Aquaculture 624 (2026) 744248

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

The potential of culture supernatant from *Bacillus* sp. isolated from sea cucumber (*Stichopus* sp.) as *Vibrio parahaemolyticus* and *Vibrio harveyi* biofilm inhibitors on *Penaeus vannamei*

Dyah Wulandari [a,d,*,1], Severus R. Wisastra [a,1], Anto Budiharjo [d,e,**], R. Haryo B. Setiarto [b,c], Chumporn Soowannayan [f,g], A.H. Condro Haditomo [h], Ahmad Ni'matullah Al-Baarri [i]

[a] Department of Food Technology, Faculty of Agricultural Technology, Soegijapranata Catholic University, Semarang, Central Java, Indonesia
[b] Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Cibinong, Bogor 16911, Indonesia
[c] Research Collaboration Center for Traditional Fermentation, National Research and Innovation Agency (BRIN), Cibinong, Bogor 16911, Indonesia
[d] Molecular and Applied Microbiology Laboratory, Central Laboratory of Research and Service, Diponegoro University, Semarang, Central Java 50275, Indonesia
[e] Biotechnology Study Program, Faculty of Science and Mathematics, Diponegoro University, Semarang, Central Java 50275, Indonesia
[f] Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok 10400, Thailand
[g] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Thailand Science Park, Klong Luang, Patumthani 12120, Thailand
[h] Aquaculture Department, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Central Java 50275, Indonesia
[i] Department of Food Technology, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java 50275, Indonesia

ARTICLE INFO

Keywords:
Acute hepatopancreatic necrosis disease
Biofilm inhibitor
Feed supplement
Postbiotics

ABSTRACT

Acute Hepatopancreatic Necrosis Disease is a shrimp bacterial infection caused by particular isolates of *Vibrio* spp., especially *Vibrio parahaemolyticus* and *Vibrio harveyi*, which carry the pVA1 plasmid responsible for expressing the pirAB toxins. These pathogens initially establish biofilms in the shrimp forestomach before releasing toxins that destroy hepatopancreatic tissue. Therefore, biofilm inhibitors are a promising prevention approach to antibiotics. This study evaluated the biofilm-inhibitory potential of cell-free supernatants (CFS) from four *Bacillus* strains, *Bacillus* sp., *B. amyloliquefaciens*, *B. licheniformis*, *and B. safensis* isolated from sea cucumbers (*Stichopus* sp). Using microtiter-plate assays, each CFS was analyzed for its ability to suppress the growth and biofilm development of AHPND-causing *V. parahaemolyticus* and *V. harveyi*, employing in vitro assays on uncoated (growth) and 0.4% chitosan-coated (biofilm) 96-well plates. All four *Bacillus* CFS can significantly reduce biofilm formation up to 90.4% and 74.9% inhibition for *V. parahaemolyticus* and *V. harveyi*, respectively. Results also indicate that the probiotic supernatants may exert *quorum*-quenching effects by reducing AI-2 synthesis and activity. Two out of four bacteria isolates were used for in vivo study by mixing it with feed by 1:1 ratio. Shrimp were given either CFS supplemented test feeds or buffer-supplemented control feed for seven days prior to the immersion challenge with the respective pathogens at 10^6 CFU/mL. The result showed that the addition of *Bacillus* CFS can reduce shrimp mortality when infected with *V. parahaemolyticus* and *V. harveyi*. Confirmatory histology also showed that it reduces epithelial cells sloughing into the shrimp's hepatopancreas lumen.

1. Introduction

Shrimp is one of the most frequently exported commodities by volume and value, often produced via aquaculture (Henriksson et al., 2019; Xuan et al., 2021; Setyawan et al., 2022). There are many shrimp species, but the most cultured shrimp are white-leg shrimp (*Penaeus vannamei*). To meet the demand, large-scale farming and intensification are often implemented. However, both practices have led to a rise in the

* Corresponding author at: Department of Food Technology, Faculty of Agricultural Technology, Soegijapranata Catholic University, Semarang, Central Java, Indonesia.
** Corresponding author at: Molecular and Applied Microbiology Laboratory, Central Laboratory of Research and Service, Diponegoro University, Semarang, Central Java 50275, Indonesia.
E-mail addresses: dyahwulandari@unika.ac.id (D. Wulandari), anto.budiharjo@live.undip.ac.id (A. Budiharjo).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.aquaculture.2026.744248
Received 4 February 2026; Received in revised form 22 May 2026; Accepted 27 May 2026
Available online 28 May 2026

occurrence of infections caused by pathogenic bacterial strains, mostly the *Vibrio* strain that resulted in mortalities and economic loss (Prachumwat et al., 2019; Amatul-Samahah et al., 2020). Moreover, compared to other seafood, shrimp farming produces high greenhouse gas (GHG), thus reduced mortality rate can improve sustainability and productivity (Bianchi et al., 2022).

Infection by pathogenic *Vibrio* species can cause vibriosis, with Acute Hepatopancreatic Necrosis Disease (AHPND) representing the most severe infection (Devadas et al., 2019). This infection predominantly affects shrimp at the post-larval stage and is a major contributor to high mortality during early development (Sirikharin et al., 2015; Phiwsaiya et al., 2017; Kumar et al., 2021). The initial stage of AHPND pathogenesis involves bacterial adhesion to host tissues, followed by biofilm formation. During this process, the bacteria secrete toxins that damage and slough off hepatopancreatic epithelial cells (Restrepo et al., 2021; Tran et al., 2013). Biofilm development is one of several virulence mechanisms expressed by bacteria once their population reaches a critical density, a process regulated by cell-to-cell signaling known as *quorum sensing* (QS) (Kareb and Aïder, 2020; Papenfort and Bassler, 2016).

Preventing vibriosis is therefore essential to reduce economic losses in aquaculture systems. A range of management strategies has been adopted, including lowering stocking densities, routine removal of organic waste, and the application of antibiotics. Nevertheless, the use of antibiotics is no longer recommended, primarily because of their diminishing effectiveness against vibriosis, but more significantly, their usage has been discouraged due to the residual antibiotic residues that could harm the environment and consumers by potentially causing allergies or adverse reactions to the drugs (ADR) (Sahandi et al., 2019). Moreover, the use of antibiotics resulted in antibiotics-resistant *Vibrio* species due to genetic mutation and horizontal gene transfer (Ashrafudoulla et al., 2019; Ventola, 2015).

Investigating alternative strategies to suppress biofilm formation without relying on antibiotics has emerged as a promising and effective approach for controlling bacterial infections, while also presenting fewer safety and environmental concerns (Sanches-Fernandes et al., 2022). Various probiotic strains, including *Lactobacillus, Bacillus, Pseudomonas,* and *Pediococcus*, are commonly used in aquaculture (Abdel-Latif et al., 2022; Yilmaz et al., 2022). These probiotics contribute to the establishment of a balanced microbial community in shrimp and help limit the growth of pathogenic bacteria, particularly *Vibrio* spp. However, because probiotics are live microorganisms, their use carries the potential risk of serving as reservoirs for antibiotic resistance genes (Montassier et al., 2021; Wong et al., 2015).

To address these limitations, the present study focuses on the use of probiotic-derived metabolites, referred to as postbiotics, obtained from culture supernatants. Unlike live cells, culture supernatants reduce the risk of transferring virulence factors or antibiotic resistance genes and offer advantages such as greater stability and extended shelf life (Liang and Xing, 2023). This research aims to identify novel probiotics isolated from sea cucumber, evaluate the potential of their postbiotics in reducing vibriosis in shrimp aquaculture, and delve into its mechanism through *quorum sensing* analysis.

2. Materials and methods

2.1. Probiotics isolation and identification

Probiotic bacterial strains were isolated from sea cucumbers collected and processed at Diponegoro University. The isolation procedure was conducted according to (Wulandari et al., 2024). Briefly, 3 g of soft coral polyps were first rinsed with sterile seawater to remove surface contaminants and then homogenized. Approximately 1 g of the homogenized sample was suspended in 9 mL of sterile distilled water and serially diluted up to 10^{-8}. Aliquots from dilutions 10^{-6} to 10^{-8}were spread onto Marine Agar plates using the spread plate method. Following incubation, distinct colonies were purified by repeated streak plating. Pure isolates were subsequently maintained on Marine Agar slants for further use. The obtained isolates were screened through a series of enzymatic activity assays. Based on their performance in the selection assays, four bacterial isolates were selected for further analysis.

Table 1
List of probiotic species and growth media salinity.

Probiotic Isolates	Salinity	Code	GenBank Accession Number
Bacillus sp.	20 ppt	1(8)	PZ287847.1
Bacillus amyloliquefaciens	30 ppt	BSSM7	PZ287849.1
Bacillus licheniformis	40 ppt	1(5)	PZ287846.1
Bacillus safensis	40 ppt	4(8)	PZ287848.1

The selected isolates were subjected to molecular identification using partial 16S rRNA gene sequencing. Genomic DNA was extracted from each isolate and used as a template for PCR amplification with universal 16S rRNA gene primers. The amplified PCR products were purified and verified by gel electrophoresis prior to sequencing. Raw sequencing data were quality-checked, trimmed, and converted into FASTA format. Reference 16S rRNA gene sequences of closely related *Bacillus* species, along with *Vibrio parahaemolyticus* as an outgroup, were retrieved from the GenBank database (KM073271.1). All sequences were aligned using the MUSCLE algorithm in MEGA version 12, followed by manual inspection and trimming. Phylogenetic analysis was performed using the Maximum Likelihood method based on the Kimura 2-parameter substitution model. The robustness of the inferred tree topology was evaluated by bootstrap analysis with 1000 replicates. The resulting phylogenetic tree was visualized and edited using MEGA.

2.2. Bacteria strain and cultivation

The probiotics used in this study were grown on Tryptic Soy Broth (TSB; Difco, USA) +3% NaCl (Sigma-Aldrich, USA) diluted with salt water with respective salinity (Table 1.). The different pathogenic bacteria strains (Table 2.) were obtained from the Center of Excellence for Shrimp Molecular Biology and Biotechnology, Mahidol University, and the American Type Culture Collection. The pathogenic bacteria were grown on Mueller-Hinton Broth (MHB) supplemented with 3% NaCl. All bacteria were added with 20% of 80% glycerol and stored at −80 °C for further analysis.

2.3. Probiotics cell free supernatant preparation

2.3.1. TSB media

The preparation of probiotics cell-free culture supernatant was done according to the method described by Koohestani et al. (2018) with modification. A single colony bacterium, grown on Tryptic Soy Agar (TSA) with 3% NaCl was inoculated into a 50 mL centrifuge tube containing 25 mL of TSB with 3% NaCl. The cultures were incubated at 37 °C with 200 rpm overnight. The supernatants from the probiotic culture were separated by centrifugation at 4 °C and 10,000 xg for 8 min. The supernatants were collected carefully into a new sterile tube. To obtain cell-free culture supernatants (CFSs), the supernatants were

Table 2
List of pathogenic bacteria strain.

Strains	Autoinducers	Sensor	ATCC Catalog number
V. harveyi VH1	1+; 2+	1+; 2+	
V. harveyi BAA-1116 (BB120)	1+; 2+	1+; 2+	ATCC® BAA-1116™
V. harveyi BAA-1119 (BB152)	1-; 2+	1-; 2+	ATCC® BAA-1119™
V. harveyi BAA-1121 (MM32)	1+; 2-	1-; 2+	ATCC® BAA-1121™
V. harveyi BAA-2363 (KM413)	1-; 2-	1+; 2+	ATCC® BAA-2363™
V. parahaemolyticus 3HP	n/d	n/d	

filtered through 0.2 μm filter membranes and stored at 4 °C until further use.

2.3.2. 5% soybean media

The preparation of CFS in 5% soybean is similar to the procedure above, however, 100 μl of the overnight culture was cultured again into a new tube containing 5 mL of TSB + 3% NaCl and incubated until Optical Density (OD) reached 1–2 measured on 600 nm. The new subcultures were diluted until OD_{600} was 0.05 with TSB + 3% NaCl added into 100 mL 5% soybean media (1 mL) with different salinity for different probiotic isolates. The cultures were incubated at 37 °C with 200 rpm overnight. The supernatants from the probiotic culture were separated through centrifugation at 4 °C and 10,000 xg for 12 min using a centrifuge. The supernatants were carefully filtered with Whatman paper before being filtered using 0.2 μm membranes to obtain the CFSs. The CFS were stored at 4 °C until further use.

2.4. Preparation of pathogenic Vibrio isolates culture

Two lyophilized cells of shrimp pathogenic bacteria were revived by plating in MHA + 3% NaCl plates and were incubated at 30 °C overnight. To prepare the starter culture for growth and biofilm inhibition test, a single colony of VH1 or 3HP was inoculated into 3 mL of MHB + 3% NaCl in sterile 15 mL centrifuge tubes. The tubes will be incubated at 30 °C overnight with 200 rpm agitation. The overnight cultures were subcultured again using the same media incubated at the same conditions and stopped until OD_{600} reached 1–2. The cultures with OD_{600} 1–2 will be diluted until OD_{600} 0.1 with the same medium before being diluted 10 more times. The media for the growth inhibition assay were MHB + 3% NaCl, while for the biofilm assay, the media was added with an additional 1% glycerol.

2.5. Vibrio growth inhibition assays

The method for the *Vibrio* growth inhibition assay was done according to the protocol described by Soowannayan et al. (2019). The 96-well microplate was seeded with 180 μl of diluted *Vibrio* starter culture before 20 μl of CFSs from probiotic bacteria were added into each well of the seeded microplate (Fig. 1.). For the negative control wells, the seeded microplate was added with either 20 μl TSB or 5% soybean media instead. All microplates will be incubated overnight at 30 °C in an incubator with 200 rpm agitation. The growth inhibition activity was measured using a SpectraMax® iD3 microplate reader at 600 nm light wavelength. *Vibrio* growth inhibition was expressed as mean relative inhibition (%) calculated using the following formula:

$$\textit{Mean Relative Inhibition}\ (\%) = \left[1 - \left(\frac{\textit{Sample Mean absorbance at } 600\ nm}{\textit{Negative Control Mean Absorbance at } 600\ nm}\right)\right] \times 100$$

2.6. Vibrio biofilm inhibition assays

The *Vibrio* biofilm inhibition assay was done with a similar protocol described in Section 2.5, except the microplate wells were coated with 0.4% chitosan and let dry overnight in a biosafety cabinet. The coated microplate was seeded with 180 μl of diluted *Vibrio* starter culture and 20 μl of CFSs and was incubated for 24 h at 30 °C in a static incubator (Fig. 1.). After that, the culture medium was removed and washed with sterile water, then the microplates were air-dried in a fume hood. To stain the formed biofilm, the air-dried microplates were added with 200

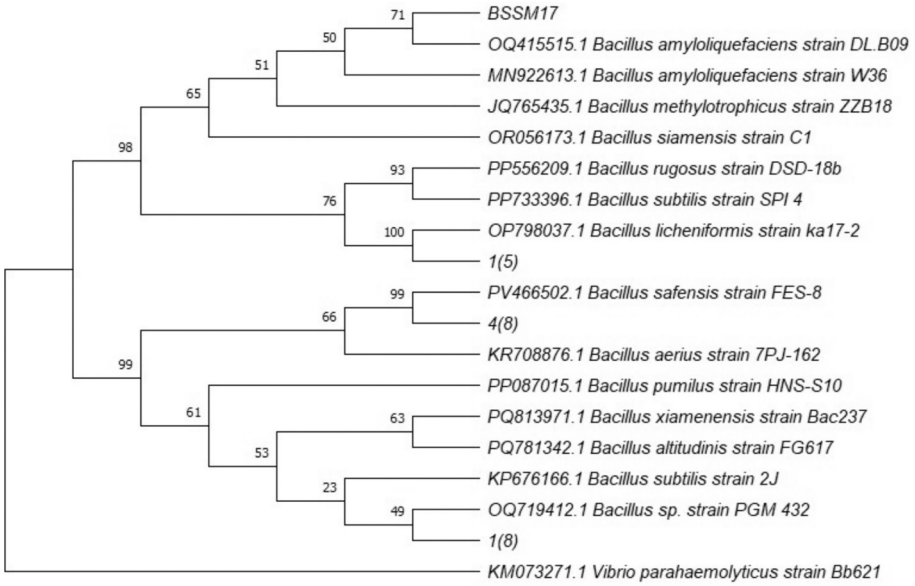


Fig. 1. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). and are in the units of the number of base substitutions per site. This analysis involved 19 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1474 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

μl of 0.3% crystal violet for 15 min. The excess stain was removed and rinsed with tap water and was allowed to dry overnight. To dissolve the stained biofilm, 200 μl of 33% acetic acid was added to each well of the microplate and was allowed to stand for 15 min at room temperature, after which an absorbance at 600 nm was measured using a microplate reader. The mean absorbance at 600 nm was also calculated into the mean percentage of relative inhibition using the formula mentioned in Section 2.5.

2.7. Bioluminescence inhibition assay

2.7.1. Screening for bioluminescence inhibitors

To determine the effect of the CFS on bacteria *quorum sensing*, a bioluminescence assay was employed according to Karnjana et al. (2019) with modifications. A single colony of the reference wild-type strain *V. harveyi* BAA-1116 (BB-120) was grown on MHA + 3% NaCl media and then cultured into autoinducer bioassay media (AB ATCC 2746) overnight at 30 °C. The overnight culture was subcultured again in AB medium until OD_{600} reached 1, after which it was adjusted to OD_{600} 0.05 and then distributed to each well (180 μL) of a 96-well black-bottomed microtiter plate. Different CFS were then added onto each well (20 μL) or media as the controls. The microtiter plates were incubated at 30 °C for 6 h at 200 rpm. After that the bioluminescence inhibition activity was measured using a SpectraMax® iD3 microplate reader. Some wells needed to be skipped to avoid any light interference between samples.

2.7.2. CFS effect on autoinducers activities

2.7.2.1. Autoinducers synthesis. *Vibrio harveyi* mutant strains BAA-1119 (BB152), in which the luxM gene has been deleted, and BAA-1121 (MM32), a luxS deletion mutant, were used as external sources of AI-2 and AI-1, respectively. To determine whether the addition of CFSs affected autoinducer production, these strains were cultured in AB medium supplemented with 5% (*v*/v) CFS at 30 °C for 6 h with agitation at 200 rpm. Cultures were then centrifuged at 12,000 rpm for 10 min to obtain supernatants containing the autoinducers, which were subsequently sterilized by filtration through 0.2 μm syringe filters to remove residual bacterial cells. The *V. harveyi* mutant strain BAA-2363 (KM413), which lacks both luxS and luxM, was employed as the reporter strain. BAA-2363 was grown overnight in AB medium and diluted to an OD_{600} of 0.05 before being dispensed into black-bottom 96-well microplates. Autoinducer-containing supernatants (10%, v/v) were then added. Wells containing BAA-2363 supplemented with autoinducer supernatants derived from media-treated cultures served as the negative control. The plates were incubated at 30 °C for 6 h with shaking at 200 rpm.

2.7.2.2. Autoinducers activity. To evaluate autoinducer activity, *V. harveyi* mutant strains BAA-1119 (BB152) and BAA-1121 (MM32) were employed as external sources of AI-2 and AI-1, respectively. These strains were cultivated in AB medium at 30 °C for 24 h with agitation at 160 rpm. Cultures were subsequently centrifuged at 12,000 rpm for 5 min to obtain cell-free supernatants containing the respective autoinducers. The supernatants were sterilized by filtration through 0.2 μm syringe filters to remove residual bacterial cells. The *V. harveyi* mutant strain BAA-2363 (KM413), which lacks both luxS and luxM, was used as the reporter strain. BAA-2363 was grown overnight in AB medium and diluted to an optical density at 600 nm (OD_{600}) of 0.05 before being dispensed into black-bottom 96-well microplates. Autoinducer-containing supernatants (5%, *v*/v) were then added, followed by the addition of CFS at 10% (*v*/v). Wells containing BAA-2363 and the supernatant containing the autoinducer added with media served as the negative control. The plates were incubated at 30 °C for 6 h with shaking at 200 rpm.

2.8. Shrimp challenge experiments

2.8.1. Feed preparation

The supplemented feeds were obtained by mixing CFS that has the strongest biofilm inhibition against *Vibrio* species with a commercial feed on a clean petri dish. In this research, *B. amyloliquefaciens* and *B. safensis* grown on 5% soybean media were selected for supplementation. The commercial feed was thoroughly mixed with a ratio of 1 mL of CFS to 1 g of dry feed. For the control, feed was supplemented with 5% soybean media with 30 or 40 ppt salinity. The blend was desiccated within a fume hood. Subsequently, the resulting product was placed into clean plastic bags and stored at 4 °C until it was ready for use.

2.8.2. Animal husbandry

Pathogen-free post-larvae (PL) of *Penaeus vannamei* were put into a tank containing artificial seawater (15 ppt), with an air stone for aeration, and acclimatized for 3 days before the experiment began. A total of 20 PLs were put into jars filled with 300 mL artificial seawater (15 ppt), additionally, each jar was added with an air stone to aerate and maintain the dissolved oxygen (DO). The jars were placed in a plastic tank containing water. To maintain a good quality of water in each jar, shrimps' waste was sucked periodically using a plastic pipette, and 50% of the water was replaced with new artificial seawater (pre-aerated overnight) every 3 days.

2.8.3. Challenge experiment (survival rate)

The groups were divided into negative control, positive control, and experimental groups. In the negative control group, the shrimps were given normal feed. The positive control group was given normal feed or 5% 30 ppt or 40 ppt soybean-supplemented feed, and the experimental group was given CFSs supplemented feed that had biofilm biofilm-inhibiting effect against the *Vibrio* isolate.

Within the first 7 days, all the shrimps were fed accordingly, twice a day with 10% body weight per meal. On day 7, the shrimp in the positive and experimental groups were added with VH1 or 3HP for the bacteria immersion challenge. The negative control jars were added with sterile MHB + 3% NaCl. The feeding continued and the mortalities of each group will be monitored.

2.8.4. Growth performance

The shrimps were weighed before and after 7 days of feeding (prior to bacterial infection) to determine the average daily gain (ADG; g/day), total weight gain (WG %), and specific daily growth (SGR; %/day) using the formula below:

$$ADG = \frac{final\ weight}{days\ of\ feeding}$$

$$WG = \left[\frac{(final\ mean\ body\ weight - initial\ mean\ body\ weight)}{initial\ mean\ body\ weight}\right] \times 100$$

$$SGR = \left[\frac{ln\ (final\ mean\ body\ weight) - ln(initial\ body\ weight)}{days\ of\ feeding}\right] \times 100$$

2.8.5. Histopathology preparation and staining

Moribund shrimp were fixed in Davidson's solution for 24 h and subsequently preserved in 70% ethanol. Samples were then processed for histopathological examination following the paraffin-embedding protocol of Bell and Lightner (1988). The tissues were first dehydrated through a graded ethanol series, cleared in xylene, and infiltrated with molten paraffin. After embedding, the blocks were chilled and sectioned at a thickness of 5 μm.

Prior to staining, the sections were deparaffinized in xylene and rehydrated through a descending alcohol series. Hematoxylin and eosin staining was then applied. Following staining, the slides were dehydrated again using an ascending alcohol series, air-dried, and finally

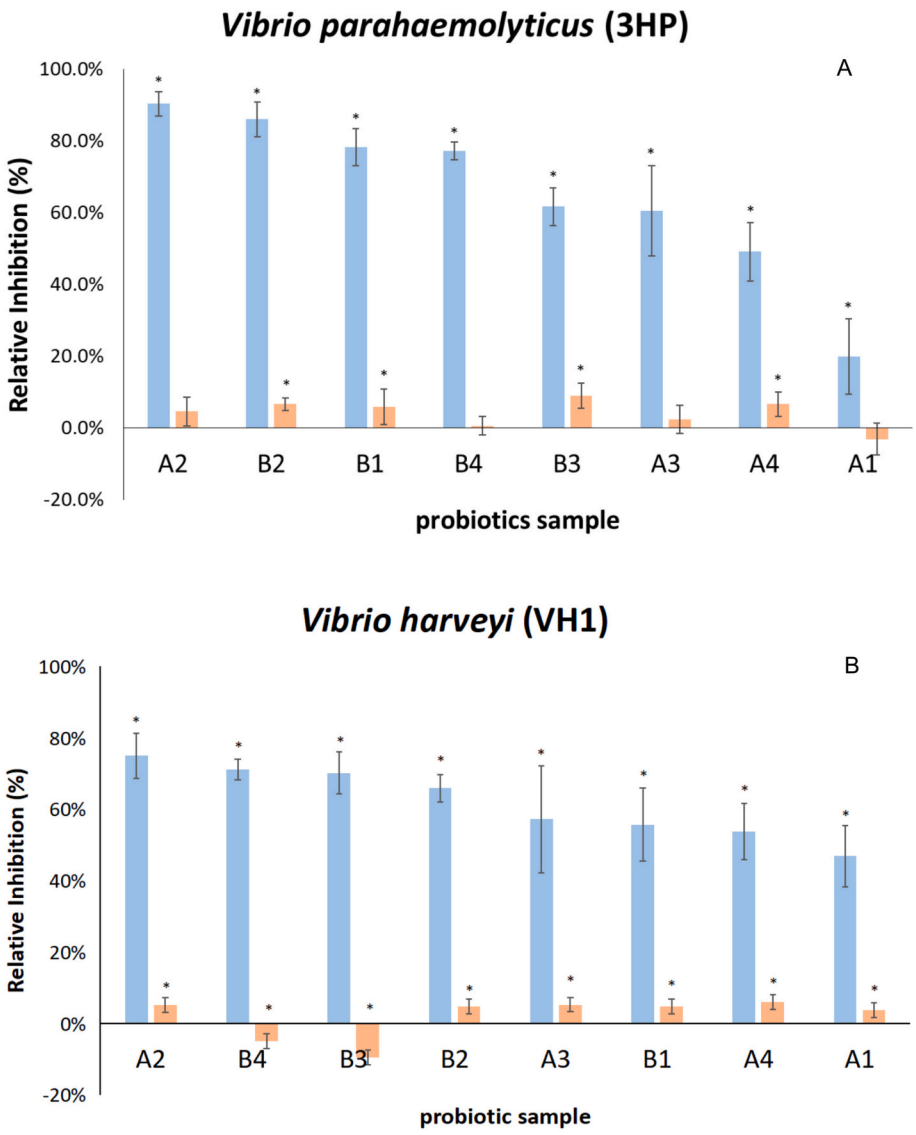


Fig. 2. Growth and biofilm inhibition of bacillus CFS on (A) *Vibrio parahaemolyticus* 3HP and (B) *Vibrio harveyi* VH1.
Blue bar: biofilm inhibition, orange bar: growth inhibition.
A1: *Bacillus* sp. on TSB + 3% NaCl; A2: *Bacillus amyloliquefaciens* on TSB + 3% NaCl; A3: *Bacillus licheniformis* on TSB + 3% NaCl; A4: *Bacillus safensis* on TSB + 3% NaCl; B1: *Bacillus* sp. on 5% soybean; B2: *Bacillus amyloliquefaciens* on 5% soybean; B3: *Bacillus licheniformis* on 5% soybean; B4: *Bacillus safensis* on 5% soybean (*) indicates a significant difference in mean absorbance between the test sample well and the control well. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

immersed in xylene. A drop of mounting medium was added before cover slipping, and the prepared slides were examined under a light microscope.

2.9. Data analysis

The data obtained was statistically analyzed using SPSS. For most datasets, differences among groups were evaluated using one-way ANOVA followed by Tukey's post hoc test, while independent *t*-tests were applied where appropriate. Specifically, t-tests were used to compare B1 and B2 samples with the control, as these comparisons involved fewer than three groups. Prior to analysis, assumptions of normality and homogeneity of variance were verified. The test was done with a confidence interval (CI) of 95%. Statistical differences were found between samples at $p < 0.05$.

Table 3
Summary of probiotic isolates and its effect on growth and biofilm formation.

Vibrio Strain	Growth	Biofilm	Sample Code
3HP	Inhibit	Inhibit	A2, A3, A4, B1, B2, B3, B4
	Promote	Inhibit	A1
VH1	Inhibit	Inhibit	A1, A2, A3, A4, B1, B2
	Promote	Inhibit	B4, B3

3. Results

3.1. Probiotics isolation and identification

Phylogenetic analysis of partial 16S rRNA gene sequences (Fig. 1) showed that all bacterial isolates clustered within the *Bacillus subtilis* group, indicating a close evolutionary relationship with the reference strain. The short branch lengths and strong bootstrap support suggest a

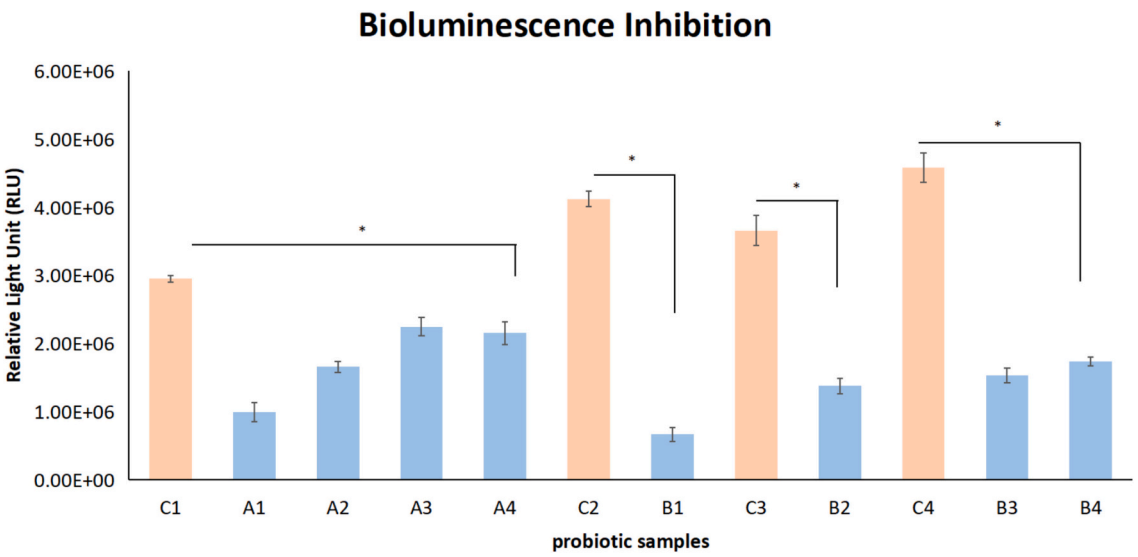


Fig. 3. Bioluminescence Inhibition of *V. harveyi* WT added with Bacillus CFS.
Orange bar: control; C1: TSB + 3% NaCl, C2: 5% soybean 20 ppt, C3: 5% soybean 30 ppt, C4: 5% soybean 40 ppt. Blue bar: sample; A1: *Bacillus* sp. on TSB + 3% NaCl; A2: *Bacillus amyloliquefaciens* on TSB + 3% NaCl; A3: *Bacillus licheniformis* on TSB + 3% NaCl; A4: *Bacillus safensis* on TSB + 3% NaCl; B1: *Bacillus* sp. on 5% soybean; B2: *Bacillus amyloliquefaciens* on 5% soybean; B3: *Bacillus licheniformis* on 5% soybean; B4: *Bacillus safensis* on 5% soybean (*) indicates a significant difference in mean absorbance between the test sample well and the control well. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

high degree of genetic similarity among the isolates. The clear separation of *Vibrio parahaemolyticus* as an outgroup confirms the robustness and proper rooting of the phylogenetic tree.

3.2. Probiotics CFSs inhibit growth and biofilm formation of *V. parahaemolyticus and V. harveyi*

The growth inhibition assay significantly ($p < 0.001$) inhibits the growth of *V. parahaemolyticus* 3HP (Fig. 2A), except for sample code A2, A3 and B4 ($p = 0.138$; $p = 0.675$; $p = 0.947$). In contrast, sample code A1 promotes 3HP growth but is not significant ($p = 0.466$). The growth inhibition ranges from 0.58% to 8.94%. As for the biofilm-inhibiting properties, all probiotic isolates can inhibit the biofilm formation of 3HP significantly ($p < 0.001$) compared to the negative control. The biofilm inhibition ranges from 19.9% to 90.4%. Similar results are also found in *V. harveyi* VH1 (Fig. 2B), the growth inhibition ranges from 3.71% to 5.97% and the biofilm inhibition ranges from 46.9% to 74.9%. Some probiotic strains also perform better when grown on 5% soybean media compared to TSB + 3% media. The summary of growth and biofilm inhibition is shown on Table 3.

3.3. Probiotics CFSs induce AI-1 while suppress AI-2

Bacteria virulence factors are often controlled by the surrounding densities which utilize the secretion of autoinducers that act as signaling molecules. The autoinducers synthesis and activity of *V. harveyi* VH1 is a complex process. Based on Fig. 3. *V. harveyi* bioluminescence is significantly lower in the probiotics CFSs group compared to control ($p < 0.001$). In-depth analysis of individual variables shows that the CFSs reduce the AI-2 synthesis and activity (Fig. 4 CD) significantly ($p < 0.001$). However, a significant increase is observed in AI-1 activity ($p = 0.000$; $p = 0.030$ for sampe B2) alongsideAI-1 synthesis ($p < 0.001$). However, post hoc analysis indicated that samples A1 ($p = 0.079$), and B4 ($p = 0.142$) were not significantly different from the control (Fig. 4 AB).

3.4. Probiotics CFSs have no effect on shrimps' growth

Shrimp growth performance was monitored for seven days prior to bacterial infection to assess the effects of the different feed treatments. No significant differences were observed among experimental groups ($p > 0.05$) (Fig. 5). Analysis of individual growth parameters, including weight gain (WG; $p = 0.156$), specific growth rate (SGR; $p = 0.104$), and average daily gain (ADG; $p = 0.389$), further confirmed the absence of significant variation. This result indicates that the addition of probiotics CFS does not interfere with shrimp growth performance.

3.5. Probiotics CFSs reduce shrimps' mortality

The negative control group showed the highest mean survival rate at 81.7%. In contrast, shrimp challenged with 3HP displayed significantly lower survival ($P < 0.001$), the positive control groups fed with normal feed, 30 ppt soybean, and 40 ppt soybean had survival rates of 17.7%, 25.9%, and 48.5%, respectively (Fig. 6 A).

Among the CFS-supplemented treatments, *B. amyloliquefaciens* achieved a survival rate of 57.3%, providing moderate protection compared with the positive control groups receiving normal feed and 30 ppt soybean ($P < 0.05$). *B. safensis* also offered protection, maintaining 66.8% survival up to day 9 post-infection. However, a sharp mortality occurred on day 10 (reducing survival to 6%), likely caused by insufficient aeration rather than the infection itself. Interestingly, the positive control group fed with 40 ppt soybean also exhibited reduced mortality; however, histological analysis revealed severe epithelial sloughing in the hepatopancreas (Fig. 7), which may be attributed to inconsistent bacterial concentrations during periodic tank cleaning.

For shrimp challenged with VH1, the negative control group again showed significantly higher survival ($P < 0.001$) compared with the positive controls. The positive control groups fed with normal feed, 30 ppt soybean, and 40 ppt soybean had survival rates of 18.8%, 15.3%, and 26.7%, respectively (Fig. 6B). Both *B. amyloliquefaciens* and *B. safensis* supplementation improved survival, yielding rates of 53.6% and 64.1%, respectively, demonstrating protective effects against VH1.

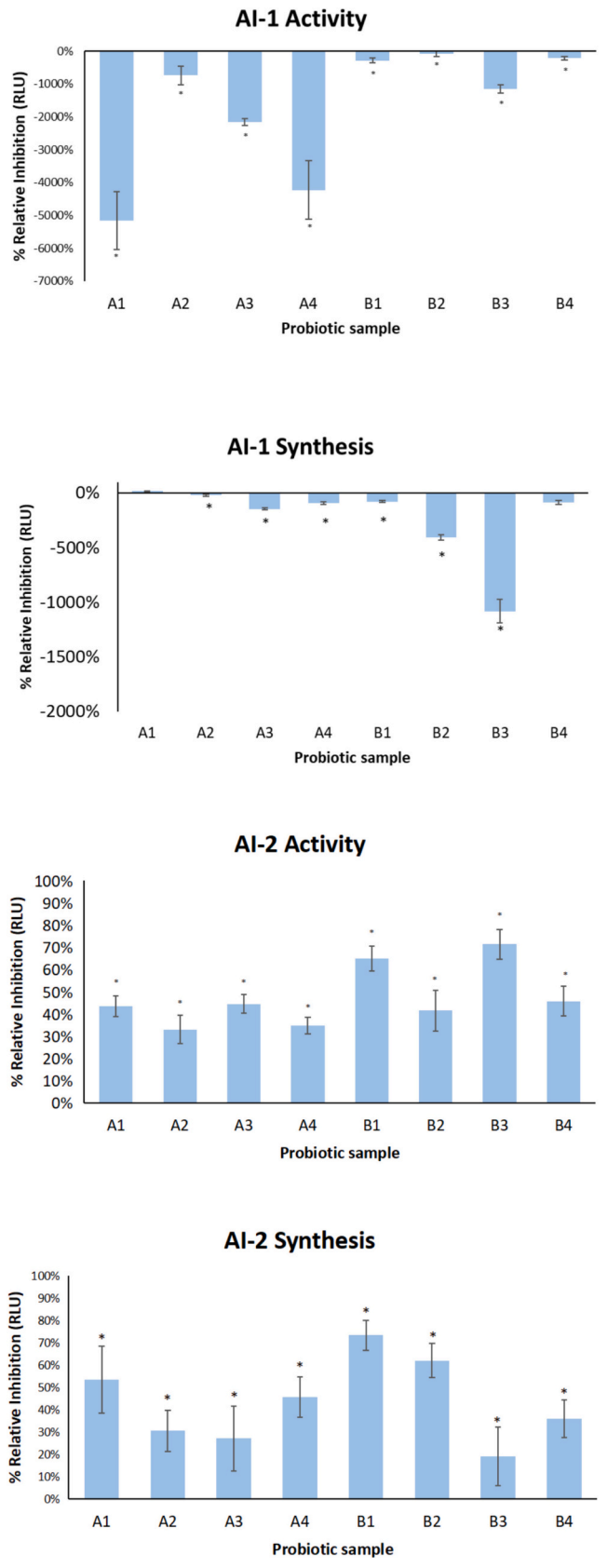


Fig. 4. *Quorum sensing* inhibition mechanism analyzed through their activity and synthesis.
(*) indicates a significant difference in mean absorbance between the test sample well (VH1 + CFS) and the control well (VH1 + probiotic media)

4. Discussion

Probiotics can produce a wide range of bioactive compounds primarily through their metabolic activities. Such compounds include bacteriocins, short-chain fatty acids (SCFAs), amino acids, vitamins, and enzymes (Martínez Cruz et al., 2012; Pandiyan et al., 2013; El-Saadony et al., 2021; You et al., 2022). However, the application of probiotics as live microorganisms poses certain risks. Probiotic strains may acquire antibiotic resistance genes from the surrounding environment and subsequently transfer these genes to other bacteria, potentially compromising their beneficial effects and contributing to the spread of antimicrobial resistance (Wong et al., 2015; Montassier et al., 2021). To mitigate these concerns, the present study focuses exclusively on probiotic-derived metabolites, referred to as postbiotics, obtained in the form of CFS.

Results from in vitro assays demonstrated that all four *Bacillus* spp. CFSs exhibited biofilm inhibitory activity. Specifically, the addition of CFS significantly reduced biofilm thickness on microplates coated with 0.4% chitosan. Chitosan is a deacetylated derivative of chitin, which constitutes a major structural component of shrimp cuticles (Rinaudo, 2006; Rocha et al., 2017; Yang et al., 2020). The chitosan coating was applied to simulate the shrimp shell and gastrointestinal tract, both of which are lined with cuticular structures that facilitate bacterial adhesion. This environment also supports biofilm formation and growth of *V. harveyi* VH1 and *V parahaemolyticus* 3HP, as these species can utilize chitin as a carbon source (Regmi and Boyd, 2019).

Based on the findings, CFSs produced in soybean-based medium exhibited stronger biofilm inhibitory activity than those derived from TSB. This observation indicates that all probiotic strains were able to grow effectively in soybean medium. Although the precise mechanism underlying the superior performance of soybean medium remains unclear, the enhanced biofilm inhibition does not appear to be directly associated with increased bacterial growth. Instead, we hypothesize that soybean medium, as a natural and cost-effective substrate, provides a nutrient profile that favors the synthesis of bioactive metabolites rather than rapid cell proliferation. Soybeans are rich in amino acids with compositions comparable to animal-derived proteins, including phenylalanine, methionine, threonine, valine, isoleucine, leucine, tryptophan, and lysine (Kudełka et al., 2021). Importantly, bacterial growth does not necessarily correlate with elevated bacteriocin production (Bogovič-Matijašić and Rogelj, 1998). The biosynthesis of bacteriocins is influenced by multiple environmental and nutritional factors, such as pH, temperature, and medium composition (Sandiford, 2017; Lajis, 2020). Amino acid availability plays a critical role, as the amino acid sequence ultimately determines bacteriocin structure and biological activity (Harwood et al., 2018; Lajis, 2020). Supporting this hypothesis, Dominguez et al. (2007) demonstrated that supplementation with soybean protein (20 g/L) enabled the production of cerein 8 A, a *Bacillus cereus* bacteriocin, at levels of 1600 AU/mL. This result is comparable to yields obtained using brain heart infusion (BHI) medium. Furthermore, Yi et al. (2013) reported that the addition of exogenous glycine and cysteine increased the production of lac-B23 bacteriocin from *Lactobacillus paracasei* J23 by 250% and 325%, respectively. The nutritional requirements for optimal bacteriocin production vary depending on bacteriocin type, as some amino acids can be synthesized endogenously, whereas others require external supplementation (Kiss et al., 2008).

While the present study did not quantify or characterize individual bioactive compounds within the CFS, the results suggest that soybean-based media may offer significant potential for supporting the scalable production of bioactive metabolites, thereby facilitating their commercial application in biotechnology (Xu et al., 2014).

Common bacteriocins and functional peptides produced by *Bacillus* species include iturin, amylocyclicin, bacilysin, pumilacidin, fengycin, surfactin, and plipastatin (Harwood et al., 2018; Kim et al., 2010; Scholz et al., 2014; Vater et al., 2002). Several studies have shown that these compounds can impair bacterial motility, including swimming and

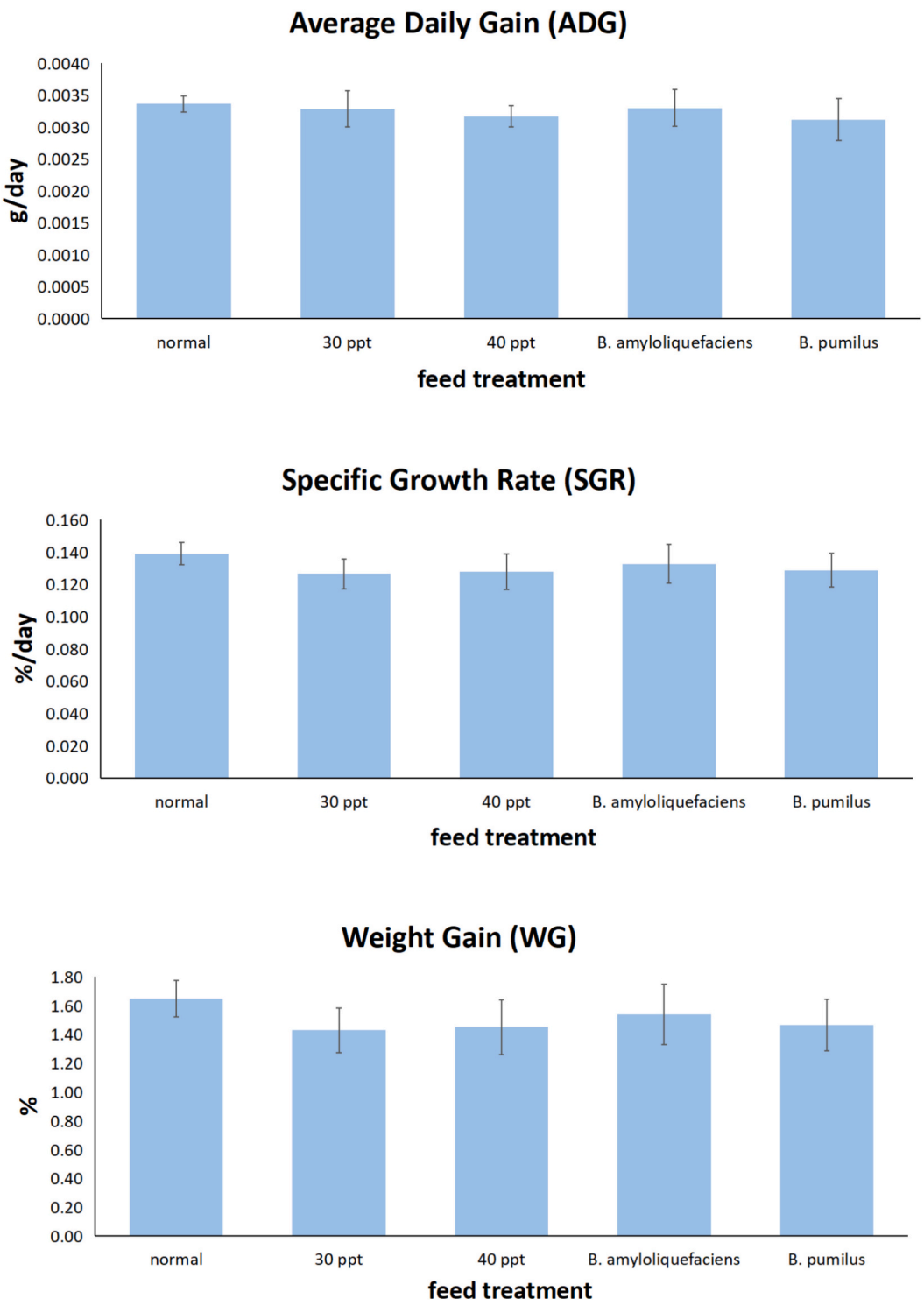
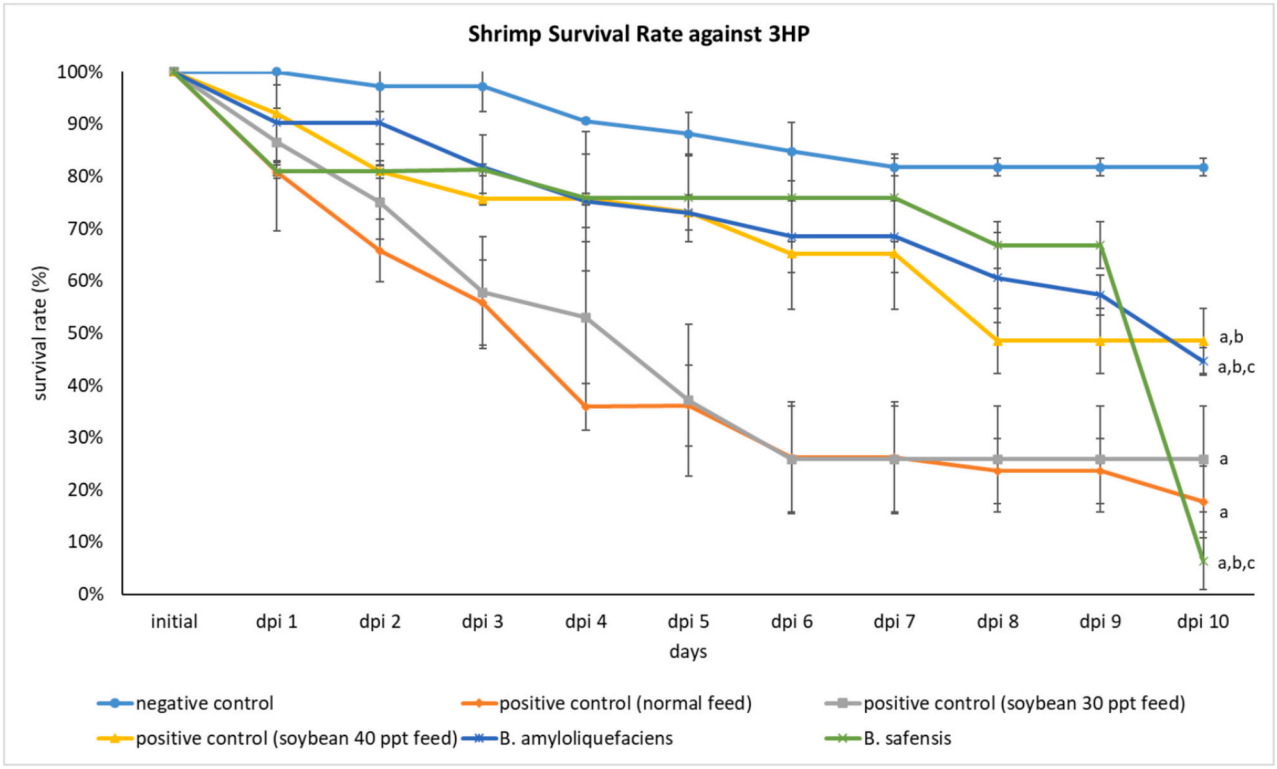


Fig. 5. Shrimp growth performance based on ADG, WG, and SGR indicators between unsupplemented and supplemented feeds with probiotics media or probiotics CFSs.

swarming behaviors. For example, Thongjun et al. (2016) reported that iturin A and surfactin produced by *B. amyloliquefaciens* reduced *V. parahaemolyticus* adhesion by approximately 60% at a crude extract concentration of 500 μg/mL. Similarly, CFS from *B. licheniformis* achieved up to 80% inhibition of *V. harveyi* biofilm formation on polystyrene surfaces (Hamza et al., 2016). Bacterial motility, mediated primarily by flagella, plays a crucial role in surface colonization and biofilm development (Letchumanan et al., 2014). Consequently, interference with swimming and swarming behaviors leads to reduced biofilm formation (Sathiyamoorthi et al., 2023). In addition to impairing motility, biofilm inhibition may also occur through disruption of bacterial *quorum sensing* systems, which regulate collective behaviors essential for biofilm development.

Quorum sensing in *V. harveyi* and *V. parahaemolyticus* is mediated by three signaling molecules, harveyi AI-1 (HAI-1), AI-2, and cholera AI-1 (CAI-1) that are synthesized by *LuxM*, *LuxS*, and *cqsA* genes and bind to *LuxN*, *LuxPQ*, and *cqsS* respectively. (Papenfort and Bassler, 2016). HAI-1 is an acyl homoserine-lactone that is known as an intraspecies or



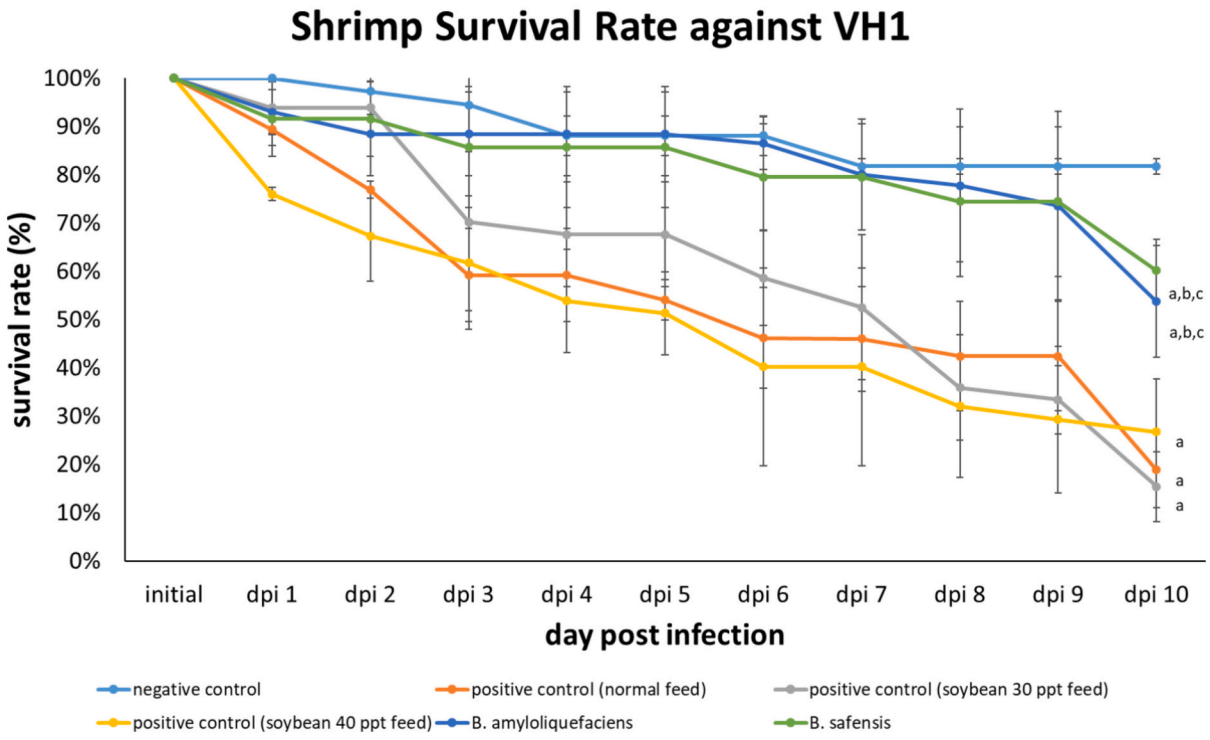


Fig. 6. average survival rate (%) of shrimp during feeding experiment.
Lines represent mean ± standard deviation, (a) indicates a significant difference between negative control; (b) indicates a significant difference between positive control normal feed; (c) indicates a significant difference between positive control 30 ppt or 40 ppt with $p < 0.05$.

species-specific autoinducer, while AI-2 is known to be a universal autoinducer (Schauder and Bassler, 2001), therefore HAI-1 is rarely found and only produced by several bacteria species and its near relative, in contrast to AI-2, which more commonly produced. Studies found that the use of biosurfactants can inhibit biofilm formation by reducing the gene expression of biofilm formation genes. Crude biosurfactants produced from L. *acidophilus* show *quorum*-quenching activities on *C. violaceum, S. marcescens,* and *P. aeruginosa* (Adnan et al., 2023). Algburi et al. (2017) report that subtilosin produced by *B.subtilis is able* to repress AI-2 production in *G. vaginalis.*

Our result found that the addition of CFS only inhibits the AI-2 while being able to induce AI-1. Additionally, our study also found that AI-1 is more responsible for producing bioluminescence compared to AI-2. Our result also aligned with Mok et al. (2003) that found the addition of external AI-1 on *LuxM* and *LuxS* mutant has higher light production compared to external AI-2, 10^4 and 10^2, respectively. Henke and Bassler (2004) also reported where they found that the light produced by the *LuxM* mutant is dark, while the light produced by the *luxS* mutant is similar to WT levels of light, both are found on agar and liquid media. Disruption in *quorum sensing* can occur via different mechanisms (1) AI synthesis inhibition; (2) AI degradation; (3) competition with AI receptor sites (Wang et al., 2022). We found that *Bacillus* CFS can act as a quorum quenching agent in AI-2 through inhibition of their synthesis and activity.

Following in vitro screening, two of the four *Bacillus* species, *B. amyloliquefaciens* and *B. safensis* were selected for in vivo evaluation based on their pronounced biofilm inhibitory activity against *V. parahaemolyticus* 3HP and *V. harveyi* VH1. In vivo experiments demonstrated that oral administration of CFSs derived from either *Bacillus* species effectively reduced shrimp mortality and provided protection against AHPND caused by both 3HP and VH1. These outcomes were consistent with the in vitro findings, which indicated strong biofilm inhibition and *quorum*-quenching activity.

Histopathological examination further confirmed the protective effects of *B. amyloliquefaciens* and *B. safensis* supernatants. Shrimp treated with these CFS exhibited preserved hepatopancreatic structure, in contrast to the positive control group, which showed extensive epithelial sloughing and tissue necrosis (Fig. 7). These observations support the role of Bacillus-derived metabolites in mitigating pathogen-induced tissue damage.

Comparable findings have been reported in related studies. For instance, *Bacillus velezensis* isolated from fermented soybean paste (cheonggukjang) was shown to secrete a wide array of secondary metabolites, including bacillibactin, amylocyclicin, paenibactin, difficidin, fengycin, plipastatin, bacillomycin D, mycosubtilin, iturin, paenilarvins, bacillaene, macrolactin, surfactin, and bacilysin. Supplementation with *B. velezensis* significantly enhanced the survival of *Penaeus vannamei* challenged with *Vibrio campbellii* (Jeon et al., 2022). Similarly, postbiotics containing cell lysates from *Bacillus*, *Lactobacillus*, and *Saccharomyces* species improved growth performance, increased survival against *V. parahaemolyticus*, and enhanced antioxidant capacity and non-specific immune responses in *Macrobrachium nipponense* (Wang et al., 2023).

Overall, the application of postbiotics represents a promising alternative to antibiotics for disease prevention and management in aquaculture, with potential applicability across other sectors requiring sustainable and effective antimicrobial strategies.

5. Conclusion

This study demonstrates that CFS derived from *Bacillus* spp. isolated from sea cucumber possess strong antibiofilm and *quorum*-quenching

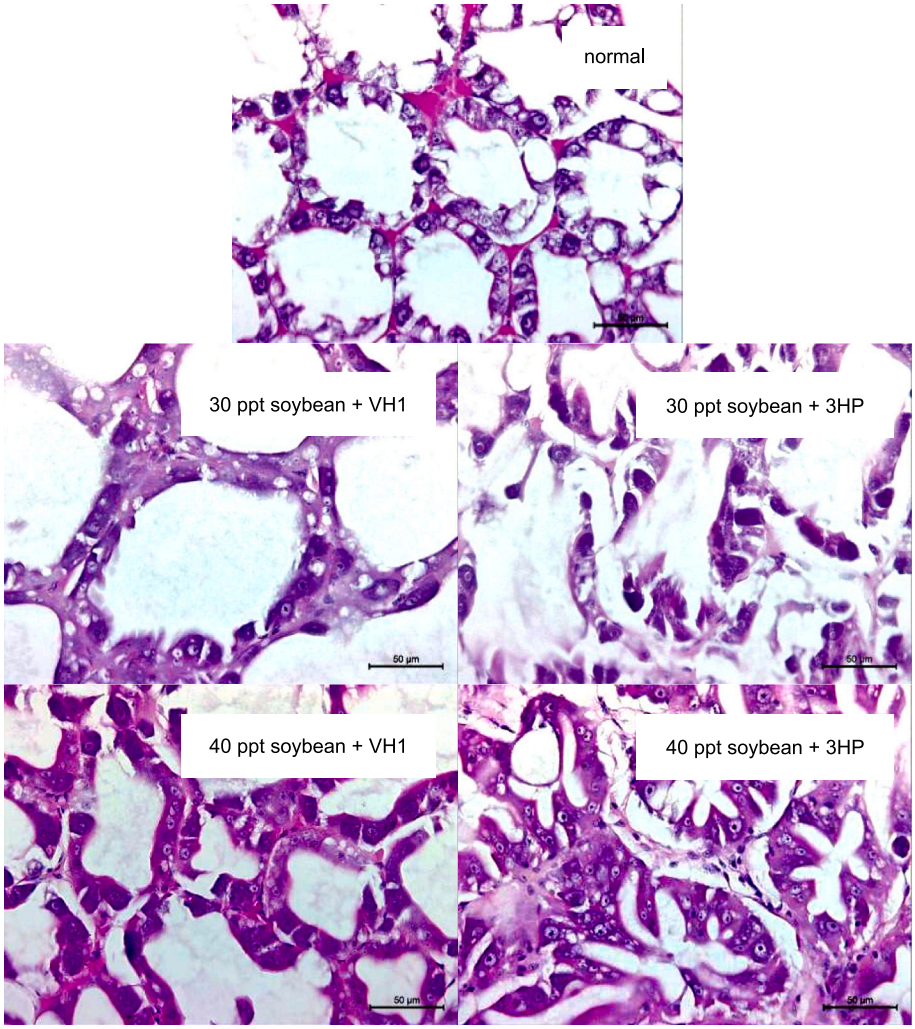


Fig. 7. Hematoxylin and Eosin (H&E) stained shrimps' hepatopancreas tissue.

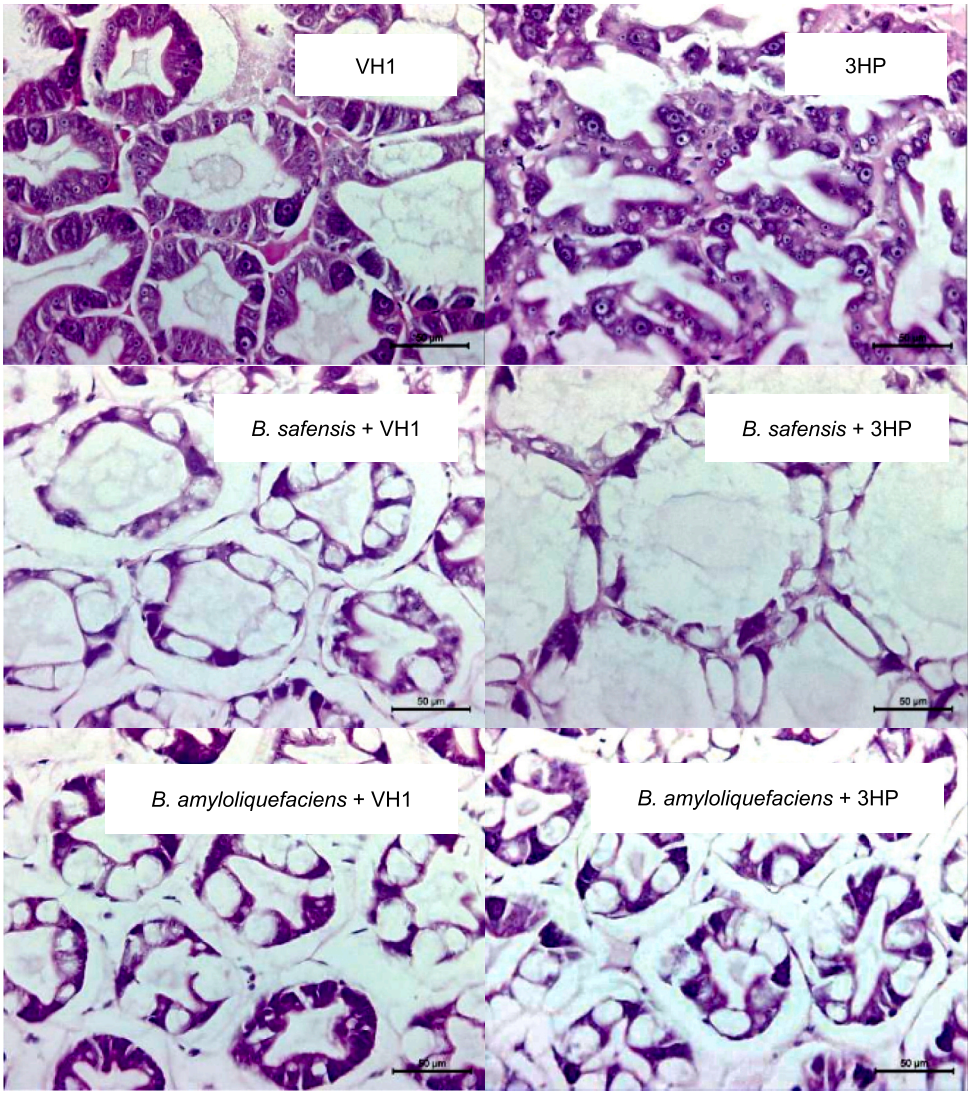


Fig. 7. (*continued*).

activities against *Vibrio parahaemolyticus* and *Vibrio harveyi*, the primary causative agents of AHPND in *Penaeus vannamei*. *Quorum-sensing* analysis reveals that these CFS significantly suppress AI-2 synthesis and activity as main factor to disrupt biofilm formation, but simultaneously induce AI-1 signaling. This selective disruption of *quorum sensing* highlights AI-2 as a critical regulatory target for biofilm development and virulence expression in *Vibrio* spp. and provides insight into how *Bacillus*-derived metabolites interfere with pathogen communication systems.

In vivo feeding trials further confirmed the protective potential of the postbiotics. Dietary supplementation with CFS from *B. amyloliquefaciens* and *B. safensis* significantly improved shrimp survival following immersion challenges with both pathogens, without negatively affecting growth performance. Histopathological analysis supported these results by showing reduced epithelial sloughing and preservation of hepatopancreatic tissue integrity in CFS-treated shrimp.

Overall, the findings highlight Bacillus-derived postbiotics, particularly when produced in soybean-based media, as promising, sustainable alternatives to antibiotics for controlling AHPND in shrimp aquaculture.

Future research should focus on identifying the specific bioactive compounds responsible for quorum-quenching activity and validating their performance under farm-scale conditions. Such efforts will be critical to bridging the gap between laboratory findings and commercial implementation, ultimately contributing to more sustainable and resilient shrimp aquaculture practices.

CRediT authorship contribution statement

Dyah Wulandari: Writing – review & editing, Supervision, Data curation, Conceptualization. **Severus R. Wisastra:** Writing – original draft, Investigation, Formal analysis. **Anto Budiharjo:** Writing – review & editing, Supervision. **R. Haryo B. Setiarto:** Writing – review & editing. **Chumporn Soowannayan:** Validation, Supervision. **A.H. Condro Haditomo:** Writing – review & editing, Methodology. **Ahmad Ni'matullah Al-Baarri:** Writing – review & editing.

Funding

This study was funded by (i) BIMA Fundamental research: Pemetaan Mikrobiota dan Senyawa Bioaktif dalam Makanan Fermentasi Dengke Naniura melalui Pendekatan Multi-Omics, Grant No: 08/LL6/AL.04.03/PL-MULTITAHUN LANJUTAN/2026, 00953/H.2/LPPM/05/2026. (ii) AB would like to thank Diponegoro University for the RPI Grant No: 222-515/UN7D2/PP/2IV/2025 and Equity Grant No. 463/UN7.A/HK/XI/2025.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The author would like to extend their deepest gratitude to all parties who have contributed to the completion of this research.

Data availability

All data generated or analysed during this study are included in this published article

References

Abdel-Latif, H.M., Yilmaz, E., Dawood, M.A., Ringø, E., Ahmadifar, E., Yilmaz, S., 2022. Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: a review. Aquaculture 551, 737951. https://doi.org/10.1016/j.aquaculture.2022.737951.

Adnan, M., Siddiqui, A.J., Noumi, E., Ashraf, S.A., Awadelkareem, A.M., Hadi, S., Snoussi, M., Badraoui, R., Bardakci, F., Sachidanandan, M., Patel, M., 2023. Biosurfactant derived from probiotic *Lactobacillus acidophilus* exhibits broad-spectrum antibiofilm activity and inhibits the quorum sensing-regulated virulence. Biomolecules and Biomedicine 23, 1051. https://doi.org/10.17305/bb.2023.9324.

Algburi, A., Zehm, S., Netrebov, V., Bren, A.B., Chistyakov, V., Chikindas, M.L., 2017. Subtilosin prevents biofilm formation by inhibiting bacterial quorum sensing. Probiotics and Antimicrobial Proteins 9, 81–90. https://doi.org/10.1007/s12602-016-9242-x.

Amatul-Samahah, M.A., Omar, W.H.H.W., Ikhsan, N.F.M., Azmai, M.N.A., Zamri-Saad, M., Ina-Salwany, M.Y., 2020. Vaccination trials against vibriosis in shrimp: a review. Aquaculture Reports 18, 100471. https://doi.org/10.1016/j.aqrep.2020.100471.

Ashrafudoulla, M., Mizan, M.F.R., Park, H., Byun, K.H., Lee, N., Park, S.H., Ha, S.D., 2019. Genetic relationship, virulence factors, drug resistance profile and biofilm formation ability of *Vibrio parahaemolyticus* isolated from mussel. Front. Microbiol. 10, 513. https://doi.org/10.3389/fmicb.2019.00513.

Bell, T.A., Lightner, D.V., 1988. A Handbook of Normal Penaeid Shrimp Histology. Arizona Cooperative Extension, Tucson.

Bianchi, M., Hallström, E., Parker, R.W., Mifflin, K., Tyedmers, P., Ziegler, F., 2022. Assessing seafood nutritional diversity together with climate impacts informs more comprehensive dietary advice. Commun. Earth Environ. 3, 188. https://doi.org/10.1038/s43247-022-00516-4.

Bogovič-Matijašić, B., Rogelj, I., 1998. Bacteriocin complex of Lactobacillus acidophilus LF221—production studies in MRS media at different pH values and effect against Lactobacillus helveticus ATCC 15009. Process Biochem. 33 (3), 345–352.

Devadas, S., Banerjee, S., Yusoff, F.M., Bhassu, S., Shariff, M., 2019. Experimental methodologies and diagnostic procedures for acute hepatopancreatic necrosis disease (AHPND). Aquaculture 499, 389–400. https://doi.org/10.1016/j.aquaculture.2018.06.042.

Dominguez, A.P.M., Bizani, D., Cladera-Olivera, F., Brandelli, A., 2007. Cerein 8A production in soybean protein using response surface methodology. Biochem. Eng. J. 35, 238–243. https://doi.org/10.1016/j.bej.2007.01.019.

El-Saadony, M.T., Alagawany, M., Patra, A.K., Kar, I., Tiwari, R., Dawood, M.A., Dhama, K., Abdel-Latif, H.M., 2021. The functionality of probiotics in aquaculture: an overview. Fish Shellfish Immunol. 117, 36–52. https://doi.org/10.1016/j.fsi.2021.07.007.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Hamza, F., Kumar, A.R., Zinjarde, S., 2016. Antibiofilm potential of a tropical marine *Bacillus licheniformis* isolate: role in disruption of aquaculture associated biofilms. Aquacult. Res. 47, 2661–2669. https://doi.org/10.1111/are.12716.

Harwood, C.R., Mouillon, J.M., Pohl, S., Arnau, J., 2018. Secondary metabolite production and the safety of industrially important members of the *Bacillus subtilis* group. FEMS Microbiol. Rev. 42, 721–738. https://doi.org/10.1093/femsre/fuy028.

Henke, J.M., Bassler, B.L., 2004. Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. J. Bacteriol. 186, 6902–6914. https://doi.org/10.1128/JB.186.20.6902-6914.2004.

Henriksson, P.J.G., Banks, L.K., Suri, S.K., Pratiwi, T.Y., Fatan, N.A., Troell, M., 2019. Indonesian aquaculture futures—identifying interventions for reducing environmental impacts. Environ. Res. Lett. 14, 124062. https://doi.org/10.1088/1748-9326/ab4b79.

Jeon, H.J., Song, J.W., Lee, C., Kim, B., Park, S.Y., Kim, J.H., Han, J.E., Park, J.H., 2022. Antibacterial activity of *Bacillus* strains against acute hepatopancreatic necrosis disease-causing *Vibrio campbellii* in Pacific white leg shrimp. Fishes 7, 287. https://doi.org/10.3390/fishes7050287.

Kareb, O., Aïder, M., 2020. Quorum sensing circuits in the communicating mechanisms of bacteria and its implication in the biosynthesis of bacteriocins by lactic acid bacteria: a review. Probiotics and Antimicrobial Proteins 12, 5–17. https://doi.org/10.1007/s12602-019-09555-4.

Karnjana, K., Soowannayan, C., Wongprasert, K., 2019. Ethanolic extract of red seaweed Gracilaria fisheri and furanone eradicate *Vibrio harveyi* and *Vibrio parahaemolyticus* biofilms and ameliorate the bacterial infection in shrimp. Fish Shellfish Immunol. 88, 91–101. https://doi.org/10.1016/j.fsi.2019.01.058.

Kim, P.I., Ryu, J., Kim, Y.H., Chi, Y.T., 2010. Production of biosurfactant lipopeptides iturin a, fengycin and surfactin a from *Bacillus subtilis* CMB32 for control of *Colletotrichum gloeosporioides*. J. Microbiol. Biotechnol. 20, 138–145.

Kiss, A., Balikó, G., Csorba, A., Chuluunbaatar, T., Medzihradszky, K.F., Alföldi, L., 2008. Cloning and characterization of the DNA region responsible for megacin A-216 production in *Bacillus megaterium* 216. J. Bacteriol. 190, 6448–6457. https://doi.org/10.1128/JB.00557-08.

Koohestani, M., Moradi, M., Tajik, H., Badali, A., 2018. Effects of cell-free supernatant of *Lactobacillus acidophilus* LA5 and *Lactobacillus casei* 431 against planktonic form and biofilm of *Staphylococcus aureus*. Veterinary Research Forum 9, 301–306. https://doi.org/10.30466/vrf.2018.33086.

Kudełka, W., Kowalska, M., Popis, M., 2021. Quality of soybean products in terms of essential amino acids composition. Molecules 26, 5071. https://doi.org/10.3390/molecules26165071.

Kumar, V., Roy, S., Behera, B.K., Bossier, P., Das, B.K., 2021. Acute hepatopancreatic necrosis disease (AHPND): virulence, pathogenesis and mitigation strategies in shrimp aquaculture. Toxins 13, 524. https://doi.org/10.3390/toxins13080524.

Lajis, A.F.B., 2020. Biomanufacturing process for the production of bacteriocins from Bacillaceae family. Bioresour. Bioprocess. 7, 1–26. https://doi.org/10.1186/s40643-020-0295-z.

Letchumanan, V., Chan, K.G., Lee, L.H., 2014. *Vibrio parahaemolyticus*: a review on the pathogenesis, prevalence, and advance molecular identification techniques. Front. Microbiol. 5, 705. https://doi.org/10.3389/fmicb.2014.00705.

Liang, B., Xing, D., 2023. The current and future perspectives of postbiotics. Probiotics and Antimicrobial Proteins 15, 1626–1643. https://doi.org/10.1007/s12602-023-10045-x.

Martínez Cruz, P., Ibáñez, A.L., Monroy Hermosillo, O.A., Ramírez Saad, H.C., 2012. Use of probiotics in aquaculture. International Scholarly Research Notices 2012, 916845. https://doi.org/10.5402/2012/916845.

Mok, K.C., Wingreen, N.S., Bassler, B.L., 2003. *Vibrio harveyi* quorum sensing: a coincidence detector for two autoinducers controls gene expression. EMBO J. 22, 870–881. https://doi.org/10.1093/emboj/cdg085.

Montassier, E., Valdés-Mas, R., Batard, E., Zmora, N., Dori-Bachash, M., Suez, J., Elinav, E., 2021. Probiotics impact the antibiotic resistance gene reservoir along the human GI tract in a person-specific and antibiotic-dependent manner. Nat. Microbiol. 6, 1043–1054. https://doi.org/10.1038/s41564-021-00920-0.

Pandiyan, P., Balaraman, D., Thirunavukkarasu, R., George, E.G.J., Subaramaniyan, K., Manikkam, S., Sadayappan, B., 2013. Probiotics in aquaculture. Drug Invent. Today 5, 55–59. https://doi.org/10.1016/j.dit.2013.03.003.

Papenfort, K., Bassler, B.L., 2016. Quorum sensing signal-response systems in gram-negative bacteria. Nat. Rev. Microbiol. 14, 576–588. https://doi.org/10.1038/nrmicro.2016.89.

Phiwsaiya, K., Charoensapsri, W., Taengphu, S., Dong, H.T., Sangsuriya, P., Nguyen, G.T. T., Pham, H.Q., Amparyup, P., Sritunyalucksana, K., Taengchaiyaphum, S., Chaivisuthangkura, P., Longyant, S., Sithigorngul, P., Senapin, S., 2017. A natural *Vibrio parahaemolyticus* ΔpirAVpΔpirBVp mutant kills shrimp but produces neither PirVp toxins nor acute hepatopancreatic necrosis disease lesions. Appl. Environ. Microbiol. 83, e00680-17. https://doi.org/10.1128/AEM.00680-17.

Prachumwat, A., Taengchaiyaphum, S., Mungkongwongsiri, N., Aldama-Cano, D.J., Flegel, T.W., Sritunyalucksana, K., 2019. Update on early mortality syndrome/acute hepatopancreatic necrosis disease by April 2018. J. World Aquacult. Soc. 50, 5–17. https://doi.org/10.1111/jwas.12559.

Regmi, A., Boyd, E.F., 2019. Carbohydrate metabolic systems present on genomic islands are lost and gained in *Vibrio parahaemolyticus*. BMC Microbiol. 19, 1–20. https://doi.org/10.1186/s12866-019-1487-6.

Restrepo, L., Domínguez-Borbor, C., Bajaña, L., Betancourt, I., Rodríguez, J., Bayot, B., Reyes, A., 2021. Microbial community characterization of shrimp survivors to AHPND challenge test treated with an effective shrimp probiotic (*Vibrio diabolicus*). Microbiome 9, 1–20. https://doi.org/10.1186/s40168-021-01043-8.

Rinaudo, M., 2006. Chitin and chitosan: properties and applications. Prog. Polym. Sci. 31, 603–632. https://doi.org/10.1016/j.progpolymsci.2006.06.001.

Rocha, M.A.M., Coimbra, M.A., Nunes, C., 2017. Applications of chitosan and their derivatives in beverages: a critical review. Curr. Opin. Food Sci. 15, 61–69. https://doi.org/10.1016/j.cofs.2017.06.008.

Sahandi, J., Sorgeloos, P., Xiao, H., Wang, X., Qi, Z., Zheng, Y., Tang, X., 2019. The use of selected bacteria and yeasts to control *Vibrio* spp. in live food. Antibiotics 8, 95. https://doi.org/10.3390/antibiotics8030095.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sanches-Fernandes, G.M.M., Sá-Correia, I., Costa, R., 2022. Vibriosis outbreaks in aquaculture: addressing environmental and public health concerns and preventive therapies using gilthead seabream farming as a model system. Front. Microbiol. 13, 904815. https://doi.org/10.3389/fmicb.2022.904815.

Sandiford, S., 2017. Perspectives on lantibiotic discovery—where have we failed and what improvements are required? Expert Opin. Drug Discovery 10, 315–320. https://doi.org/10.1517/17460441.2015.1016496.

Sathiyamoorthi, E., Lee, J.H., Tan, Y., Lee, J., 2023. Antimicrobial and antibiofilm activities of formylchromones against *Vibrio parahaemolyticus* and *Vibrio harveyi*. Front. Cell. Infect. Microbiol. 13, 1234668. https://doi.org/10.3389/fcimb.2023.1234668.

Schauder, S., Bassler, B.L., 2001. The languages of bacteria. Gene Dev. 15 (12), 1468–1480.

Scholz, R., Vater, J., Budiharjo, A., Wang, Z., He, Y., Dietel, K., Schwecke, T., Herfort, S., Lasch, P., Borriss, R., 2014. Amylocyclicin, a novel circular bacteriocin produced by *Bacillus amyloliquefaciens* FZB42. J. Bacteriol. 196, 1842–1852. https://doi.org/10.1128/JB.01474-14.

Setyawan, P., Imron, I., Gunadi, B., van den Burg, S., Komen, H., Camara, M., 2022. Current status, trends, and future prospects for combining salinity tolerant tilapia and shrimp farming in Indonesia. Aquaculture 561, 738658. https://doi.org/10.1016/j.aquaculture.2022.738658.

Sirikharin, R., Taengchaiyaphum, S., Sanguanrut, P., Chi, T.D., Mavichak, R., Proespraiwong, P., Nuangsaeng, B., Thitamadee, S., Flegel, T.W., Sritunyalucksana, K., 2015. Characterization and PCR detection of binary, Pir-like toxins from *Vibrio parahaemolyticus* isolates that cause acute hepatopancreatic necrosis disease (AHPND) in shrimp. PloS One 10, e0126987. https://doi.org/10.1371/journal.pone.0126987.

Soowannayan, C., Boonmee, S., Puckcharoen, S., Anatamsombat, T., Yatip, P., Ng, W.-K., Thitamadee, S., Tuchinda, P., Munyoo, B., Chabang, N., Nuangsaeng, B., Sonthi, M., Withyachumnarnkul, B., 2019. Ginger and its component shogaol inhibit *Vibrio* biofilm formation in vitro and orally protect shrimp against acute hepatopancreatic necrosis disease (AHPND). Aquaculture 504, 139–147. https://doi.org/10.1016/j.aquaculture.2019.02.007.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101, 11030–11035.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA 11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. https://doi.org/10.1093/molbev/msab120.

Thongjun, J., Tansila, N., Panthong, K., Tanskul, S., Nishibuchi, M., Vuddhakul, V., 2016. Inhibitory potential of biosurfactants from *Bacillus amyloliquefaciens* derived from mangrove soil against *Vibrio parahaemolyticus*. Ann. Microbiol. 66, 1257–1263. https://doi.org/10.1007/s13213-016-1216-4.

Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K., Lightner, D.V., 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Dis. Aquat. Organ. 105, 45–55. https://doi.org/10.3354/dao02621.

Vater, J., Kablitz, B., Wilde, C., Franke, P., Mehta, N., Cameotra, S.S., 2002. Matrix-assisted laser desorption ionization–time of flight mass spectrometry of lipopeptide biosurfactants in whole cells and culture filtrates of *Bacillus subtilis* C-1 isolated from petroleum sludge. Appl. Environ. Microbiol. 68, 6210–6219. https://doi.org/10.1128/AEM.68.12.6210-6219.2002.

Ventola, C.L., 2015. The antibiotic resistance crisis: part 1: causes and threats. P&T 40, 277–283.

Wang, Y., Sun, M., Cui, X., Gao, Y., Lv, X., Li, J., Zhou, K., 2022. Peptide LQLY3-1, a novel *Vibrio harveyi* quorum sensing inhibitor produced by *Lactococcus lactis* LY3-1. LWT 170, 114093. https://doi.org/10.1016/j.lwt.2022.114093.

Wang, J., Li, S., Jian, Y., Song, J., Zheng, J., Zhou, D., Ding, Z., 2023. Dietary postbiotics supplementation improves growth, survival rate, antioxidant capacity, non-specific immunity and gut health of juvenile oriental river prawn (*Macrobrachium nipponense*). Aquaculture Reports 33, 101771.

Wong, A., Ngu, D.Y.S., Dan, L.A., Ooi, A., Lim, R.L.H., 2015. Detection of antibiotic resistance in probiotics of dietary supplements. Nutr. J. 14, 1–6. https://doi.org/10.1186/s12937-015-0084-2.

Wulandari, D., Amatullah, L.H., Lunggani, A.T., Pratiwi, A.R., Budiharjo, A., 2024. Antibacterial activity and molecular identification of soft coral *Sinularia* sp. symbiont bacteria from Karimunjawa Island against skin pathogens *Propionibacterium acnes* and *Staphylococcus epidermidis*. In: Bio Web of Conferences, vol. 92. EDP Sciences, p. 02001. https://doi.org/10.1051/bioconf/20249202001.

Xu, H.M., Rong, Y.J., Zhao, M.X., Song, B., Chi, Z.M., 2014. Antibacterial activity of the lipopeptides produced by *Bacillus amyloliquefaciens* M1 against multidrug-resistant *Vibrio* spp. isolated from diseased marine animals. Appl. Microbiol. Biotechnol. 98, 127–136. https://doi.org/10.1007/s00253-013-5291-1.

Xuan, B.B., Sandorf, E.D., Ngoc, Q.T.K., 2021. Stakeholder perceptions towards sustainable shrimp aquaculture in Vietnam. J. Environ. Manage. 290, 112585. https://doi.org/10.1016/j.jenvman.2021.112585.

Yang, F., Li, X., Li, S., Xiang, J., Li, F., 2020. A novel cuticle protein involved in WSSV infection to the Pacific white shrimp *Litopenaeus vannamei*. Dev. Comp. Immunol. 102, 103491. https://doi.org/10.1016/j.dci.2019.103491.

Yi, H., Han, X., Yang, Y., Liu, W., Liu, H., Zhang, Y., Sun, K., Zhang, L., Ma, F., 2013. Effect of exogenous factors on bacteriocin production from *Lactobacillus paracasei* J23 by using a resting cell system. Int. J. Mol. Sci. 14, 24355–24365. https://doi.org/10.3390/ijms141224355.

Yilmaz, S., Yilmaz, E., Dawood, M.A., Ringø, E., Ahmadifar, E., Abdel-Latif, H.M., 2022. Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: a review. Aquaculture 547, 737514. https://doi.org/10.1016/j.aquaculture.2021.737514.

You, S., Ma, Y., Yan, B., Pei, W., Wu, Q., Ding, C., Huang, C., 2022. The promotion mechanism of prebiotics for probiotics: a review. Front. Nutr. 9, 1000517. https://doi.org/10.3389/fnut.2022.1000517.