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Isolation of *Pediococcus pentosaceus* to compete *Vibrio harveyi* in the shrimp *Litopenaeus vannamei* hatchery

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Technology Research Development, PT. Central Proteina Prima. Tbk. Jl. HR Rasuna Said Kav. H1-H2, Kuningan, Karet, South Jakarta 12920, Jakarta, Indonesia. ♥email: rifky.rizkiantino@cpp.co.id

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Abstract. *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.* Shrimp is an aquaculture commodity with high economic value, and is widely cultured in Asia and South America. Among diseases in the shrimp hatchery, vibriosis is considered as the main problem, with emphasize on *Vibrio harveyi* infection. Probiotics are life microbes with defense mechanism against pathogens, and have been used in aquaculture as an alternative strategy in disease management through application into the water or through the feed. In order to renew the current probiotics available, exploration of probiotic candidate isolates with ability to compete with the luminous bacteria has been done in mangrove area of Merak Belantung, Kalianda, Lampung Province, Indonesia, and also from shrimp intestine, raised in the tank of research facility. Nine bacteria isolates were obtained from this exploration, and based on the references, four isolates had the potential to compete *Vibrio harveyi*. Based on the 16S rRNA sequencing result, those four isolates were *Bacillus cereus*, *B. aryabhattai*, *Weisella paramesenteroides*, and *Pediococcus pentosaceus*. Further analysis of in vitro test using agar-well diffusion inhibition method and in vivo test showed that among those four isolates, only *P. pentosaceus* had strong antibacterial activity against *V. harveyi* and could be used as probiotic in shrimp rearing with optimal dose at 5-10 µL L^{-1}.

Keywords: Inhibition zone, *Litopenaeus vannamei*, *Pediococcus pentosaceus*, probiotic, *Vibrio harveyi*

INTRODUCTION

Whiteleg shrimp (*Litopenaeus vannamei*) is an aquaculture commodity with high economic value, and widely cultured in Asia and South America, and more recently in Africa (Khushbu et al. 2022). It is one of the world's most delicious and nutritious seafood, and the demand is increasing (Toma et al. 2019). Shrimp accounts for about 42% of the country's fish trade balance, ranking first in Indonesia's fish exports, with high quantity and export value (Yolandika et al. 2022), thus the shrimp culture plays an important role in the contribution of revenue in Indonesia (Budhiman et al. 2005), and also one of important penaeid species farmed worldwide (Balakrishnan et al. 2011).

Farmers usually raise the whiteleg shrimp for 90 to 120 days before harvested, while the post-larvae (PL) is mainly obtained from the shrimp hatcheries (Jannat et al. 2017; Azizah et al. 2020). During cultured, both in the hatchery and in the farm, shrimp is vulnerable to number of diseases. Furthermore, disease infestation has become one of the many constraints on shrimp production at present. Some of the main diseases in shrimp culture are white spot syndrome virus (WSSV), taura syndrome virus (TSV), infectious myonecrosis virus (IMNV), vibriosis, and acute hepatopancreatic necrosis disease (AHPND) (Amelia et al. 2021).

Vibriosis is one of bacterial disease that is responsible for mortality of cultured shrimp worldwide, frequently occur in the hatcheries, but also commonly occur in the shrimp pond (Annam 2015). Various *Vibrio* bacteria have been associated with high mortality in shrimp larval culture (Newman 2015), especially the luminous *Vibrio* bacteria. The use of antibiotics in shrimp culture may cause development of antibiotics resistance (Holmström et al. 2003), and on the other hand, probiotics have been used in aquaculture as an alternative strategy in disease management (Ninawe and Selvin 2009). Generally, probiotics in aquaculture may help to improve water quality and also to improve animal performance, therefore the application of probiotics in aquaculture may be through direct application into the water, as well as through feed (van Hai and Fotedar 2010). As for the probiotic candidates currently available, especially in the Indonesian aquaculture industry, many come from the lactic acid bacteria (LAB) group of the genera *Bacillus* and *Lactobacillus.* Lactic acid bacteria themselves have an abundance of very diverse genera with a total of approximately 20 genera with 13 genera, including *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Tetrageonococcus*, *Vagococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Fructobacillus*, *Oenococcus*, *Weissella*, *Lactococcus*, and *Streptococcus* (von Wright and Axelsson 2019). Therefore, an in-depth exploration of

LABs that have potential as probiotic candidate is needed so that the choice of probiotics that can be used in shrimp farming can be more varied and effective in reducing the incidence of vibriosis cases due to luminescent bacteria, which can be detrimental to shrimp farmers. Isolation of bacteria from marine environment, including mangrove area, is a good approach to obtain potential effective probiotics for aquaculture, since mangrove ecosystem has abundance of microorganism diversity (Thatoi et al. 2013), as well as isolation of probiotic candidate from animal gut (Ganguly et al. 2018). Analysis of microbial diversity in mangrove ecosystem helps to isolate and identify new and potential microorganism for various applications, such as isolating the lactic acid bacteria with antimicrobial activity (Hwanhlem et al. 2014). The objective of this study was to explore and isolate probiotic candidates from the mangrove environment and from the shrimp gut raised in the research facility, to compete *Vibrio harveyi*. Potentially, the mangrove environment has the same aquatic environmental conditions as the vannamei shrimp culture, particularly a high enough salinity parameter. So, it is hoped that the probiotic candidates found in these environments can be utilized directly in actual shrimp farming and hatchery conditions.

MATERIALS AND METHODS

Isolation of probiotic candidates from mangrove environment

The process of bacterial exploration was conducted in August 2021. Isolation of probiotic candidates was conducted at mangrove area and research facility of Merak Belantung, Kalianda, Lampung Province, Indonesia, as shown in Figure 1. Samples were taken from mangrove area as a mixture of water and mud, collected using a micropipette and placed in a separate microtube for further processing at the Laboratory of Marine Research Center, Technology Research Development, PT. Central Proteina Prima, Tbk., Suak, Lampung, Indonesia. Samples were then diluted in series of 10^{-1} and 10^{-2}, and then 100 µL of each dilution was spread on de Man, Rogosa and Sharpe (MRS) Agar (Merck, Germany), and incubated for 48 h at 37°C. The isolates were then purified on MRS Agar by streak plate method, as described by Sanders (2012).

Isolation of probiotic candidates from shrimp intestine

As many as three of whiteleg shrimp (*Litopenaeus vannamei*) (mean body weight of 5.2±0.4 g at 25 days of culture) were collected from the shrimp rearing tanks in the company research facility. The whiteleg shrimp were rinsed with sterile distilled water. Aseptically, 0.1 g of shrimp intestine from each sample was taken and then diluted in 0.9 mL of 0.85% NaCl physiology solution. Samples were then diluted in the series of 10^{-1} and 10^{-2}, and 100 µL of each dilution was spread on MRS Agar, and incubated for 48 h at 37°C. The isolates were purified on MRS Agar by streak plate method, as described by Sanders (2012).

Identification of probiotic candidates

Bacterial isolates were cultured and purified on Difco™ tryptic soy agar (Becton, Dickinson and Company, MD, USA) and incubated at 35°C for 24 hours. The growing bacterial colonies were identified using the 16S rRNA gene sequencing method. DNA extraction was performed to obtain a DNA template. The extraction method used IQ Real DNA Extraction kit (GeneReach Biotechnology Corp., Taichung City, Taiwan) according to the manufacturer's instructions. The primer pairs used were forward primer (63F) 5′-CAGGCCTAACACATGCAAGTC-3′ and reverse primer (1387R) 5′-GGGCGGWGTGTACAAGGC-3′ with an amplicon length of 1324 bp. GoTaq® Master Mix (Promega, Madison, Wisconsin, USA) was used as the PCR master mix according to the manufacturer's instructions with a total reaction volume of 50 µL.

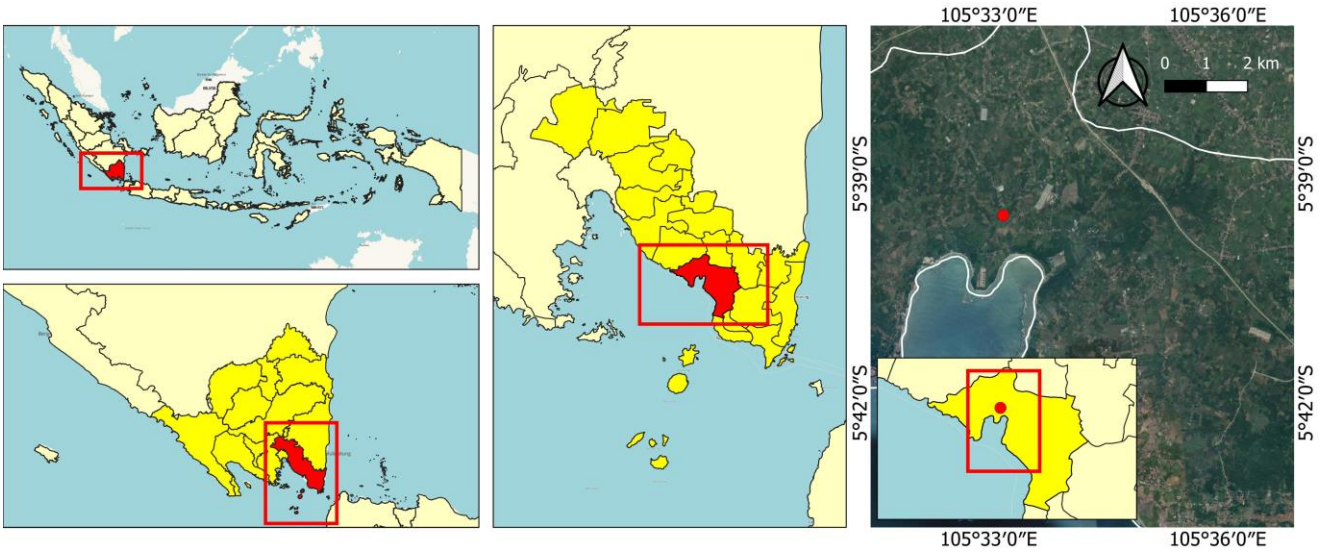

Figure 1. Sampling location in Merak Belantung, Kalianda, Lampung Province, Indonesia (5°41'40.1"S 105°31'28.9"E) (red pinned)

The PCR amplification process consisted of 30 cycles of initial denaturation (94°C, 5 minutes), denaturation (94°C, 30 seconds), annealing (55°C, 30 seconds), elongation (72°C, 1 minute 15 seconds), and final elongation (72°C, 5 min). The PCR products were then sent for sequencing through a third party (1st BASE, Singapore) using Sanger sequencing technology. The sequencing results were then analyzed using MEGA software version 11.0 (Pennsylvania State University, USA), and the 16S rRNA gene sequences were compared with data in GenBank® (National Library of Medicine, MD, USA) to identify each of the isolates obtained.

Based on the bacterial identification results, reference study was then conducted to determine the isolates with competition capacity against *Vibrio harveyi*. Selected isolates were used in the agar-well diffusion inhibition test to confirm their competition capacity against *Vibrio harveyi*. Isolate of *Vibrio harveyi* for challenge test (agar-well diffusion inhibition and in vivo test) was obtained from PLs of a commercial shrimp *Litopenaeus vannamei* hatchery in Kalianda, Lampung Province, Indonesia. Isolation was conducted in Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar (Merck, Germany), and the colony used for test on this study was the green colony.

In vitro test of probiotic isolate candidates (Agar-well diffusion inhibition test)

The test was performed in duplo by swabbing *Vibrio harveyi* bacteria with a concentration of 10^7 CFU/mL on Mueller Hinton Agar (MHA) media (Oxoid, Thermo Fisher Scientific Inc., Massachusetts, USA). Four wells with each volume of 40 µL were made in the MHA media, then 2 wells were filled with bacterial suspension, one well with sterile 0.9% NaCl solution as a negative control and one well with enrofloxacin antibiotic (5 mg mL^{-1}) as a positive control. The plates were incubated for 24 hours at 35°C. The inhibition zone formed was measured and compared with the positive control. Isolates with inhibition capability produced a clear halo near the well, as described by Morales et al. (2003).

In vivo test of probiotic isolate candidates (challenge test)

Candidates only showed inhibition activity in the in vitro inhibition test was used in the in vivo test. A total of 7 L of sterile seawater was filled into jar with capacity of 8 L. Nauplii of whiteleg shrimp was obtained from commercial shrimp hatchery in Kalianda, Lampung Province, Indonesia, and was put into the jar with density of 125 pcs L^{-1}. Water quality parameters were monitored and kept at: dissolved oxygen (DO) minimum of 4 mg L^{-1}, water pH as 7.9-8.2, while the water temperature was at 31-33°C. Artificial feed, algae, and *Artemia* sp. were used as feed, with feeding frequency of 8 times day^{-1}.

One öse loop of colony *Pediococcus pentosaceus* was enriched into 10 mL of MRS Broth (Merck, Germany) respectively, and incubated for 24 h at 37°C. After 24 h incubation, the density of bacteria was 10^8 CFU mL^{-1}. The bacterial density was checked with McFarland Standard 3 as described by Souza et al. (2020). The probiotic candidate isolates were put into trial jars during Zoea 1 to PL 9 stages, with dose of 0, 1, 5, and 10 µL L^{-1}, 5 replication respectively. At Mysis 3 (M 3) stage, a challenge test was carried out using *Vibrio harveyi* with density of 10^3 CFU mL^{-1}. The negative control was the treatment group which was not infected with *V. harveyi* and was not given *P. pentosaceus* probiotic bacteria candidate. The positive control was the treatment group which was infected by *V. harveyi* and was not given the *P. pentosaceus* probiotic bacteria candidate (dose of 0 µL L^{-1}). At PL 10, trial was terminated and parameters of survival rate (%), biomass (g), mean body weight (MBW) (g), and total length (mm) were checked. Total *Vibrio* count (TVC), yellow *Vibrio* colony (YVC), green *Vibrio* colony (GVC), total lactic acid bacteria count (TLABC), and total bacteria count (TBC) ($\times 10^3$ CFU mL^{-1}) from water samples were checked at stages M 3, PL 5, and PL 10 (end of the experiment) for each trial group, respectively.

Data analysis

Data were analyzed by one-way ANOVA, and multiple comparisons were performed using Duncan's post hoc test, with differences considered at p 0.05. All statistical analysis was performed using SAS software version 9.00.

RESULTS AND DISCUSSION

Isolation and identification of probiotic isolates candidates

Nine different isolates were obtained from the mangrove area (three isolates), research facility (two isolates) of Merak Belantung, Kalianda, Lampung Province, Indonesia and shrimp intestine (four isolates). In MRS Agar, colony with clear halo was suspected as lactic acid bacteria, as shown in Figure 2. Based on BLAST analysis result, nine isolates were belonged to six genera, namely *Pediococcus*, *Klebsiella*, *Bacillus*, *Vibrio*, *Staphylococcus*, and *Weissella* (Table 1). From the literature study, four isolates were selected for challenge test against *Vibrio harveyi*, both in vitro and in vivo.

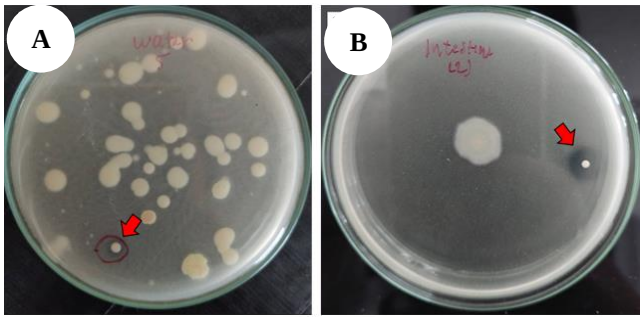

Figure 2. Colony of lactic-acid bacteria (red arrow) on de Man, Rogosa, and Sharpe (MRS) Agar from water of mangrove area (A) and shrimp intestine (B)

In vitro and in vivo test of probiotic isolate candidates

The results of in vitro challenge test using agar-well diffusion are presented in Table 2 and Figure 3. Considering the presence of clear halo in the agar near well, among the four probiotic isolates, only *Pediococcus pentosaceus* isolate showed strong inhibition activity against *Vibrio harveyi*. The halo zone with diameter of 13.00±0.00 mm was notice clearly near the well *Pediococcus pentosaceus* was the only candidate showing antibacterial effect, so it was further used in the in vivo test. The in vivo challenge test result is shown in Table 3. Bacterial parameters, such as YVC, GVC, TVC, TLABC, and TBC results are shown in Tables 4, 5, and 6.

From Table 3, it could be seen that application of 5 µL L^{-1} *Pediococcus pentosaceus* resulted in the highest SR value, even though statistically there was no significant difference compared to others treatments. The other parameters, such as total length, MBW, and the biomass also showed no significant differences among treatment groups. In the hatchery operation, the main production parameter was survival rate. *Pediococcus pentosaceus* used in this research was isolated from the tank water in the research facility.

Discussion

A total of nine bacterial colonies with morphological characteristics with clear halo were found, but only four of nine candidate isolates were chosen to be used in challenge test against *Vibrio harveyi*. Four out of nine candidates were selected on the basis of literature studies. In the agar-well diffusion inhibition test among four probiotic isolates, only *Pediococcus pentosaceus* showed strong inhibition activity against *Vibrio* harveyi. Probiotic candidates were isolated and cultured on differential selective media, such as de Man, Rogosa and Sharpe (MRS) in order to facilitate screening based on their morphological characteristics. Colony with clear halo in MRS Agar was suspected as lactic acid bacteria (Morales et al. 2003; Kurniati et al. 2021). Based on literature studies, *Bacillus cereus* (Barman et al. 2017), *Bacillus aryabhattai* (Tepaamorndech et al. 2018), *Weisella paramesenteroides* (Pal and Ramana 2010), and *Pediococcus pentosaceus* (Hong et al. 2022) have potential and safe to use as probiotics in aquaculture activity.

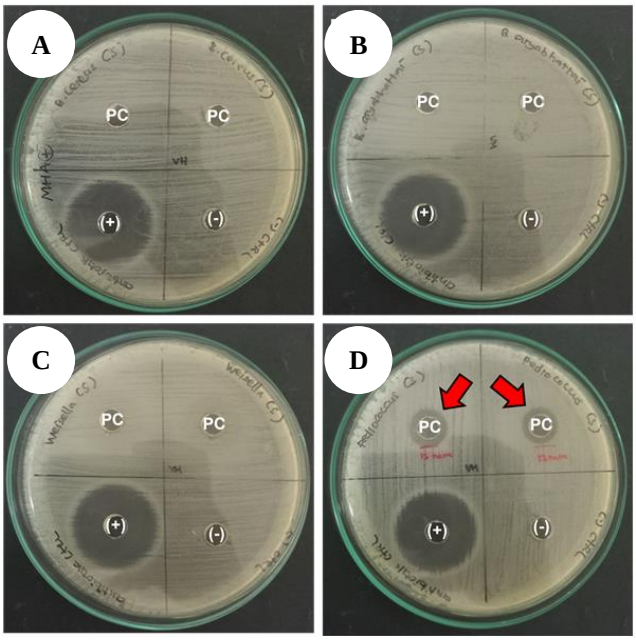

Figure 3. Agar-well diffusion inhibition test of four probiotic isolates. A. *Bacillus cereus*. B. *Bacillus aryabhattai*. C. *Weissella paramesenteroides*. D. *Pediococcus pentosaceus*. PC. Probiotics candidates. (+). Positive control. (-). Negative control. Red arrow showed the clear halo zone in agar with *Pediococcus pentosaceus* isolate

Table 1. BLAST results of nine probiotic isolate candidates

Isolates code	Sampling locations	BLAST results	Query coverage	E-value	Percentage identity
MR 3.1	Mangrove root	*Bacillus cereus**	100%	0.0	100.00%
MW 5.2	Mangrove water	*Vibrio porteresiae*	100%	0.0	99.69%
MW 5.1	Mangrove water	*Bacillus aryabhattai**	100%	0.0	100.00%
Intestine T3.1	Research facility	*Staphylococcus saprophyticus*	100%	0.0	99.92%
Intestine T3.2	Research facility	*Staphylococcus edaphicus*	100%	0.0	99.92%
Intestine T1.1	Research facility	*Weissella paramesenteroides**	100%	0.0	100.00%
Tank water	Research facility	*Pediococcus pentosaceus**	100%	0.0	100.00%
Intestine	Research facility	*Klebsiella pneumoniae*	100%	0.0	99.80%
Tank water	Research facility	*Staphylococcus haemolyticus*	100%	0.0	100.00%

Annotation = *) Isolate candidates used in probiotic in vitro testing

Table 2. In vitro agar-well diffusion inhibition test of four probiotic isolate candidates

Isolates code	Probiotic isolate candidates	Inhibition zone (mm) (Mean±SD, n = 2)
Tank water	*Pediococcus pentosaceus*	13.00±0.00
MR 3.1	*Bacillus cereus*	0.00±0.00
MW 5.1	*Bacillus aryabhattai*	0.00±0.00
Intestine T1.1	*Weissella paramesenteroides*	0.00±0.00

Table 3. In vivo test of *Pediococcus pentosaceus* against *Vibrio harveyi*

Treatments	Survival rate (SR) (%)	Total length (mm)	Mean body weight (MBW) (g)	Biomass (g)
Negative control	54.2±13.5[a]	9.6±0.2[a]	4.5±0.6[a]	4.0±0.5[a]
Positive control	41.1±4.5[a]	9.4±0.5[a]	4.9±1.5[a]	4.3±1.3[a]
Pediococcus pentosaceus 1 µL L^{-1}	49.0±11.8[a]	9.4±0.4[a]	3.9±0.9[a]	3.4±0.7[a]
Pediococcus pentosaceus 5 µL L^{-1}	56.2±6.9[a]	9.2±0.3[a]	4.0±0.7[a]	3.5±0.6[a]
Pediococcus pentosaceus 10 µL L^{-1}	50.2±14.3[a]	9.2±0.4[a]	5.1±0.8[a]	4.5±0.7[a]
p-value	0.375	0.482	0.267	0.274

Note: Different superscript letters in the same column indicate significant differences

Table 4. Results of yellow *Vibrio* colony (YVC), green *Vibrio* colony (GVC), total *Vibrio* count (TVC), total lactic acid bacteria count (TLABC), and total bacteria count (TBC) in mysis 3 (M3) stage

Treatments	Bacterial colony parameters (×10^3 CFU mL^{-1})				
	YVC	GVC	TVC	TLABC	TBC
Negative control	0.50±0.32[a]	0.08±0.07[a]	0.58±0.35[a]	0.00±0.00[c]	7.17±0.32[a]
Positive control	0.88±0.18[a]	0.20±0.19[a]	1.08±0.35[a]	0.00±0.00[c]	4.17±1.03[a]
Pediococcus pentosaceus 1 µL L^{-1}	6.60±8.20[a]	0.15±0.10[a]	6.75±8.10[a]	0.15±0.01[b]	4.47±2.29[a]
Pediococcus pentosaceus 5 µL L^{-1}	0.40±0.39[a]	1.14±1.50[a]	1.54±1.26[a]	1.07±0.13[a]	5.30±2.41[a]
Pediococcus pentosaceus 10 µL L^{-1}	0.45±0.31[a]	0.14±0.03[a]	0.59±0.29[a]	1.17±0.05[a]	5.27±2.32[a]
p-value	0.4148	0.5120	0.4428	<0.0001	0.7389

Note: Different superscript letters in the same column indicate significant differences

Table 5. Results of yellow *Vibrio* colony (YVC), green *Vibrio* colony (GVC), total *Vibrio* count (TVC), total lactic acid bacteria count (TLABC), and total bacteria count (TBC) in post-larvae 2 (PL 2) stage

Treatments	Bacterial colony parameters (×10^3 CFU mL^{-1})				
	YVC	GVC	TVC	TLABC	TBC
Negative control	0.07±0.04[b]	0.01±0.02[b]	0.08±0.06[a]	0.00±0.00[b]	0.00±0.00[a]
Positive control	1.73±0.13[b]	0.12±0.02[b]	1.86±0.11[a]	0.00±0.00[b]	22.00±0.82[a]
Pediococcus pentosaceus 1 µL L^{-1}	10.14±5.91[a]	0.04±0.06[b]	10.18±5.89[a]	0.13±0.03[b]	58.83±40.59[a]
Pediococcus pentosaceus 5 µL L^{-1}	0.07±0.05[b]	8.92±5.19[a]	8.99±5.23[a]	1.18±0.07[a]	45.00±24.83[a]
Pediococcus pentosaceus 10 µL L^{-1}	0.07±0.05[b]	8.92±5.19[a]	8.99±5.22[a]	1.11±0.12[a]	31.17±10.14[a]
p-value	0.0133	0.0265	0.1139	<0.0001	0.1523

Note: Different superscript letters in the same column indicate significant differences

Table 6. Results of yellow *Vibrio* colony (YVC), green *Vibrio* colony (GVC), total *Vibrio* count (TVC), total lactic acid bacteria count (TLABC), and total bacteria count (TBC) in post-larvae 10 (PL 10) stage

Treatments	Bacterial colony parameters (×10^3 CFU mL^{-1})				
	YVC	GVC	TVC	TLABC	TBC
Negative control	10.35±1.30[a]	0.00±0.00[a]	10.35±1.30[a]	0.00±0.00[c]	363.27±227.87[a]
Positive control	4.00±2.51[a]	8.75±6.02[a]	12.75±3.68[a]	0.00±0.00[c]	511.00±307.33[a]
Pediococcus pentosaceus 1 µL L^{-1}	5.16±4.84[a]	7.95±5.59[a]	13.11±1.58[a]	0.11±0.01[c]	71.77±11.44[a]
Pediococcus pentosaceus 5 µL L^{-1}	5.38±2.39[a]	7.47±3.89[a]	12.85±1.61[a]	1.60±0.37[b]	46.43±28.23[a]
Pediococcus pentosaceus 10 µL L^{-1}	2.85±3.32[a]	5.24±5.73[a]	8.08±5.15[a]	1.12±0.06[a]	275.40±284.70[a]
p-value	0.2235	0.4150	0.4460	<0.0001	0.2272

Note: Different superscript letters in the same column indicate significant differences

The current study showed that *Pediococcus pentosaceus* at a dose of 5-10 µL L^{-1} can increase survival rate, MBW, and biomass and can reduce YVC, GVC, and TVC in the early phase (M 3) and late period of infection (PL 10) by *V. harveyi*, although it was not statistically significant. Previous research conducted by Le et al. (2022) showed that the addition of *P. pentosaceus* in shrimp feed could enhance the immunity of whiteleg shrimp. Adel et al. (2016), Won et al. (2020), and Thao et al. (2021) also reported that a concentration of 10^8 CFU g^{-1} of *P. pentosaceus* to shrimp increased survival rate, growth performance, digestive enzyme activity, immunity, histological conditions of the digestive tract, immune gene expression, and their tolerance to *V. anguillarum* and *V. parahaemolyticus* infections and was able to reduce TVC. The phenomenon of increased tolerance for *Vibrio* infection in shrimp is thought to be due to the antioxidant effect produced by *P. pentosaceus*, which reduces stress

levels in shrimp, including oxidative stress that can occur at the cellular level. Bacteriocin produced by *P. pentosaceus* can also become antimicrobial peptides that can kill pathogenic bacteria by damaging cell membranes (Ladha and Jeevaratnam 2020).

On the contrary, *Bacillus cereus*, *B. aryabhattai*, and *Weisella paramesenteroides*, isolated from mangrove environments and shrimp intestines, showed no inhibition zones in vitro tests against *V. harveyi* at a concentration of 10^7 CFU mL^{-1}. This is thought to occur due to the influence of different strains and species in inhibitory potency test against *Vibrio* bacteria. Masitoh et al. (2016) reported that *B. cereus* and *B. thuringiensis* produced zones of inhibition against *V. harveyi* of 16.47-31.70 mm and 18.60-35.97 mm, respectively. However, Nakayama et al. (2009) reported that *B. licheniformis* and *B. megaterium* have more potential against *V. harveyi* attacks because they can significantly reduce hemolysin activity. Vaseeharan and Ramasamy (2003) and Zokaeifar et al. (2014) also stated another finding that *B. subtilis* reduced mortality by 90% in shrimp infected with *V. harveyi* and enhances the water quality, growth performance, immune response, and resistance against *V. harveyi*. Paz et al. (2016) stated that *B. aryabhattai* is more usable as waste biodegradation and/or revalorization.

Papagianni and Papamichael (2011) stated that *W. paramesenteroides* has potential as a probiotic candidate as it can produce bacteriocin in the form of weissellin A, which is able to show various levels of activity against all Gram-positive bacteria tested, but is inactive against *Salmonella enterica Enteritidis*, which is Gram-negative bacteria. This is thought to be the reason why *W. paramesenteroides*, which was successfully isolated and then tested against *V. harveyi* as Gram-negative bacteria, could not produce inhibition zone during in vitro test. Based on the results obtained, further research is still needed on the potential of *Bacillus cereus*, *B. aryabhattai*, and *W. Paramesenteroides* against other pathogenic *Vibrio* bacterial species that their growth may inhibit.

In conclusion, the use of *Pediococcus pentosaceus* is safe in shrimp culture, and the application of *P. pentosaceus* with a dose of 5 µL L^{-1} showed the highest survival rate, while a dose of 10 µL L^{-1} can decreased YVC, GVC, and TVC in the early (M 3) and late (PL 10) infection period phase.

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