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Impact of *Streptomyces* and *Bacillus* on Ammonia, Nitrite, *Vibrio* Counts and Growth of *Penaeus vannamei* in Recirculating Aquaculture System

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Abstract

This study evaluates the effects of supplementing whiteleg shrimp (*Penaeus vannamei*) feed with *Streptomyces* strains (TM_1, TM_2, TM_7, TM_{21}, TM_{22}), *Bacillus* strains (AQ_1, BIO_2, BAL_3), and their combinations at different densities (10^6, 10^7, and 10^8 CFU/g) on growth performance, immune response, water quality, and *Vibrio* control. Two experiments were conducted over 36 days: the first tested individual *Bacillus* and *Streptomyces* consortia at varying doses, and the second assessed combined treatments. Survival was monitored after shrimp were fed probiotics and subsequently challenged with *Vibrio parahaemolyticus*. Results showed significant improvements in final body weight (FBW), daily weight gain (DWG), feed conversion ratio (FCR), survival rate (SR), and total hemocyte count (THC) in all probiotic treatments, with greater benefits at higher probiotic doses. Combined probiotic treatments consistently produced greater numerical enhancements in growth performance than single-strain applications, indicating synergistic effects between *Streptomyces* and *Bacillus* consortia. Water quality responses differed between probiotic types, with *Streptomyces* treatments providing rapid early reductions in total ammonia nitrogen (TAN) and nitrite, whereas *Bacillus*-supplemented treatments achieved delayed but more stable and higher removal efficiencies toward the end of the experiment. The combination treatment B8S8 (*Bacillus* and *Streptomyces* at 10^8 CFU/g each) yielded the greatest growth enhancement (approximately 143% FBW and 149% DWG increase relative to the control) and effectively reduced *Vibrio* counts, achieving 2.9 and 3.1 log reductions in water (B6S8 and B8S8, respectively) and 3.5 and 3.6 log reductions in the shrimp gastrointestinal tract. In combined treatments, TAN and nitrite removal efficiencies exceeded 95% by Day 36, with no significant differences among probiotic combinations. This probiotic supplementation strategy enhances shrimp health and growth in recirculating aquaculture systems at 100 shrimp/100 L, suggesting that combined *Streptomyces* and *Bacillus* consortia are a promising approach for improving *P. vannamei* aquaculture productivity.

Keyword: *Streptomyces, Bacillus, Penaeus Vannamei,* Whiteleg Shrimp, RAS, *Vibrio parahaemolyticus,* Total Ammonia Nitrogen, Nitrite, Total Hemocyte Count, Growth Performance

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INTRODUCTION

The role of aquaculture in food security also indirectly impacts GDP by ensuring the stability of food prices and availability, particularly in countries where seafood is a major part of the diet. Therefore, aquaculture can be considered a stabilizing force in the national economy, helping to balance trade and contributing to the overall economy.[1] One innovative solution to achieving this sustainability is the use of Recirculating Aquaculture Systems (RAS), which offer advantages over traditional open systems by efficiently managing water resources and improving environmental control.[2] RAS can reuse more than 90% of the water in a closed-loop system, minimizing water use and reducing environmental impacts while maintaining optimal conditions for aquaculture.[3,4] This system not only supports higher stocking densities of cultured species but also allows for better management of water quality parameters, making it an increasingly popular choice for intensive aquaculture.[5] In the 1990s period, various research projects were carried out in the USA aimed at cultivating *Litopenaeus vannamei* with the use of recirculating raceway systems.[6] For the last three decades, researchers have continually explored different RAS techniques for rearing *Litopenaeus vannamei*. The technologies cover a variety of RAS types, such as raceway RAS,[7] elevated pond systems,[8] fully enclosed land-based setups,[9,10] constructed wetland systems,[11] and small experimental RAS facilities.[12-14] These systems use industrial methods to regulate water conditions in culture tanks, improving the environment for shrimp growth and survival.[14]

Bacterial diseases remain one of the most significant challenges in aquaculture, especially those caused by *Vibrio* species.[15] These bacteria are commonly associated with infectious diseases in marine organisms, particularly shrimp. The first 30 days of shrimp culture are a particularly vulnerable period, as postlarvae are in the early stages of development and more prone to infection.[16] Various species of *Vibrio*, including *V. harveyi*, *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. penaeicida*, play a significant role in the occurrence of vibriosis in aquatic organisms, especially under certain environmental conditions.[17,18] Shrimp infected with AHPND often exhibit visible symptoms, including a distended stomach and a reduced size of the hepatopancreas (HP). The mortality rate tends to rise significantly within the first three days of infection, and disease outbreaks generally occur within 8 to 45 days post-stocking.[19] The rise and spread of diseases in shrimp have restricted the growth of shrimp farming areas and global productivity, leading to excessive antibiotic use by farmers to reduce disease-related losses. However, in recent years, concerns over the long-term environmental effects and potential risks to human health have led to a backlash against antibiotic use. In 2020, global antimicrobial usage reached approximately 99,500 tons, and it is projected to grow by 8.0%, reaching about 107,500 tons by 2030. The bulk of this usage is concentrated in Asia, accounting for nearly 67% of the total, while Africa uses less than 1%. These trends point to an increase in global antimicrobial consumption by 2030, surpassing earlier 2017 forecasts, with significant growth expected in Asia/Oceania and the Americas.[20] The widespread use of antibiotics exerts selection pressure, driving the development of resistance in bacteria. These bacteria adapt to this pressure, primarily through horizontal gene transfer, which can lead to the unclear flow of resistance genes. Resistance mechanisms can emerge through the acquisition of chromosomal material, although these genetic alterations are not transferable to other bacterial cells. Furthermore, some bacterial pathogens are capable of acquiring resistance via plasmid-based mechanisms.[20,21]

The 2001 FAO/WHO report defines probiotics as beneficial live microbes that, when ingested in sufficient quantities, contribute to the health of the host by modulating the microbial balance. Studies have shown that probiotics can improve nutrition, enhance feed efficiency, strengthen immunity, and increase disease resistance.[20] In their 2014 study, Lazado and Caipang emphasized the practical benefits of using probiotics in aquaculture, with their initial demonstration of this potential dating back to 1986.[22] Probiotics operate through multiple mechanisms and are essential for promoting the well-being of aquatic species.[23]

Among the many probiotics, *Bacillus* and *Streptomyces* have attracted particular attention

due to their beneficial effects in aquaculture. *Bacillus* species are recognized for producing a range of bioactive compounds, including antimicrobial peptides and enzymes, which help control harmful bacteria, enhance water quality, and support the growth of aquatic species. Similarly, *Streptomyces*, well-known for its antibiotic production, has demonstrated potential in controlling various marine pathogens, including *Vibrio*. *Bacillus licheniformis* DAHB1, isolated from shrimp ponds, is capable of producing an AHL-lactonase enzyme that destroys the communication signal molecule involved in the biofilm formation of *Vibrio parahaemolyticus* DAHP1.[24] *Bacillus thuringiensis* QQ1 and *Bacillus cereus* QQ2 have high potential to degrade both chemically synthesized and naturally biosynthesized AHL signal molecules from *Vibrio harveyi* and *Vibrio alginolyticus.*[25] The bacteriocin CAMT2, which is synthesized by *Bacillus amyloliquefaciens* isolated from *Epinephelus areolatus*, demonstrates antibacterial effects against *Vibrio parahaemolyticus*.[26] *Bacillus* sp. Lts40, obtained from shrimp ponds, exhibits antibacterial properties against *Vibrio harveyi*, the pathogen responsible for shrimp diseases, by producing bacteriocins.[27] Approximately 70% of antibiotics used today are derived from actinomycetes.[28,29] These *Streptomyces* strains produce siderophores that bind iron in the aquatic environment, inhibiting the growth of pathogenic *Vibrio* strains.[30] *Streptomyces griseus* and *Streptomyces nigrescens* demonstrate bacteriocin-mediated inhibition of pathogenic bacteria, including *Vibrio parahaemolyticus.*[31] Although both *Bacillus* and *Streptomyces* have been studied individually in aquaculture, limited research has explored the combined application of these two genera in recirculating aquaculture systems (RAS) to control *Vibrio* infections and improve shrimp health. This study aimed to assess the effects of integrating *Bacillus* and *Streptomyces* strains in a recirculating aquaculture system for controlling *Vibrio* levels, improving water quality, and supporting the development of *Penaeus vannamei* over a 36 day culture period.

MATERIALS AND METHODS

Tank preparation and shrimp

The study was carried out at Lien Hiep Phat Science and Technology Co. Ltd., which is located in Ho Chi Minh City, Vietnam. Twelve-day-old whiteleg shrimp postlarvae (PL_{12}) were purchased from Viet Uc Seafood Corporation. The shrimp were acclimatized for 3 days in a 400L HDPE plastic tank. Water parameters in the acclimation tank were regulated to match hatchery standards, with temperature ranging from 28 to 30 °C, salinity at 10‰, dissolved oxygen between 4 and 6 ppm, pH from 7.8 to 8.2, and alkalinity between 140 and 160 ppm. Shrimp were given Tomboy Aquafeed JSC commercial feed, with a daily feeding amount equivalent to 3% of their body weight. After acclimation, the shrimp were moved to the designated RAS, with a stocking density of 1 shrimp per liter.

A RAS unit consists of a 100 L glass rearing tank designed for shrimp farming and connected to a biological filtration system with a working volume of 36 L, resulting in a total operational water volume of 136 L per system. Solid waste is removed using a polyester matting sponge (Biopro, Japan), followed by a moving-bed biofilter containing Kaldnes media (Small Boss, China) and a fixed-bed biofilter with ceramic noodles and volcanic stones as carrier materials (Biopro, Japan). RAS units are illuminated by sunlight through solar panels on the roof. Oxygen is supplied to the system through continuous aeration in both the rearing tanks and the biological filtration units.

Culture water circulates continuously through the system using a 28 W pump (Atman, China) with a nominal flow rate of 2000 L h^{-1}, corresponding to a hydraulic retention time (HRT) of approximately 4 minutes per circulation cycle. Water parameters in the RAS units were adjusted similarly to those during the acclimation period. Prior to shrimp stocking, all RAS units were operated continuously for 14 days to allow biofilter maturation. No external microbial inoculum was added during this period, as the experiment aimed to evaluate the effects of diets supplemented with *Bacillus* spp. and *Streptomyces* spp. on nitrogen compound dynamics in the culture water.

Biofilter performance during the experimental period was assessed based on total ammonia nitrogen (TAN) and nitrite (NO_2^--N) concentrations measured from the day of shrimp stocking onward. No routine water exchange was applied during the experiment, except for

the addition of freshwater to compensate for evaporative losses.

Bacterial strains

The microorganism strains utilized in this project include *Streptomyces* sp. TM_1, *Streptomyces* sp. TM_2, *Streptomyces* sp. TM_7, *Streptomyces* sp. TM_{21}, and *Streptomyces* sp. TM_{22}, which were isolated and selected from the water and bottom mud of whiteleg shrimp ponds in previous research.[32] Additionally, *Bacillus* sp. AQ_1, *Bacillus* sp. BIO_2, and *Bacillus* sp. BAL_3 were isolated and selected from the mud of Can Gio mangrove forest, Vietnam.[33] *Vibrio parahaemolyticus* was isolated from diseased shrimp provided by Lien Hiep Phat Sci-Tech Company, Ltd.

Vibrio parahaemolyticus and *Bacillus* strains were cultivated in Tryptone Soy Broth (TSB) medium with 1.5% NaCl at 37 °C for 24-48 hours, while *Streptomyces* strains were cultured on Gause I medium with 1.5% NaCl at 37 °C for a duration of 7 days. Following cultivation, the centrifugation process was carried out for 20 minutes at a speed of 6000 rpm for the cultures, and the resulting pellet was washed three times with saline (1.5% w/v). After washing, the pellet was resuspended in 1.5% saline to prepare a bacterial stock, with concentrations of 5.0 x 10^8 CFU/mL for *Bacillus* strains[34,35] and 1.0 x 10^9 CFU/mL for *Streptomyces* strains,[36,37] determined by measuring the optical density using a spectrophotometer. The bacterial suspensions, with optical density measured at 600 nm, were quantified by counting the colonies formed after plating on solid media, using the spread plate method. *Streptomyces* strains were cultured on Gause I, while *Bacillus* strains and *Vibrio parahaemolyticus* were cultivated on Tryptone Soy Agar (TSA).[35,38]

Probiotic formulation

Commercial shrimp feed (Tomboy, Skretng Vietnam) was mixed with bacterial stocks prepared as described in the Bacterial strains section, so that the CFU/g feed density reached approximately three levels: 1 x 10^6, 1 x 10^7, and 1 x 10^8 of *Bacillus* spp.[36,37] and *Streptomyces* spp.[39-41] The bacterial cultures were evenly applied to the surface of the feed pellets, which were then left to dry at 40 °C for 24 hours. In the control group, an equal volume of sterile seawater was used in place of the bacterial suspension. To confirm the presence of bacteria in the feed, quantitative analysis was performed using NA with 1.5% NaCl (Nutrient Agar) for *Bacillus* and Gause I agar for *Streptomyces*.

Experimental design

Impact of *Bacillus* spp. and *Streptomyces* spp. in Feed on Water Quality, Intestinal *Vibrio* Levels, and Growth Performance of Shrimp

The experiment was conducted over 5 weeks with 21 Recirculating Aquaculture System (RAS) units, divided into 7 treatments, each replicated 3 times. The treatments included B6, B7, B8 (*Bacillus* spp. AQ_1, BIO_2, BAL_3 in a 1:1:1 ratio), and S6, S7, S8 (*Streptomyces* spp. TM_1, TM_2, TM_7, TM_{21}, and TM_{22} in a 1:1:1:1:1 ratio), corresponding to feed formulas containing 1 × 10^6, 1 × 10^7, and 1 × 10^8 CFU/g feed. The control treatment (C) consisted of feed without probiotics.

The shrimp were provided food four times a day, at intervals of 4 hours, with a feeding rate of 5%-10% of their body weight.[35,42,43] Water was not replaced during the experiment, except for water lost through evaporation, which was replenished as necessary.

After 5 weeks, the shrimp from the experimental tanks were collected, measured, and tallied to assess SR (Survival rate, %), biomass, FBW (Final body weight, g), DWG (Daily weight gain, g/day), and FCR (feed conversion ratio). These parameters were calculated based on previously described methods.[44-46]

The number of strains and their internal ratios within each probiotic group were kept constant across treatments, while the total bacterial density (CFU/g feed) was used as the primary experimental variable. Equal ratios among *Bacillus* strains (1:1:1) and *Streptomyces* strains (1:1:1:1:1) were applied to ensure uniform representation of each strain and to minimize additional sources of variation. This experimental design allowed the evaluation of dose-dependent effects of *Bacillus* spp. and *Streptomyces* spp. supplementation rather than effects related to strain composition or ratio.

Effect of Combining *Bacillus* spp. and *Streptomyces* spp. in feed at screened densities on water quality, intestinal *Vibrio* density, and shrimp growth parameters

Following the results of the initial experiment, the optimal *Bacillus* spp. and *Streptomyces* spp. densities for improving water quality, reducing *Vibrio* densities in the gut, and enhancing shrimp growth performance were determined. The optimal feed formulas for each bacterium were selected for further combination in the next phase of the study.

The feed formulas will be prepared as described in the Probiotic formulation section, ensuring that both *Bacillus* spp. and *Streptomyces* spp. are present simultaneously in the feed at the selected densities and inoculation ratios, similar to those used in the initial experiment. This experiment will be conducted with another batch of shrimp, of similar age to the initial batch, purchased from Viet Uc Seafood Corporation. Feeding procedures, experimental duration, and evaluation criteria will remain the same as in the initial experiment.

In the combined treatments, the internal strain ratios of *Bacillus* spp. and *Streptomyces* spp. were maintained as defined in the initial experiment. This approach was applied to preserve the previously screened strain composition and to avoid introducing additional experimental variables, thereby allowing the evaluation of the combined effects of *Bacillus* spp. and *Streptomyces* spp. supplementation.

Water quality analysis

Fifteen milliliters of water were collected from just beneath the surface, approximately 20 cm deep, in each tank using a transparent, aseptic borosilicate vial with a tight-sealing lid (manufactured by Pyrex, USA). This was accomplished with a plastic Pasteur pipette to analyze ammonia and nitrite levels, and to assess the density of *Bacillus*, actinomycetes, and *Vibrio*. The quantification of TAN and Nitrite was performed using APHA 4500 NH_3 F – Phenate method and APHA 9245 B – Multiple tube method, respectively.[47] Bacterial counts were performed using the spread plate method. The water samples were diluted tenfold using saline (0.9% NaCl), and 100 µL aliquots of these dilutions were spread on different types of agar: nutrient agar (NA) with 1.5% NaCl for total heterotrophic count, Gause I agar for actinomycetes detection, and thiosulfate-citrate-bile-salt-sucrose (TCBS) agar for enumerating *Vibrio* counts. *Bacillus* enumeration was carried out using the heat-cold shock method,[48] where the sample was heated to 85 °C for 15 minutes to eliminate vegetative cells and promote spore formation, followed by rapid cooling on ice for 1-2 minutes. The total *Bacillus* count was determined by plating on NA medium with 1.5% NaCl.

Determination of bacterial counts in shrimp gut

After completing the experiment, the digestive systems of the shrimp, encompassing the stomach, hepatopancreas, and intestines, were extracted from three shrimp chosen at random from each experimental tank. After weighing and homogenizing the samples in saline, a tenfold dilution was performed. The diluted samples were analyzed for microbial populations, such as *Vibrio*, *Bacillus*, heterotrophic bacteria, and actinomycetes, employing techniques outlined in the Water Quality Analysis section.

Total hemocyte count

Three shrimp from each experimental tank were randomly selected for blood collection to assess the total hemocyte count at the end of the experiment. A 27 gauge needle was used to collect 50 µL of hemolymph from the first abdominal segment's ventral side. The sample was then mixed with 450 µL of an anticoagulant solution (EDTA 1×10^{-2} M, trisodium citrate 3×10^{-2} M, citric acid 2.6×10^{-2} M, NaCl 45×10^{-2} M, and glucose 10×10^{-2} M), and the solution's pH was adjusted to 4.6 using HCl (1 M).[49,50] This mixture was stored on ice and fixed with a 6% formaldehyde solution in a 1:3 ratio. The post-fixed blood mixture was then combined with 20 µL of Rose Bengal (1.2% in 50% ethanol solution) and allowed to incubate for 20 minutes.[49] The total hemocyte count was assessed with a Neubauer counting chamber under a transmission microscope, with the results expressed in cells/mL.[51,52]

Challenge with *Vibrio parahaemolyticus*

The challenge was conducted through immersion using a suspension of *Vibrio parahaemolyticus* (1.0 x 10^6 CFU/mL and 1.0 x 10^8 CFU/mL).[53] This challenge was administered following a 5 week period of probiotic administration. There were two experiments conducted. In Experiment 1, a total of seven treatments, including the control group, were tested at each challenge density, with each group consisting of ten animals and three replicates. After obtaining the results from Experiment 1, selected treatments were chosen for further testing in Experiment 2, which followed a similar setup with the same challenge densities and animal groups. Survival rates were recorded daily over a four-day period.

The *Vibrio* infection experiments were conducted under strictly controlled conditions. All equipment, including tanks, water containers, and sampling tools, was sterilized with a 70% ethanol solution before and after each use. To prevent cross-contamination, personnel handling the shrimp wore gloves, lab coats, safety glasses, and masks. Access to the experimental area was restricted to minimize the risk of introducing external pathogens.

The culture water was initially treated with sodium hypochlorite (5% - Xilong, China) at a concentration of 30 ppm. After a 12 hour incubation period, the residual chlorine concentration was measured using a Sera Chlorine Test Kit to ensure complete neutralization of chlorine before stocking the shrimp. These biosecurity measures were implemented to maintain the integrity of the study and ensure the safety of the experimental environment.

Statistical methods

One-way ANOVA was applied to assess the differences between groups, with statistical significance determined at a significance level of $P < 0.05$. Following this, pairwise comparisons were conducted using Fisher's test (Statgraphics Centurion XV) to identify specific treatment effects.

RESULTS

Impact of *Bacillus* spp. and *Streptomyces* spp. in feed on water quality, intestinal *Vibrio* levels, and growth performance of shrimp

Bacterial parameters and total hemocyte count

Supplementary Figure S1 shows the progression of the total *Vibrio* count (TVC) during the experiment. At the start of the experiment, TVC varied from 1.059-1.905 log CFU/mL. TVC increased over the first two weeks (D15), with values ranging from 2.626-3.395 log CFU/mL across treatments, while the control had a value of 3.950 log CFU/mL. From week 4 (D22) onward, TVC in the *Streptomyces*–fed treatments decreased and stabilized, with log CFU/mL values of 3.094 ± 0.056 (S6), 3.011 ± 0.061 (S7), and 2.948 ± 0.021 (S8), showing no significant differences among them. In the *Bacillus*–added treatments, TVC remained stable from week 4 onwards, with log CFU/mL values of 3.827 ± 0.065 (B6), 3.825 ± 0.046 (B7), and 3.576 ± 0.035 (B8). Treatment B8 showed a significantly lower count compared to B7 and B6, but significantly higher than S6, S7, and S8. The TVC in all treatments was notably lower than that in the control treatment (5.303 ± 0.125 log CFU/mL). For total actinomycetes count (TAMC), actinomycetes appeared in the culture water after 1 week of feeding with *Streptomyces*–containing feed, but not in the control or *Bacillus* treatments. After 4 weeks, actinomycetes were present in all treatments, with higher densities in those supplemented with probiotics (Figure S2). By the end of the experiment, the log CFU/mL values were 1.651 ± 0.090 (Control), 3.946 ± 0.095 (S6), 4.122 ± 0.037 (S7), 4.310 ± 0.087 (S8), 2.822 ± 0.144 (B6), 2.680 ± 0.208 (B7), and 2.504 ± 0.115 (B8), with S6, S7, and S8 having significantly higher actinomycete density than the other treatments. Regarding the total heterotrophic plate count (TPC), no considerable variations were observed among treatments throughout the experimental period (Figure S3). In terms of total Bacillus count (TBC), Bacillus density tended to increase over time in all treatments (Figure S4). At the end of the experiment, Bacillus-fed treatments (B6-B8) showed significantly higher TBC values compared

to the other treatments, with log CFU/mL values of 2.881 ± 0.076 (Control), 3.098 ± 0.024 (S6), 3.088 ± 0.047 (S7), 3.144 ± 0.068 (S8), 3.477 ± 0.106 (B6), 3.400 ± 0.072 (B7), and 3.608 ± 0.133 (B8). Figure 1 illustrates the overall levels of TVC, TPC, TBC, and TAMC in the shrimp gastrointestinal tract. TVC in the probiotic treatments was markedly lower than the control group (6.304 ± 0.179 log CFU/g). No notable variations in TVC were observed with values of log CFU/g across B6 (4.774 ± 0.045), B7 (4.689 ± 0.045), and B8 (4.454 ± 0.086), while S6 (4.368 ± 0.094) showed similar values to B8 and S7 (4.134 ± 0.083). TVC in S8 (3.823 ± 0.043 log CFU/g) was considerably lower than that in all other treatments. TAMC (log CFU/g) was significantly higher in the *Streptomyces* treatments, particularly in S7 (5.172 ± 0.142) and S8 (5.698 ± 0.026). *Bacillus* supplemented treatments (B6, B7, B8) showed no significant difference from the control, with TAMC values of 2.922-2.855 log CFU/g. TBC was significantly higher in B6 (4.467 ± 0.145), B7 (4.711 ± 0.111), and B8 (4.997 ± 0.116) compared

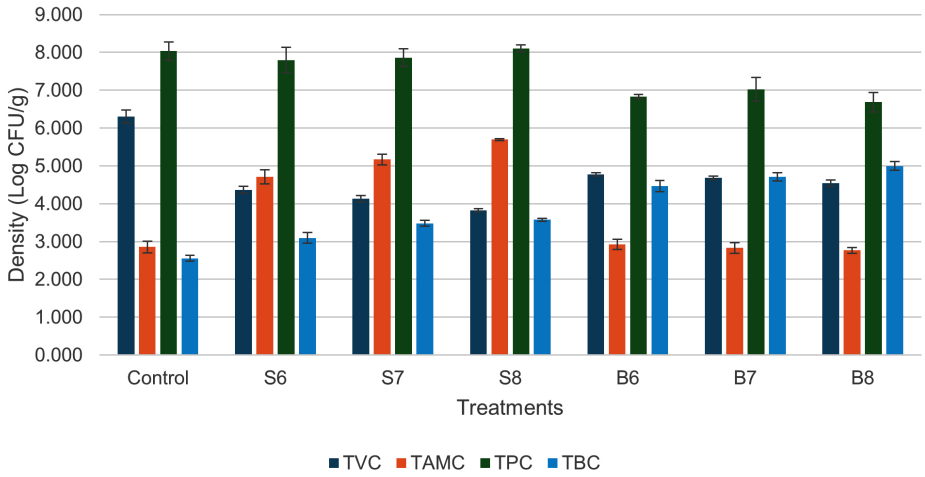

Figure 1. Bacterial counts in shrimp gastrointestinal tract after 36 days of feeding different densities of *Bacillus* spp. and *Streptomyces* spp.

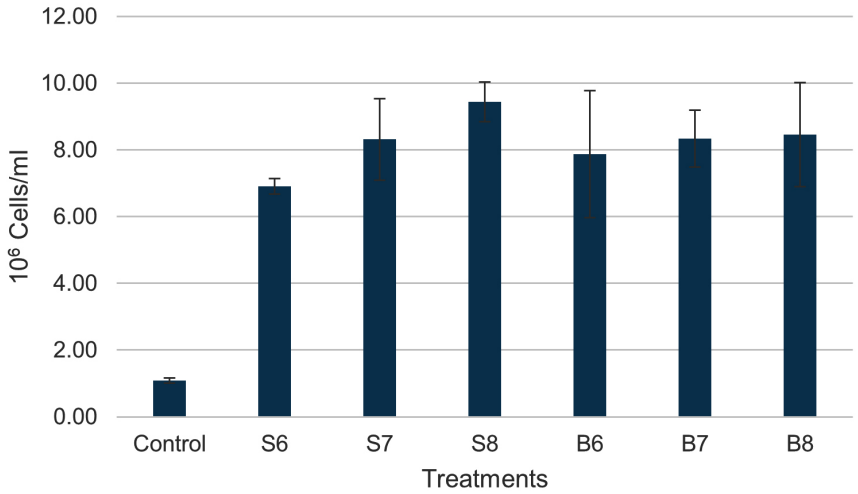

Figure 2. Total hemocyte count of shrimp after 36 days of feeding different densities of *Bacillus* spp. and *Streptomyces* spp.

Table 1. Growth performance of shrimp after 36 days of feeding different densities of *Bacillus* spp. and *Streptomyces* spp.

Treatment	Growth Performance			
	FBW (g)	DWG (g/day)	FCR	SR (%)
Control	0.27 ± 0.04^{a}	0.0071 ± 0.0010^{a}	1.82 ± 0.25^{b}	64.67 ± 2.19^{a}
S6	0.35 ± 0.02^{b}	0.0095 ± 0.0005^{b}	1.24 ± 0.05^{a}	94.67 ± 0.88^{b}
S7	0.43 ± 0.02^{d}	0.0118 ± 0.0005^{cd}	1.10 ± 0.03^{a}	96.67 ± 1.45^{b}
S8	0.53 ± 0.02^{e}	0.0144 ± 0.0004^{e}	1.06 ± 0.03^{a}	97.00 ± 0.58^{b}
B6	0.36 ± 0.02^{bc}	0.0097 ± 0.0005^{bc}	1.28 ± 0.06^{a}	94.33 ± 0.33^{b}
B7	0.41 ± 0.00^{cd}	0.0113 ± 0.0001^{bcd}	1.19 ± 0.02^{a}	95.67 ± 1.45^{b}
B8	0.44 ± 0.02^{d}	0.0119 ± 0.0005^{d}	1.09 ± 0.02^{a}	96.00 ± 0.58^{b}

The data are shown as mean ± standard error, with different letters after the standard error representing statistically significant differences ($P < 0.05$)

to the other treatments, while the control group showed the lowest count (2.554 ± 0.075 log CFU/g). The addition of *Bacillus* reduced TPC in the gastrointestinal tract, with B6 (6.834 ± 0.059), B7 (7.023 ± 0.313), and B8 (6.687 ± 0.256) showing significantly lower values compared to S6 (7.797 ± 0.336), S7 (7.864 ± 0.227), S8 (8.109 ± 0.095), and the control (8.032 ± 0.239).

After 36 days of the experiment, both *Streptomyces* spp. and *Bacillus* spp. notably elevated the total hemocyte count (THC) in shrimp, with observed values (10^6 cells/ml) of 6.91 ± 0.23 (S6), 8.31 ± 1.22 (S7), 9.44 ± 0.60 (S8), 7.88 ± 1.90 (B6), 8.34 ± 0.86 (B7), and 8.45 ± 1.56 (B8), all of which were considerably higher than those of the control group (1.08 ± 0.08) (Figure 2). Nonetheless, no notable variations in THC were found among the treatments.

Concentrations of Total Ammonia Nitrogen (TAN) and Nitrite in the Rearing Water

The TAN concentration in the rearing water of the control group increased throughout the experiment, reaching 5.770 ± 0.397 ppm by D36 (Figure S5A). In treatments with *Streptomyces*, TAN remained stable until D26 (0.085-0.089 ppm) and gradually increased by D36, reaching 0.865 ± 0.233 ppm (S6), 0.958 ± 0.113 ppm (S7), and 0.973 ± 0.154 ppm (S8). In contrast, TAN levels in *Bacillus* supplemented treatments spiked on D8 (1.085 ± 0.039 ppm for B6, 0.971 ± 0.113 ppm for B7, and 0.942 ± 0.027 ppm for B8), then decreased after D12 and stabilized with values ranging from 0.037 to 0.115 ppm by D36. When TAN removal efficiency (RE%) for probiotic treatments was calculated using the accumulated TAN trend of the control as the baseline, clear time-dependent differences were observed between probiotic groups (Figure S5B). The *Streptomyces* treatments (S6-S8) showed high RE% early in the trial (D4-D8; approximately 55.00%-88.86%) and generally maintained high performance through the mid-phase (D12-D26; approximately 75.16%-96.48%); however, RE% declined markedly from D29 onward, reaching approximately 35.64%-42.32% at D36. In contrast, the *Bacillus* treatments (B6-B8) exhibited low to negative RE% at the beginning (D4-D8; -31.51% to -0.09%), but RE% increased substantially from D12 and remained consistently high thereafter, exceeding approximately 89.34% from D15-D36 and reaching approximately 95.74%-97.07% at D36. Overall, *Streptomyces* produced a rapid but less persistent reduction pattern, whereas *Bacillus* achieved a delayed yet more stable and superior TAN removal toward the end of the experiment.

Similarly, the nitrite concentration in the control group increased over time, reaching 5.770 ± 0.397 ppm on D36 (Figure S6A). In *Streptomyces* fed treatments, nitrite levels began increasing from D8 and peaked at 0.917 ± 0.244 ppm on D36 in S6, while S7 and S8 showed stable levels from D22 to D36. In *Bacillus* spp. fed treatments, nitrite levels peaked on D12 (2.515 ± 0.267 ppm for B6, 2.049 ± 0.200 ppm for B7, and 1.834 ± 0.084 ppm for B8), before declining by D19 and stabilizing for the remainder of the study, with concentrations

Table 2. Relative improvement (%) in growth performance of shrimp compared with the control after 36 days of feeding different densities of *Bacillus* spp. and *Streptomyces* spp.

Treatment	Relative Improvement (%) In Growth Performance			
	FBW improvement (%)	DWG improvement (%)	FCR reduction (%)	SR improvement (%)
S6	38.99 ± 25.31[a]	40.67 ± 26.43[a]	28.33 ± 13.57[a]	49.11 ± 6.18[a]
S7	70.65 ± 29.83[a]	73.49 ± 31.29[a]	36.64 ± 10.91[a]	52.33 ± 7.25[a]
S8	107.21 ± 34.45[a]	111.36 ± 36.26[a]	38.83 ± 9.81[a]	52.75 ± 5.79[a]
B6	40.87 ± 25.93[a]	42.54 ± 27.12[a]	26.59 ± 11.89[a]	48.53 ± 5.28[a]
B7	62.09 ± 22.80[a]	64.48 ± 23.94[a]	31.87 ± 9.90[a]	50.61 ± 5.63[a]
B8	69.03 ± 16.82[a]	71.60 ± 17.76[a]	37.78 ± 8.19[a]	51.08 ± 4.39[a]

Data are expressed as mean ± standard error. Percentage improvement was calculated relative to the control. Different letters indicate significant differences among treatments ($P < 0.05$)

ranging from 0.036-0.180 ppm. Nitrite removal efficiency (RE%) increased over time in all probiotic treatments, but the magnitude and stability differed by probiotic type (Figure S6B). In the *Streptomyces* treatments (S6-S8), RE% was modest and more variable at the beginning (D4-D8), then rose sharply by D12 and remained consistently high through the end of the trial, ranging from 67.23%-88.73% at later sampling points. The *Bacillus* treatments (B6-B8) started with scattered responses, including negative RE% in some cases, yet demonstrated a pronounced improvement after D12. From D19 onward, *Bacillus* treatments maintained near-complete nitrite removal (RE% between 92.77% and 99.33%), staying above 96% at D36. Starting from Day 22, *Bacillus* treatments had significantly higher nitrite removal efficiency than the *Streptomyces* treatments.

Shrimp growth performance

The growth performance of shrimp fed diets supplemented with single strains of *Bacillus* spp. and *Streptomyces* spp. at different densities is presented in Table 1. All probiotic treatments significantly enhanced shrimp growth compared with the control group ($P < 0.05$). Final body weight (FBW) increased progressively with increasing probiotic density, with the highest value observed in the S8 treatment (0.53 ± 0.02 g), which was significantly higher than all other treatments. FBW in S6 was comparable to B6, while S7 showed similar values to B7 and B8.

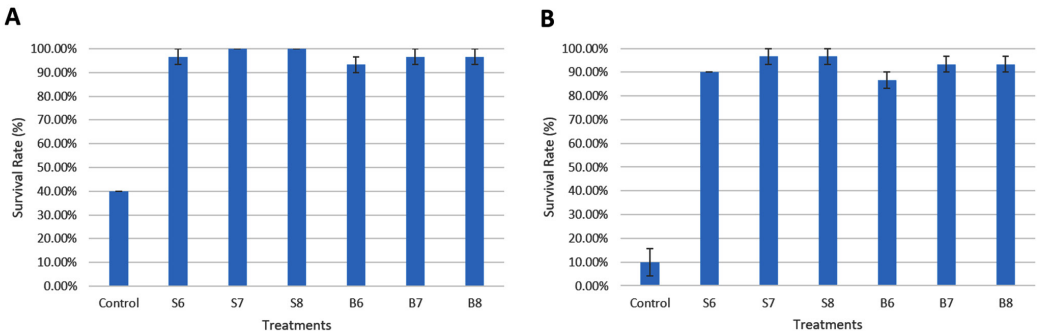

Figure 3. Survival rate of shrimp after 4 days of exposure to *Vibrio parahaemolyticus* at 10^6 CFU/mL (A) and 10^8 CFU/mL (B) following 36 days of feeding with different densities of *Bacillus* spp. and *Streptomyces* spp.

Daily weight gain (DWG) followed a similar pattern, increasing with probiotic supplementation. The S8 group exhibited the highest DWG (0.0144 ± 0.0004 g/day), which was significantly greater than those of all other treatments. DWG in S6 did not differ significantly from B6 and B7, while S7 showed comparable performance to *Bacillus*-supplemented diets. In contrast, no significant differences in feed conversion ratio (FCR) and survival rate (SR) were observed among probiotic treatments, although all probiotic-fed shrimp exhibited significantly lower FCR and higher SR than the control ($P < 0.05$).

To further quantify the magnitude of growth enhancement, relative improvement percentages compared with the control are summarized in Table 2. Probiotic supplementation resulted in substantial increases in FBW and DWG, with FBW improvement ranging from 38.99%-107.21% and DWG improvement from 42.54%-111.36%. The greatest numerical improvements were consistently observed in the S8 treatment, indicating that higher densities of *Streptomyces* spp. exerted the strongest growth-promoting effect among single-strain applications.

Feed utilization efficiency was markedly improved in all probiotic treatments, as reflected by FCR reductions of 26.59%-38.83% relative to the control. Survival rate also increased considerably, showing improvements of approximately 48%-53%.

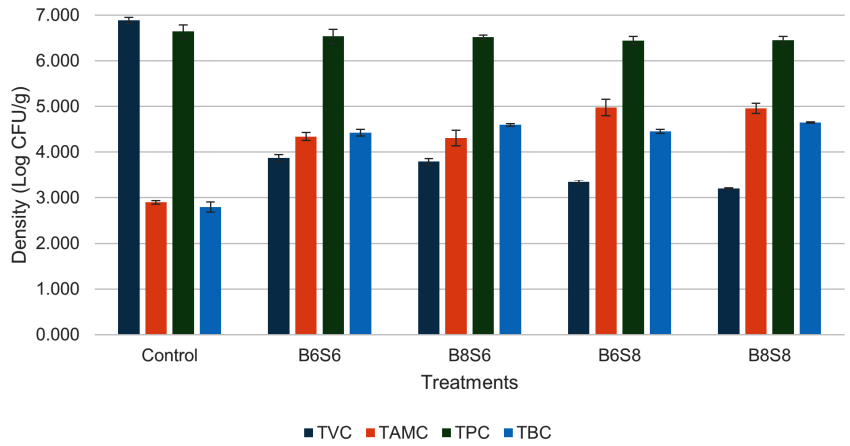

Figure 4. Bacterial counts in shrimp gastrointestinal tract after 36 days of feeding with combined *Bacillus* spp. and *Streptomyces* spp.

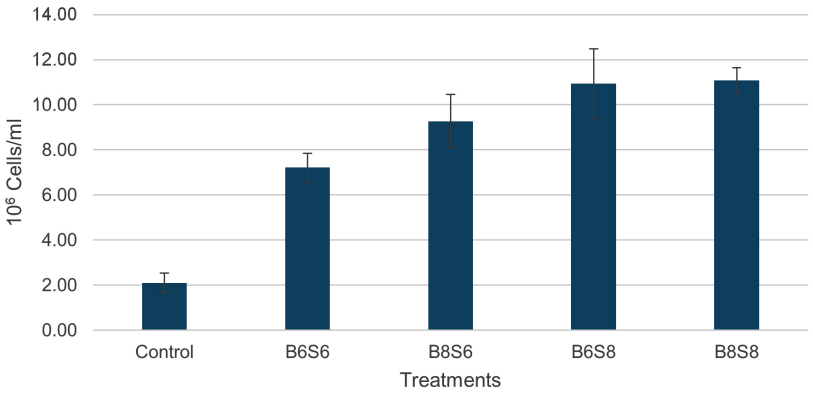

Figure 5. Total hemocyte count of shrimp after 36 days of feeding with combined *Bacillus* spp. and *Streptomyces* spp.

Table 3. Growth performance of shrimp after 36 days of feeding with combined *Bacillus* spp. and *Streptomyces* spp.

Treatment	Growth Performance			
	FBW (g)	DWG (g/day)	FCR	SR (%)
Control	0.27 ± 0.02[a]	0.0072 ± 0.0006[a]	1.40 ± 0.07[c]	82.00 ± 1.73[a]
B6S6	0.39 ± 0.03[b]	0.0106 ± 0.0009[b]	1.17 ± 0.03[b]	92.33 ± 0.88[b]
B8S6	0.51 ± 0.01[c]	0.0139 ± 0.0004[c]	1.02 ± 0.01[a]	96.67 ± 0.88[c]
B6S8	0.52 ± 0.02[c]	0.0143 ± 0.0004[c]	1.00 ± 0.01[a]	97.33 ± 0.33[c]
B8S8	0.64 ± 0.05[d]	0.0174 ± 0.0014[d]	1.02 ± 0.01[a]	96.67 ± 1.45[c]

The data are shown as mean ± standard error, with different letters after the standard error representing statistically significant differences ($P < 0.05$)

However, no statistically significant differences were detected among probiotic treatments for any relative improvement parameter ($P > 0.05$), suggesting that both *Bacillus* spp. and *Streptomyces* spp. effectively enhanced shrimp growth and survival across the tested densities.

Challenge with *Vibrio parahaemolyticus*

Following 36 days of feeding shrimp with diets containing *Streptomyces* spp. and *Bacillus* spp., the shrimp in the treatments were exposed to a challenge with *Vibrio parahaemolyticus* at 10^6 CFU/mL and 10^8 CFU/mL. After a 4 day challenge, the survival rates of shrimp in the probiotic-supplemented groups at 10^6 CFU/mL were 96.67% (S6), 100.00% (S7), 100.00% (S8), 93.33% (B6), 96.67% (B7), and 96.67% (B8), which were notably greater than the control group, which had a survival rate of 40.00%. For the 10^8 CFU/mL challenge, the survival rates were 90.00% (S6), 96.67% (S7), 96.67% (S8), 86.67% (B6), 93.33% (B7), and 93.33% (B8), with the control group showing a survival rate of 10.00% (Figure 3). No notable variations in survival rates were found across the treatments supplemented with probiotics for either concentration.

Effect of Combining *Bacillus* spp. and *Streptomyces* spp. in Feed at Screened Densities on Water Quality, Intestinal *Vibrio* Density, and Shrimp Growth Parameters

Based on the findings from the first experiment, the densities of *Streptomyces* spp. and *Bacillus* spp. were chosen for inclusion in the shrimp diet at concentrations of 10^6 CFU/g and 10^8 CFU/g, respectively. The experiment was designed with five treatments, including a control group with no probiotic supplementation. The probiotic treatments were added to the feed as follows: B6S6 (*Streptomyces* consortium with 10^6 CFU/g and *Bacillus* consortium with 10^6 CFU/g), B6S8 (*Streptomyces* consortium with 10^6 CFU/g and *Bacillus* consortium with 10^8 CFU/g), and B8S8 (*Streptomyces* consortium with 10^8 CFU/g and *Bacillus* consortium with 10^8 CFU/g). The experimental procedure followed the protocol established in the previous experiment. Water quality parameters, shrimp growth performance, survival rates after infection with *Vibrio parahaemolyticus*, and total hemocyte counts were measured upon completion of the 5 week experimental period.

Bacterial parameters and Total hemocyte count

The TVC (log CFU/mL) in the control treatment increased from 1.661 ± 0.276 at D0 to 4.991 ± 0.003 at D36 (Figure S7). Treatments with higher levels of *Streptomyces* spp. (B6S8 and B8S8) showed significantly lower TVC than those with lower levels (B6S6 and B8S6) by D36, with log CFU/mL values of 2.039 ± 0.029, 1.872 ± 0.075, 3.275 ± 0.010, and 3.275 ± 0.068 for B6S8, B8S8, B6S6, and B8S6. No significant differences in TPC were observed across treatments, with values ranging from 5.345-5.676 log CFU/mL (Figure S8). In treatments supplemented with high levels of *Bacillus* spp. and *Streptomyces* spp. (10^8 CFU/g), both TBC and TAMC were notably elevated compared to the control (Figures S9 and S10). At D36, TBC (log CFU/mL) was highest in B8S6 (3.570 ± 0.016) and B8S8 (3.591 ± 0.050), significantly higher than B6S6 (3.357 ± 0.081) and

Table 4. Relative improvement (%) in growth performance of shrimp compared with the control after 36 days of feeding with combined *Bacillus* spp. and *Streptomyces* spp.

Treatment	Relative Improvement (%) In Growth Performance			
	FBW (g)	DWG (g/day)	FCR	SR (%)
B6S6	49.69 ± 23.38[a]	51.79 ± 24.49[a]	16.41 ± 5.22[a]	12.72 ± 2.88[a]
B8S6	92.54 ± 17.04[ab]	96.27 ± 17.97[ab]	26.78 ± 3.03[a]	18.04 ± 3.55[a]
B6S8	96.48 ± 9.42%[ab]	100.33 ± 10.09[ab]	28.20 ± 2.87[a]	18.80 ± 2.53[a]
B8S8	142.67 ± 37.12[b]	148.50 ± 39.07[b]	27.01 ± 3.98[a]	19.17 ± 2.00[a]

Data are expressed as mean ± standard error. Percentage improvement was calculated relative to the control. Different letters indicate significant differences among treatments ($P < 0.05$)

B6S8 (3.331 ± 0.040). TAMC (log CFU/mL) was highest in B6S8 (4.069 ± 0.075) and B8S8 (4.057 ± 0.062), significantly different from B6S6 (3.741 ± 0.060) and B8S6 (3.597 ± 0.144).

The microbial density in the shrimp digestive tract showed that probiotic-supplemented diets reduced TVC by approximately 3 log compared to the control (Figure 4). High-level *Streptomyces* spp. treatments (B6S8 and B8S8) had significantly lower TVC values (3.346 ± 0.046 and 3.205 ± 0.015 log CFU/g) compared to low-level treatments (3.867 ± 0.076 and 3.791 ± 0.065 log CFU/g). TPC showed consistent values, ranging from 6.445-6.641 log CFU/g, with no significant changes observed. TAMC and TBC in probiotic-supplemented treatments showed significantly higher values than the control group. B6S8 and B8S8 had significantly higher TAMC values (4.974 ± 0.179 and 4.958 ± 0.110 log CFU/g) than B6S6 and B8S6 (4.338 ± 0.088 and 4.306 ± 0.167 log CFU/g), while TBC in B8S8 was highest (4.645 ± 0.015 log CFU/g), significantly higher than B6S6 (4.423 ± 0.074 log CFU/g). The combination of *Streptomyces* spp. and *Bacillus* spp. significantly increased total hemocyte count (THC), with values of 2.09 ± 0.44 (Control), 7.22 ± 0.63 (B6S6), 9.27 ± 1.19 (B8S6), 10.94 ± 1.55 (B6S8), and 11.08 ± 0.56 (B8S8). THC in B6S8 and B8S8 was significantly higher than in B6S6 but not different from B8S6 (Figure 5).

Concentrations of Total Ammonia Nitrogen (TAN) and Nitrite in the Rearing Water

Figures S11A and S12A show that TAN and nitrite levels in the control treatment gradually increased throughout the experiment, with TAN values reaching up to 1.605 ppm and nitrite values up to 2.694 ppm by the end of the experiment. In the probiotic-supplemented treatments, TAN and nitrite also increased gradually, peaking on Day 15, before decreasing gradually from Day 19 until the end of the experiment. No significant differences

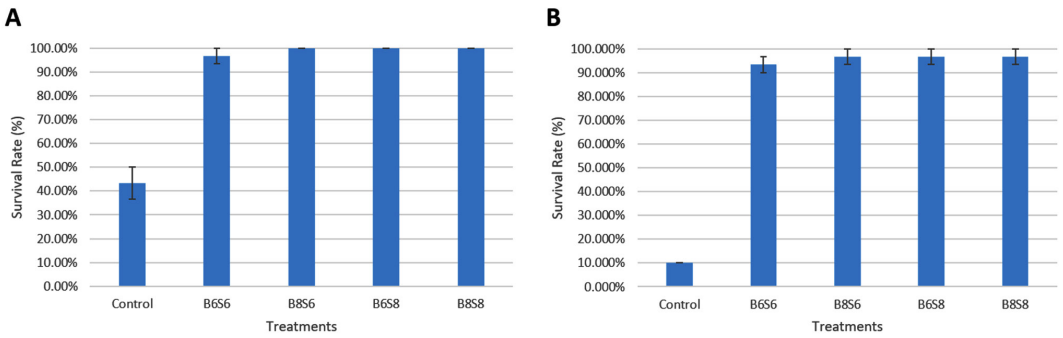

Figure 6. Survival rate of shrimp after 4 days of exposure to *Vibrio parahaemolyticus* at 10^6 CFU/mL (A) and 10^8 CFU/mL (B) following 36 days of feeding with combined *Bacillus* spp. and *Streptomyces* spp.

were observed among these treatments. By Day 36, the TAN and nitrite values in the probiotic treatments ranged from 0.055-0.070 ppm and 0.107-0.124 ppm, respectively.

In terms of TAN removal efficiency (RE%) (Figure S11B), all probiotic treatments (B6S6, B8S6, B6S8, and B8S8) showed substantial improvements in TAN removal, particularly on Days 12, 15, and 19. B8S6 and B8S8 demonstrated a peak TAN removal efficiency of 98.61 ± 0.35% and 98.36 ± 0.37% on Day 22, respectively. At Day 36, the TAN removal efficiencies in the probiotic treatments ranged from 95.63%-96.58%. Notably, there were no significant statistical differences among the four probiotic treatments (B6S6, B8S6, B6S8, and B8S8), with all treatments demonstrating similar removal efficiencies. Overall, B8S6 and B8S8 exhibited better and more stable TAN removal efficiency compared to B6S6 and B6S8 throughout the experiment. Similarly, for nitrite removal efficiency (RE%) (Figure S12B), the probiotic treatments also exhibited increased removal efficiency over time, with B8S6 showing the highest removal efficiency at 99.09 ± 0.02% on Day 29. However, this was not significantly different from B8S8, which had a RE% of 98.53 ± 0.35%. By Day 36, nitrite removal efficiencies in the probiotic treatments ranged from 95.35% to 96.05%, with no significant statistical differences between the treatments.

In summary, the supplementation of *Bacillus* spp. and *Streptomyces* spp. (at concentrations of 10^6 and 10^8 CFU/g) significantly improved both TAN and nitrite removal efficiencies in the rearing water.

Shrimp growth performance

After 36 days, diets supplemented with combined *Bacillus* spp. and *Streptomyces* spp. significantly enhanced shrimp growth performance compared with the control (Table 3). Treatments containing higher probiotic densities (B8S6, B6S8, and B8S8) resulted in significantly greater FBW and DWG than the low-density treatment (B6S6) ($P < 0.05$). Among all combinations, B8S8 exhibited the highest FBW (0.64 ± 0.05 g) and DWG (0.0174 ± 0.0014 g/day), indicating superior growth stimulation when both probiotics were applied at high density. Feed utilization efficiency was also improved in probiotic-fed groups, as reflected by significantly lower FCR values compared with the control, while survival rate was significantly increased across all combined treatments ($P < 0.05$).

Relative improvement analysis further confirmed the growth-promoting effects of probiotic combinations (Table 4). FBW and DWG increased by 49.69%-142.67% and 51.79%-148.50%, respectively, compared with the control. The greatest numerical improvements were consistently observed in B8S8, which showed significantly higher FBW and DWG enhancement than B6S6 ($P < 0.05$), while exhibiting comparable performance to B8S6 and B6S8. Feed conversion ratio was reduced by 16.41%-28.20%, and survival rate improved by 12.72%-19.17% across treatments, with no significant differences among combined probiotic groups ($P > 0.05$).

Challenge with *Vibrio parahaemolyticus*

Following 36 days of feeding with combined *Streptomyces* spp. and *Bacillus* spp., shrimp were exposed to *Vibrio parahaemolyticus* at 10^6 CFU/mL and 10^8 CFU/mL. After 4 days of exposure, survival rates in the probiotic-supplemented treatments at 10^6 CFU/mL were 96.67% (B6S6), 100.00% (B8S6), 100.00% (B6S8), and 100.00% (B8S8), all of which were notably higher than the control group, which had a survival rate of 43.33%. For the 10^8 CFU/mL challenge, survival rates were 93.33% (B6S6), 96.67% (B8S6), 96.67% (B6S8), and 96.67% (B8S8), significantly higher than the control group, which had a survival rate of 10.00% (Figure 6). No significant variation in survival rates was observed between the probiotic treatments for either concentration.

DISCUSSION

Probiotics are among the most widely proven solutions for controlling and antagonizing pathogens in various host species, including *Vibrio* spp.[54] Several probiotic bacterial strains have been identified that can control pathogenic *Vibrio* in aquaculture, including *Bacillus* spp.[55,56] and *Streptomyces* spp.[57] *Bacillus* and *Streptomyces* strains are capable of controlling *Vibrio* through various mechanisms, such as quorum quenching,[24,58] bacteriocins,[26,31] and other secondary compounds, such as siderophores,[30] quinone antibiotics,[59] peptide antibiotics,[60] aminoglycoside antibiotics,[61]

and macrolide antibiotics.[62] In the present study, we did not conduct direct assays (e.g., AHL degradation, gene expression, enzyme activity, or metabolite profiling) to verify these mechanisms; therefore, they should be considered as plausible explanations based on prior literature rather than confirmed pathways in our experimental system.

This study showed that shrimp diets enriched with *Bacillus* spp., *Streptomyces* spp., or a mix of both effectively reduced TVC in the culture water and TVC in the gastrointestinal tract. Additionally, incorporating a mix of *Streptomyces* spp. and *Bacillus* spp. into the feed at three varying concentrations (10^6, 10^7, and 10^8 CFU/g feed) proved effective in lowering TVC in the culture water after 2 weeks of the experiment. This reduction was maintained consistently throughout the remainder of the study, suggesting a sustained effect of the microbial supplementation. The *Streptomyces* consortium reduced the TVC in both rearing water (by 2.2-2.3 log CFU/mL) and the shrimp digestive tract (by 1.9-2.4 log CFU/g) compared to the control group. In comparison, the *Bacillus* consortium led to reductions of 1.4 to 1.7 log units in rearing water and 1.5-1.7 log units in the shrimp digestive tract. These results are consistent with earlier research, which reported that the addition of *Bacillus megaterium* and *Streptomyces fradiae* to the diet of *Penaeus monodon* postlarvae led to a reduction in *Vibrio* density, with decreases of approximately 0.87 and 0.89 log units in the rearing water and 1.36 and 1.40 log units in the shrimp postlarvae, respectively.[63] When combining two consortia of *Streptomyces* spp. and *Bacillus* spp. at densities of 10^6 and 10^8 CFU/g of feed, the higher-density consortia (B6S8 and B8S8) showed greater effectiveness in controlling total *Vibrio* in water after just 1 week, reducing the maximum TVC by 2.9 to 3.1 log units in water and 3.5 to 3.6 log units in the shrimp digestive tract. These reductions were higher compared to the lower-density consortia (B6S6 and B8S6), which resulted in a reduction of TVC in water by 1.7-2.5 log CFU/mL and in the shrimp digestive tract by 1.6-1.7 log CFU/g. Previous studies have also demonstrated that adding a combination of *Bacillus* consortium and *Streptomyces* consortium to the diet of whiteleg shrimp resulted in a reduction of TVC in the rearing water (by 4.1 log units) and in the hepatopancreas (by 3.6 log units), relative to the control group.[52] These findings suggest that, in terms of reducing TVC, this combination in shrimp diets effectively controls TVC in both the rearing water and the gastrointestinal tract of the shrimp, outperforming the use of individual probiotics. Furthermore, these probiotics did not alter the density of total heterotrophic bacteria in the culture water or shrimp digestive tract, suggesting that they do not negatively impact water quality and help maintain a stable bacterial population in the shrimp digestive tract. The presence of total heterotrophic bacteria is essential in the process of decomposing and mineralizing organic matter.[64] The counts of total actinomycetes and *Bacillus* in both the water and the shrimp digestive tract were higher than those in the control group, with the density increasing in correlation to the probiotic levels in the feed. Therefore, the addition of a *Bacillus* consortium combined with a *Streptomyces* consortium to the shrimp diet had a stable effect in controlling TVC in both the digestive tract and the rearing water, contributing to the maintenance of shrimp health and preventing diseases caused by *Vibrio* infections. Taken together, our results demonstrate a consistent reduction in TVC; however, the specific inhibitory mechanisms remain to be elucidated in future work using targeted functional assays.

The increased TAMC and TBC in the culture water may explain the effective reduction of TAN and nitrite levels. Based on the observed trends, the *Bacillus* spp. treatment provided strong and stable nitrogen removal, achieving approximately 98% TAN removal efficiency (TAN RE%) and ~99% nitrite removal efficiency (Nitrite RE%). In contrast, the *Streptomyces* spp. treatment reached approximately 96% TAN RE% and ~89% Nitrite RE%. Notably, despite its relatively high peak performance, the *Streptomyces* spp. treatment showed weaker TAN control toward the end of the experiment, as evidenced by the decline in TAN RE% to the lowest range (~36%-43%). These findings align with earlier research on *Bacillus* sp. strains, including BIO_2, BAL_3, and AQ_1, which have shown their capacity to reduce TAN and nitrite levels in both *in vitro* and *in vivo* environments.[33,35] Several studies have shown that *Streptomyces* strains are capable of metabolizing various nitrogen sources. For instance, He et al. reported that the *S. mediolani* EM-B2 strain achieved

maximum removal rates of ammonia, nitrite, and nitrate of 3.46, 1.71, and 1.73 ppm·h^{-1}, respectively. Furthermore, enzymes associated with nitrogen metabolism were identified in this strain, such as ammonia monooxygenase-AMO, hydroxylamine oxidoreductase-HAO, nitrate reductase-NR, and nitrite reductase-NiR.[65] Thus, the decrease in the TAN control rate towards the end of the experiment in treatments supplemented with only *Streptomyces* spp. may be due to the accumulation of TAN in the water, surpassing the metabolic capacity of these strains. Alternatively, it could result from the assimilation process, where these strains use nitrite and nitrate as nitrogen sources, converting them into ammonia through enzymes such as nasA (nitrate reductase assimilatory, $NO_3^- \rightarrow NO_2^-$) and nirBD (nitrite reductase assimilatory, $NO_2^- \rightarrow NH_4^+$), with the support of the nitrate transport protein (Nark-type NO_3^- transporter).[66-68] These genus-specific patterns provide a clear rationale for co-supplementation, as *Bacillus* spp. ensured robust baseline TAN/nitrite control, whereas *Streptomyces* spp. contributed broader nitrogen metabolic functions but appeared more vulnerable to late-stage constraints. Accordingly, the combined *Bacillus* spp. and *Streptomyces* spp. treatment was expected to improve functional complementarity and stability. Consistent with this expectation, all combined formulations (B6S6, B8S6, B6S8, and B8S8) achieved near-complete peak removal and maintained high end-point performance for both TAN and nitrite (Figures S11 and S12). The combined treatments also showed similar removal efficiencies across formulations, with no significant differences detected. Notably, unlike the *Streptomyces* spp. treatment, co-supplementation did not exhibit late-stage deterioration in TAN control, supporting improved robustness under prolonged rearing conditions. Overall, co-supplementation at 10^6-10^8 CFU/g achieved near-ceiling peak TAN/nitrite removal while maintaining stable end-point efficiencies, supporting its advantage over genus-only treatments for sustained nitrogen management in rearing water.

Numerous studies have demonstrated that probiotics can enhance the immune response of shrimp to *Vibrio* infection. The total hemocyte count (THC) serves as a key indicator of the shrimp's immune response to pathogens, such as *Vibrio*.[69,70] In this study, both the *Streptomyces* consortium and the *Bacillus* consortium significantly increased THC of shrimp, with the combination of both consortia showing the greatest effect in comparison to the control group. THC showed an increasing trend with higher probiotic content in the feed, reaching its peak when *Streptomyces* spp. was combined with *Bacillus* spp. These results align with earlier research, which showed that a 0.4% concentration of *Bacillus cereus* significantly increased total hemocyte numbers (259×10^5 cells/mL), 47% higher than the control in *P. monodon*. Furthermore, the THC increased with higher *Bacillus cereus* density in the shrimp diet.[71] The *Bacillus subtilis* S12 strain significantly increased THC to 4.71×10^6 cells/mL, 27% higher than the control in whiteleg shrimp.[72] Additionally, *Bacillus licheniformis* has demonstrated its ability to boost the immune response in shrimp, raising THC to 45.8×10^5 cells/mL, a 145% increase compared to the control group.[73] *Streptomyces* sp. (RL8 or N7), and combinations of N7 and RL8, or *Bacillus* and *Streptomyces* strains, increased THC in shrimp by 43% (4.0 million cells/mL), 111% (5.9 million cells/mL), 279% (10.6 million cells/mL), and 200% (8.4 million cells/mL), respectively, relative to the control group.[52]

An increase in THC improves the health of whiteleg shrimp by enhancing phagocytic capacity, which is vital for defending the shrimp against microbial threats.[74,75] All treatments supplemented with probiotics at varying concentrations, either alone or in combination, provided strong protection against *Vibrio parahaemolyticus* challenge in whiteleg shrimp. The increased TBC and TAMC in the shrimp digestive tract, with their ability to control *Vibrio* through antibacterial compounds and stimulate immune response via increased THC, contributed to this enhanced protection. This finding is consistent with the research by Bernal et al., which reported that treatments containing *Streptomyces* or a *Streptomyces-Bacillus* combination enhanced the survival of whiteleg shrimp following infection with *Vibrio parahaemolyticus*.[52] Moreover, it has been demonstrated that the application of *Streptomyces* sp. CLS-28 notably enhanced the survival rate of black tiger shrimp after infection with *Vibrio harveyi*.[76]

It has been demonstrated that *Streptomyces* and *Bacillus* improve the growth performance of different livestock species.[77,78] In this study, varying dietary supplementation concentrations of the *Bacillus* consortium, *Streptomyces* consortium, or particularly their combination, significantly enhanced the growth parameters (FBW, FCR, DWG) and survival of whiteleg shrimp relative to the control group. A clear dose-dependent response was observed, with higher probiotic concentrations resulting in greater growth improvement. Notably, combined supplementation consistently produced the highest numerical enhancements in growth performance, indicating a synergistic interaction between the two probiotic consortia rather than a merely additive effect. This is consistent with previous studies, where the addition of *Streptomyces* strains at 1% concentration to the feed improved growth parameters in *Penaeus monodon* after 15 days.[76] Additionally, *Streptomyces fradiae* and *Bacillus megaterium* at 10^9 CFU/g of feed improved the weight of *P. monodon* after 60 days.[63] Supplementing the feed with *Streptomyces* strains (1.0×10^8 CFU/g) alone or together with *Bacillus* strains (1.0×10^6 CFU/g) improved the growth and survival of *Litopenaeus vannamei.*[52] The observed growth enhancement is likely associated with extracellular digestive enzymes produced by probiotic strains in the shrimp gastrointestinal tract, including protease, amylase, and lipase, which facilitate nutrient breakdown and absorption.[79] Both *Streptomyces* and *Bacillus* species synthesize a broad range of exoenzymes such as protease, amylase, cellulase, and lipase, thereby improving feed utilization efficiency and digestive capacity.[77,78,80] Furthermore, *Bacillus* strains act as a source of amino acids, vitamins, and macro- and micronutrients, contributing further to shrimp health.[77] The synergistic growth-promoting effect observed in combined treatments may arise from complementary enzyme production profiles and expanded substrate degradation capacity, leading to more efficient nutrient utilization than that achieved by individual probiotic strains. In a previous study, *Streptomyces* sp. strains used in the present research demonstrated strong exoenzyme production, including strains TM_1, TM_2, TM_7, TM_{21}, and TM_{22}, along with pronounced antagonistic activity against several *Vibrio* species and the production of protease, amylase, and cellulase.[33] The multifunctional enzyme production and antimicrobial potential of these strains likely contributed to the enhanced growth performance of whiteleg shrimp observed in this study.

In summary, the results of this research demonstrated that, regardless of the varying densities of individual *Bacillus* and *Streptomyces* consortia in the feed, they effectively controlled TVC, enhanced growth, and increased THC in response to pathogenic *Vibrio* infection. Nonetheless, the pairing of *Streptomyces* and *Bacillus* proved to be one of the most promising alternatives. The *Streptomyces* consortium outperformed the *Bacillus* consortium in reducing TVC in both the rearing water and shrimp digestive tract, with higher supplementation densities leading to a more significant decrease in TVC. In contrast, the *Bacillus* consortium was more effective at lowering TAN and nitrite concentrations in the rearing water. Furthermore, when *Streptomyces* and *Bacillus* were combined at high densities (10^8 CFU/g feed), the best shrimp growth, as well as the most effective control of *Vibrio* in the rearing water and digestive tract, along with the best control of TAN and nitrite, were observed. Therefore, the combination of *Streptomyces* and *Bacillus* offers functional complementarity, providing comprehensive water quality control and promoting more effective growth of whiteleg shrimp. The combined effect of *Streptomyces* and *Bacillus* strains aligns with previous research, which suggests that probiotics containing multiple strains or species can be more effective than those with a single strain.[35,52,81]

CONCLUSION

This study showed that the combination of *Streptomyces* strains (TM_1, TM_2, TM_7, TM_{21}, and TM_{22}) and *Bacillus* strains (AQ_1, BIO_2, and BAL_3) supplemented into feed at a density of 10^8 CFU/g can effectively improve growth parameters (FCR, FBW, DWG), survival rate, and total hemocyte count (THC), helping shrimp resist *Vibrio parahaemolyticus* infection. It also controls TVC, TAN, and nitrite concentrations in the culture water, as well as TVC in the shrimp digestive system. This combination can be considered as a supplementary feed for *P. vannamei* postlarvae

at a density of 100 shrimp/100 L in a recirculating aquaculture system.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.20.1.60

Additional file: Figure S1-S12.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

TDP conceived and designed the experiments. TDP, BTHL, PTTD, HTTL, and NTNH carried out the experiments. TDP analyzed the data PMT performed data validation. TDP wrote the manuscript. NTH guided the study. PMT and NTH supervised the study. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Martinell DP, Vergara-Solana FJ, Padilla MEA, Garza FA. *An Introduction to Sustainable Aquaculture*. 2024.
2. Cressey D. Aquaculture: Future fish. *Nature*. 2009;458(7237):398-400. doi: 10.1038/458398a
3. Colt J, Plesha P, Huguenin J. Impact of net positive suction head on the design and operation of seawater pumping systems for use in aquaculture. *Aquac Eng*. 2006;35(3):239-257. doi: 10.1016/j.aquaeng.2006.03.001
4. Ebeling JM, Timmons MB. Recirculating Aquaculture Systems. *Aquaculture Production Systems*. 2012:245-277.
5. Du Y, Xu J, Zhou L, Chen F, Qiu T, Sun J. Retrofitting Sea Cucumber Nursery Tanks to Recirculating Aquaculture Systems for Highly Intensive *Litopenaeus vannamei* Aquaculture. *Appl Sci*. 2021;11(20):9478. doi: 10.3390/app11209478
6. Reid B, Arnold CR. The intensive culture of the penaeid shrimp *Penaeus vannamei* Boone in a recirculating raceway system. *J World Aquac Soc*. 1992;23(2):146-153. doi: 10.1111/j.1749-7345.1992.tb00763.x
7. Davis DA, Arnold CR. The design, management and production of a recirculating raceway system for the production of marine shrimp. *Aquac Eng*. 1998;17(3):193-211. doi: 10.1016/S0144-8609(98)00015-6
8. Yang JG, C, Song H, Liu X, Gu Z, Guo Y. Design and test of mass balance-based recirculating aquaculture system for higher place shrimp pond. *Trans Chin Soc Agric Eng*. 2017;33:217-222. doi: 10.11975/j.issn.1002-6819.2017.14.030
9. Yang JN, Q, Zhang, Y, Xu, B. Construction technology on RAS for shrimp culture. *Trans Chin Soc Agric Eng*. 2010;26(8):136-140. doi: 10.3969/j.issn.1002-6819.2010.08.023
10. Suantika GS, ML, Kurniawan JB, Pratiwi SA, et al. Development of a zero water discharge (ZWD)-Recirculating aquaculture system (RAS) hybrid system for super intensive white shrimp (Litopenaeus vannamei) culture under low salinity conditions and its industrial trial in commercial shrimp urban farming in Gresik, East Java, Indonesia. *Aquac Eng* 2018;82:12-24. doi: 10.1016/j.aquaeng.2018.04.002
11. Shi YHZ GY, Liu JZ, Zhu YZ, Xu JB. Performance of a constructed wetland in treating brackish wastewater from commercial recirculating and super-intensive shrimp growout systems. *Bioresour Technol*. 2011;102(20):9416-9424. doi: 10.1016/j.biortech.2011.07.058
12. Fleckenstein LJ, Tierney TW, Fisk JC, Ray AJ. Effects of supplemental LED lighting on water quality and Pacific white shrimp (*Litopenaeus vannamei*) performance in intensive recirculating systems. *Aquaculture*. 2019;504:219-226. doi: 10.1016/j.aquaculture.2019.01.066
13. Fleckenstein LJ, Tierney TW, Ray AJ. Comparing biofloc, clear-water, and hybrid recirculating nursery systems (Part II): Tilapia (*Oreochromis niloticus*) production and water quality dynamics. *Aquacultural Engineering*. 2018;82:80-85. doi: 10.1016/j.aquaeng.2018.06.006
14. Chen Z, Chang Z, Zhang L, et al. Effects of water recirculation rate on the microbial community and water quality in relation to the growth and survival of white shrimp (*Litopenaeus vannamei*). *BMC Microbiol*. 2019;19(1):192. doi: 10.1186/s12866-019-1564-x

15. Yazid SHM, Daud HM, Azmai MNA, Mohamad N, Mohd Nor N. Estimating the Economic Loss Due to Vibriosis in Net-Cage Cultured Asian Seabass (*Lates calcarifer*): Evidence From the East Coast of Peninsular Malaysia. Original Research. *Front Vet Sci*. 2021;8;644009. doi: 10.3389/fvets.2021.644009
16. Morales V, Cuéllar-Anjel J. Technical Guide: Pathology and Immunology of Penaeid Shrimp [Guía Técnica: Patología e Inmunología de Camarones Penaeidos; in Spanish]. OIRSA; 2014:382
17. Brock JA, Lightner DV. Chapter 3: Diseases of Crustacea. *Diseases of Marine Animals*. Biologische Anstalt Helgoland. 1990:245-424.
18. Ishimaru KA-M, M, Muroga, K. *Vibrio penaeicida* sp. nov, a Pathogen of Kuruma Prawns (*Penaeus japonicus*). *Int J Syst Bacteriol*. 1995;45(1):134-138. doi: 10.1099/00207713-45-1-134
19. Hoa TTT, Fagnon MS, Thy DTM, Chabrillat T, Trung NB, Kerros S. Growth Performance and Disease Resistance against *Vibrio parahaemolyticus* of Whiteleg Shrimp (*Litopenaeus vannamei*) Fed Essential Oil Blend (Phyto AquaBiotic). *Animals (Basel)*. 2023;13(21):3320. doi: 10.3390/ani13213320
20. Rahayu S, Amoah K, Huang Y, et al. Probiotics application in aquaculture: its potential effects, current status in China and future prospects. *Front Marine Sci*. 2024;11:1455905. doi: 10.3389/fmars.2024.1455905
21. Romano N, Koh C-B, Ng W-K. Dietary micro-encapsulated organic acids blend enhances growth, phosphorus utilization, immune response, hepatopancreatic integrity and resistance against *Vibrio harveyi* in white shrimp, *Litopenaeus vannamei. Aquaculture*. 2015;435:228-236. doi: 10.1016/j.aquaculture.2014.09.037
22. Lazado CC, Caipang CMA. Mucosal immunity and probiotics in fish. *Fish Shellfish Immunol*. 2014;39(1):78-89. doi: 10.1016/j.fsi.2014.04.015
23. Schar D, Klein EY, Laxminarayan R, Gilbert M, Van Boeckel TP. Global trends in antimicrobial use in aquaculture. *Sci Rep*. 2020;10(1):21878. doi: 10.1038/s41598-020-78849-3
24. Vinoj G, Vaseeharan B, Thomas S, Spiers AJ, Shanthi S. Quorum-quenching activity of the AHL-lactonase from *Bacillus licheniformis* DAHB1 inhibits *Vibrio* biofilm formation *in vitro* and reduces shrimp intestinal colonisation and mortality. *Mar Biotechnol (NY)*. 2014;16(6):707-15. doi: 10.1007/s10126-014-9585-9
25. Ghanei-Motlagh R, Mohammadian T, Gharibi D, et al. Quorum Quenching Properties and Probiotic Potentials of Intestinal Associated Bacteria in Asian Sea Bass Lates calcarifer. *Mar Drugs*. 2019;18(1):23. doi: 10.3390/md18010023
26. An J, Zhu W, Liu Y, et al. Purification and characterization of a novel bacteriocin CAMT2 produced by *Bacillus amyloliquefaciens* isolated from marine fish *Epinephelus areolatus. Food Control*. 2015;51:278-282. doi: 10.1016/j.foodcont.2014.11.038
27. Rusmana I, Isramilda I, Akhdiya A. Characteristics of anti-*Vibrio harveyi* compounds produced by *Bacillus* spp. isolated from shrimp ponds. *Biodiversitas Journal of Biological Diversity*. 2021;22(11):221120. doi: 10.13057/biodiv/d221120
28. Genilloud O. Actinomycetes: still a source of novel antibiotics. *Nat Prod Rep*. 2017;34(10):1203-1232. doi: 10.1039/c7np00026j
29. Behie SW, Bonet B, Zacharia VM, McClung DJ, Traxler MF. Molecules to Ecosystems: Actinomycete Natural Products In situ. *Front Microbiol*. 2016;7:2149. doi: 10.3389/fmicb.2016.02149
30. Hariharan S, Dharmaraj S. Selection of New Probiotics: The Case of *Streptomyces*. *Therapeutic, Probiotic, and Unconventional Foods*. 2018:27-54.
31. Hernandez-Saldana OF, Barboza-Corona JE, Bideshi DK, Casados-Vazquez LE. New bacteriocin-like substances produced by *Streptomyces* species with activity against pathogens. *Folia Microbiol (Praha)*. Aug 2020;65(4):669-678. doi: 10.1007/s12223-020-00770-z
32. Phuc TD, Huong NTT, Dan PTT, Phuong TTM. Isolation and Screening of *Actinomycetes* Against *Vibrio* spp. and Producing Extracellular Enzymes. *Ho Chi Minh City University of Education Journal of Science*. 2021;18(6):1016-1027. doi: 10.54607/hcmue.js.18.6.2934(2021)
33. Phuc TD, Huong NT, Thong DH, Tung CV, Dan PTT, Phuong TTM. Investigation of Ability to Remove NH_3 and NO_2 By Combination of *Yucca Schidigera* Extract and *Bacillus* Strains. *IOSR J Biotechnol Biochem*. 2021;7(4):01-17. doi: 10.9790/264X-0704010117
34. Kewcharoen W, Srisapoome P. Probiotic effects of *Bacillus* spp. from Pacific white shrimp (*Litopenaeus vannamei*) on water quality and shrimp growth, immune responses, and resistance to *Vibrio parahaemolyticus* (AHPND strains). *Fish Shellfish Immunol*. 2019;94:175-189. doi: 10.1016/j.fsi.2019.09.013
35. Phuc TD, Huong NT, Dan PTT, Linh BTH, Mui TV. Effect from Combinations of *Yucca schidigera* extract with *Bacillus* Strains on the Growth of White Leg Shrimp (*Penaeus vannamei*), Density of *Vibrio* sp, and on the Ammonia and Nitrite Content of Culture Water. *J Pure Appl Microbiol*. 2023;17(3):1444-1457. doi: 10.22207/jpam.17.3.06
36. Luis-Villasenor IE, Macias-Rodriguez ME, Gomez-Gil B, Ascencio-Valle F, Campa-Cordova A. Beneficial effects of four *Bacillus* strains on the larval cultivation of *Litopenaeus vannamei*. *Aquaculture*. 2011;321(1):136-144. doi: 10.1016/j.aquaculture.2011.08.036
37. Abasolo-Pacheco F, Saucedo PE, Mazon-Suastegui JM, et al. Isolation and use of beneficial microbiota from the digestive tract of lions-paw scallop Nodipecten subnodosus and winged pearl oyster Pteria sterna in oyster aquaculture. *Aquac Res*. 2016;47(10):3042-3051. doi: 10.1111/are.12754
38. Gopalakrishnan S, Vadlamudi S, Bandikinda P, et al. Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. *Microbiol Res*. 2014;169(1):40-48. doi: 10.1016/j.micres.2013.09.008
39. Li L-m, Zheng T, Chen Y, et al. The antagonistic mechanisms of *Streptomyces sioyaensis* on the growth and metabolism of poplar canker pathogen Valsa sordida. *Biological Control*. 2020;151:104392. doi: 10.1016/j.biocontrol.2020.104392
40. Phuc TD, Huong NT, Dan PTT, Linh BTH, Loan VTT, Hang

NTB. Dual Role Of *Streptomyces* Strains: Reduction of *Aeromonas* Counts In Both Culture Water And The Intestine, And Growth Enhancement of Discus Fish (*Symphysodon* sp.). *IOP Conf Ser Earth Environ Sci*. 2024;1340(1):012016. doi: 10.1088/1755-1315/1340/1/012016

41. Newaj-Fyzul A, Austin B. Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. *J Fish Dis*. 2015;38(11):937-955. doi: 10.1111/jfd.12313
42. Sha Y, Wang L, Liu M, Jiang K, Xin F, Wang B. Effects of lactic acid bacteria and the corresponding supernatant on the survival, growth performance, immune response and disease resistance of *Litopenaeus vannamei*. *Aquaculture*. 2016;452:28-36. doi: 10.1016/j.aquaculture.2015.10.014
43. Yang Q-h, Tan B-p, Dong X-h, Chi S-y, Liu H-y. Effects of different levels of *Yucca schidigera* extract on the growth and nonspecific immunity of Pacific white shrimp (*Litopenaeus vannamei*) and on culture water quality. *Aquaculture*. 2015;439:39-44. doi: 10.1016/j.aquaculture.2014.11.029
44. Abdel-Tawwab M, Khalil RH, Nour AM, Elkhayat BK, Khalifa E, Abdel-Latif HMR. Effects of *Bacillus subtilis*-fermented rice bran on water quality, performance, antioxidants/oxidants, and immunity biomarkers of White leg shrimp (*Litopenaeus vannamei*) reared at different salinities with zero water exchange. *Journal of Applied Aquaculture*. 2020;34(2):332-357. doi: 10.1080/10454438.2020.1844110
45. Novriadi R, Albasri H, Wahyudi AE, Fadhilah R, Ali A, Trullas C. Effects of the addition of oak (*Quercus robur* L.) and yucca (*Yucca schidigera*) on the water quality and growth performance of pacific white shrimp (*Litopenaeus vannamei*) cultured intensively in concrete tanks. *J World Aquac Soc*. 2022;53(5):984-994. doi: 10.1111/jwas.12871
46. Felix N, Sudharsan M. Effect of glycine betaine, a feed attractant affecting growth and feed conversion of juvenile freshwater prawn Macrobrachium rosenbergii. *Aquac Nutr*. 2004;10(3):193-197. doi: 10.1111/j.1365-2095.2004.00292.x
47. APHA. Standard Methods for the Examination of Water and Wastewater (23rd ed.). Washington DC: American Public Health Association; 2017.
48. Sookchaiyaporn N, Srisapoome P, Unajak S, Areechon N. Efficacy of *Bacillus* spp. isolated from Nile tilapia *Oreochromis niloticus* Linn. on its growth and immunity, and control of pathogenic bacteria. *Fisheries Science*. 2020;86(2):353-365. doi: 10.1007/s12562-019-01394-0
49. Chotikachinda R, Lapjatupon W, Chaisilapasung S, Sangsue D, Tantikitti C. Effect of inactive yeast cell wall on growth performance, survival rate and immune parameters in Pacific White Shrimp (*Litopenaeus vannamei*). *Songklanakarin J Sci Technol*. 2008;30(6):687-692.
50. Owens L, O'Neill A. Use of a clinical cell flow cytometer for differential counts of prawn Penaeus monodon haemocytes. *Dis Aquat Organ*. 1997;31:147-153. doi: 10.3354/dao031147
51. Muralisankar T, Kalaivani P, Thangal SH, Santhanam P. Growth, biochemical, antioxidants, metabolic enzymes and hemocytes population of the shrimp *Litopenaeus vannamei* exposed to acidified seawater. *Comp Biochem Physiol C Toxicol Pharmacol*. Jan 2021;239:108843. doi: 10.1016/j.cbpc.2020.108843
52. Bernal MG, Marrero RM, Campa-Cordova AI, Mazon-Suastegui JM. Probiotic effect of *Streptomyces* strains alone or in combination with *Bacillus* and *Lactobacillus* in juveniles of the white shrimp *Litopenaeus vannamei*. *Aquaculture International*. 2016;25(2):927-939. doi: 10.1007/s10499-016-0085-y
53. Hamsah H, Widanarni W, Alimuddin A, Yuhana M, Junior MZ, Hidayatullah D. Immune response and resistance of Pacific white shrimp larvae administered probiotic, prebiotic, and synbiotic through the bio-encapsulation of *Artemia* sp. *Aquac Int*. 2019;27(2):567-580. doi: 10.1007/s10499-019-00346-w
54. Mustafa MF, Bunga M, Achmad M. Use of probiotics to fight bacterial populations of *Vibrio* sp. on vaname shrimp cultivation (*Litopenaeus vannamei*). *Torani: JFMarSci*. 2019;2(2):69-76. doi: 10.35911/torani.v2i2.7056
55. Nimrat S, Boonthai T, Vuthiphandchai V. Effects of probiotic forms, compositions of and mode of probiotic administration on rearing of Pacific white shrimp (*Litopenaeus vannamei*) larvae and postlarvae. *Anim Feed Sci Technol*. 2011;169(3):244-258. doi: 10.1016/j.anifeedsci.2011.07.003
56. Proespraiwong P, Mavichak R, Imaizumi K, Hirono I, Unajak S. Evaluation of *Bacillus* spp. as Potent Probiotics with Reduction in AHPND-Related Mortality and Facilitating Growth Performance of Pacific White Shrimp (*Litopenaeus vannamei*) Farms. *Microorganisms*. 2023;11(9). doi: 10.3390/microorganisms11092176
57. Tan LT-H, Chan K-G, Lee L-H, Goh B-H. *Streptomyces* Bacteria as Potential Probiotics in Aquaculture. Mini Review. *Front Microbiol*. 2016;7L79. doi: 10.3389/fmicb.2016.00079
58. Miao L, Xu J, Yao Z, et al. The anti-quorum sensing activity and bioactive substance of a marine derived *Streptomyces*. *Biotechnol Biotechnol Equip*. 2017;31(5):1007-1015. doi: 10.1080/13102818.2017.1348253
59. Hayashi M, Unemoto T, Minami-Kakinuma S, Tanaka H, Omura S. The mode of action of nanaomycins D and A on a gram-negative marine bacterium *Vibrio alginolyticus*. *J Antibiot (Tokyo)*. 1982;35(8):1078-85. doi: 10.7164/antibiotics.35.1078
60. Castillo UF, Strobel GA, Ford EJ, et al. Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on Kennedia nigriscans. *Microbiology (Reading)*. 2002;148(Pt 9):2675-2685. doi: 10.1099/00221287-148-9-2675
61. Yang N, Sun C. The Inhibition and Resistance Mechanisms of Actinonin, Isolated from Marine *Streptomyces* sp. NHF165, against *Vibrio anguillarum*. *Front Microbiol*. 2016;7:1467. doi: 10.3389/fmicb.2016.01467
62. Rateb ME, Houssen WE, Harrison WT, et al. Diverse metabolic profiles of a *Streptomyces* strain isolated from a hyper-arid environment. *J Nat Prod*.

2011;74(9):1965-71. doi: 10.1021/np200470u
63. Aftabuddin SK, M Abul; Kader, M Abdul; Sikder, M Nurul Azim; Hakim, M Abdul. Use of *Streptomyces fradiae* and *Bacillus megaterium* as probiotics in the experimental culture of tiger shrimp *Penaeus monodon* (Crustacea, Penaeidae). *AACL BIOFLUX*. 2013;6(3):253-267.
64. Thompson KD, Rodkhum C, Bunnoy A, et al. Addressing Nanovaccine Strategies for Tilapia. *Vaccines*. 2023;11(8):1356. doi: 10.3390/vaccines11081356
65. He T, Zhang M, Ding C, et al. New insight into the nitrogen removal capacity and mechanism of *Streptomyces mediolani* EM-B2. *Bioresour Technol*. 2022;348:126819. doi: 10.1016/j.biortech.2022.126819
66. Tiffert Y, Supra P, Wurm R, Wohlleben W, Wagner R, Reuther J. The *Streptomyces coelicolor* GlnR regulon: identification of new GlnR targets and evidence for a central role of GlnR in nitrogen metabolism in actinomycetes. *Mol Microbiol*. 2008;67(4):861-80. doi: 10.1111/j.1365-2958.2007.06092.x
67. Amin R, Reuther J, Bera A, Wohlleben W, Mast Y. A novel GlnR target gene, nnaR, is involved in nitrate/nitrite assimilation in *Streptomyces coelicolor. Microbiology*. 2012;158(Pt 5):1172-1182. doi: 10.1099/mic.0.054817-0
68. Fischer M, Alderson J, van Keulen G, White J, Sawers RG. The obligate aerobe *Streptomyces coelicolor* A3(2) synthesizes three active respiratory nitrate reductases. *Microbiology (Reading)*. Oct 2010;156(Pt 10):3166-3179. doi: 10.1099/mic.0.042572-0
69. Ji PF, Yao CL, Wang ZY. Immune response and gene expression in shrimp (*Litopenaeus vannamei*) hemocytes and hepatopancreas against some pathogen-associated molecular patterns. *Fish Shellfish Immunol*. 2009;27(4):563-570. doi: 10.1016/j.fsi.2009.08.001
70. Zubaidah A, Yuhana M, Widanarni. Encapsulated Synbiotic Dietary Supplementation at Different Dosages to Prevent Vibriosis in White Shrimp, *Litopenaeus vannamei. HAYATI J Biosci.* 2015;22(4):163-168. doi: 10.1016/j.hjb.2015.10.007
71. NavinChandran M, Iyapparaj P, Moovendhan S, et al. Influence of probiotic bacterium *Bacillus cereus* isolated from the gut of wild shrimp *Penaeus monodon* in turn as a potent growth promoter and immune enhancer in P. monodon. *Fish Shellfish Immunol*. Jan 2014;36(1):38-45. doi: 10.1016/j.fsi.2013.10.004
72. Liu H, Li Z, Tan B, et al. Isolation of a putative probiotic strain S12 and its effect on growth performance, non-specific immunity and disease-resistance of white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol*. 2014;41(2):300-307. doi: 10.1016/j.fsi.2014.08.028
73. Li K, Zheng T, Tian Y, et al. Beneficial effects of *Bacillus licheniformis* on the intestinal microflora and immunity of the white shrimp, *Litopenaeus vannamei. Biotechnol Lett*. 2007;29(4):525-530. doi: 10.1007/s10529-006-9291-4
74. Daniel K, & Sari, P. D. W. Feeding management of vannamei shrimp (*Litopenaeus vannamei*) with immunostimulant addition. *IOP Conf. Series: Earth and Environmental Science*. 2023:
75. Chiu CH, Guu YK, Liu CH, Pan TM, Cheng W. Immune responses and gene expression in white shrimp, *Litopenaeus vannamei,* induced by *Lactobacillus plantarum*. *Fish Shellfish Immunol*. 2007;23(2):364-77. doi: 10.1016/j.fsi.2006.11.010
76. Das S, Ward LR, Burke C. Screening of marine *Streptomyces* spp. for potential use as probiotics in aquaculture. *Aquaculture*. 2010;305(1-4):32-41. doi: 10.1016/j.aquaculture.2010.04.001
77. James G, Das BC, Jose S, Vattringal Jayadradhan RK. *Bacillus* as an aquaculture friendly microbe. *Aquac Int*. 2021;29(1):323-353. doi: 10.1007/s10499-020-00630-0
78. James G, Prasannan Geetha P, Thavarool Puthiyedathu S, Vattringal Jayadradhan RK. Applications of Actinobacteria in aquaculture: prospects and challenges. *3 Biotech*. Feb 2023;13(2):42. doi: 10.1007/s13205-023-03465-7
79. Reda RM, Selim KM. Evaluation of *Bacillus amyloliquefaciens* on the growth performance, intestinal morphology, hematology and body composition of Nile tilapia, *Oreochromis niloticus*. *Aquac Int*. 2015;23(1):203-217. doi: 10.1007/s10499-014-9809-z
80. Jagannathan SV, Manemann EM, Rowe SE, Callender MC, Soto W. Marine Actinomycetes, New Sources of Biotechnological Products. *Mar Drugs*. 2021;19(7):365. doi: 10.3390/md19070365
81. Timmerman HM, Koning CJM, Mulder L, Rombouts FM, Beynen AC. Monostrain, multistrain and multispecies probiotics—A comparison of functionality and efficacy. *Int J Food Microbiol*. 2004;96(3):219-233. doi: 10.1016/j.ijfoodmicro.2004.05.012