively delivering probiotics to lobsters is essential. A deeper understanding of the genetic and physiological mechanisms underlying the stress tolerance of LAB will help identify strains that can thrive in marine environments. Comparative studies with other aquatic species can also provide valuable insights for optimizing probiotic use across various aquaculture systems (Rahayu et al. 2024).

While this study provides a strong foundation for the use of LAB in lobster aquaculture, many factors must still be considered to ensure successful implementation in the field. Probiotics have the potential to revolutionize sustainable aquaculture practices, but a multidisciplinary approach combining microbiology, aquaculture technology, and regulatory science is essential to achieve this goal (Hancz 2022).

The findings of this study indicate that probiotics with robust multienzymatic capabilities have significant potential for application in aquaculture practices. Probiotics that produce extracellular enzymes with the capacity to efficiently hydrolyze proteins, starches and lipids can enhance nutrient digestibility and bioavailability, which in turn can lead to improved growth rates in cultured lobsters (Perera and Simon 2015). The optimization of feed utilization through the use of probiotics can result in a reduction in feed waste, thereby lowering production costs and minimizing environmental impacts. This, in turn, contributes to the development of more sustainable aquaculture practices.

Furthermore, probiotics with such capabilities can play a pivotal role in disease management. The production of bioactive metabolites and the competitive exclusion of pathogenic bacteria by probiotics can facilitate the establishment of a healthy gut microbiome, thereby reducing the incidence of diseases in lobster farming. This is of

particular significance in intensive aquaculture systems, where disease outbreaks present a significant challenge. Moreover, the utilization of probiotics as feed supplements can diminish the necessity for antibiotics in aquaculture, addressing concerns about antimicrobial resistance and satisfying the increasing demand for environmentally friendly and secure aquaculture products (Indira et al. 2019). In general, incorporating probiotics with high enzymatic activity into lobster farming practices has the potential to enhance growth performance, augment disease resistance and advance sustainability, aligning with the global shift towards more responsible and eco-friendly aquaculture approaches.

In conclusion, this study identified seven out of 14 bacterial isolates from the digestive tract of spiny lobsters as lactic acid bacteria (LAB). These isolates included SP1, SP3, SP4, SP6, SU3, SU4, and SU7, which were classified into the genera *Pediococcus* (SP1, SP3, SP6, and SU4), *Enterococcus* (SP4), and *Lactobacillus* (SU3 and SU7). Most of the LAB isolates demonstrated the ability to hydrolyze proteins, starch, and fats, and exhibited antimicrobial activity against *V. harveyi*. Among them, SP6 emerged as the most promising probiotic candidate, due to its exceptional protein and fat hydrolysis capabilities, along with its strong inhibition of *V. harveyi* growth.

REFERENCES

Agustin R, Amin M, Lamid M. 2023. The effect of formulated diets with different protein sources on feed consumption, feed conversion ratio, and nutrient retention of scalloped spiny lobster (*Panulirus homarus*). IOP Conf Ser: Earth Environ Sci 1273 (1): 012047. DOI: 10.1088/1755-1315/1273/1/012047.

Anee IJ, Alam S, Begum RA, Shahjahan RM, Khandaker AM. 2021. The role of probiotics on animal health and nutrition. J Basic Appl Zool 82: 52. DOI: 10.1186/s41936-021-00250-x.

Axelsson L. 2004. Lactic acid bacteria: Microbiological and functional aspect. Rev Bras Cienc Farm 42 (3). DOI: 10.1590/S1516-93322006000300018.

Cappucino JG, Sherman N. 2002. Microbiology: A Laboratory Manual. 6th Edition, Pearson Education Inc., San Francisco.

Chandra P, Enespa, Singh R, Arora PK. 2020. Microbial lipases and their industrial applications: A comprehensive review. Microb Cell Fact 19 (1): 169. DOI: 10.1186/s12934-020-01428-8.

Chizhayeva A, Amangeldi A, Oleinikova Y, Alybaeva A, Sadanov A. 2022. Lactic acid bacteria as probiotics in sustainable development of aquaculture. Aquat Living Resour 35: 10. DOI: 10.1051/alr/2022011.

Daniels CL, Merrifield DL, Ringø E, Davies SJ. 2013. Probiotic, prebiotic and synbiotic applications for the improvement of larval European lobster (*Homarus gammarus*) culture. Aquaculture 416-417: 396-406. DOI: 10.1016/j.aquaculture.2013.08.001.

Derunets AS, Selimzyanova AI, Rykov SV, Kuznetsov AE, Berezina OV. 2024. Strategies to enhance stress tolerance in lactic acid bacteria across diverse stress conditions. World J Microbiol Biotechnol 40 (4): 126. DOI: 10.1007/s11274-024-03905-3.

Dida G. 2018. Isolation and characterization of starch degrading rhizobacteria from soil of Jimma University Main Campus, Ethiopia. Afr J Microbiol Res 12 (32): 788-795. DOI: 10.5897/ajmr2018.8873.

El-Haroun ER, Goda AS, Kabir Chowdhury MA. 2006. Effect of dietary probiotic biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia, *Oreochromis niloticus* (L.). Aquacult Res 37: 1473-1480. DOI: 10.1111/j.1365-2109.2006.01584.x.

Elida M, Agustina A, Ermiati E, Desminarti S. 2022. Isolate characterization and amylolytic properties of lactic acid bacteria from traditional fermented dadih. IOP Conf Ser Earth Environ Sci 1097 (1): 012025. DOI: 10.1088/1755-1315/1097/1/012025.

Erlania E, Radiarta IN, Sugama K. 2014. Dinamika kelimpahan benih lobster (*Panulirus* spp.) di perairan Teluk Gerupuk, Nusa Tenggara Barat: Tantangan pengembangan teknologi budidaya lobster. Jurnal Riset Akuakultur 9: 475-486. DOI: 10.15578/jra.9.3.2014.475-486. [Indonesian]

Fachri M, Amoah K, Huang Y, Cai J, Alfatat A, Ndandala CB, Shija VM, Jin X, Bissih F, Chen H. 2024. Probiotics and paraprobiotics in aquaculture: A sustainable strategy for enhancing fish growth, health and disease prevention-a review. Front Mar Sci 11: 1499228. DOI: 10.3389/fmars.2024.1499228.

Faturrahman, Ismiati I, Nurhasanah A. 2019. Distribusi bakteri penghasil enzim ekstraseluler pada saluran pencernaan lobster mutiara (*Panulirus ornatus*). Jurnal Sains Teknologi dan Lingkungan 5 (2): 71-82. DOI: 10.29303/jstl.v5i2.129. [Indonesian]

Fauziah PN, Nurhajati J, Chrysanti. 2015. Daya antibakteri filtrat asam laktat dan bakteriosin *Lactobacillus bulgaricus* KS1 dalam menghambat pertumbuhan *Klebsiella pneumoniae* Strain ATCC 700603, CT1538, dan S941. Majalah Kedokteran Bandung 47 (1): 35-41. DOI: 10.15395/mkb.v47n1.395. [Indonesian]

Finanda A, Mukarlina, Rahmawati. 2021. Isolasi dan karakterisasi genus bakteri asam laktat dari fermentasi daging buah pisang kepok (*Musa paradisiaca* L.). Protobiont 10 (2): 37-41. DOI: 10.26418/protobiont.v10i2.53897. [Indonesian]

Furini G, Berger JS, Campos JA, Sand STVD, Germani JC. 2018. Production of lipolytic enzymes by bacteria isolated from biological effluent treatment systems. An Acad Bras Ciênc 90 (3): 2955-2965. DOI: 10.1590/0001-3765201820170952.

Haliman RW, Kurnia MD, Iman MN, Kusuma SA, Halalludin B, Rizkiantino R, Sari PP, Rahayu M, Fitriana RN, Panjaitan BV, Jacinda AK, Laiman H. 2023. Isolation of *Pediococcus pentosaceus* to compete *Vibrio harveyi* in the shrimp *Litopenaeus vannamei* hatchery. Biodiversitas 24: 4514-4520. DOI: 10.13057/biodiv/d240853.

Hamidah MN, Rianingsih L, Romadhon R. 2019. Aktivitas antibakteri isolat bakteri asam laktat dari peda dengan jenis ikan berbeda terhadap *E. coli* dan *S. aureus*. Jurnal Ilmu dan Teknologi Perikanan 1 (2): 11-21. DOI: 10.14710/jitpi.2019.6742. [Indonesian]

Hancz C. 2022. Application of probiotics for environmentally friendly and sustainable aquaculture: A review. Sustainability 14 (22): 15479. DOI: 10.3390/su142215479.

Indira M, Venkateswarulu TC, Abraham Peele K, Nazneen Bobby M, Krupanidhi S. 2019. Bioactive molecules of probiotic bacteria and their mechanism of action: A review. 3 Biotech 9 (8): 306. DOI: 10.1007/s13205-019-1841-2.

Khushboo, Karnwal A, Malik T. 2023. Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. Front Microbiol 14: 1170725. DOI: 10.3389/fmicb.2023.1170725.

Kieliszek M, Pobiega K, Piwowarek K, Kot AM. 2021. Characteristics of the proteolytic enzymes produced by lactic acid bacteria. Molecules 26 (7): 1858. DOI: 10.3390/molecules26071858.

Kivanc M, Acu E. 2022. Production of extracellular lipase by *Enterococcus faecium* E68 with olive oil waste as substrate. Biotecnia 24 (3): 87-93. DOI: 10.18633/biotecnia.v24i3.1750.

Lein EY, Lal MTM,Maran BAV, Ch'ng CL, Hamasaki K, Sano M, Tuzan AD. 2022. Gastrointestinal microbiota of spiny lobster: A review. Fishes 7 (3): 108. DOI: 10.3390/fishes7030108.

Maharani SD, Amin M, Lamid M. 2023. Potency of formulated diets with different protein on feed consumption, feed conversion ratio, feed efficiency and nutrient retention of scalloped spiny lobster (*Panulirus homarus*). IOP Conf Ser: Earth Environ Sci 1273 (1): 012075. DOI: 10.1088/1755-1315/1273/1/012075.

Manoharan M, Balasubramanian TS. 2022. An extensive review on production, purification, and bioactive application of different classes of bacteriocin. J Trop Biodivers Biotechnol 7 (3): jtbb72735. DOI: 10.22146/jtbb.72735.

Maryati Y, Nuraida L, Hariyadi RD. 2021. Production of organic acid and Short-Chain Fatty Acids (SCFA) from lactic acid bacteria isolate on oligosaccharide media. Jurnal Kimia Sains Aplikasi 24 (6): 213-221. DOI: 10.14710/jksa.24.6.213-221.

Maťátková O, Pospíšilová D, Michailidu, Jaroš P, Masák J. 2019. Effect of subinhibitory concentration of antibiotics on *Rhodococcus erythropolis* and *Pseudomonas fluorescens* biofilm formation. Chem Pap 73: 1113-1119. DOI: 10.1007/s11696-018-0662-9.

Muñoz-Atienza E, Gómez-Sala B, Araújo C, Campanero C, del Campo R, Hernández PE, Herranz C, Cintas LM. 2013. Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of

aquatic origin intended for use as probiotics in aquaculture. BMC Microbiol 13: 15. DOI: 10.1186/1471-2180-13-15.
Nangin D, Sutrisno A. 2015. Enzim amilase pemecah pati mentah dari mikroba: Kajian pustaka. Jurnal Pangan dan Agroindustri 3 (3): 1032-1039. [Indonesian]
Nguyen VD, Pham TT, Nguyen THX, Nguyen TTX, Hoj L. 2014. Screening of marine bacteria with bacteriocin-like activities and probiotic potential for ornate spiny lobster (*Panulirus ornatus*) juveniles. Fish Shellfish Immunol 40: 49-60. DOI: 10.1016/j.fsi.2014.06.017.
O'toole GA. 2016. Classic spotlight: How the gram stain works. J Bacteriol 198 (23): 3128. DOI: 10.1128/jb.00726-16.
Perera E, Simon C. 2015. Digestive physiology of spiny lobsters: Implications for formulated diet development. J Rev Aquacult 7 (4): 243-261. DOI: 10.1111/raq.12066.
Phieter AC, Chrisnasari R, Pantjajani T. 2020. Karakterisasi enzim pemecah pati dari malt serelia. Keluwih: J Sci Technol 1 (1): 38-48. DOI: 10.24123/saintek.v1i1.2773. [Indonesian]
Prastiyanto ME, Wardoyo FA, Wilson W, Darmawati S. 2020. Antibacterial activity of various extracts of *Averrhoa bilimbi* against multidrug resistant bacteria. Biosaintifika 12 (2): 163-168. DOI: 10.15294/biosaintifika.v12i2.23600.
Rahayu HM, Setiadi AE. 2023. Isolation and characterization of indigenous lactic acid bacteria from Pakatikng Rape, Dayak's Traditional Fermented. Food Nat Sci Educ Res J 9 (2): 920-925. DOI: 10.29303/jppipa.v9i2.2801.
Rahayu S, Amoah K, Huang Y, Cai J, Wang B, Shija VM, Jin X, Anokyewaa MA, Jiang M. 2024. Probiotics application in aquaculture: Its potential effects, current status in China and future prospects. Front Mar Sci 11: 1455905. DOI: 10.3389/fmars.2024.1455905.
Riadi S, Setiyawati D, Situmeang S. 2020. Isolasi dan uji potensi bakteri asam laktat asal kimchii dan teh kombucha dalam menghambat bakteri patogen. Jurnal Kesmas Prima Indonesia 4 (1): 25-29. DOI: 10.34012/jkpi.v2i1.891. [Indonesian]
Ringø E, Doan HV, Lee S, Song SK. 2020. Lactic acid bacteria in shellfish: possibilities and challenges. Rev Fish Sci Aquac 28 (2): 139-169. DOI: 10.1080/23308249.2019.1683151.
Ringø E, Hoseinifar SH, Ghosh K, Doan HV, Beck BR, Song SK. 2018. Lactic acid bacteria in finfish-An update. Front Microbiol 9: 1818. DOI: 10.3389/fmicb.2018.01818.
Ruiz Rodríguez LG, Mohamed F, Bleckwedel J, Medina R, Vuyst LD, Hebert EM, Mozzi F. 2019. Diversity and functional properties of lactic acid bacteria isolated from wild fruits and flowers present in Northern Argentina. Front Microbiol 10: 1091. DOI: 10.3389/fmicb.2019.01091.
Rumampuk YB, Wowor PM, Mambo CD. 2017. Uji daya hambat ekstrak spons laut (*Callyspongia aerizusa*) terhadap pertumbuhan bakteri *Salmonella typhi* dan *Streptococcus pyogenes*. eBiomedik 5 (2). DOI: 10.35790/ebm.5.2.2017.18480. [Indonesian]
Sandi FM, Subagiyo S. 2022. Aktifitas antibakteri isolat bakteri asam laktat saluran pencernaan kuda laut (*Hippocampus kuda* Bleeker, 1852) terhadap *Vibrio harvey*. Jurnal Kelautan Tropis 25 (2): 241-248. [Indonesian]
Sari M. 2018. Kemampuan antimikroba bakteri asam laktat (BAL) dari bekasam dalam menghambat *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, dan *Salmonella* sp. [Thesis]. Universitas Sumatera Utara, Medan. [Indonesian]
Silitonga LR, Nursyirwani, Effendi I. 2019. Isolation, identification and sensivity of amilolitic bacteria from mangrove ecosystem sediment in Purnama Marine Station Dumai on the phatogenic bacteria. Asian J Aquat Sci 2 (3): 257-266. DOI: 10.31258/ajoas.2.3.257-266.
Suciati P, Tjahjaningsih W, Masithah ED, Pramono H. 2016. Activity enzymatic of isolate lactic acid bacteria from the digestive tract of mud crab (*Scylla* spp.) as a candidate probiotics. Jurnal Ilmiah Perikanan Kelautan 8 (2): 94-108. DOI: 10.20473/jipk.v8i2.11182.
Sumarsih S, Sulistiyanto B, Sutrisno CI, Rahayu ES. 2012. Peran probiotik bakteri asam laktat terhadap produktivitas unggas. Jurnal Litbang Provinsi Jawa Tengah 10 (1): 1-9. [Indonesian]
Thierry A, Collins YF, Mukdsi MCA, McSweeney PLH, Wilkinson MG, Spinnler HE. 2017. Lipolysis and metabolism of fatty acids in cheese. In: McSweeney PLH, Fox PF, Cotter PD, Everett DW (eds). Cheese. Academic Press, London. DOI: 10.1016/B978-0-12-417012-4.00017-X.
Torres-Maravilla E, Parra M, Maisey K, Vargas RA, Cabezas-Cruz A, Gonzalez A, Tello M, Bermúdez-Humarán LG. 2024. Importance of probiotics in fish aquaculture: Towards the identification and design of novel probiotics. Microorganisms 12 (3): 626. DOI: 10.3390/microorganisms12030626.
Truc LNT, Ngoc AT, Hong TTT, Thanh TN, Kim HH, Kim LP, Truong GH, Quoc PT, Ngoc TNT et al. 2019. Selection of lactic acid bacteria (LAB) antagonizing *Vibrio parahaemolyticus*: The pathogen of acute hepatopancreatic necrosis disease (AHPND) in whiteleg shrimp (*Penaeus vannamei*). Biology 8: 91. DOI: 10.3390/biology8040091.
Valente CS, Wan AH. 2021. *Vibrio* and major commercially important vibriosis diseases in decapod crustaceans. J Invertebr Pathol 181: 107527. DOI: 10.1016/j.jip.2020.107527.
Veronica N, Liew CV, Heng PWS. 2022. Impact of amylose-amylopectin ratio of starches on the mechanical strength and stability of acetylsalicylic acid tablets. AAPS PharmSciTech 23 (5): 118. DOI: 10.1208/s12249-022-02266-0.
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. DOI: 10.3389/fbioe.2021.612285.
Wei C, Luo K, Wang M, Li Y, Pan M, Xie Y, Tian X. 2022. Evaluation of potential probiotic properties of a strain of *Lactobacillus plantarum* for shrimp farming: From beneficial functions to safety assessment. Front Microbiol 13: 854131. DOI: 10.3389/fmicb.2022.854131.
Wihartati LD, Permana IDGM, Hapsari NMI, Puspawati NN. 2022. Antibacterial activity of *Lactobacillus plantarum* 1 RN9 against *Escherichia coli* ATCC 25922. J Itepa 11 (4): 669-687. DOI: 10.24843/itepa.2022.v11.i04.p08.
Wu D, Dai M, Shi Y, Zhou Q, Li P, Gu Q. 2022. Purification and characterization of bacteriocin produced by a strain of *Lacticaseibacillus rhamnosus* ZFM216. Front Microbiol 13: 1050807. DOI: 10.3389/fmicb.2022.1050807.
Zhang F, Zhou K, Xie F, Zhao Q. 2022. Screening and identification of lactic acid bacteria with antimicrobial abilities for aquaculture pathogens in vitro. Arch Microbiol 204 (12): 689. DOI: 10.1007/s00203-022-03285-y.