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Enhancing the Growth Performance of Whiteleg Shrimp, *Litopenaeus vannamei*, by Salt-Tolerant *Bacillus* sp.

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Abstract

Probiotics are vital in aquaculture for maintaining water quality, boosting aquatic species' health, and enhancing growth rates. This study investigated the effects of probiotics, namely salt-tolerant *Bacillus velezensis* MT50 and *Bacillus amyloliquefaciens* MT51, on the water quality and performance of whiteleg shrimp (*Litopenaeus vannamei* PL) cultured in tanks. The experimental design included two bacterial treatments and one control treatment (without *Bacillus*), with each treatment replicated three times. The results indicated that temperature, pH, and total alkalinity varied within the ranges of 27.8 to 28.9°C, 7.81 to 7.94, and 98.5 to 114.7 mg $CaCO_3$ L^{-1}, respectively, and all were maintained at appropriate levels. Additionally, other parameters such as dissolved oxygen (DO), chemical oxygen demand (COD), total suspended solids (TSS), and total ammonia nitrogen (TAN) exhibited less fluctuation in the treatments supplemented with *Bacillus* sp. compared to the control. Furthermore, the densities of pathogenic agents (e.g., *Vibrio*) in tanks with the addition of MT50 and MT51 bacteria (10^2 and 10^1 CFU mL^{-1}, respectively) were significantly lower than in the control tanks (10^4 CFU mL^{-1}). The survival rates of shrimp treated with MT50 (70.0 ± 5.3%) and MT51 (86.7 ± 3.1%) were significantly higher (P <0.05) compared to the control group (65.3 ± 3.1%). These findings suggest the potential application of *B. velezensis* MT50 and *B. amyloliquefaciens* MT51 as probiotics for sustainable aquaculture practices.

Keywords

Bacillus, *Litopenaeus vannamei*, probiotics, Vibrio, water quality

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Introduction

The seafood industry in Vietnam, particularly in Nam Dinh, is experiencing significant development due to various investment opportunities. However, recent years have seen disease outbreaks in

aquatic animals due to intensive high-density farming and erratic climate conditions. Furthermore, water quality and the cultural environment have suffered from pollution, which has adversely affected production. The frequent and improper use of antibiotics to treat aquatic diseases has led to the emergence of antibiotic-resistant bacterial strains (Rupasinghe *et al.*, 2024). The global increase in chemical usage across various industries is impacting human health, prompting a growing interest in replacing harmful chemicals with environmentally friendly alternatives. Consequently, there is a pressing need for solutions to enhance the quality of the cultural environment without harming aquatic animals or humans (Cabello *et al.*, 2013; Madhana *et al.*, 2021; Mondal *et al.*, 2022).Currently, the application of beneficial microorganisms, known as probiotics, in aquaculture is a widely adopted solution to address these challenges. According to Onianwah (2018), sustainable aquaculture systems require the presence of beneficial bacteria. Several species of probiotic microbes, such as *Bacillus subtilis*, *B. licheniformis*, *B. megaterium*, *Nitrobacter* sp., *Nitrosomonas* sp., *Lactobacillus plantarum*, and *L. fermentum*, have been utilized (Butt *et al.*, 2021). These bacteria are non-toxic, have no side effects, do not persist in the environment, and are not resistant to antibiotics. They are effective in improving the environment, boosting the immune systems of aquatic animals, reducing stress, and maintaining the equilibrium of the aquatic ecosystem (Hoseinifar *et al.*, 2018; Madhana *et al.*, 2021).

Bacteria play a crucial role in producing bioactive compounds, and *Bacillus* strains are particularly known for degrading organic compounds in soil and water through their biological activities. Enzymes such as protease, amylase, and cellulase contribute to closing the material cycle in nature (Madhana *et al.*, 2021). This capability is also leveraged in waste processing and decomposition.

Throughout their lifecycle, bacteria secrete numerous biologically active substances that can resist various microbial species, including fungi and bacteria. Our previous study showed that two *Bacillus* strains, MT50 and MT51, exhibited high efficiency in ammonium removal, and optimally thrived at pH 7 and in high salinity conditions (4% NaCl). Laboratory tests on their capability to improve shrimp aquaculture wastewater revealed that the two *Bacillus* strains removed 72.25% and 78.85% of the ammonium by the seventh day, respectively. Notably, strain MT50, when combined with unsterilized shrimp wastewater, demonstrated the highest ammonium removal efficiency of 79.91% with a 1% cell suspension by the fourth day. Additionally, the experiment showed the production of nitrite and nitrate, which were subsequently removed by the selected *Bacillus* species. These findings suggest that the two isolated *Bacillus* strains could be utilized as heterotrophic nitrification-aerobic denitrification species. Thus, developing new methods of using these two bacterial strains with high adaptability for aquaculture applications is essential.

Materials and Methods

Shrimp aquaculture water preparation

The seawater source with a salinity of 30‰, obtained from Xuan Thuy, Nam Dinh, was mixed with tap water to achieve a final salinity of 16‰. This water was then treated with chlorine at a concentration of 30mg L^{-1}, followed by adding $Na_2S_2O_3$ to neutralize any excess chlorine, and aerated for approximately 12-24 hours. One hundred-liter composite tanks, prepared for the experiment, were placed in a closed room (room temperature was kept at 25°C) and arranged according to an open system with continuous aeration. Before the experiment, each tank was disinfected with chlorine at a concentration of 30ppm for 30 minutes to prevent bacterial contamination.

Preparation of the two endophytic *Bacillus* inoculants

The two ammonia-oxidizing endophytic bacteria used in this study, *Bacillus velezensis* MT50 (MT50) and *Bacillus amyloliquefaciens* MT51 (MT51), were isolated from Man Trau grass (*Eleusine indica*) collected from shrimp farms in Binh Thuan (Trung *et al.*, 2022). These *Bacillus* strains have demonstrated salt tolerance

and ammonium removal capabilities in synthesized shrimp aquaculture wastewater. The strains were initially cultured in LB media and then proliferated in liquid LB media. Following proliferative culture, the bacterial cells were collected via centrifugation, and resuspended in sterile water, and their density was determined by measuring the optical density (OD) at a wavelength of 600nm on a UV/Vis spectrophotometer (Spectroquant®Prove 300, Merck, Germany).

Evaluation of the effects of the *Bacillus* inoculants on the shrimp culture water quality

Whiteleg shrimp (*Litopenaeus vannamei* PL 9) were used in the experiment. The shrimp were kept in a tank for 30 days until they reached an average weight of 1g. Before the experiment, 20 shrimp were measured and weighed. The shrimp were stocked at a density of approximately 0.5 shrimp per liter (500 shrimp m^{-3}).

The experiment consisted of three treatments: T1 (Control), with no addition of bacterial culture; T2, with the addition of *Bacillus velezensis* MT50; and T3, with the addition of *Bacillus amyloliquefaciens* MT51. The optical density (OD) of the bacterial cultures was 10^6 CFU mL^{-1}. The experiment was conducted in 100-L composite tanks filled with 100L of prepared saline water (salinity of 16‰, pH 7.5) as described above. The treatments were arranged in a completely randomized design and repeated three times at room temperature (25°C).

Shrimp were fed with Grow Feed four times a day at 06:00, 11:00, 16:00, and 21:00. The sediment was siphoned out of each tank twice daily, in the morning and the afternoon, and aeration was continuous. Throughout the experimental procedure, the water was not replaced but was instead replenished to compensate for the volume siphoned. The duration of the experiment was 60 days.

Throughout the experiment, water quality indicators (temperature, pH, total soluble solids (TSS), chemical oxygen demand (COD), total ammonia nitrogen (TAN), and total alkalinity), shrimp survival rate, and bacterial density were monitored. Water samples were collected 20-30 cm below the water surface, refrigerated at 4°C, and analyzed within two hours. pH and temperature were measured twice daily (at 06:00 and 15:00) using a Milwaukee Mi805 Portable pH/EC/TDS/Temperature Meter (Mi805 Milwaukee, CO, USA).

TSS, COD, TAN, and alkalinity were checked every five days using the detergent solution provided with a multi-parameter photometer (HI83399-02, Hana, Romania) as per the manufacturer's instructions. After each test, if the total alkalinity was low, $NaHCO_3$ was used to stabilize the alkalinity levels.

Bacterial samples were collected before the addition of bacteria and every five days thereafter until the end of the experiment. Bacterial density was determined by the colony counting method (Jett, 1997). The density of *Vibrio* spp. was determined by the colony counting method on Thiosulfate Citrate Bile Salts Sucrose (TCBS) selective agar medium (Merck, Germany), incubated at 30ºC for 24 hours. For the total number, density was measured by counting colonies on Nutrient Agar medium (Merck, Germany), incubated at 30ºC for 48 hours. *Bacillus* spp. was confirmed by Gram-positive and catalase-positive tests.

The following formula was used to calculate the growth rate in terms of shrimp length:

Growth rate in length (%) = [(Lf – Li)/Li] × 100

where Li is the length of 20 shrimp at the beginning of the experiment (cm) and Lf is the length of 20 shrimp at the end of the experiment (cm).

The following formula was used to calculate the shrimp weight:

Weight gain (g) = Wf – Wi

where Wi is the weight of 20 shrimp before the experiment (g) and Wf is the weight of 20 shrimp at the end of the experiment (g).

The survival rate of the shrimp was determined at the end of the experiment using the formula:

Survival rate (%) = (Final numbers/Initial numbers) × 100

Daily weight gain (g day^{-1}) = (Final weight – Initial weight)/number of days

Specific growth rate (% day^{-1}) = ([ln(final wt) – ln(initial wt)]/number of days) × 100

Statistical analysis

The data were calculated and statistically described using Excel software. The data were compared via single-factor ANOVA statistics and the DUNCAN test using SPSS version 16.0. The level of statistical significance was set at 0.05.

Results and Discussion

Temperature of water

In this study, the water temperature in the treatments ranged from 27.8 to 28.9°C (**Table 1**), which were quite similar to the initial temperature (27.47°C) and were within the suitable range for shrimp farming as reported by Whetstone *et al.* (2002) and Boyd *et al.* (2002). Previous research has shown that *P. vannamei* is able to tolerate a wide range of temperatures, from 7.5 to 42.0°C, with optimal temperatures being 30°C for smaller shrimp and 27°C for larger shrimp (Millard *et al.*, 2020). The findings of this study aligned with these reported data.

pH of water

Overall, the pH of the water in the treatments exhibited minimal fluctuations compared to the initial pH (7.5), with a slight increase observed mid-experiment. This increase could be attributed to the accumulation of organic matter from leftover feed and shrimp manure, as well as fluctuations in the dissolved oxygen (DO) content. Notably, pH variation ranged from 7.80 to 7.95 in the experimental groups (**Table 1**), with morning values ranging from 7.80 to 7.85 and afternoon values from 7.90 to 7.95. According to Chanratchakool *et al.* (1995), pond pH is crucial as it directly or indirectly affects shrimp development, with the optimal range for shrimp growth being 7.8 to 8.2. Additionally, Briggs & Funge-Smith (1994) reported that a pH of 7.5-8.5 was ideal for the growth of nitrifying bacteria. Therefore, the pH levels in this study were suitable for shrimp growth.

Total alkalinity

The alkalinity in the treatments ranged from 98.43-115.01 mg CaCO3 L^{-1} (**Figure 1**). Specifically, in the non-bacterial treatment (T1), total alkalinity ranged between 98.43-114.12 mg CaCO3 L^{-1}. In the groups supplemented with *Bacillus* MT50 (T2) and *Bacillus* MT51 (T3), the alkalinity ranged from 98.1-114.03 mg CaCO3 L^{-1}, and 98.2-114.05 mg CaCO3 L^{-1}, respectively. The treatments were not statistically different (P >0.05).

The observed increase in alkalinity during the experiment can be attributed to the maintenance of stable alkalinity levels by adding $CaCO_3$ to the culture tanks. According to Tran Ngoc Hai *et al.* (2004), the optimal alkalinity for shrimp growth is between 80 and 120 mg L^{-1}. These results demonstrate that the alkalinity levels across the tanks were consistent and within the optimal range for shrimp growth.

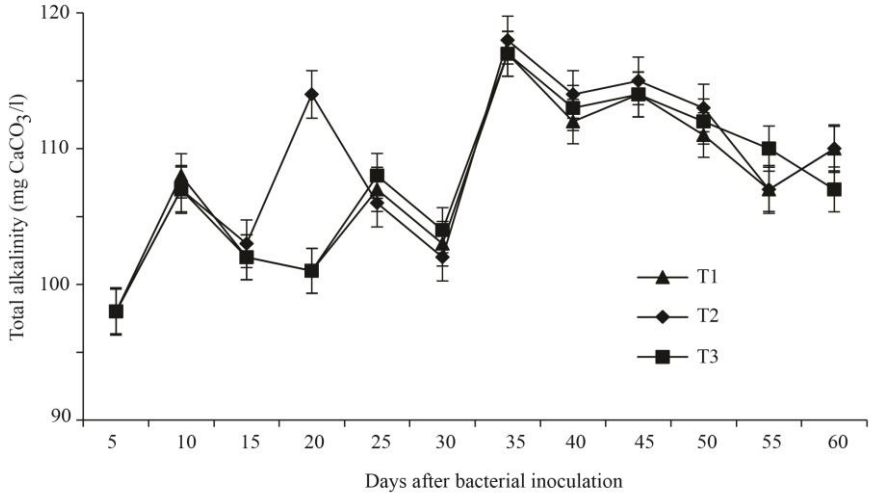
Total suspended solids (TSS)

The findings revealed a gradual increase in total suspended solids (TSS) throughout the culture period, peaking at 392.01 mg L^{-1} (**Figure 2**). Additionally, TSS exhibited fluctuations in the T1, T2, and T3 treatments, ranging from 18.37 to 392.01 mg L^{-1}, 20.01 to 304.37mg L^{-1}, and 19.34 to 302.19 mg L^{-1}, respectively. Particularly, T3 demonstrated significantly lower TSS levels compared to T2 (P <0.05).

Table 1. Mean water temperature and pH of treated shrimp aquaculture water collected from the different experimental groups after 60 days.

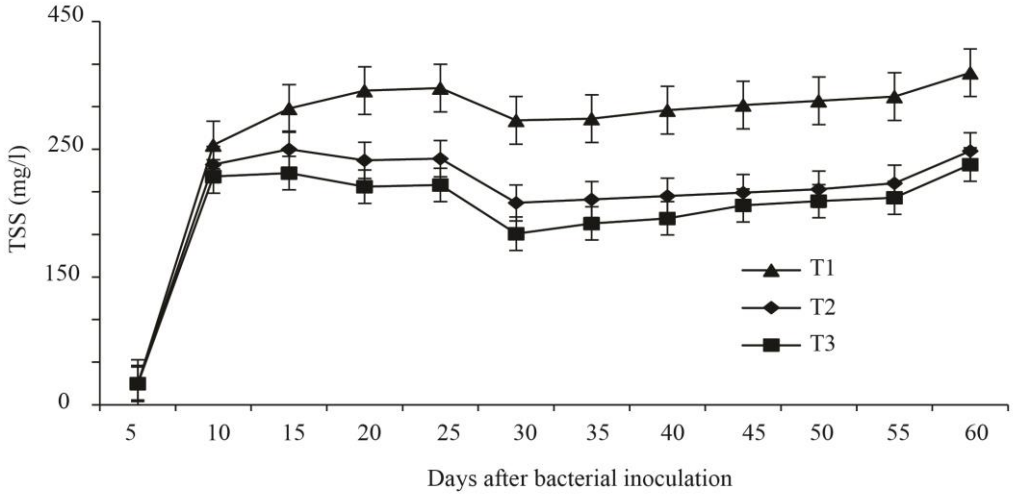
Parameters	T1	T2	T3
Temperature (°C)	27.8 ^{c}	28.16 ^{a}	28.9 ^{a}
pH	7.80 ^{c}	7.81^{a}	7.95 ^{a}

Note: Data presented as mean. Values in the same row with different letters are statistically different (P <0.01). T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51.



Note: T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51.

Figure 1. Fluctuation in total alkalinity among the different experimental groups for 60 days



Note: T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51.

Figure 2. Fluctuations in total suspended solids among the different experimental groups for 60 days

In another study by Pham *et al*. (2011), shrimp cultured in tanks exhibited TSS levels comparable to those observed in our study (384 mg L^{-1}). In contrast, shrimp cultured in ponds without beneficial bacterial additions, as reported by Nguyen & Vo (2008), showed much higher TSS accumulation (746 mg L^{-1}). Our findings indicate a consistent rise in TSS, with the highest recorded value of 390 mg L^{-1} observed in the control group. Furthermore, our data underscored a notable decrease in TSS levels in tanks supplemented with *Bacillus* bacteria. This reduction can be attributed to the effective breakdown of organic compounds by the MT51

strain, resulting in significantly lower TSS levels compared to the control treatment.

Dissolved oxygen (DO)

The dissolved oxygen (DO) content ranged from 3.71 to 6.62 mg L^{-1} during the experiment. Initially, the oxygen content increased in the first three measurements and then stabilized within the range of 5-6 mg L^{-1}. This stability was attributed to continuous aeration in the tank, which minimized fluctuations in the dissolved oxygen levels. In the T3 treatment, the dissolved oxygen content (5.14 ± 0.3 mg L^{-1}) was slightly lower compared to T1 (5.31 ± 0.4 mg L^{-1}). However, the difference in DO content between the treatments was not statistically significant (P >0.05).

It is well-documented that the optimal range of dissolved oxygen for shrimp growth is above 5 mg L^{-1} and should not exceed 15 mg L^{-1} (Whetstone *et al.*, 2002; Millard *et al.*, 2020). Therefore, the observed fluctuations in dissolved oxygen levels during the experiment were suitable for supporting shrimp growth. These fluctuations may have been influenced by bacterial activity within the tanks and the aeration process.

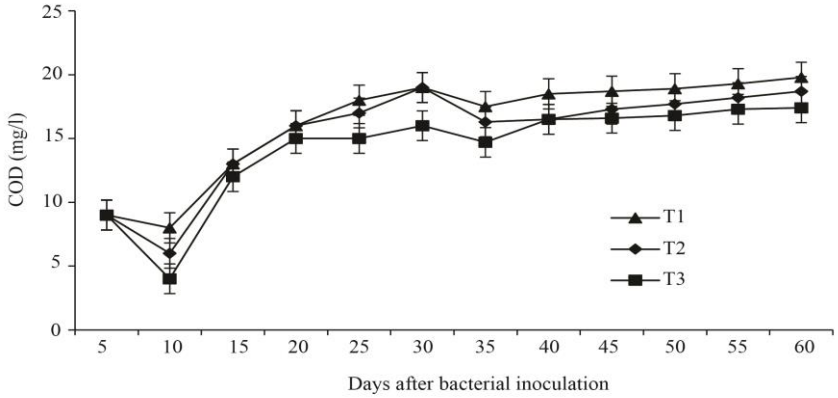
Chemical oxygen demand (COD)

COD fluctuations in the control treatment (T1) were notably higher compared to the bacteria-assisted treatments, T2 and T3 (**Figure 3**). Among the bacteria-assisted treatments, T3 exhibited the lowest COD levels (13.01 ± 0.34 mg L^{-1}), indicating effective decomposition of organic matter by the bacteria present in this tank. However, COD in T2 (15.04 ± 0.32 mg L^{-1}) was also statistically different from T1 (16.78 ± 1.04 mg L^{-1}). Overall, the COD levels ranged from 4.37 to 19.84 mg L^{-1} and tended to increase gradually towards the end of the experiment, reflecting the accumulation of excess feed and shrimp waste over time, necessitating more oxygen for decomposition.

Similar trends in dissolved oxygen (DO) were observed for COD across all treatments, indicating a higher organic content in T1 compared to T2 and T3. The addition of bacteria in T2 and T3 played significant roles in decomposing organic matter, thereby reducing contamination levels in the solutions.

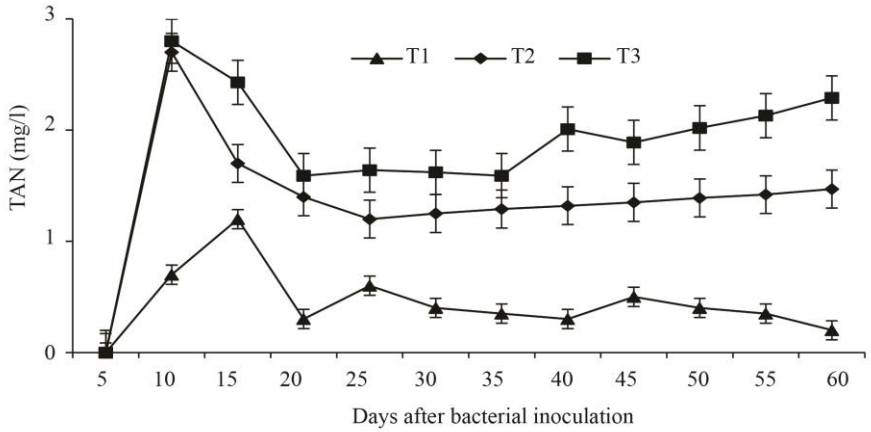
Total ammonia nitrogen (TAN)

Figure 4 illustrates the fluctuations in total ammonia nitrogen (TAN) across the different experimental groups. The TAN content ranged from 0.03 to 2.34 mg L^{-1}, peaking at the second sampling point before decreasing to within permissible limits. Particularly, TAN levels in T3 were notably higher compared to the control (T1), indicating the enhanced role of MT51 bacteria in decomposing organic matter within the culture.



Note: T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51.

Figure 3. Fluctuations in COD among the different experimental groups for 60 days



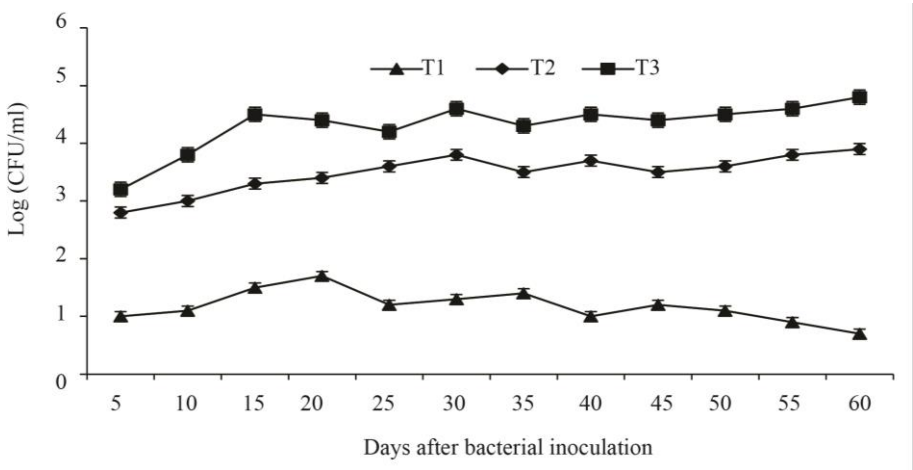
Note: T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51.

Figure 4. Fluctuations in TAN among the different experimental groups for 60 days

A critical concern in aquaculture is ammonia toxicity, and the ammonia values in the experiment initially showed significant fluctuation. This can be attributed to the strong metabolism of organic proteins by *Bacillus* bacteria, resulting in the production of NH_4^+ and NH_3 as by-products of cellular development. These findings aligned with earlier studies. Whetstone *et al.* (2002) suggested that shrimp can thrive within TAN levels ranging from 0.02 to 2 mg L^{-1}, while Boyd *et al.* (2002) emphasized that TAN levels in shrimp pond environments should not exceed 3 mg L^{-1}.

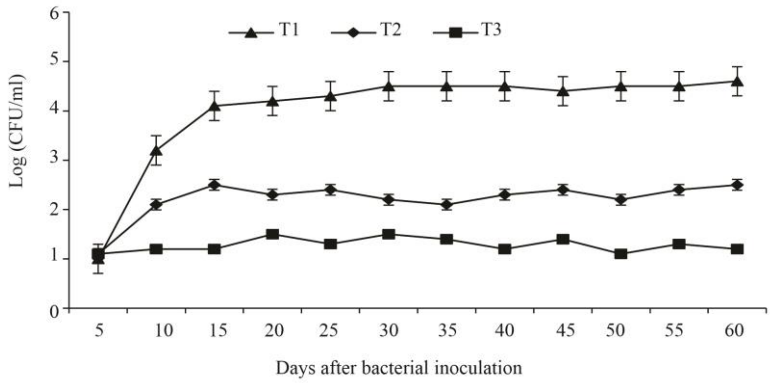
Bacillus density fluctuations

The results revealed varying densities of *Bacillus* ranging from 3.4 to 8.6 × 10^4 CFU mL^{-1} (**Figure 5**). Particularly in the T3 treatment, the *Bacillus* density was the highest (1.66 × 10^3 to 3.13 × 10^4 CFU mL^{-1}), while the lowest values were observed in the T1 treatment (8.2 to 27.85 ± 2.31 CFU mL^{-1}). Moreover, among the two treatments supplemented with *Bacillus* bacteria, the T2 treatment (0.53 × 10^3 to 4.37 × 10^3 CFU mL^{-1}) showed statistically significantly lower results (P <0.05) compared to T3, indicating that



Note: T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51.

Figure 5. Fluctuations in the density of *Bacillus* bacteria in the water among the different experimental groups for 60 days



Note: T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51.

Figure 6. Fluctuations in the density of *Vibrio* bacteria in the water from the different experimental groups for 60 days

the bacterial strain used in T2 struggled to adapt to the environment.

Overall, in the T2 and T3 treatments, in which *Bacillus* bacteria were added, the densities consistently exceeded those in the control tank (T1) by 2-3 log units, maintaining levels between 10^3 and 10^4 CFU mL^{-1}. This clearly demonstrates the effectiveness of the added bacteria in achieving the desired bacterial density ($<10^7$ CFU mL^{-1}).

Vibrio density fluctuations

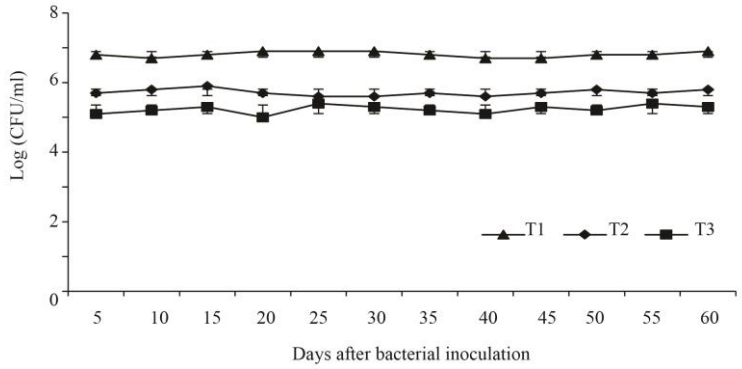
Throughout the 12 sampling points, the *Vibrio* density in the water samples from the treatments supplemented with bacteria ranged approximately from 10^1 to 10^2 CFU mL^{-1}, whereas, in the control treatment (T1), it ranged from 10^3 to 10^4 CFU mL^{-1} (**Figure 6**). This indicates that the *Vibrio* density in the bacterial supplemented treatments was consistently 10-100 times lower compared to the non-supplemented treatment. Upon comparing the bacterial-supplemented experiments, the *Vibrio* densities were measured at 10^2 CFU mL^{-1} for T2 and 10^1 CFU mL^{-1} for T3. Notably, MT51 bacteria exhibited higher resistance to *Vibrio* compared to MT50, as evidenced by the lower *Vibrio* density observed in T3 than T2. Conversely, in the control treatment (T1) without *Bacillus* supplementation, the *Vibrio* density was higher at 10^4 CFU mL^{-1}.

Figure 6 illustrates the fluctuations in *Vibrio* bacterial density across the different experimental groups. It is evident that the density of *Vibrio* bacteria remained within permissible limits throughout the study period. However, in the absence of *Bacillus* bacteria supplementation (T1), the *Vibrio* density exceeded permissible limits at times. Despite this, *Vibrio* bacteria are not exclusively pathogenic, allowing the shrimp to thrive throughout the experiment.

Fluctuations in total bacterial density

The findings indicated that the bacterial density in the water ranged between 10^5-10^6 CFU mL^{-1} (**Figure 7**). In the experimental tanks supplemented with bacteria (T2 and T3), the total bacterial count was consistently one logarithmic unit (10 times) lower compared to the control tank (T1). Specifically, the bacterial density in T1 was significantly higher than in T2 and T3 (P <0.05). Fluctuations in the total bacterial density in the *Bacillus*-supplemented tanks depended on the timing and dosage of bacterial additions. Conversely, the total bacterial density in the control tank (T1) gradually increased due to the accumulation of shrimp feed and waste.

Notably, contrasting trends were observed between *Bacillus* and *Vibrio* bacteria in the experiments. The relatively stable bacterial density in the *Bacillus*-supplemented tanks may be attributed to periodic additions of *Bacillus*



Note: T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51.

Figure 7. Fluctuations in the density of total bacteria in the water among the different experimental groups for 60 days

bacteria throughout the experiment, which potentially inhibited *Vibrio* growth. Conversely, the increase observed in the control tank was facilitated by the accumulation of shrimp waste and excess feed, creating favorable conditions for *Vibrio* proliferation. These results underscored that *Bacillus* supplementation effectively reduced *Vibrio* bacterial density within the aquaculture system (refer to **Figure 6**). This finding is consistent with Moriarty's(1998) report suggesting that *Bacillus* supplementation can enhance shrimp survival by controlling *Vibrio* pathogens in water. Moriarty (1999) also noted that *Vibrio* densities exceeding 10^3 CFU mL^{-1} could be detrimental to shrimp. Additionally, fluctuations in the total bacterial density observed in aquaculture may be attributed to the inhibitory effects of *Bacillus* bacteria, which potentially curb the growth of other bacterial species. Anderson (1993) indicated that in clean water, total bacterial counts are typically below 10^3 CFU mL^{-1}, with counts exceeding 10^7 CFU mL^{-1} posing risks to shrimp development and environmental cleanliness. Overall, the total bacterial density in the treatments remained within acceptable limits throughout the study.

Bacillus isolates enhanced the growth rate of shrimp length and weight

The growth rates in length and weight gain observed in the treatments were statistically significant (P <0.05), as presented in **Table 2** and **Table 3**, respectively. **Table 2** data reveal that the shrimp cultured in the T3 tank exhibited the highest growth rate in length (44.73%), whereas the control group (T1) showed the lowest growth rate in length.

Additionally, significant differences were observed between the T3 and T1 treatments. In the T1 treatment, the shrimp's weight gain was 5.92 ± 0.2 g $shrimp^{-1}$, whereas in the T3 treatment, it was 1.5 times higher at 8.92 ± 0.1 g $shrimp^{-1}$. Importantly, there was no statistically significant difference noted between the T2 and T1 treatments.

Under identical conditions, the growth rates of shrimp differed significantly between the treatments supplemented with *Bacillus* (T2 and T3) and the control (T1) (**Table 3**). The introduction of *Bacillus* species into the tanks to enhance the aquatic environment had a positive impact on shrimp growth. These bacteria reduced toxic substances harmful to shrimp and mitigated

Table 2. Growth rate in the length of shrimp cultured in the different experimental groups after 60 days

Formula	T1	T2	T3
Growth rate in the length of shrimp (%)	31.35	36.56^b	43.53^c

Note: Data presented as mean. Values in the same row with different letters are statistically different (P <0.01). T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51

Table 3. Growth rate in the weight of shrimp cultured in the different experimental groups after 60 days

Experiments	Average Weight before the experiment (g shrimp^{-1})	Average Weight after the experiment (g shrimp^{-1})	Weight gain (g)	Daily weight gain (g day^{-1})	Specific growth rate (% day^{-1})
T1	0.90 ^{a}	6.82 ^{a}	5.92 ^{a}	0.10 ^{a}	2.78 ^{a}
T2	0.96 ^{a}	7.37 ^{a}	6.41 ^{a}	0.10 ^{a}	3.39 ^{a}
T3	0.93^{a}	9.85 ^{b}	8.92 ^{b}	0.15 ^{b}	3.93 ^{b}

Note: Data presented as mean. Values in the same column with different letters are statistically different (P <0.01). T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51

Table 4. The survival rate of shrimp after 60 days of culture under different growth conditions

Formula	T1	T2	T3
Survival rate of shrimp (%)	66.26^{a}	71.12^{b}	87.62^{c}

Note: Data presented as mean. Values in the same row with different letters are statistically different (P <0.01). T1: shrimp culture with no addition of bacteria culture, T2: shrimp culture with the addition of Bacillus velezensis MT50, and T3: shrimp culture with the addition of Bacillus amyloliquefaciens MT51

pathogenic attacks. Regular additions of *Bacillus* facilitated the breakdown of excessive organic compounds in the shrimp culture tanks, thereby maintaining stable conditions and enhancing feed utilization and shrimp growth.

Furthermore, the results demonstrated notable enhancements in shrimp growth and survival rates in the tanks supplemented with either MT50 or MT51 bacteria compared to the control. This finding aligns with previous studies indicating that beneficial *Bacillus* species not only enhance organic matter degradation and stabilize aquatic environments but also boost productivity nearly twofold compared to untreated ponds (Butt *et al*., 2021). Moreover, the addition of beneficial bacterial strains improves water quality, minimizes sediment pollution, suppresses harmful bacterial strains like *Vibrio*, and concurrently facilitates the release of essential digestive enzymes for the host, thereby promoting higher growth and survival rates (Butt *et al*., 2021).

Shrimp survival rate

The results are presented in **Table 4**.

The data in **Table 4** indicate the survival rate of the shrimp in the experiment varied from 65.53-86.7% with statistically significant differences between the treatments with the addition of bacteria (T2 and T3) and the control (T1). In the two treatments with the addition of bacteria, the survival rate of shrimp collected from the T3 treatment (86.7%) differed statistically significantly from the T2 treatment (70.0%).

Conclusions

In the two experimental treatments (T2 and T3), most of the water quality indicators showed significant improvement compared to the control (T1). The introduction of *Bacillus* strains positively impacted both water quality and shrimp culture. Among the strains tested, MT51 demonstrated superior treatment efficiency over MT50, as evidenced by enhanced water quality, higher survival rates, and improved growth rates. The total bacterial and *Vibrio* densities in the water were consistently lower in the treatments supplemented with *Bacillus* strains compared to the control. Further research is warranted to develop these *Bacillus* strains as probiotics for a sustainable fishery industry.

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