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Pseudoalteromonas flavipulchra as a dual-functional probiotic for aquaculture: enhancing microalgae growth and antagonizing *Vibrio* pathogens

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Vibrio disease is a prevalent bacterial infection in aquaculture, and using bacteria with antagonistic properties against *Vibrio* species as probiotics has emerged as a promising method for disease prevention. Additionally, low biomass productivity of microalgae feed remains a significant bottleneck in bivalve aquaculture. Therefore, it is essential to screen for bacteria that both enhance microalgae growth and inhibit *Vibrio* pathogens. In this study, seven bacterial strains capable of promoting microalgae growth were screened for their ability to inhibit three *Vibrio* pathogens, i.e., *Vibrio vulnificus, Vibrio parahaemolyticus* and *Vibrio cholerae*, and thus serve as a dual-functional probiotic for aquaculture. The antagonistic mechanism of these bacteria was further investigated by analyzing the inhibitory effects of their extracellular products (ECP) on *Vibrio* species. Results indicated that *Pseudoalteromonas flavipulchra* exhibited antagonistic effects against all three *Vibrio* species tested. The ECP of *P. flavipulchra* displayed stable antibacterial activity, though this ability was lost after the ECP was treated with heat, alkali, or proteinase K. The proteinaceous fraction isolated from the ECP by precipitation with 90% saturated ammonium sulfate demonstrated concentration-dependent antibacterial activity. These findings suggest that *P. flavipulchra* could serve as a promising dual-functional probiotic for aquaculture, warranting further research to optimize its application in this field.

KEYWORDS

microalgae growth-promoting bacteria, *Pseudoalteromonas flavipulchra*, *Vibrio*, antibacterial activity, antibacterial substance

1 Introduction

Due to high-density farming, modern aquaculture is increasingly beleaguered by frequent disease outbreaks that cause substantial losses (Jin et al., 2024). Vibriosis is one of the most prevalent diseases in aquaculture and it affects marine fish, shellfish, crustaceans, and many other species. Common pathogenic *Vibrio* species include *Vibrio*

parahaemolyticus, *Vibrio alginolyticus*, *Vibrio harveyi*, etc. Among *Vibrio* pathogens, *Vibrio vulnificus* is considered as the most virulent, and it causes significant mortality in the aquaculture of *Cynoglossus semilaevis* (Hu et al., 2020; Oliver, 2006). Infections caused by *V. parahaemolyticus* create a health risk to humans through the consumption of contaminated seafood (Osborne et al., 2023). *Vibrio* pathogens produce toxins that can lead to acute hepatopancreatic necrosis disease (AHPND) in bivalves and crustaceans (López-Cervantes et al., 2021), and the AHPND induced by *V. parahaemolyticus* is one of the most devastating diseases in the global shrimp aquaculture industry (Jun et al., 2017).

The prevention and management of vibriosis in aquaculture has long relied on the use of antibiotics, but the selective pressure due to the excessive use of antibiotics has created persistent antibiotic-resistant bacteria that are spreading rapidly. The situation calls for a shift in disease management strategies to ensure environmental and food safety and protect the aquaculture industry (Yilmaz et al., 2022). Probiotics are now recognized as a safe and effective alternative for controlling aquatic diseases. In shellfish aquaculture, various probiotics that can inhibit *Vibrio* growth, improve host health, and reduce disease incidence have been identified. For instance, *Phaeobacter inhibens* S4 can protect larval eastern oysters from pathogenic *Vibrio* infections without affecting growth (Takyi et al., 2024). Similarly, the addition of the probiotic *Bacillus pumilus* RI06-95 to a hatchery of eastern oyster larvae provides protection against *Vibrio coralliilyticus* RE22 (Stevick et al., 2019), since it alters the abundance and composition of *Vibrio* populations and decreases the relative abundance of pathogenic species. Muñoz-Cerro et al. isolated three probiotic strains from the scallop veliger larvae exhibiting antibacterial activity against *Vibrio bivalvicida in vitro* and found that they protect the larvae against infection (Muñoz-Cerro et al., 2023).

Microalgae are a key food source in aquaculture because they are rich in proteins, polyunsaturated fatty acids, vitamins, and other valuable nutrients (Nagappan et al., 2021), and species such as *Isochrysis* sp., *Nannochloropsis oculata*, *Skeletonema* sp., *Phaeodactylum tricornutum*, and *Chlorella* sp. are widely used as microalgae feed (Zhang et al., 2023). However, the current bivalve aquaculture industry is limited by the biomass productivity of microalgae feed, as the growth of the algae struggles to catch up with the escalating need of the expanding bivalve aquaculture. The algae-bacteria relationship and its impact on ecosystems are now hot research areas. Recent studies found that some bacteria can strongly enhance the biomass productivity of microalgae. For example, *Sphingobacteria* and *Flavobacteria* isolated from *Nannochloropsis oceanica* have been shown to promote the growth of *N. oceanica* and increase the accumulation of eicosapentaenoic acid (EPA) in the algae (Liu et al., 2020). Analogously, *Algoriphagus* sp. and *Oceanicaulis* sp. enhance the growth of *Isochrysis galbana* and boost the content of docosahexaenoic acid (DHA) in the algae (Wu et al., 2023). Most studies focused on the effects of bacteria on the growth and metabolism of microalgae themselves to screen microalgae growth-promoting bacteria (MGPB). However, these MGPB will also enter the bivalve aquaculture systems, along with the microalgae feed, and their impact on shellfish culture systems deserves attention, particularly with regard to antagonistic effects on pathogenic vibrios (Di Costanzo et al., 2023; Li et al., 2023; Seymour et al., 2017). Therefore, it is essential to screen for microalgae growth-promoting bacteria (MGPB) that can both promote the microalgae growth and inhibit pathogenic vibrios. This would not only improve microalgae biomass yield but also promote the healthy development of shellfish larvae.

While there has been considerable research on probiotics in aquaculture, the antagonistic effects of phycospheric bacteria on *Vibrios* pathogens are scarcely studied. In this study, seven MGPB were screened for antibacterial activity against three major pathogenic *Vibrio* species, namely *V. vulnificus*, *V. parahaemolyticus* and *Vibrio cholerae*. The antibacterial substances produced by the MGPB were further investigated and analyzed. Through co-cultivating microalgae and bacteria, this study aims to enhance the microalgae growth while effectively inhibiting the proliferation of pathogenic vibrios. The use of MGPB with *Vibrio* antagonism could provide a foundation for the future biological control of bacterial diseases in aquaculture.

2 Material and methods

2.1 Culture of microalgae and bacteria

The *I. galbana* strain (NMBjih021-2) used in this study was obtained from the Marine Biotechnology Laboratory of Ningbo University, China. The microalgae were cultured in NMB3 medium (Peng et al., 2020), which contained KNO_3 (100 mg/L), KH_2PO_4 (10 mg/L), $MnSO_4{\cdot}H_2O$ (2.5 mg/L), $FeSO_4{\cdot}7H_2O$ (2.5 mg/L), EDTA-Na_2 (10 mg/L), vitamin B_1 (6 mg/L), and vitamin B_{12} (0.05 mg/L). The microalgae were cultivated at 25°C with a 12 h/12 h light/dark cycle in a light-controlled incubator (GXZ-280B, China), and the light intensity was 100 µmol photon m^{-2} s^{-1}. Axenic *I. galbana* was maintained as described by Cao et al. (2019).

Seven bacterial strains, i.e., *Alteromonas* sp., *Oceanicaulis* sp., *Dinoroseobacter* sp., *Pseudoalteromonas flavipulchra*, *Alteromonas macleodii*, *Marinobacter* sp., and *Bacillus jeotgali*, were isolated from the cultures of *I. galbana* in the exponential growth phase (Supplementary Table S1, Supplementary Figure S1). *Vibrio vulnificus*, *V. parahaemolyticus* and *V. cholerae* were isolated from the water samples collected at the Dalai Experimental Base in the Luoyuan County of Fujian, China (Supplementary Table S1, Supplementary Figure S2). All bacteria were freshly plated on 2216E agar plates in a biochemical incubator (SPX-50, China) and grown in 2216E medium at 28°C with shaking at 220 rpm in a thermostatic shaking incubator (THZ-103B, China).

2.2 Co-culture experiment

The cultures of the seven bacterial strains (OD_{600} = 0.4–0.6) were centrifuged at 8,000 rpm for 7 min and washed twice with sterile NMB3 medium, and the collected bacteria were added to the axenic culture of *I. galbana* in the exponential phase, whose cell density was approximately 1 × 10^6 cells/mL, to give a co-culture

with a bacteria/algae ratio of 1:1. The mixture was cultivated at 25°C for 14 days under fluorescent light (4000 lux) with a 12 h/12 h light/dark cycle, and the algal growth was assessed every two days by cell counting (Qin et al., 1999). All experiments were carried out in triplicate. Axenic *I. galbana* culture was used as the control.

2.3 Screening bacterial strains for *Vibrio* antagonism

The seven bacterial strains were tested for their antagonistic activities against *V. vulnificus, V. parahaemolyticus*, and *V. cholerae* using the disc diffusion method (Bauer et al., 1966). The isolates of the seven bacterial strains were prepared in a liquid 2216E medium, and sterilized filter paper discs were soaked in the culture medium of the bacterial strains for 1 h. The discs were then spread on plates containing solidified 2216E medium that was pre-inoculated with fresh *Vibrio* spp. cultures (100 µL, about 24 h old). The growth inhibition zones (GIZ) around each disc were measured after incubation at 28°C for 24–48 h. Cephalosporin and 2216E liquid medium were used as the positive and negative controls, respectively. To further verify the inhibitory effect of the bacteria on *Vibrio* growth, the co-cultures of *Vibrio* spp. with the bacterial strains were incubated in 2216E liquid medium at 28°C for 24 h. The cultures from each group were sampled and spread evenly on thiosulfate citrate bile salts sucrose agar plates, and the colonies of *Vibrio* spp. were counted.

2.4 Isolation and analysis of the extracellular products (ECP) of *P. flavipulchra*

The culture of *P. flavipulchra* was centrifuged (TGL-18M, China) at 4°C and 8000 rpm for 10 min, and the supernatant was passed through a sterilized 0.22 µm Millipore filter. The obtained solution of the *P. flavipulchra* ECP was stored at 4°C until use. To measure the inhibitory effect of the ECP on *Vibrio* spp., a 1:1 v/v mixture of the ECP solution and the culture of *Vibrio* spp. was prepared. The mixture was incubated at 28°C with shaking at 220 rpm, and OD_{600} was measured every 3 h using a spectrophotometer (TU-180, China). The control group used the mixture of 2216E liquid medium and the culture of *Vibrio* spp. To evaluate the effect of ECP concentration on inhibition effect, the ECP solution (1–6 mL) was added into the culture of *Vibrio* spp. (10 mL). The mixtures were incubated at 28°C with shaking at 220 rpm, and OD_{600} was measured using a spectrophotometer every 3 h up to 24 h. The control group used the *Vibrio* spp. culture alone with the addition of ECP.

2.5 Sensitivity of antibacterial substance to heat, alkali, and proteinase K treatment

The following treatments were applied separately to the *P. flavipulchra* ECP: (1) heating at 100°C in a water bath for 30 min, (2) adjustment of the solution pH 12 using 3 M NaOH, (3) incubation with proteinase K (1 mg/mL) at 37°C for 2 h. After adjusting to pH 7 (if needed), the treated ECP was then added to *Vibrio* spp. cultures to assess the antibacterial activity.

2.6 Precipitation of proteins from *P. flavipulchra* ECP

Proteins were isolated from the *P. flavipulchra* ECP through ammonium sulfate precipitation using a solution of $(NH_4)_2SO_4$ at 70%, 80%, or 90% saturation. The mixture was maintained at 4°C for 12 h, then centrifuged at 4°C and 8,000 rpm for 10 min. The collected precipitates were resuspended in phosphate-buffered saline (PBS) and dialyzed using a dialysis membrane. The antibacterial activity of the dialysate was tested by adding it to the culture of *Vibrio* spp. The mixture was incubated at 28°C with shaking at 220 rpm for 24 h, and OD_{600} was measured using a spectrophotometer. The precipitates obtained using 90% ammonium sulfate were subsequently added into the culture of *Vibrio* spp. at varying concentrations, and the antibacterial activity was measured.

2.7 Statistical analysis

Statistical analysis was carried out using SPSS 22.0 (SPSS Inc., USA). All experiments were run in three replicates, and the mean value and standard deviation were calculated accordingly. Differences were considered statistically significant when $P < 0.05$.

3 Results

3.1 Effects of the seven bacterial strains on *I. galbana* growth

All seven bacterial strains effectively promoted the growth of *I. galbana*. *Oceanicaulis* sp. and *Marinobacter* sp. significantly promoted the growth of *I. galbana* starting from day 2, whereas *B. jeotgali*, *Alteromonas* sp., *P. flavipulchra*, and *A. macleodii* promoted the microalgae growth starting from day 4. *Dinoroseobacter* sp. enhanced the growth of *I. galbana* starting from day 8 (Figure 1). The growth of axenic *I. galbana* started to plateau after day 6, but the growth of *I. galbana* co-cultured with bacteria either did not slow down over the 14-day experimental period or had an inflection point at a much later time.

3.2 Screening bacterial strains with *Vibrio* antagonism

All seven bacterial strains were further tested for their antagonistic activities against *V. vulnificus*, *V. parahaemolyticus*, and *V. cholerae* using the disc diffusion method. Only the discs soaked with *P. flavipulchra* or cephalosporin produced clear growth inhibition zones (Figure 2), suggesting that *P. flavipulchra* was the only bacterial strain that suppressed the growth of *V. cholerae*, *V.*

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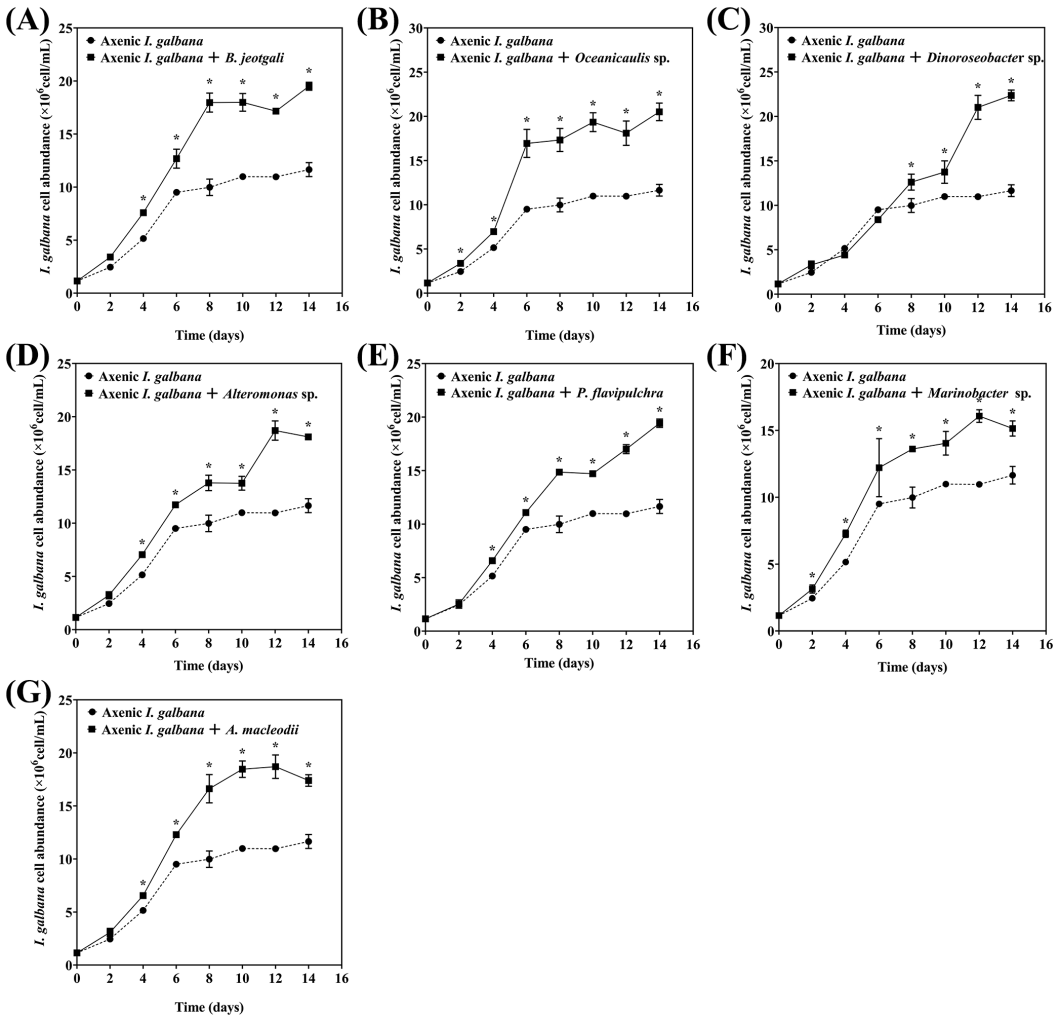

FIGURE 1
Effects of seven phycospheric bacterial strains on the growth of *Isochrysis galbana*. **(A)** *Bacillus jeotgali*, **(B)** *Oceanicaulis* sp., **(C)** *Dinoroseobacter* sp., **(D)** *Alteromonas* sp., **(E)** *Pseudoalteromonas flavipulchra*, **(F)** *Marinobacter* sp., **(G)** *Alteromonas macleodii*. Error line represented standard deviation (SD), * represented significant difference, $p < 0.05$.

vulnificus, and *V. parahaemolyticus*. To further verify the inhibitory effect of the bacteria on *Vibrio* growth, the co-cultures of *Vibrio* spp. with different bacterial strains were grown in 2216E liquid medium. The cell densities of *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* were significantly reduced compared to the control upon co-culturing with *P. flavipulchra* (Figure 3). The results corroborated the findings from the disc diffusion screening and confirmed that *P. flavipulchra* could inhibit *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus*.

3.3 The antibacterial activity of the *P. flavipulchra* ECP

To further analyze whether antibacterial activity of *P. flavipulchra* is due to the secretion of extracellular antibacterial substances, the *P. flavipulchra* ECP was added to *Vibrio* spp. cultures. The growth of *V. vulnificus*, *V. cholerae*, and *V. parahaemolyticus* was significantly inhibited by the addition of the *P. flavipulchra* ECP (Figures 4A–C), and the inhibitory effect increased with rising ECP concentrations. The inhibition rate reached 35.2%, 18.1%, and 30.7% for *V. vulnificus*, *V. cholerae*, and *V. parahaemolyticus*, respectively, when 6 mL ECP was added into 10 mL *Vibrio* culture (Figures 4D–F).

3.4 Effects of heat, alkali and proteinase K treatments on the antibacterial activity of the *P. flavipulchra* ECP

Next, *V. parahaemolyticus* was selected as a representative substrate to analyze the effects of heat, alkali, and proteinase K treatments on the antibacterial activity of the *P. flavipulchra* ECP (Figure 5). The *P. flavipulchra* ECP completely lost its antibacterial activity against *V. parahaemolyticus* after it was heated, exposed to alkali, or treated with proteinase K. Thus, the antibacterial substance in the ECP of *P. flavipulchra* likely had proteinaceous characteristics.

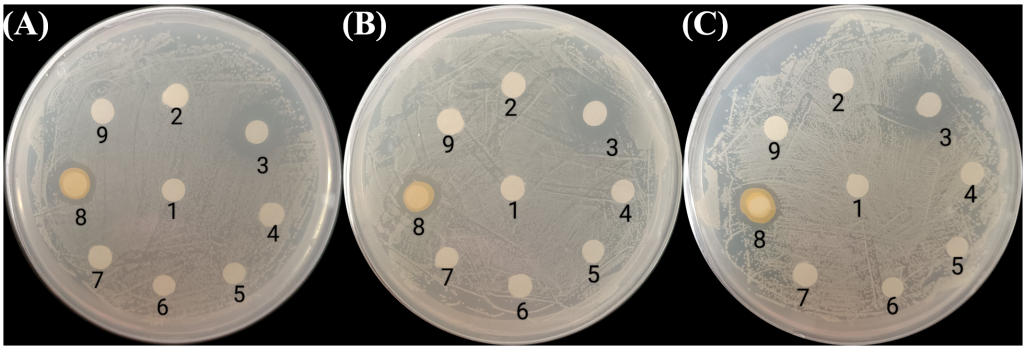

FIGURE 2
Screening bacteria with *Vibrio* antagonism against **(A)** *Vibrio vulnificus*, **(B)** *Vibrio cholerae*, and **(C)** *Vibrio parahaemolyticus* using the disc diffusion method. The discs were (1) 2216E (negative control), (2) *Alteromonas macleodii*, (3) cephalothin (positive control, 0.1 mg/mL), (4) *Bacillus jeotgali*, (5) *Oceanicaulis* sp., (6) *Dinoroseobacter* sp., (7) *Alteromonas* sp., (8) *Pseudoalteromonas flavipulchra*, and (9) *Marinobacter* sp.

3.5 The antibacterial activity of the proteins isolated from the *P. flavipulchra* ECP

To further determine whether the antibacterial substances in *P. flavipulchra* ECP are proteins, the proteins in the *P. flavipulchra* ECP were isolated by precipitation using ammonium sulfate. The proteins precipitated using 90% ammonium sulfate had the highest antibacterial activity and were used for further assessments (Figure 6A). Further testing of these protein fractions revealed a concentration-dependent increase in antibacterial activity against on *V. parahaemolyticus*, demonstrating that higher protein concentrations enhanced the antibacterial effect (Figure 6B).

4 Discussion

4.1 The importance of microalgae growth-promoting bacteria

To date, many MGPB have been identified. They demonstrate important regulatory effects on the growth and metabolism of microalgae. For instance, co-culturing *Pseudomonas putida* and *Chlorella vulgaris* significantly increases the cell density of *C. vulgaris* (Shen et al., 2017), and *Rhizobium* strain 1011 enhances the chlorophyll levels and lipid content in microalgae by supplying vitamin B_{12} (Do Nascimento et al., 2013). Nevertheless, the impact of MGPB on the aquaculture animals feeding on microalgae is underexplored, particularly concerning their antagonistic effects against *Vibrio* species. This work thus aimed to screen MGPB capable of *Vibrio* antagonism and analyze the antibacterial substances from the MGPB.

4.2 The role of probiotics in disease prevention in aquaculture

Vibrio species are widely recognized as the major causes of mass mortality in marine aquaculture (Wang et al., 2022). Although antibiotics have long been employed to prevent *Vibriosis*, the excessive application of antibiotics has led to environmental degradation and the emergence of antibiotic-resistant bacteria (Rico et al., 2017). This situation has heightened the need for alternative

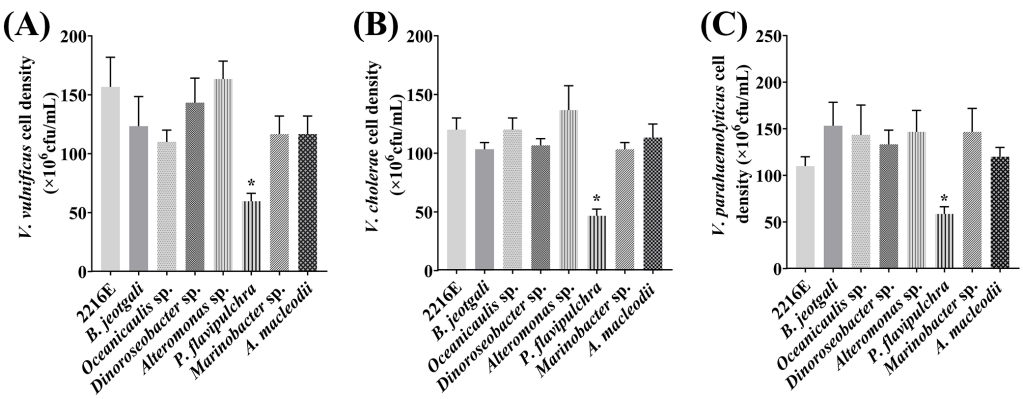

FIGURE 3
Analysis of *Vibrio* antagonism of seven phycospheric bacterial strains co-cultured with **(A)** *Vibrio vulnificus*, **(B)** *Vibrio cholerae*, and **(C)** *Vibrio parahaemolyticus*. Error line represented standard deviation (SD), * represented significant difference, $p < 0.05$.

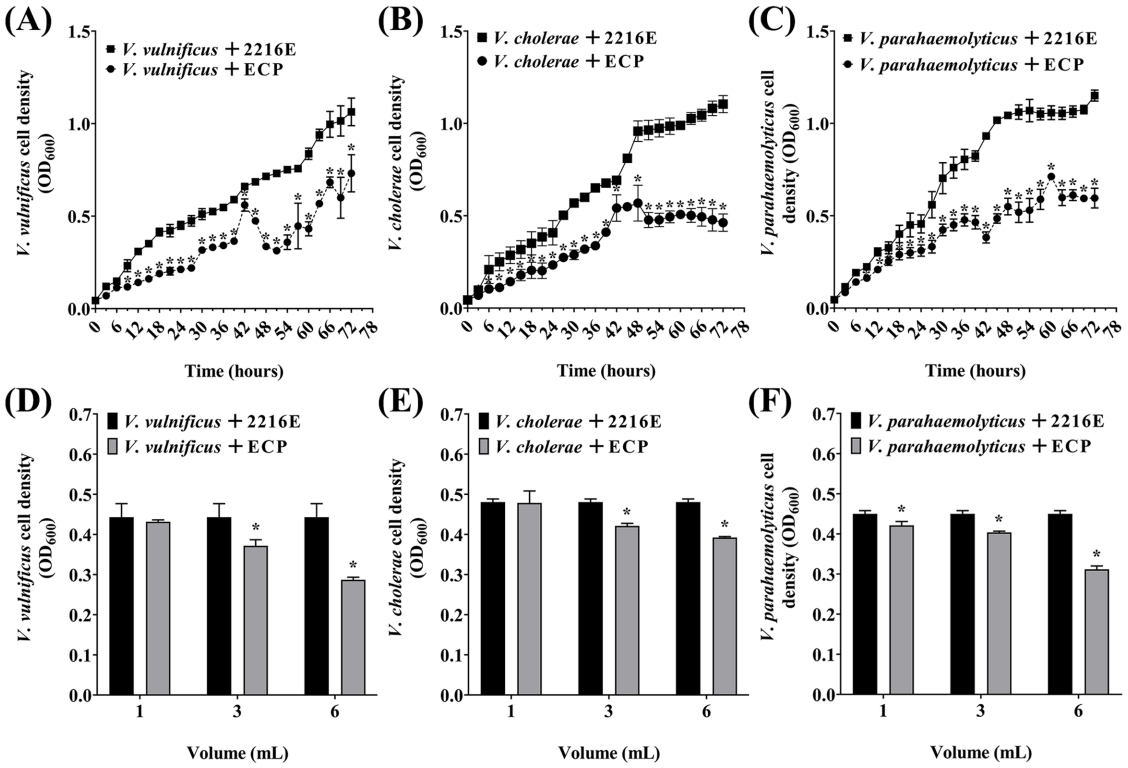

FIGURE 4
Analysis of antagonism of *Pseudoalteromonas flavipulchra* extracellular products (ECP) against **(A, D)** *Vibrio vulnificus*, **(B, E)** *Vibrio cholerae*, **(C, F)** *Vibrio parahaemolyticus*. Error line represented standard deviation (SD), * represented significant difference, $p < 0.05$.

approaches. Probiotics have emerged as a promising option for disease prevention in aquaculture (Dawood and Koshio, 2016; Lieke et al., 2020; Wang et al., 2008; Doan et al., 2020). Recent studies have demonstrated that probiotics can improve growth performance (Li et al., 2024), enhance immunological responses (Costa et al., 2024), and inhibit pathogenic microorganisms (Wang et al., 2020a). However, most studies on probiotics focus on the isolation and screening of bacterial strains from healthy aquaculture animals, and limited attention is given to the antagonistic effects of MGPB against pathogens such as *Vibrio* species. The present study revealed that *P. flavipulchra* simultaneously promoted the growth of *I. galbana* and inhibited *V. vulnificus*, *V. cholerae*, and *V. parahaemolyticus*, suggesting that it might be a promising probiotic for shellfish aquaculture. Nevertheless, the application of *P. flavipulchra* as a probiotic in aquaculture faces several challenges. For instance, variations in environmental conditions, such as temperature and salinity, can influence the activity and survival of the bacterium, and further study is needed to evaluate if *P. flavipulchra* can maintain its anti-*Vibrio* activity despite these fluctuations. In addition, it may not be straightforward to deliver *P. flavipulchra* in a way that ensures high survival and activity, and methods like freeze-drying or incorporation into the shellfish feed may reduce bacterial viability. Therefore, the application of *P. flavipulchra* as a probiotic in aquaculture still requires additional investigation.

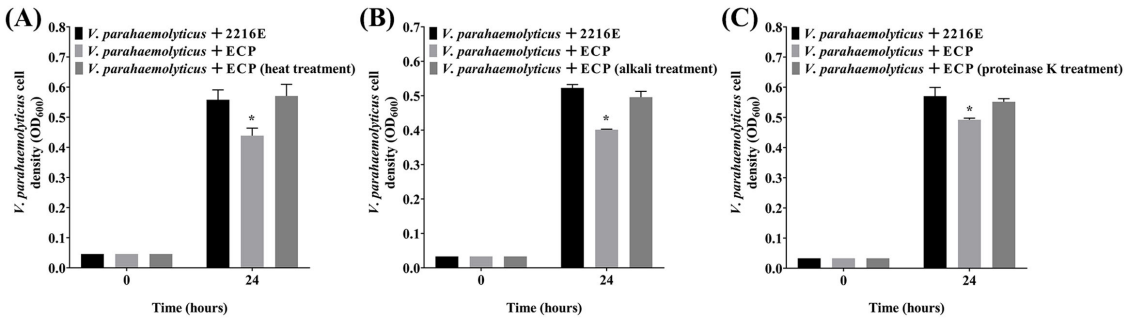

FIGURE 5
Effects of **(A)** heat, **(B)** alkali and **(C)** proteinase K treatments on the antibacterial activity of the *Pseudoalteromonas flavipulchra* ECP. Error line represented standard deviation (SD), * represented significant difference, $p < 0.05$.

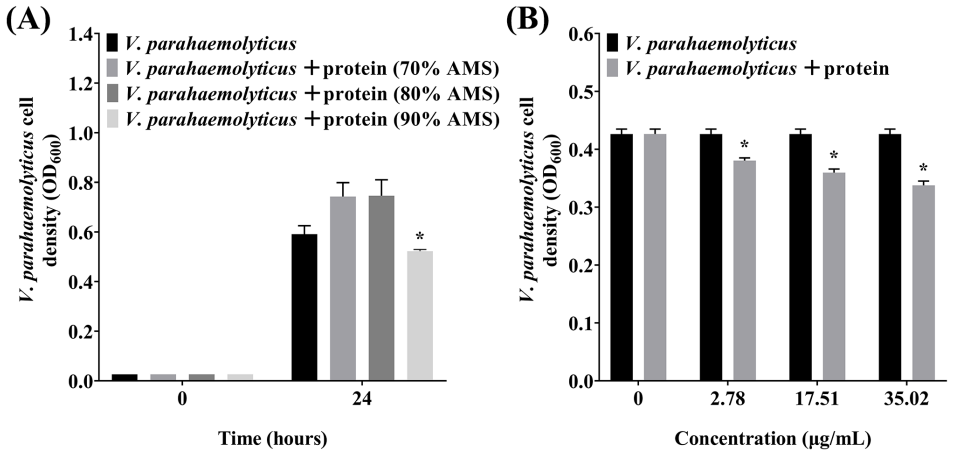

FIGURE 6
The antibacterial activity of the proteins precipitated from the *Pseudoalteromonas flavipulchra* ECP. **(A)** The antibacterial activity of proteins precipitated using ammonium sulfate at different levels of saturation. **(B)** The antibacterial efficacy from using rising amounts of the proteins precipitated with ammonium sulfate at 90% saturation. Error line represented standard deviation (SD), * represented significant difference, $p < 0.05$.

4.3 Mechanisms of *P. flavipulchra* antagonism against *Vibrio* species through antibacterial substances

Several mechanisms have been proposed for the inhibition of various *Vibrio* species by probiotics, including competition for adhesion sites, competition for nutrients, immune system stimulation, disruption of quorum sensing (QS), and production of inhibitory substances (Alcaide et al., 2005; Chauhan and Singh, 2019). Most commonly, the probiotics synthesize and secrete antibiotics, antibacterial agents, lysozymes, proteases, bacteriocins, butyric acid, small molecules, or organic acids that have antibacterial properties (Kesarcodi-Watson et al., 2008; Wang et al., 2019). For example, *Weissella cibaria* KY10 completely inhibits the growth of *V. parahaemolyticus* T11.1 through the secretion of various antibacterial substances, primarily organic acids (Kanjan et al., 2022). *Bacillus subtilis* produces amicoumacin A to inhibit the growth of *V. parahaemolyticus* and other *Vibrio* species, thereby reducing the risk of vibriosis outbreaks (Wang et al., 2020b; Chen et al., 2024). *Lactobacillus plantarum* W2 inhibits seven pathogenic bacteria, including *V. parahaemolyticus*, and organic acids are believed to be active antibacterial agents (Wei et al., 2022). In this study, the ECP isolated from *P. flavipulchra* exhibited antibacterial effects against *V. parahaemolyticus*, but its potency was lost after treatment with heat, alkali, or proteinase K. Analogously, Fontoura et al. reported that the purified antibacterial substance from *Pseudomonas* sp. strain 4B is partially inactivated by proteinase K or trichloroacetic acid and suggested that a protein moiety is involved in the antibacterial activity (Fontoura et al., 2008). Thus, the antibacterial substances in the ECP of *P. flavipulchra* are likely proteinaceous.

Many studies have demonstrated that bacteria can produce proteinaceous substances to inhibit *Vibrio* species. For instance, *Lactobacillus* sp. M31 produces a novel iturin, known as iturin V, which exhibits antibacterial activity against *Vibrio* species (Singh et al., 2021). *Pseudoalteromonas sluteoviolacea* produces a 100 kDa protein that possesses L-amino acid oxidase activity and exerts antibacterial activity against *Vibrio* species such as *V. parahaemolyticus* (Gómez et al., 2008). *Pseudoalteromonas* sp. strain X153 generates an 87 kDa antimicrobial protein that can protect bivalve larvae against *Vibrio* (Longeon et al., 2004). In this study, the proteins precipitated from the ECP of *P. flavipulchra* using 90% ammonium sulphate exhibited strong antibacterial activity and inhibited the growth of *V. parahaemolyticus* in a concentration-dependent manner. However, further investigation is needed to identify and fully characterize the exact protein responsible for the antibacterial property.

4.4 Emerging antibacterial mechanisms of *Pseudoalteromonas*

Pseudoalteromonas is garnering increasing attention in aquaculture due to its ability to produce a myriad of antibacterial compounds, including chitinase, pigments, and antibiotics (Bosi et al., 2017; Richards et al., 2017). In addition, *P. flavipulchra* has been noted to produce vesicle-like structures, which may be involved in some novel antibacterial mechanism (Wang et al., 2021). *Vibrio* spp. typically use QS to regulate virulence, biofilm formation, bioluminescence, sporulation, swarming motility, host colonization, and other population behaviors (Milton, 2006). *Pseudoalteromonas* sp. strain DL3 exhibits not only broad-spectrum antibacterial activity but also the ability to quench QS signal molecules (Zhao et al., 2023). Xu et al. reported that healthy cuttlefish harbored both the pathogenic *V. alginolyticus* and the antagonistic *Pseudoalteromonas piscicida* and attributed the inhibition of *V. alginolyticus* to chemotaxis rather than the production of antibacterial substances (Xu et al., 2024). While the ECP of *P. flavipulchra* contained proteinaceous material that was

antimicrobial, further research is necessary to find out whether *P. flavipulchra* also utilizes other mechanisms to inhibit *Vibrio* species.

5 Conclusion

This study demonstrated the potential of *P. flavipulchra* as a dual-functional probiotic for aquaculture, with both antibacterial activity against *Vibrio* pathogens and the ability to promote microalgae growth. The antagonistic effects of *P. flavipulchra* against *V. vulnificus*, *V. parahaemolyticus*, and *V. cholerae* were likely mediated by the proteinaceous compounds in the extracellular products, as the antibacterial activity was lost after exposure to heat, alkali, or enzymatic degradation. The concentration-dependent antibacterial efficacy of the purified substances underscored their potential use in disease prevention. It seems rewarding to further investigate if *P. flavipulchra* can be applied as a probiotic to create healthier and more productive aquaculture systems, thanks to its ability to both inhibit *Vibrio* pathogens and improve microalgal biomass productivity.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

M-NW: Conceptualization, Data curation, Investigation, Writing – original draft. Y-JX: Data curation, Investigation, Writing – original draft. M-MS: Data curation, Writing – review & editing. Z-YW: Data curation, Writing – review & editing. J-YC: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. J-LX: Conceptualization, Funding acquisition, Resources, Writing – review & editing.

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Conflict of interest

Author J-LX was employed by the company Fujian Dalai Seed Science and Technology Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1492142/full#supplementary-material

References

Alcaide, E., Blasco, M. D., and Esteve, C. (2005). Occurrence of drug-resistant bacteria in two European Eel Farms. *Appl. Environ. Microb.* 71, 3348–3350. doi: 10.1128/AEM.71.6.3348-3350.2005

Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45, 493–496. doi: 10.1093/ajcp/45.4_ts.493

Bosi, E., Fondi, M., Orlandini, V., Perrin, E., Maida, I., de Pascale, D., et al. (2017). The pangenome of (Antarctic) *Pseudoalteromonas* bacteria: evolutionary and functional insights. *BMC Genomics* 18, 93. doi: 10.1186/s12864-016-3382-y

Cao, J. Y., Kong, Z. Y., Zhang, Y. F., Ling, T., Xu, J. L., Liao, K., et al. (2019). Bacterial community diversity and screening of growth-affecting bacteria from *Isochrysis galbana* following antibiotic treatment. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.00994

Chauhan, A., and Singh, R. (2019). Probiotics in aquaculture: a promising emerging alternative approach. *Symbiosis* 77, 99–113. doi: 10.1007/s13199-018-0580-1

Chen, Y. A., Chiu, W. C., Wang, T. Y., Wong, H. C., and Tang, C. T. (2024). Isolation and characterization of an antimicrobial *Bacillus subtilis* strain O-741 against *Vibrio parahaemolyticus*. *PLoS One* 19, e0299015. doi: 10.1371/journal.pone.0299015

Costa, T. S., Copatti, C. E., da Silva, J. J., Marchao, R. S., da Silva, R. C., Rocha, A. D., et al. (2024). Use of *Bacillus subtilis* multiplicate in the water used for biofloc formation: Growth, hemato-biochemistry, intestinal bacteria colonies, and bacterial resistance evaluations of Nile tilapia. *Aquaculture* 590, 741039. doi: 10.1016/j.aquaculture.2024.741039

Dawood, M. A. O., and Koshio, S. (2016). Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture* 454, 243–251. doi: 10.1016/j.aquaculture.2015.12.033

Di Costanzo, F., Di Dato, V., and Romano, G. (2023). Diatom-bacteria interactions in the marine environment: Complexity, heterogeneity, and potential for biotechnological applications. *Microorganisms* 11, 2967. doi: 10.3390/microorganisms11122967

Doan, H. V., Hoseinifar, S. H., Ringo, E., Esteban, M. A., Dadar, M., and Dawood, M. A. O. (2020). Host-associated probiotics: A key factor in sustainable aquaculture. *Rev. Fish. Sci. Aquac.* 28, 16–42. doi: 10.1080/23308249.2019.1643288

Do Nascimento, M., Dublan, M. D., Ortiz-Marquez, J. C. F., and Curatti, L. (2013). High lipid productivity of an *Ankistrodesmus-Rhizobium* artificial consortium. *Bioresour. Technol.* 146, 400–407. doi: 10.1016/j.biortech.2013.07.085

Fontoura, R., Spada, J. C., Silveira, S. T., Tsai, S. M., and Brandelli, A. (2008). Purification and characterization of an antimicrobial peptide produced by *Pseudomonas* sp. strain 4B. *World J. Microb. Biot.* 25, 205–213. doi: 10.1007/s11274-008-9882-4

Gómez, D., Espinosa, E., Bertazzo, M., Lucas-Elío, P., Solano, F., and Sanchez-Amat, A. (2008). The macromolecule with antimicrobial activity synthesized by *Pseudoalteromonas luteoviolacea* strains is an l-amino acid oxidase. *Appl. Microbiol. Biot.* 79, 925–930. doi: 10.1007/s00253-008-1499-x

Hu, Y. R., Li, Y. Z., Li, Z. M., Chen, C. S., Zang, J. J., Li, Y. W., et al. (2020). Novel insights into the selective breeding for disease resistance to vibriosis by using natural outbreak survival data in Chinese tongue sole (*Cynoglossus semilaevis*). *Aquaculture* 529, 735670. doi: 10.1016/j.aquaculture.2020.735670

Jin, W. Y., Jiang, L. H., Hu, S., and Zhu, A. Y. (2024). Effects of *Lactobacillus plantarum* and *Bacillus subtilis* on growth, immunity and intestinal flora of largemouth bass (*Micropterus salmoides*). *Aquaculture* 583, 740581. doi: 10.1016/j.aquaculture.2024.740581

Jun, J. W., Han, J. E., Giri, S. S., Tang, K. F. J., Zhou, X. H., Aranguren, L. F., et al. (2017). Phage application for the protection from acute hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei*. *Indian J. Microbiol.* 28, 114–117. doi: 10.1007/s12088-017-0694-9

Kanjan, P., Kimtun, A., Chaimongkol, S., and Sakpetch, P. (2022). Probiotic *Weissella cibaria* KY10 derived from digestive tract of healthy shrimp exhibits strong antibacterial effects against *Vibrio parahaemolyticus* causing AHPND in shrimp. *Aquac. Res.* 53, 2587–2607. doi: 10.1111/are.15777

Kesarcodi-Watson, A., Kaspar, H., Lategan, M. J., and Gibson, L. (2008). Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture* 274, 1–14. doi: 10.1016/j.aquaculture.2007.11.019

Li, Z., Du, Q. Y., Jiao, T. Y., Zhu, Z. Y., Wan, X. L., Ju, C. W., et al. (2024). Effects of probiotic supplementary bioflocs (*Rhodospirillum rubrum*, *Bacillus subtilis*, *Providencia rettgeri*) on growth, immunity, and water quality in cultures of Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture* 591, 741141. doi: 10.1016/j.aquaculture.2024.741141

Li, S. N., Zhang, C. F., Li, F. H., Ren, N. Q., and Ho, S. H. (2023). Recent advances of algae-bacteria consortia in aquatic remediation. *Crit. Rev. Environ. Sci. Technol.* 53, 315–339. doi: 10.1080/10643389.2022.2052704

Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L., and Steinberg, C. E. W. (2020). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Rev. Aquacult.* 12, 943–965. doi: 10.1111/raq.12365

Liu, B. L., Eltanahy, E. E., Liu, H. W., Chua, E. T., Thomas-Hall, S. R., Wass, T. J., et al. (2020). Growth-promoting bacteria double eicosapentaenoic acid yield in microalgae. *Bioresour. Technol.* 316, 123916. doi: 10.1016/j.biortech.2020.123916

Longeon, A., Peduzzi, J., Barthélemy, M., Corre, S., Nicolas, J. L., and Guyot, M. (2004). Purification and partial identification of novel antimicrobial protein from marine bacterium *Pseudoalteromonas* species strain X153. *Mar. Biotechnol.* 6, 633–641. doi: 10.1007/s10126-004-3009-1

López-Cervantes, G., Alvarez-Ruiz, P., Luna-Suárez, S., Luna-González, A., Esparza-Leal, H. M., Castro-Martínez, C., et al. (2021). Temperature and salinity modulate virulence and PirA gene expression of *Vibrio parahaemolyticus*, the causative agent of AHPND. *Aquacult. Int.* 29, 743–756. doi: 10.1007/s10499-021-00654-0

Milton, D. L. (2006). Quorum sensing in vibrios: Complexity for diversification. *Int. J. Med. Microbiol.* 296, 61–71. doi: 10.1016/j.ijmm.2006.01.044

Muñoz-Cerro, K., González, R., Mercado, A., Lira, G., Rojas, R., Yáñez, C., et al. (2023). Scallop larvae resistant to a pathogenic *Vibrio* harbor host-associated bacteria with probiotic potential. *Aquaculture* 579, 740217. doi: 10.1016/j.aquaculture.2023.740217

Nagappan, S., Das, P., AbdulQuadir, M., Thaher, M., Khan, S., Mahata, C., et al. (2021). Potential of microalgae as a sustainable feed ingredient for aquaculture. *J. Biotechnol.* 341, 1–20. doi: 10.1016/j.jbiotec.2021.09.003

Oliver, J. D. (2006). "Vibrio vulnificus," in *The biology of vibrios*. Eds. F. L. Thompson, B. Austin and J. Swings (American Society of Microbiology, Washington, DC), 349–366.

Osborne, B., Siboni, N., Seymour, J. R., Ralph, P., and Pernice, M. (2023). Exploring the potential of algae-bacteria interactions in the biocontrol of the marine pathogen *Vibrio parahaemolyticus*. *J. Appl. Phycol.* 35, 2731–2743. doi: 10.1007/s10811-023-03071-3

Peng, H. X., de-Bashan, L. E., Bashan, Y., and Higgins, B. T. (2020). Indole-3-acetic acid from *Azosprillum brasilense* promotes growth in green algae at the expense of energy storage products. *Algal Res.* 47, 101845. doi: 10.1016/j.algal.2020.101845

Qin, J. G., Pearson, L., and Carragher, J. F. (1999). Response of *Tetraselmis suecica* to nutrient and grazer manipulation. *J. Appl. Phycol.* 11, 481–485. doi: 10.1023/A:1008116530154

Richards, G. P., Watson, M. A., Needleman, D. S., Uknalis, J., Boyd, E. F., and Fay, J. P. (2017). Mechanisms for *Pseudoalteromonas piscicida*-induced killing of vibrios and other bacterial pathogens. *Appl. Environ. Microb.* 83, 171–175. doi: 10.1128/AEM.00175-17

Rico, A., Jacobs, R., Van den Brink, P. J., and Tello, A. (2017). A probabilistic approach to assess antibiotic resistance development risks in environmental compartments and its application to an intensive aquaculture production scenario. *Environ. pollut.* 231, 918–928. doi: 10.1016/j.envpol.2017.08.079

Seymour, J. R., Amin, S. A., Raina, J. B., and Stocker, R. (2017). Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. *Nat. Microbiol.* 2, 17065. doi: 10.1038/nmicrobiol.2017.65

Shen, Y., Gao, J. Q., and Li, L. S. (2017). Municipal wastewater treatment via co-immobilized microalgal-bacterial symbiosis: Microorganism growth and nutrients removal. *Bioresour. Technol.* 243, 905–913. doi: 10.1016/j.biortech.2017.07.041

Singh, S. S., Akhtar, M. N., Sharma, D., Mandal, S. M., and Korpole, S. (2021). Characterization of iturin V, a novel antimicrobial lipopeptide from a potential probiotic strain *lactobacillus* sp. M31. *Probiotics Antimicro.* 13, 1766–1779. doi: 10.1007/s12602-021-09796-2

Stevick, R. J., Sohn, S., Modak, T. H., Nelson, D. R., Rowley, D. C., Tammi, K., et al. (2019). Bacterial community dynamics in an oyster hatchery in response to probiotic treatment. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.01060

Takyi, E., Stevick, R. J., Witkop, E. M., Gregg, L., Chesler-Poole, A., Small, J. M., et al. (2024). Probiotic treatment modulates the bacterial microbiome of larval eastern oysters, *Crassostrea virginica*, in hatcheries. *Aquaculture* 583, 740624. doi: 10.1016/j.aquaculture.2024.740624

Wang, D., Flint, S. H., Palmer, J. S., Gagic, D., Fletcher, G. C., and On, S. L. W. (2022). Global expansion of *Vibrio parahaemolyticus* threatens the seafood industry: Perspective on controlling its biofilm formation. *Lwt-Food Sci. Technol.* 158, 113182. doi: 10.1016/j.lwt.2022.113182

Wang, R. X., Guo, Z. H., Tang, Y. P., Kuang, J. W., Duan, Y. F., Lin, H. Z., et al. (2020a). Effects on development and microbial community of shrimp Litopenaeus vannamei larvae with probiotics treatment. *AMB Express* 10, 109. doi: 10.1186/s13568-020-01041-3

Wang, Y. B., Li, J. R., and Lin, J. D. (2008). Probiotics in aquaculture: Challenges and outlook. *Aquaculture* 281, 1–4. doi: 10.1016/j.aquaculture.2008.06.002

Wang, D. D., Li, J. H., Zhu, G. L., Zhao, K., Jiang, W. W., Li, H. D., et al. (2020b). Mechanism of the potential therapeutic candidate *Bacillus subtilis* BSXE-1601 against shrimp pathogenic Vibrios and multifunctional metabolites biosynthetic capability of the Strain as predicted by genome analysis. *Front. Microbiol.* 11. doi: 10.3389/fmicb.2020.581802

Wang, A. R., Ran, C., Wang, Y. B., Zhang, Z., Ding, Q. W., Yang, Y. L., et al. (2019). Use of probiotics in aquaculture of China-a review of the past decade. *Fish Shellfish Immunol.* 86, 734–755. doi: 10.1016/j.fsi.2018.12.026

Wang, H. L., Sun, B. C., Xie, G. S., Wan, X. Y., Huang, J., and Song, X. L. (2021). Spotlight on a novel bactericidal mechanism and a novel SXT/R391-like integrative and conjugative element, carrying multiple antibiotic resistance genes, in *Pseudoalteromonas flavipulchra* strain CDM8. *Microbiol. Res.* 242, 126598. doi: 10.1016/j.micres.2020.126598

Wei, C., Luo, K., Wang, M. Y., Li, Y. M., Pan, M. J., Xie, Y. M., et al. (2022). Evaluation of potential probiotic properties of a strain of *lactobacillus plantarum* for shrimp farming: from beneficial functions to safety assessment. *Front. Microbiol.* 13. doi: 10.3389/fmicb.2022.854131

Wu, M. N., Cao, J. Y., Xu, Y. J., Xu, S. M., Zhou, Z. B., Shao, M. M., et al. (2023). Impact of three phycospheric bacterial strains on the growth and fatty acid composition of *Isochrysis galbana*. *Alagl Res.* 74, 103183. doi: 10.1016/j.algal.2023.103183

Xu, L. T., Jiang, M. W., Peng, R. B., Jiang, X. M., Wang, S. Y., Han, Q. X., et al. (2024). The pathogen Vibrio alginolyticus H1 and its antagonist *Pseudoalteromonas piscicida* H2 associated with the health status of cuttlefish *Sepia pharaonis*. *Comp. Immunol. Rep.* 6, 200141. doi: 10.1016/j.cirep.2024.200141

Yilmaz, S., Yilmaz, E., Dawood, M. A. O., Ringo, E., Ahmadifar, E., and Abdel-Latif, H. M. R. (2022). Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. *Aquaculture* 547, 737514. doi: 10.1016/j.aquaculture.2021.737514

Zhang, J. X., Ran, Z. S., Xie, H. X., Kong, F., Zhang, M. Q., Zhou, Y., et al. (2023). A systematic analysis and evaluation of nutritional composition of 23 strains of marine microalgae commonly used in aquaculture. *Algal Res.* 72, 103122. doi: 10.1016/j.algal.2023.103122

Zhao, S. S., Zhang, H. J., Cai, X. L., Wang, L. R., Shen, X. R., Cao, Z., et al. (2023). Study on antibacterial and quorum quenching activity of a Pseudoalteromonas sp. DL3. *Acta Agriculturae Zhejiangensis* 35, 50–57. doi: 10.3969/j.issn.1004-1524.2023.01.06