Bacteriocin-producing lactic acid bacteria in shrimp intestine as a potential probiotic consortium

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Abstract. Shrimp is one of Indonesia's fishery export commodities that has provided a quite massive foreign exchange income for the country. The objective of this study was to obtain bacteriocin-producing lactic acid bacteria from the stomach contents of several types of shrimp from the Karimunjawa waters, Central Java, Indonesia. The research method used was descriptive, explorative, and also experimental. The sample collection was carried out in Karimunjawa waters, Central Java, Indonesia. The research phase consisted of: (1) Screening of bacteriocin-producing lactic acid bacteria. (2) Optimization of bacterial culture from the digestive tract of shrimp. (3) Acid resistance test, steel plate attachment test, and pathogenicity test. (4) Identification of lactic acid 4 types of shrimp species were collected from the waters of Karimunjawa: *Metapenaeus affinis*, *Harpiosquilla raphidea*, *Parapenaeopsis sculptilis*, and *Penaeus merguiensis*. The bacterial screening of the collected specimens found 32 bacterial isolates based on the pathogenic bacteria test results (activity against *Vibrio harveyi, Vibrio alginolitycus, Vibrio parahemolitycus*, and *Vibrio vulnivicus* pathogens). 2 isolates of candidate bacteria were resistant to acid, able to stick and colonize on steel plates, and not pathogenic. Based on the optimization of the probiotic production and on the probiotic dosage formulation, 2 types of bacteria were selected, namely the isolate K.15, which was related to *Lactobacillus plantarum* No. MW693210 (99.3%), and the isolate K.28, which was related to the *Lactococcus garviae* bacteria No. MH0572260 (99.71%).
Key Words: bacteriocin, *L. garviae*, *L. plantarum*, lactic acid, shrimp.

Introduction. Shrimp has provided a massive foreign exchange income for the country. Indonesia increases shrimp exports through aquaculture activities. However, a production decline occurred in shrimp farming in Indonesia due to the accumulation of organic feed waste, which resulted in pond water quality damage (Zhang et al 2022), low shrimp growth (Li et al 2022), and vibriosis disease (Supono et al 2019; de Souza Valente & Wan 2021). Disease control in Indonesia has been based on the use of drugs and chemicals. However, the application of antibiotic drugs increases the risk of antibiotic accumulation in the pond ecosystem, causing the resistance of pathogenic bacteria to antibiotics. The right solution to the problem is to take preventive measures using probiotics. However, there are some issues: the commercial probiotics available in the community are only of a single isolate type with restrained abilities, coming from a single source (Ansari et al 2019; Koirala & Anal 2021). Therefore, this research proposes a basic material to obtain probiotics in the form of a bacterial consortium producing extracellular enzymes, such as proteases, amylase, cellulose and lipase, and also active antibacterial compounds against vibriosis pathogens (Pringgenies et al 2020). The results showed that marine bacterial isolates had protein, fat, and carbohydrate degrading activity and produced anti-bacterial and antifungal compounds (Pringgenies et al 2016; Pringgenies & Setyati 2021).

Research also showed that the consortium of bacteria in the stomach content of sea cucumbers can reduce the number of bacteria (CFU mL^{-1}) in fish (Djunaedi et al 2021) and the impact of mixed seaweed feed on the immune response of *Oreochromis niloticus* (Susanto et al 2023). The sea cucumber compounds and bacterial symbionts can be used as raw materials for the marine aquaculture (Santosa et al 2020), also improving the fish meat quality (Pringgenies et al 2021a). On the other hand, bacteria originating from shrimp stomachs improve the fish immunity, due to their potential as anti-bacterial and anti-viral agents (Stan et al 2021; Balasubramaniam et al 2021). Thus, the research objective was to produce bacteriocin by lactic acid bacteria from the stomach content of several types of shrimp living in the Karimunjawa waters, Central Java, Indonesia.

Material and Method

Sample collection and shrimp identification. Samples were collected from the waters of Karimunjawa, Central Java, Indonesia. A total of 4 samples of various types of shrimp, including *Metapenaeus affinis*, *Harpiosquilla raphidea*, *Parapenaeopsis sculptilis*, and *Penaeus merguiensis*, were caught. The samples were immediately placed into an icebox for the identification and isolation of bacteria from the shrimp guts in the laboratory of the Aquaculture Study Program at Nahdlatul Ulama Islamic University, Jepara. The researchers identified the shrimp species as members of the suborder Macrura Natantia was based on a reference website (Holthuis 1980; Wardiatno & Mashar 2011).

Lactic Acid Bacterial (LAB) activity test and Total Plate Count (TPC) test. The antibacterial activity test against indicator bacteria was carried out using the disc method. The LAB isolates were grown in 5.0 mL liquid MRS medium, then vortexed until homogeneous, and then incubated at 32ºC for 24 h. The liquid culture was centrifuged at 1600xg, at 4ºC, for 15 minutes. The filtrate was neutralized to pH 6.0 using a pH meter, by adding 1 N of NaOH solution. The filtrate was sterilized with a Millipore filter to obtain an antibacterial supernatant. Then, A nutrient agar (NA) media was prepared, which previously had been mixed with the indicator bacteria. The indicator bacteria were *Vibrio harveyi*, *Vibrio alginolitycus*, *Vibrio parahemolitycus*, and *Vibrio vulnivicus*. Total Plate Count (TPC) testing was done to show the number of microbes contained in the product by counting the bacterial colonies grown on the agar medium. The scoring of the best isolate selection was done by obtaining a comparison of the inhibition zone (mm) and bacteriocin activity (AU mL^{-1}) against the test bacteria.

Selection of carbon and nitrogen sources. The nutrient selection, including carbon and nitrogen sources, used the modified methods of Polak-Berecka et al (2011) and Pringgenies et al (2021b). Experiments to optimize the concentration of carbon and nitrogen in the modified Zobell 2216E medium, with reference to (Pringgenies et al 2021b), were carried out from the carbon and nitrogen sources that had been successfully selected, based on the carbon and nitrogen source selection experiments.

Optimization of pH and salinity. The liquid Zobell 2216E medium was added with a carbon source and a nitrogen source with optimum concentrations obtained through a concentration orientation experiment in an Erlenmeyer inoculated with a starter isolate, which gave an OD of 0.01 on A_{600}, at anoptimal pH and salinity for growth (Pringgenies et al 2021b). The tested pH values were pH 3, pH 7 (normal), and pH 8.

Bacterial density measurement. The bacterial density measurement was carried out using a spectrophotometer, by inserting a blank (control) solution into the cuvette of the spectrophotometer and setting the wavelength of the spectrophotometer at 600 nm. Then, the solution that already contained the sample was put into the cuvette of the spectrophotometer. Optical density (OD) data from a spectrophotometer is used to calculate absolute growth (AG), growth rate (μ), number of generations (g), and generation time (Gt): a measure of the absorbance of light by a solution containing bacterial cells. The absolute growth is a measure of the total increase in the quantity of

bacteria over a specific period. It is calculated by subtracting the initial bacterial density (control) from the final bacterial density (experimental sample). The growth rate represents the exponential increase in the bacterial population over a specific period. Growth rate is calculated using the formula:

$$\mu = (\ln(Nf) - \ln(Ni)) / \Delta t$$

Where:
Nf - the final bacterial density;
Ni - the initial bacterial density;
Δt - the time interval.

The number of generations is a measure of how many times a bacterial population doubles during the exponential growth phase. It can be calculated using the formula:

$$g = \log2 (Nf / Ni)$$

Where:
Nf - the final bacterial density;
Ni - the initial bacterial density.

The generation time represents the average time taken by a bacterial population to double in size during exponential growth. It can be calculated using the formula (Widdel 2010):

$$Gt = \Delta t / g$$

Where:
Δt - the time interval;
g - the number of generations.

Bacterial molecular DNA identification. The selected bacteria were identified by molecular DNA methods, which included: isolation and purification of DNA (Altschul et al 1997); 16S rRNA gene amplification (Marchesi et al 1998); electrophoresis and sequencing (Marchesi et al 1998). The bacterial molecular DNA identification analysis was carried out according to Zainuddin et al (2021).

Results

Total plate count, LAB positive isolate bacteriocin-inactive and bacteriocin-active LAB. 4 types of shrimps were collected: *Metapenaeus affinis*, *Harpiosquilla raphidea*, *Parapenaeopsis sculptilis*, and P*enaeus merguiensis*. 11 of the highest total concentration (TPC) isolates were positive for LAB, at higher concentrations in *Parapenaeopsis sculptilis*, followed by *Penaeus merguiensis*, while the lowest concentrations were found in *Parapenaeopsis sculptilis* (Figure 1).

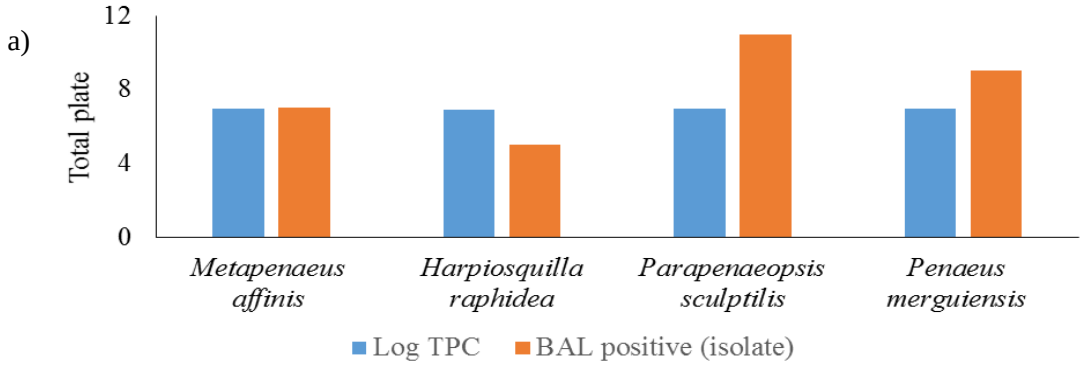

Figure 1. TPC and positive LAB isolates (a), bacteriocin-inactive LAB (b), and bacteriocin-active LAB (c).

Overall, the shrimp intestine bacteria had a LAB activity in 56% of the samples of all shrimp species, (Figure 1b). The number of LAB isolates active against the test bacteria showed no significant difference (Figure 1c). The inhibition zone of lactic acid bacteria activity is shown in Figure 2.

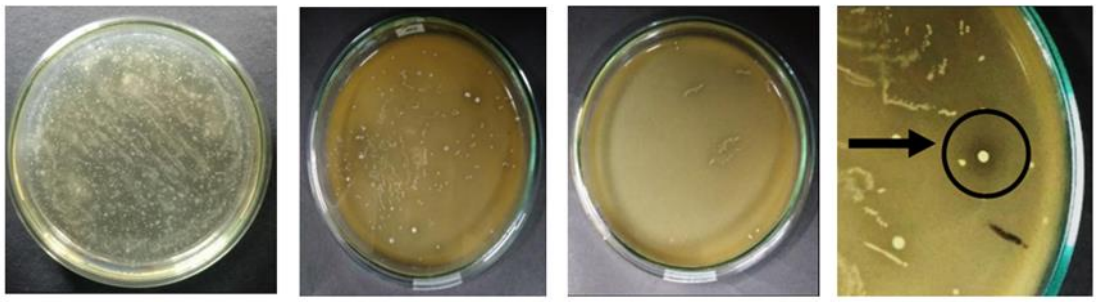

Figure 2. Lactic acid bacteria activity zone.

Bacteriocin activity ratio. The results of the inhibition zone test indicated a bacteriocin activity 32 isolates from the shrimp intestines of 4 types of shrimp. Of the 32 isolates, 2 were found to have a significant inhibition zone and bacteriocin activity. The isolate K.15 with an inhibitory zone of 4.36 mm against *V. harveyi*, 3.42 mm against *V. alginolycus*, 3.63 mm against *V. parahemolitycus*, and 3.41 mm against *V. vulnivicus*. Meanwhile, the bacteriocin activity was 1916 AU mL^{-1} against *V. harveyi*, 1278 AU mL^{-1} against *V. alginolitycus*, 1951 AU mL^{-1} against *V. parahemolitycus*, and 1955 AU mL^{-1} against *V. vulnivicus*. The isolate K.28 was only active against 2 test bacteria: *V. harveyi*, with an inhibition zone of 1.55 mm, and *V. alginolycus*, with an inhibition zone of 3.83 mm. The bacteriocin activity of the isolate K.28 was of 652 AU mL^{-1} against *V. harveyi* and 1653 AU mL^{-1} against *V. alginolitycus*. Results of the absorbance value and bacterial density in the plate attachment test showed that the K.28 isolate had a higher density than the K.15 isolate (Figure 3).

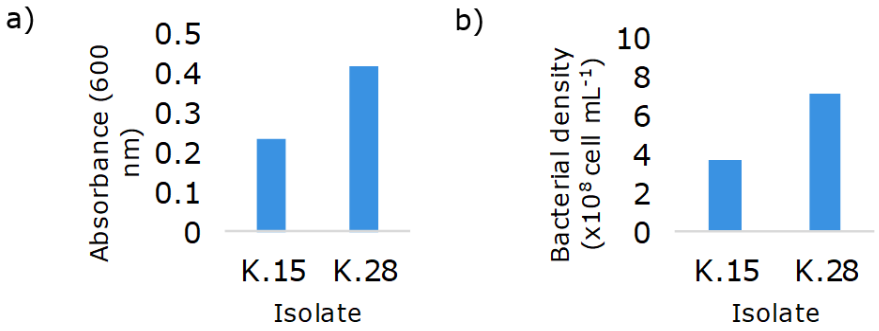

Figure 3. Absorbance value and bacterial density in the plate attachment test.

Meanwhile, the stringent response (SR) and bacterial density log values in the pathogenicity test for shrimp larvae showed that in isolates K.15 and K.28 the density

values were higher (100%) than in control (98%) and in the test bacteria isolate of *V. harveyi)* (45%). At the same time, the plate attachment test showed that densities in the isolate K.28 were higher than in the isolate K.15 (Figure 4).

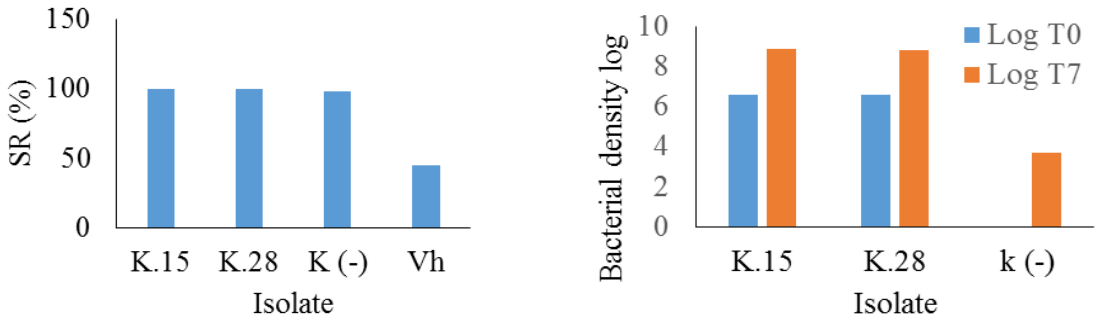

Figure 4. SR value and bacterial density log value of the pathogenicity test against shrimp larvae.

The AG and μ of bacteria showed that isolates K15 and K28 had very good C sources of glucose-fructose, sucrose, and maltose, which were >0.985 (Table 1).

Table 1

Absolute growth and growth rate of bacteria for different C sources

Isol.	*Param.*	*Growth polynomial*	*R^2*	*R*	*AG (x 10^8 cell mL^{-1})*	*μ ($hour^{-1}$)*	*g (gener.)*	*Gt (hour)*
			Source C					
K.15	Glucose	$y=-0.0004x^2+0.0209x+0.0091$	0.98	0.991	5.378^{b}	0.10^{b}	4.21^{b}	6.66^{a}
	Fructose	$y=-0.0003x^2+0.0182x+0.0216$	0.98	0.995	5.218^{b}	0.10^{ab}	4.11^{ab}	6.82^{ab}
	Sucrose	$y=-0.0003x^2+0.0187x+0.0138$	0.98	0.992	5.084^{b}	0.099^{ab}	4.02^{ab}	6.95^{ab}
	Maltose	$y=-0.0004x^2+0.0196x+0.0093$	0.98	0.993	4.402^{a}	0.094^{a}	3.83^{a}	7.31^{b}
K.28	Glucose	$y=-0.0005x^2+0.0289x-0.0137$	0.98	0.993	8.016^{b}	0.11^{b}	4.63^{b}	6.07^{a}
	Fructose	$y=-0.0004x^2+0.0263x+0.0109$	0.97	0.985	8.092^{b}	0.10^{b}	4.35^{b}	6.43^{a}
	Sucrose	$y=-0.0003x^2+0.0173x+0.0244$	0.98	0.994	5.007^{a}	0.10^{a}	4.06^{a}	6.89^{b}
	Maltose	$y=-0.0003x^2+0.0167x\ 0.0272$	0.97	0.989	4.817^{a}	0.09^{a}	3.95^{a}	7.10^{b}
			Source N					
K.15	Am chloride	$y=-0.0071x^2+0.4330x-0.6344$	0.98	0.99	6.10^{c}	0.10^{b}	4.298^{b}	6.52^{b}
	Am nitrate	$y=-0.0069x^2+0.4814x-1.2286$	0.96	0.981	7.30^{d}	0.11^{c}	4.621^{c}	6.06^{a}
	Am sulfate	$y=-0.0064x^2+0.3927x-0.8180$	0.97	0.98	5.27^{b}	0.10^{ab}	4.104^{ab}	6.82^{bc}
	Urea	$y=-0.0049x^2+0.3020x-0.3484$	0.97	0.98	4.33^{a}	0.09^{a}	3.80^{a}	7.37^{c}
K.28	Am chloride	$y=-0.0077x^2+0.5096x-1.0300$	0.97	0.98	7.64^{c}	0.11^{b}	4.68^{b}	5.98^{b}
	Am nitrate	$y=-0.0075x^2+0.5841x-1.0481$	0.98	0.99	10.26^{d}	0.12^{c}	5.00^{c}	5.58^{a}
	Am sulfate	$y=-0.0078x^2+0.4839x-0.8740$	0.99	0.99	6.59^{b}	0.11^{b}	4.48^{b}	6.23^{b}
	Urea	$y=-0.0071x^2+0.4286x-0.7418$	0.99	0.99	5.71^{a}	0.10^{a}	4.21^{a}	6.64^{c}

The letter behind the same value in the same column for each isolate indicates no significant difference in effect ($p>0.05$).

The results of the study, related to the absolute growth and bacterial growth rate on N sources, showed that isolates K.15 and K.28 had N sources from glucose, fructose, sucrose and maltose of >0.986 (Table 2). The bacterial growth rate on the optimization of glucose and ammonium nitrate, showed that isolates K.15 and K.28 had very potential outcomes the best candidates as target bacteria in this research, as shown in Table 2.

Table 2

Absolute growth and bacterial growth rate on the glucose optimization and the ammonium nitrate optimization

Isol.	*Conc. (%)*	*Growth polynomial*	*R^2*	*R*	*AG (x 10^8 cell mL^{-1})*	*μ ($hour^{-1}$)*	*g (generation)*	*Gt (hour)*
Glucose optimization								
K.15	0.05	$y=-0.0200x^2+1.3099x-2.8847$	0.98	0.99	19.84^{d}	0.13^{c}	5.54^{c}	5.05^{a}
	0.15	$y=-0.0073x^2+0.5638x-0.2919$	0.96	0.98	11.12^{c}	0.12^{b}	4.95^{b}	5.66^{b}
	0.25	$y=-0.0082x^2+0.4921x-0.0411$	0.91	0.955	7.99^{b}	0.11^{b}	4.60^{b}	6.08^{b}
	0.35	$y=-0.0061x^2+0.3921x-0.4710$	0.98	0.99	5.89^{a}	0.10^{a}	4.23^{a}	6.61^{c}
K.28	0.05	$y=-0.0182x^2+1.1663x-1.93$	0.97	0.98	18.81^{c}	$0,147^{c}$	5.949^{c}	4.70^{a}
	0.15	$y=-0.0176x^2+0.9762x-1.64$	0.97	0.99	14.24^{b}	0.13^{b}	5.38^{b}	5.20^{b}
	0.25	$y=-0.0107x^2+0.6343x-1.02$	0.94	0.96	13.43^{b}	0.12^{b}	5.08^{b}	5.50^{b}
	0.35	$y=-0.0093x^2+0.5482x-0.85$	0.97	0.99	7.89^{a}	0.11^{a}	4.47^{a}	6.26^{c}
Ammonium nitrate optimization								
K.15	0.01	$y=-0.0091x^2+0.6689x-1.3851$	0.98	0.99	11.13^{d}	0.12^{b}	5.07^{b}	5.52^{a}
	0.02	$y=-0.0082x^2+0.5463x-0.80$	0.99	0.97	8.29^{c}	0.11^{ab}	4.73^{ab}	5.95^{ab}
	0.03	$y=-0.0076x^2+0.4606x-0.29$	0.99	0.96	6.63^{b}	0.11^{ab}	4.52^{ab}	6.21^{ab}
	0.04	$y=-0.0065x^2+0.4058x-0.26$	0.97	0.98	5.93^{a}	0.10^{a}	4.20^{a}	6.68^{b}
K.28	0.01	$y=-0.0137x^2+0.8558x-1.92$	0.97	0.98	12.30^{c}	0.13^{b}	5.38^{b}	5.24^{a}
	0.02	$y=-0.0113x^2+0.7532x-1.19$	0.98	0.99	11.32^{c}	0.12^{b}	4.98^{b}	5.63^{a}
	0.03	$y=-0.0077x^2+0.4876x-0.64$	0.98	0.99	6.96^{b}	0.11^{a}	4.45^{a}	6.28^{b}
	0.04	$y=-0.0060x^2+0.3860x-0.46$	0.99	0.99	5.77^{a}	0.10^{a}	4.21^{a}	6.65^{b}

The letter behind the same value in the same column for each isolate indicates no significant difference in effect ($p>0.05$).

Results of the absolute growth and bacterial growth rates for pH 6, pH 7, pH 8, and pH 9 showed that the normal pH of isolate K.15 had the best results, while isolate K.28 showed the best results at pH 6 and pH 8 (Table 3). Meanwhile, the absolute growth and bacterial growth rates at different salinity values, of 15, 20, 25, and 30‰, showed that the isolate K15 grew better at 20‰ salinity rather than at 30% salinity (Table 3). Then, the absolute growth and bacterial growth rate in the fermenter showed that the isolate K.28 had a better growth than the isolate K.15 (Table 3). After the optimization, the fermenter is tested with the production conditions of isolate cell k.15 and 28. These conditions include 0.05% glucose, 0.01% ammonium nitrate, pH 8, salinity of 30 ppt, temperature of 35°C, and agitation at 100 rpm, as shown in Table 3.

Table 3

Absolute growth and bacterial growth rate at the pH optimization and salinity

Isol.	*Param.*	*Growth polynomial*	*R²*	*R*	*AG (x 10⁸ cell mL⁻¹)*	*μ (hour -¹)*	*g (generation)*	*Gt (hour)*
pH								
K.15	pH 6	$y=-0.0122x^2+0.7079x-0.75$	0.95	0.97	10.42^{a}	0.12^{a}	5.03^{a}	5.56^{c}
	pH 7	$y=-0,0169x^2+1.0171x-2.04$	0.98	0.99	13.77^{b}	0.13^{ab}	5.52^{ab}	5.07^{b}
	pH 8	$y=-0.0240x^2+1.6413x-4.41$	0.98	0.99	25.82^{d}	0.15^{c}	6.22^{c}	4.50^{a}
	pH 9	$y=-0.0185x^2+1.1174x-1.89$	0.95	0.97	16.34^{c}	0.14^{b}	5.67^{b}	4.93^{b}
K.28	pH 6	$y=-0.0197x^2+1.1706x-1.98$	0.98	0.99	15.66^{a}	0.140^{a}	5.69^{a}	4.92^{c}
	pH 7	$y=-0.0218x^2+1.3515x-3.28$	0.97	0.98	19.94^{b}	0.14^{ab}	5.94^{ab}	4.71^{bc}
	pH 8	$y=-0.0391x^2+2.5322x-6.76$	0.98	0.99	36.77^{d}	0.16^{c}	6.65^{c}	4.20^{a}
	pH 9	$y=-0.0177x^2+1.3766x-3.62$	0.93	0.96	25.90^{c}	0.15^{b}	6.27^{b}	4.46^{b}
Salinity optimization								
K.15	15‰	$y=-0.0166x^2+1.1117x-2.67$	0.964	0.982	17.93^{a}	0.143^{a}	5.77^{a}	4.85^{c}
	20‰	$y=-0.0212x^2+1.3197x-2.87$	0.979	0.989	19.26^{a}	0.147^{a}	5.94^{a}	470^{c}
	25‰	$y=-0.0287x^2+1.7038x-4.62$	0.961	0.980	23.96^{b}	0.155^{b}	6.26^{b}	4.47^{b}
	30‰	$y=-0.0398x^2+2.4028x-7.28$	0.953	0.976	34.063^{c}	0.165^{c}	6.67^{c}	4.19^{a}
K.28	15‰	$y=-0.0184x^2+1.5304x-5.28$	0.95	0.97	28.02^{a}	0.16^{a}	6.54^{a}	4.27^{c}
	20‰	$y=-0.0423x^2+2.8860x-1.05$	0.96	0.98	41.71^{b}	0.17^{b}	6.93^{b}	4.04^{b}
	25‰	$y=-0.0342x^2+2.5311x-8.49$	0.95	0.97	39.97^{b}	0.17^{b}	7.01^{b}	3.99^{b}
	30‰	$y=-0.0539x^2+3.5527x-10.23$	0.96	0.98	54.17^{c}	0.18^{c}	7.42^{c}	3.76^{a}
Fermenter								
K.15		$y=-0.1096x^2+7.3825x-17.034$	0.98	0.99	109.76^{b}	0.20^{b}	8.41^{b}	3.32^{c}
K.28		$y=-0.2094x^2+.600x-35.324$	0.99	0.99	227.60^{d}	0.23^{c}	9.53c	2.93^{a}

The letter behind the same value in the same column for each isolate indicates no significant difference in effect ($p>0.05$).

The bacteriocin activity of isolates K.15 and isolate K.28 in the fermenter showed that the clear zone diameter (mm), the clear zone area (mm²), and the bacteriocin activity (AU mL^{-1}) always increased at incubation (for 12, 18, 24 and 36 hours), as shown in Table 4.

The identification using molecular method showed that the isolate K.15 identified bacteria were close to the *Lactobacillus plantarum* No. MW693210 (99.3%), and the isolate K.28 identified bacteria were close to the *Lactococcus garviae* No. MH0572260 (99.71%), as shown in Figure 5 and Table 5.

Table 4

Bacteriocin activity of K.15 and K. 28 bacterial culture on the fermenter

Observation	*Incubation (hour)*				*Exponential linear regression*		
	12	*18*	*24*	*30*	*y = a + bx*	*R²*	*R*
K.15 bacterial culture on the fermenter							
Clear zone diameter (mm)	10.9[a]	11.9[a]	13[b]	13.6[c]	y=0.2336x+8.19	0.98	0.99
Clear zone area (mm²)	66[a]	84[a]	106[b]	119[c]	y=4.4657x+13.26	0.99	0.99
Bacteriocin activity (AU mL^{-1})	1107[a]	1397[a]	1762[b]	1978[c]	y=74.428x+221.03	0.99	0.99
K.28 bacterial culture on the fermenter							
Clear zone diameter (mm)	8.2[a]	8.8[ab]	9.2[b]	9.8[c]	y=0.0864x+7.1807	0.99	0.99
Clear zone area (mm²)	25[a]	32[ab]	39[b]	47[c]	y=1.2195x+9.9954	0.99	0.99
Bacteriocin activity (AU mL^{-1})	411[a]	535[a]	649[b]	779[c]	y=20.324x+166.9	0.99	0.99

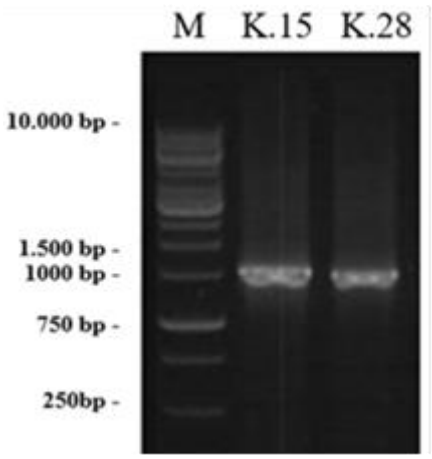

Figure 5. PCR and electrophoresis results for the K.15 and K.28 bacterial isolates.

Table 5

Molecular identification of K.15 and K.28 bacterial isolates

Isolate	*Sequence*	*Bacteria*	*Homology (%)*	*No. accession*
K.15	1476 bp	*Lactobacillus plantarum*	99.93	MW693210
K.28	1493 bp	*Lactococcus garviae*	99.71	MH057260

Discussion. *L. plantarum* is a type of bacteria that produces lactic acid compounds (LAB). This type of bacteria has been widely used in industry, especially as a natural preservative, because it can produce antibacterial compound such as bacteriocin, as has been proven by the research data. This study suggests that *L. plantarum* bacteria can be used as probiotics, reducing the use of antibiotics. The results showed that *L. plantarum* bacteria can adapt to various environmental conditions, such as differences in pH, salinity, and temperature, due to its ability to ferment various carbohydrates. In addition, it can be used as a starter culture in fermentation and as an ingredient for probiotics. Sari et al (2018) have used *L. plantarum* bacteria as a food preservative. Puspadewi et al (2011) proved that *L. plantarum* bacteria can be used to maintain the health of the digestive tract. Furthermore, Primurdia & Kusnadi (2014) stated that *L. plantarum* contains antioxidants. The *L. plantarum* has the potential to degrade carbohydrates in its growth medium into glucose and lactic acid. The presence of these acidic compounds

causes the inhibition of the population of pathogenic bacteria in the digestive tract. The strength of the activity of *L. plantarum* bacteria has been tested against the pathogenic bacteria *Escherichia coli* and *Salmonella typhimurium* (Hu et al 2019; Muhammad et al 2019). The results showed that the metabolites produced during the fermentation could inhibit the growth of the two pathogenic test bacteria.

Lactococcus garvieae is considered as a low-virulence organism that is rarely associated with human infection (Choksi & Dadani 2017). *L. garviaeae* is a bacterial pathogen often found in trout, freshwater fish, and marine fish. These bacteria belong to the gram-positive bacteria and the Streptococcaceae family. *L. garviaeae* bacteria are known to be an unusual pathogen in infective endocarditis (Malek et al 2019). *L. garviaeae* produce lactic acid compounds and bacteriocin. Ayyash et al (2020) stated that *L. garviaeae* bacteria produce Exopolysaccharides (EPS), polymers of high molecular weight, reducing sugars secreted by microorganisms into their external environment, that can be synthesized by lactic acid bacteria. EPS generally consists of monosaccharides and several non-carbohydrate substituents such as acetate, pyruvate, succinate, and phosphate (Deutscher et al 2006), as well as biomolecules such as proteins, nucleic acids, lipids and humic substances (Vu et al 2009). EPS is usually produced by lactic acid bacteria, as probiotics that have a positive effect on health (Kechagia et al 2013; Shi et al 2016). This polymer has a bioactivity with medicinal applications, such as its anti-viral and anti-inflammatory properties. In the food industry, EPS can be used as a thickener, gelling agent or emulsifier. Some EPS that have been widely used in the health sector, including β-glucan, β-mannan, xanthan, curdlan, gellan, and dextran.

Conclusions. In this study, isolates were obtained from the intestines of 4 shrimp species: *M. affinis*, *H. raphidea*, *P. sculptilis*, and *P. merguiensis*. Among the 32 bacterial isolates, 2 selected isolates of lactic acid bacteria were used as material for bacteriocin production, namely: *L. plantarum* No. MW693210 (99.3%) and *L. garviae* No. MH0572260 (99.71%). The results showed that *L. plantarum* and *L. garviae* bacteria adapted well to various environmental conditions such as the differences in pH, salinity, and temperature.

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