Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(3): 1363 – 1387 (2025)
www.ejabf.journals.ekb.eg

The Potential of Marine-Fungi (*Trichoderma reesei*) in Suppressing *Vibrio* sp. Populations in Industrial-Scale Shrimp Farming

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ARTICLE INFO

Article History:
Received: Dec. 11, 2024
Accepted: May 9, 2025
Online: May 25, 2025

Keywords:
Biocontrol, Growth Performances, *Litopenaeus vannamei*, Probiotic, Water Quality

ABSTRACT

Shrimp farming is a crucial sector for Indonesia's economy and food security, with exports reaching USD 2.23 billion in 2021. However, intensification of shrimp farming can increase the risk of diseases such as *Vibrio* sp. and acute hepatopancreatic necrosis disease (AHPND), as well as the negative impacts of antibiotic use. As an alternative, *Trichoderma reesei* shows potential as a probiotic, improving shrimp health and water quality. The aim of this study was to analyze the ability of *T. reesei* to reduce *Vibrio* sp. populations in shrimp farming ponds and its effectiveness in improving shrimp growth performance. The research was conducted at the industrial *vannamei* shrimp farm, PT. Bagja Barokah Sarerea, Cipatujah, Tasikmalaya. A completely randomized design was used with two experiments: the application of *T. reesei* (P) and without *T. reesei* (K), in six test ponds. Water quality (temperature, salinity, pH, dissolved oxygen "DO") was measured twice daily, while nitrite, ammonia, total organic matter, alkalinity, hardness, total *Vibrio* count, total *T. reesei* count, total bacterial count and shrimp performance were measured weekly. The results showed that the addition of *T. reesei* in the treatment ponds effectively controlled the growth of *Vibrio* sp., and a reduction in *Vibrio* sp. populations by 19.23%. While environmental parameters showed no significant differences between treatments (P-value > 0.05), there was potential for reduced ammonia levels in the treatment ponds (0.001–0.078mg/ L) compared to the control ponds (0.002–0.78mg/ L). Additionally, *T. reesei* probiotics improved the survival rate and productivity of *vannamei* shrimp in the treatment ponds, despite mass mortality caused by salinity differences during seed stocking. The results indicate that the potential of *T. reesei* as a probiotic has successfully improved aquaculture practices.

INTRODUCTION

The farming of *Litopenaeus vannamei* shrimp contributed 56% (6.5 million tons) to global shrimp production in 2019 **(FAO, 2021)**. In 2021, Indonesia's production of *L. vannamei* shrimp reached 768,834,605kg, with most of the production coming from intensive, traditional, and semi-intensive ponds. Additionally, smaller contributions came from offshore floating cages, still water ponds, and rice fields **(Fisheries, 2023)**. The main issue causing mass mortality in *L. vannamei* aquaculture is disease primarily caused by *Vibrio* spp. **(Baker-Austin *et al.*, 2018; Bachand *et al.*, 2020; Han *et al.*, 2020; Azhar & Yudiati, 2023)** and viruses **(Trang *et al.*, 2019; Yudiati *et al.*, 2019; Andrade *et al.*, 2022; Islam *et al.*, 2023)**. Viruses lead to significant economic losses, with an estimated annual revenue loss of US$ 238.33 million due to the white spot syndrome virus (WSSV) **(Patil *et al.*, 2021)**. Additionally, *Vibrio* species, such as *V. parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND), result in net income losses ranging from US$ –727.56 to –672.48 per hectare, with a probability of loss ranging from 95.9 to 28.1%. The impact of AHPND also includes a 60% reduction in shrimp production, causing global shrimp aquaculture losses of up to USD 43 billion **(Estrada-Perez *et al.*, 2020)**.

In shrimp farming, disease control is typically managed through the use of antibiotics and other chemicals, as they are effective in addressing disease issues in the short term. However, excessive and uncontrolled use of these substances has raised concerns about their negative impacts on environmental and human health **(Duy *et al.*, 2022)**. To address these issues and ensure the sustainability of semi-intensive shrimp farming, a more sustainable and environmentally friendly approach to disease control is needed. One promising approach is the use of probiotics as biocontrol agents **(Vargas-Albores *et al.*, 2017)**.

Probiotics are microorganisms that, when administered in the right amount, can provide health benefits to their host. The application of probiotics in shrimp farming has shown potential in enhancing shrimp immune systems, improving water quality, and inhibiting the growth of harmful pathogens **(Sánchez-Ortiz *et al.*, 2015)**. Probiotics have been proven to regulate the microbial composition in shrimp digestive tracts and boost their immune systems, making them a sustainable and eco-friendly alternative for disease management **(Goh *et al.*, 2023)**. Most research on probiotics in shrimp farming has focused on bacterial probiotics. However, there is also potential in probiotics derived from fungi, an area that remains largely unexplored.

Fungi are organisms commonly found in aquatic ecosystems, and several types of fungi have shown promising probiotic properties in research. One type of fungus with potential as a biocontrol agent to combat diseases in aquaculture caused by pathogenic *Vibrio* spp. is *Trichoderma reesei* **(Bahry *et al.*, 2021)**. *T. reesei* contributes to improved shrimp health and production by reducing pathogenic bacteria in shrimp ponds **(Insan *et***

***al.*, 2015)**. Additionally, the potential use of *T. reesei* as a probiotic also benefits water quality management **(Ali *et al.*, 2022)**. The use of *T. reesei* as a probiotic has been tested on different shrimp species, such as *Litopenaeus vannamei* and *Penaeus monodon*, demonstrating its flexibility and potential application in various shrimp farming systems **(Akter *et al.*, 2017)**. However, most research on the application of *T. reesei* as a probiotic has been limited to laboratory environments, with little development of its direct field application. The aim of this study was to analyze the ability of *T. reesei* to reduce *Vibrio* sp. populations in shrimp farming ponds and its effectiveness in improving shrimp growth performance.

MATERIALS AND METHODS

This study was conducted at the *vannamei* Shrimp Pond, PT. Bagja Barokah Sarerea, Ciheras, Cipatujah, Tasikmalaya, West Java, Indonesia (Fig. 1).

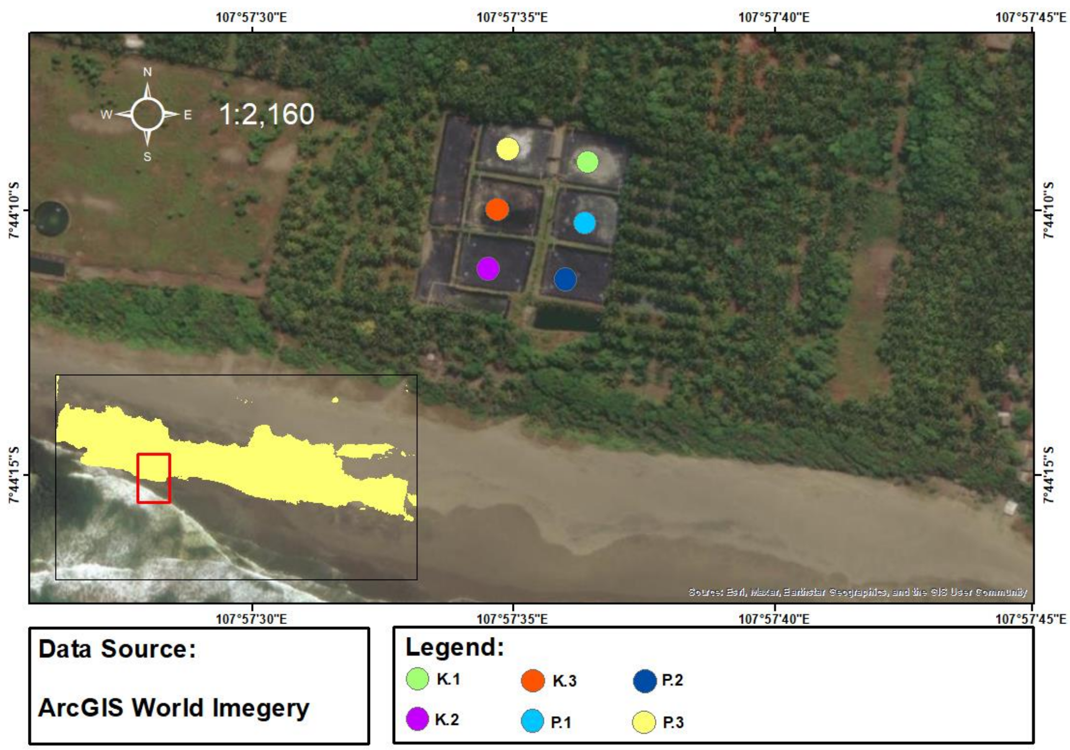

Fig. 1. Research Location at PT. Bagja Barokah Sarera (K.1, K.2, K.3: Control/without the application of *T. reesei*; P.1, P.2, P.3: with the application of *T. reesei*)

1. Experimental design

This research falls under the category of experimental studies. The aim was to determine the cause-and-effect relationship resulting from different experiments applied to the shrimp ponds at PT. Bagja Barokah Sarerea (BBS), Tasikmalaya. The research design used was a completely randomized design (CRD), with two experiments and three replications for each experiment. The experiments included the application of *T. reesei* (Treatment 1/P) and without the application of *T. reesei* (Treatment 2/K).

The layout of the test pond to be used is shown in Fig. (2). The test ponds, once confirmed to be sterile, were prepared for the next step, which was the stocking of shrimp seeds. The shrimp seeds used in this study were post-larvae 12. The shrimp population at the time of stocking in each pond ranged from 123,750 to 147,000 shrimp, with a density ranging from 115 to 130 ind/m^2.

Fig. 2. Placement setting of test ponds with each area in square meters (m²) (K.1, K.2, K.3: Control/without the application of *T. reesei*; P.1, P.2, P.3: with the application of *T. reesei*)

2. Probiotic preparation

T. reesei with a density of $\geq 1 \times 10^6$ CFU/g was mixed with 100g of calcium carbonate ($CaCO_3$) for every 100g of probiotic. For use in a 1000m² pond, a mixture of *T. reesei* and $CaCO_3$ was applied at a dosage of 0.1ppm, equivalent to 100g, and cultured or fermented in a medium containing 100g of skim milk, 1,000g of molasses dissolved in 70L of seawater overnight. This fermentation process can be used for two days across three experiment ponds, with daily applications of 10.38L for pond P1, 10.1 L for pond P2, and 10.36 L for pond P3. Probiotics are applied to the water in the grow-out pond daily. From day of culture (DOC) 1 to 30, a dosage of 0.1ppm was used, and for dissolved oxygen (DO) > 30, the dosage was increased to 0.2ppm.

3. Water quality monitoring

Water quality measurements, including temperature, pH, salinity, and DO, were conducted twice daily at 6:00 AM and 6:00 PM using the JALA Baruno device. Measurements of ammonia (NH_3) and nitrite (NO_2) were performed weekly using the spectrophotometry method. Meanwhile, measurements of alkalinity, hardness, total organic matter (TOM) were performed weekly using titrimetry.

4. *Vibrio* sp., *T. reesei*, and bacteria monitoring

Vibrio sp. and *T. reesei* density calculation followed the guidelines of Indonesian National Standard 01-2332.3-2006 on microbiological testing methods, specifically for determining the total plate count in fishery products. The bacterial growth medium used was thiosulfate-citrate-bile salts-sucrose (TCBS), which is selective for culturing *Vibrio* sp. The isolation of *Vibrio* sp. was carried out using the spread plate technique, where water samples from shrimp ponds were diluted to 10^{-1} and placed into petri dishes containing TCBS medium, spread evenly, and incubated for 24 hours. Meanwhile, *T. reesei* was isolated using fungal growth medium, potato dextrose agar, and incubated for 3 days, while the bacterial population was isolated using tryptic soy agar and incubated for 24 hours. The colonies that grew were observed and counted.

5. Growth performances analysis

Shrimp growth performance was measured weekly starting from DOC 30. The parameters measured for growth performance analysis included average body weight (ABW), average daily growth (ADG), survival rate (SR), specific growth rate (SGR), and weight gain rate (WGR), using the following formulas:

$$ABW = \frac{Total\ shrimp\ weight}{Total\ Shrimp} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

$$ADG = \frac{Current\ ABW\ - Previous\ ABW}{Sampling\ time\ intervak} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (2)$$

$$SR\ (\%) = \left(\frac{Nt}{No}\right) \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots...\ (3)$$

Nt = Number of shrimp at harvest

No = Number of initial stocking of shrimp

$$SGR\ (\%) = 100 \times \frac{ln\ [Wt\ (g)] - ln\ [Wo\ (g)]}{Duration\ (hari)} \quad \ldots\ldots\ldots\ldots\ldots...(4)$$

Wt = Final shrimp weight

Wo = Initial shrimp weight

Duration = Duration of the experiment

$$WGR\ (\%) = 100 \times \frac{Wt\ (g) - Wo\ (g)}{Wx\ (g)} \quad ..\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (5)$$

Wx = Average shrimp weight

Productivity

Productivity (kg/m^2) = $\frac{Biomass\ (kg)}{Land\ area\ (m^2)}$ ………………..…. (6)

6. Data analysis

Data were presented as mean and standard deviation. Statistical analysis was performed using non-parametric tests with SPSS for Windows. The Mann-Whitney test was used to compare significant differences between the experiment and control groups, with a P-value < 0.05 indicating a significant difference between experiment group 1 (P) and experiment group 2 (K). Subsequently, a principal component analysis (PCA) was conducted, a statistical method used to reduce data dimensions while retaining as much relevant information as possible. This technique can be used for dimensionality reduction, where PCA reduces the number of new variables, called principal components, which are linear combinations of the original variables. The PCA analysis was performed using XLSTAT 2023 software.

RESULTS

1. Water quality monitoring

Daily water quality monitoring, covering physical and chemical parameters, is shown in Table (1) as follows:

Table 1. Water quality monitoring results on the *vannamei* shrimp farm, PT. Bagja Barokah Sarerea

Water quality parameters	Experiments (average ± standard deviation)		Optimal level
	P	K	
Temperature (˚C)	30.81 ± 1.62	30.44 ± 1.78	28 – 31,5[a]
Salinity (ppt)	7.23 ± 1.14	7.38 ± 1.29	10 – 35[a]
pH	8.63 ± 0.44	8.65 ± 0.40	7,5 – 8,5[a]
DO (ppm)	5.74 ± 0.65	5.79 ± 0.64	≥ 3,0[a]
TOM (ppm)	85.15 ± 10.25	84.70 ± 9.00	≤ 90 (max)[a]
Ammonia (ppm)	0.02 ± 0.03	0.06 ± 0.17	< 0,1 (max)[a]
Nitrite (ppm)	0.05 ± 0.03	0.05 ± 0.02	< 1 (max)[a]
Alkalinity (ppm)	13.24 ± 18.70	134 ± 21.44	100 - 150[a]
Hardness (ppm)	2,059.76 ± 424.38	2,123.81 ± 464.12	–[a]

[a]: Regulation of the Minister of Marine Affairs and Fisheries Number 75 of 2016.
P: with the application of *T. reesei*
K: without the application of *T. reesei*

Based on the results of water quality parameter measurements in the experimental ponds (Table 1), the following values were obtained: the temperature in experimental ponds P and K was recorded at 30.81 ± 1.62°C and 30.44 ± 1.78°C, respectively, which are within the standard range (28–31.5°C). The salinity in both ponds averaged 7.23 ± 1.14ppt in pond P and 7.38 ± 1.29ppt in pond K, which are below the standard range for shrimp survival (10–35ppt). The pH values in pond P (8.63 ± 0.44) and pond K (8.65 ± 0.40) were slightly above the standard requirement of 7.5–8.5. DO levels were 5.74 ± 0.65ppm in pond P and 5.79 ± 0.64 ppm in pond K, both meeting the minimum standard of 3.0ppm. The TOM content was 85.15 ± 10.25ppm in pond P and 84.70 ± 9.00ppm in pond K, both within the maximum allowable limit of ≤ 90ppm. Ammonia concentrations were 0.02 ± 0.03ppm in pond P and 0.06 ± 0.17ppm in pond K, meeting the standard limit (< 0.1ppm). Meanwhile, nitrite concentrations were 0.05 ± 0.03ppm in pond P and 0.05 ± 0.02ppm in pond K, both below the maximum threshold (< 1ppm). Alkalinity in pond P was 131.24 ± 18.70ppm, while pond K recorded 134 ± 21.44ppm. Water hardness levels were 2,059.76 ± 424.38ppm in pond P and 2,123.81 ± 464.12ppm in pond K. Overall, most water quality parameters in the experimental ponds were within the standard limits set by the Ministry of Marine Affairs and Fisheries Regulation No. 75 of 2016, except for salinity, which was below the standard, and pH, which slightly exceeded the threshold.

2. *Vibrio* sp., *T. reesei* and bacteria monitoring

The results of *Vibrio* sp. abundance observations conducted in the test ponds on the *vannamei* shrimp farm of PT. Bagja Barokah Sarerea can be seen in Table (2).

Table 2. Observation of *Vibrio* sp. density in test ponds on the *vannamei* shrimp farm of PT. Bagja Barokah Sarerea

Weeks Observation	**Experiments (CFU/mL)**	
	P	**K**
1	$1.03 \times 10^2 \pm 118.78$	$1.57 \times 10^2 \pm 126.62$
2	$3.25 \times 10^2 \pm 258.65$	$4.38 \times 10^2 \pm 123.52$
3	$1.98 \times 10^2 \pm 110.72$	$3.60 \times 10^2 \pm 163.48$
4	$2.20 \times 10^2 \pm 85.44$	$5.83 \times 10^2 \pm 228.82$
5	$1.13 \times 10^2 \pm 15.28$	$6.73 \times 10^2 \pm 256.97$
6	$2.15 \times 10^2 \pm 69.46$	$5.55 \times 10^2 \pm 220.17$
7	$4.55 \times 10^2 \pm 43.30$	$5.28 \times 10^2 \pm 62.52$

P: with the application of *T. reesei*
K: without the application of *T. reesei*

Based on the Table (2), the highest result was recorded in the control group during the 5th week, with $6.73 \times 10^2 \pm 256.97$ CFU/mL. Conversely, the lowest result was found in the treatment group during the 1st week, with only $1.03 \times 10^2 \pm 118.78$ CFU/mL. In general, the control group showed a higher *Vibrio* population compared to the treatment group, especially in the 4th and 5th weeks, where the control group recorded significantly higher values. The fluctuation in *Vibrio* sp. abundance can be seen in Fig. (3).

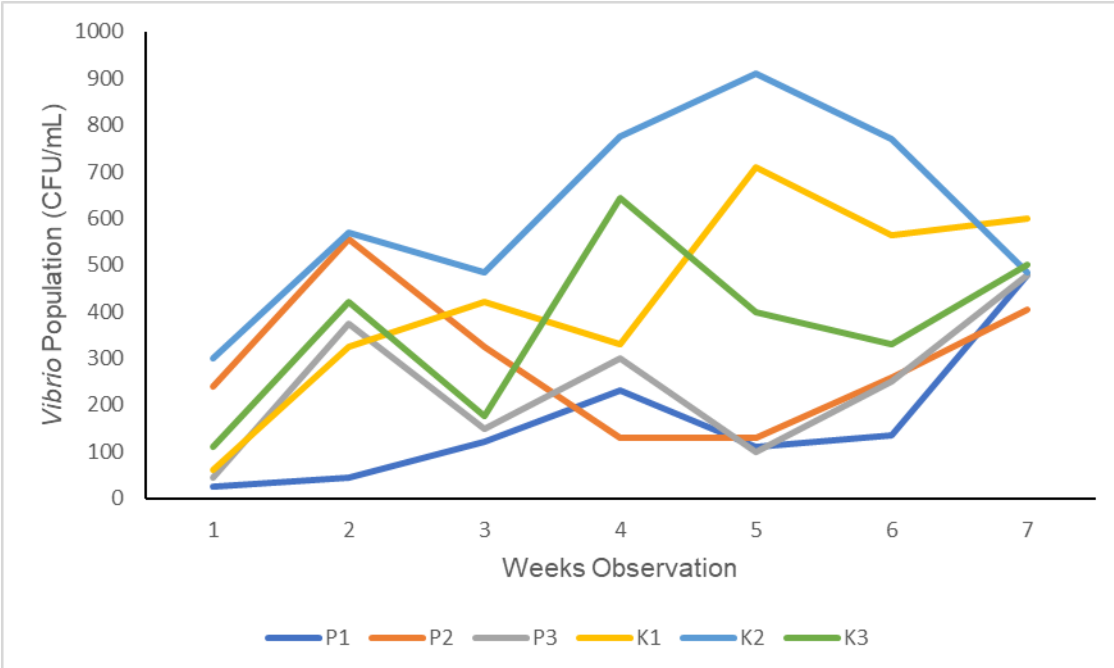

Fig. 3. Fluctuations in *Vibrio* sp. abundance during the experimental period at PT. Bagja Barokah Sarerea (K.1, K.2, K.3: Control/ without the application of *T. reesei*; P.1, P.2, P.3: with the application of *T. reesei*)

The results of *T. reesei* abundance observations conducted in the test ponds on the *vannamei* shrimp farm of PT. Bagja Barokah Sarerea are shown in Table (3).

Table 3. Observation of *T. reesei* Abundance in Test Ponds on the *vannam*ei shrimp farm of PT. Bagja Barokah Sarerea

Weeks Observation	**Experiments (CFU/mL)**	
	P	**K**
1	$0.25 \times 10^2 \pm 15$	0 ± 0
2	$0.37 \times 10^2 \pm 16{,}07$	0 ± 0
3	$0.40 \times 10^2 \pm 10$	0 ± 0
4	$0.65 \times 10^2 \pm 18{,}03$	0 ± 0
5	$0.72 \times 10^2 \pm 16{,}07$	0 ± 0
6	$0.85 \times 10^2 \pm 17{,}32$	0 ± 0
7	$0.65 \times 10^2 \pm 13{,}23$	0 ± 0

P: with the application of *T. reesei*
K: without the application of *T. reesei*

Based on Table (3), the observed *T. reesei* abundance in the test ponds ranged from 0.25×10^2 to 0.85×10^2 CFU/mL. In the treatment ponds, the lowest value was recorded in the first week at 0.25×10^2 CFU/mL, and the highest in the sixth week at 0.85×10^2 CFU/mL. Meanwhile, *T. reesei* was not detected in the control ponds throughout the observation period. The fluctuation of *T. reesei* abundance can be seen in Fig. (4).

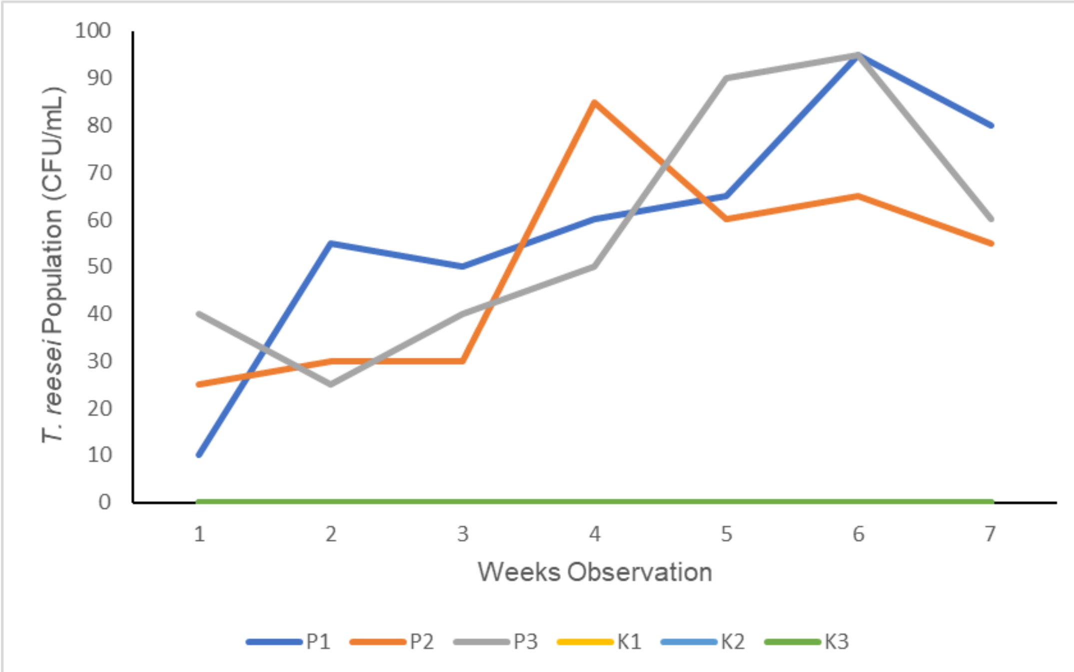

Fig. 4. Fluctuations in *T. reesei* abundance during the experimental period at PT. Bagja Barokah Sarerea (K.1, K.2, K.3: Control/ without the application of *T. reesei*; P.1, P.2, P.3: with the application of *T. reesei*)

The results of bacterial abundance observations conducted in the test ponds on the *vannamei* shrimp farm of PT. Bagja Barokah Sarerea are shown in Table (4).

Table 4. Observation of bacterial abundance in test ponds on the *vannamei* shrimp farm of PT. Bagja Barokah Sarerea

Weeks Observation	Experiments (CFU/mL)	
	P	K
1	$0.1002 \times 10^4 \pm 70.06$	$0.0708 \times 10^4 \pm 27.54$
2	$0.7567 \times 10^4 \pm 1{,}841.42$	$0.8083 \times 10^4 \pm 3{,}825.68$
3	$0.8850 \times 10^4 \pm 4{,}140.95$	$0.6283 \times 10^4 \pm 485.63$
4	$15.883 \times 10^4 \pm 4{,}919.94$	$0.7500 \times 10^4 \pm 3{,}439.48$
5	$15.283 \times 10^4 \pm 9{,}353.65$	$24.917 \times 10^4 \pm 5{,}772.64$
6	$16.467 \times 10^4 \pm 2{,}343.79$	$17.633 \times 10^4 \pm 9{,}083$
7	$22.283 \times 10^4 \pm 6{,}569.69$	$14.867 \times 10^4 \pm 9{,}975.01$

P: with the application of *T. reesei*
K: without the application of *T. reesei*

Based on Table (4), the bacterial abundance in the test ponds ranged from 0.1002×10^4 to 22.283×10^4 CFU/mL. In the treatment ponds, the lowest value was recorded in the first week at 0.1002×10^4 CFU/mL, and the highest in the seventh week at 22.283×10^4 CFU/mL. Meanwhile, the control ponds recorded the lowest value of 0.0708×10^4 CFU/mL in the first week and the highest value of 24.917×10^4 CFU/mL in the fifth week.

The growth ratio between total *Vibrio* count (TVC) and total *T. reesei* count (TTC) as well as between TVC and total bacterial count (TBC) is presented in Table (5).

Table 5. Average growth ratio between TVC and TTC and between TVC and TBC

Experiments	**TVC (cell/mL)**	**TTC (CFU/mL)**	**TBC (CFU/mL)**	**Ratio TVC/TTC (%)**	**Ratio TVC/TBC (%)**
P	2.33×10^2	0.55×10^2	12.476×10^4	80.77	1.83
K	4.71×10^2	0	11.427×10^4	100	3.96

P: with the application of *T. reesei*
K: without the application of *T. reesei*
TVC: Total *Vibrio* Count
TTC: Total *T. reesei* Count
TBC: Total Bacterial Count

Based on the Table (5), the TVC/TTC ratio in the treatment pond is 80.77%, while in the control pond, it is 100%. This indicates that in the treatment pond, where the probiotic *T. reesei* was applied, the total *Vibrio* sp. decreased by 19.23%. Meanwhile, the TVC/TBC ratio in the treatment and control ponds is recorded at 1.83% and 3.96%, respectively. This suggests that the treatment pond has a lower proportion of *Vibrio* sp. compared to the control pond.

3. Shrimp growth performance

The results of shrimp growth performance observed over 7 weeks in the test ponds on the *vannamei* shrimp farm of PT. Bagja Barokah Sarerea are shown in Table (6).

Table 6. The *vannamei* shrimp growth performance at PT. Bagja Barokah Sarerea

Parameters	**Experiments**	
	P	**K**
Initial population (ind)	128,500 ± 5,825.59	138,417 ± 7,731.16
Final population (ind)	37,728 ± 32,666.80	28,513 ± 20,312.75
SR (%)	29.91 ± 25.90	21.09 ± 15.87
SGR (%)	2.79 ± 0.75	2.93 ± 0.38
WGR (%)	48.33 ± 15.32	50.86 ± 7.98
Productivity (kg/m²)	0.20 ± 0.14	0.15 ± 0.09

P: with the application of *T. reesei*
K: without the application of *T. reesei*
SR: Survival Rate
SGR: Specific Growth Rate

WGR: Weight Growth Rate

The shrimp growth performance results presented in Table (6) show that at the beginning of the experiment, the initial shrimp population in pond P was 128,500 individuals, while in pond K, it was 138,417 individuals. After the experiment, the final population decreased to 37,728 individuals in pond P and 28,513 individuals in pond K. The SR in the treatment pond was 29.91%, higher than in the control pond at 21.09%. The SGR in the treatment pond reached 2.79%, slightly lower than the control pond at 2.93%. Meanwhile, the WGR in the treatment pond was 48.33%, also slightly lower than the control pond at 50.86%. Productivity in the treatment pond reached 0.20 kg/m², higher than the control pond at 0.15kg/ m².

4. PCA

Based on Fig. (5), the PCA analysis results indicate that two main components, F2 and F3, explain 29.15% of the total data variance. F2 (16.28%) focuses on variables such as total bacteria, alkalinity, and DO, while F3 (12.88%) highlights variables such as temperature, TOM, and total *Vibrio*. Variables such as DO, pH, ammonia, nitrate, and plankton showed a strong positive correlation with each other and contribute to F2 and F3. On the other hand, temperature showed a negative contribution to total bacteria and alkalinity. A significant negative relationship was found between total *Vibrio* and total *T. reesei*, where an increase in one tends to be followed by a decrease in the other.

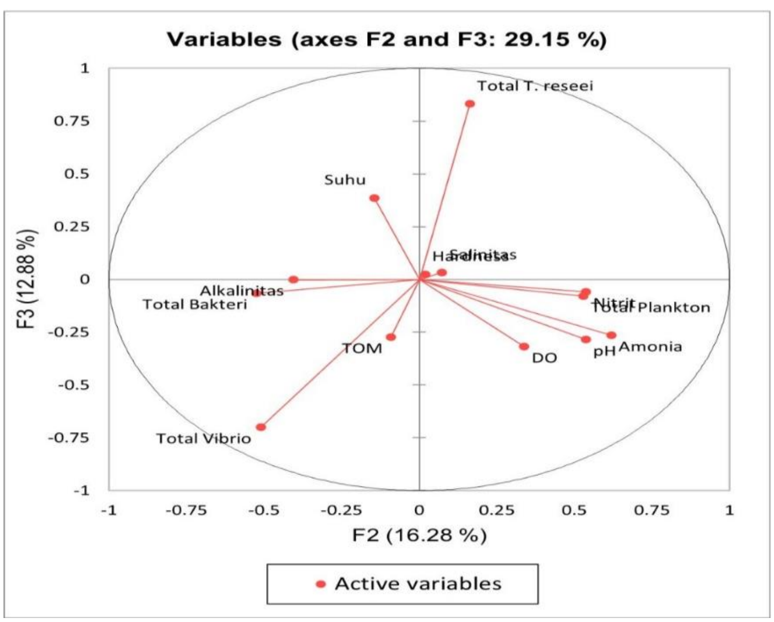

Fig. 5. The results of the principal component analysis (PCA)

DISCUSSION

The observed physical parameters included temperature and salinity, while the chemical parameters included pH, DO, TOM, ammonia, nitrite, alkalinity, and hardness. The average water temperature across all ponds ranged from 30.44 to 30.88˚C. According to the **Regulation of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia (2016)**, the optimal temperature for *vannamei* shrimp cultivation is between 28 and 31.5˚C. Based on this, it can be concluded that the temperature in both the P and K ponds met the optimal requirements for shrimp farming. However, during certain periods, the temperature dropped to 27˚C due to rainy season weather conditions during the study. Optimal temperature enhances growth and feed efficiency, whereas excessively low or high temperatures reduce shrimp growth rates **(Abdelrahman *et al.*, 2019)**. Weather can also influence temperature; during rainy conditions, lower temperatures can reduce shrimp appetite. Therefore, maintaining optimal temperature is crucial on *vannamei* shrimp farming **(Pramudia *et al.*, 2022)**.

The salinity level in Pond P averaged 7.23 ± 1.14ppt, while Pond K averaged 7.38 ± 1.29ppt. These values are significantly below the optimal salinity standard, which typically ranges between 10 and 35ppt according to Regulation No. 75 of 2016 by the Ministry of Marine Affairs and Fisheries. This lower salinity level is primarily due to the characteristics of the water source, which inherently has low salinity, and the prevailing weather conditions during the rainy season. Low-salinity environments can significantly affect the osmoregulatory processes of *vannamei* shrimp, potentially causing stress to the organisms **(Vieira-Girão *et al.*, 2015; Santanumurti *et al.*, 2019)**. This study showed that the growth and survival of *L. vannamei* can be compromised when reared in water environments with suboptimal ionic profiles. Water quality, including salinity, has been identified as a critical factor affecting various aspects of shrimp farming, such as metabolism, reproduction, osmoregulation, and stress levels **(Pine *et al.*, 2018; Rum *et al.*, 2022)**. These findings indicate that changes in salinity levels can affect *vannamei* shrimp behavior and stress levels, highlighting the importance of maintaining appropriate salinity conditions to ensure optimal shrimp health and well-being **(Huang *et al.*, 2019)**.

The pH levels during the study were relatively alkaline, ranging from 8.63 to 8.65. These values are considered unstable, and at certain times, the pH could even reach 9. The optimal pH range for maintaining semi-intensive *vannamei* shrimp ponds is 7.5–8.5 **(Ministry of Marine Affairs and Fisheries Regulation No. 75 of 2016)**. Within this optimal range, shrimp can grow well, exhibit high appetite, and have a robust immune system **(Ario & Nursani, 2024)**. Excessively alkaline pH levels can cause stress in shrimp, reduce appetite, and slow down growth rates **(Ridlo *et al.*, 2024)**. The increase in pH towards alkaline levels can also be attributed to the addition of $CaCO_3$ in the test ponds. $CaCO_3$ was introduced through the composition of *T. reesei* and additional minerals like Omyacrab, a mineral supplement for shrimp that contains $CaCO_3$. The pH levels are closely related to ammonia (NH_3) levels. When the pH is too high, ammonia levels also increase and can convert to a more toxic form that poses a threat to shrimp health **(Ge *et al.*, 2022)**. However, this effect is also influenced by temperature, which can exacerbate ammonia toxicity when both pH and temperature are high. Conversely, when pH is high but temperature is low, ammonia levels are generally considered safe.

The average DO levels across all ponds ranged from 5.74 to 5.79ppm, meeting the water quality standard for *vannamei* shrimp maintenance, which requires DO levels to be ≥ 3ppm **(Ministry of Marine Affairs and Fisheries Regulation No. 75 of 2016)**. DO is crucial for the survival of *vannamei* shrimp as it supports the respiration process, which is essential for efficient metabolism in shrimp. Adequate oxygen levels can reduce stress and prevent mass mortality in shrimp. Typically, DO levels decrease at night due to the cessation of photosynthesis, while respiration activities by other organisms continue to consume oxygen. Therefore, it is essential to monitor DO levels at night. To address low DO levels, aeration using paddle wheels can be employed. Aeration provides significant benefits in shrimp farming, especially in high-density ponds **(Franchy-Ch *et al.*, 2019)**.

The TOM values in ponds P and K were 85.15 and 84.70mg/ L, respectively. These values represent the average measurements over a 7-week observation period. The average TOM levels meet the optimal standard for shrimp growth, which is ≤ 90mg/ L, as outlined in the Ministry of Marine Affairs and Fisheries Regulation No. 75 of 2016. However, weekly fluctuations were observed, with TOM levels peaking at 104.24mg/ L in pond P and 107.07mg/ L in pond K. The increasing TOM levels are attributed to the accumulation of organic matter from uneaten feed and shrimp excreta during the observation period. By the seventh week, shrimp growth had reached a phase where feed consumption increased significantly, leading to greater amounts of leftover feed and waste. This observation aligns with findings by **Amalia *et al.* (2022)** and **Ariadi *et al.* (2023)**, who stated that as shrimp grow, their feed intake typically increases, resulting in higher volumes of residual feed and waste, directly contributing to the organic load in the water column. The accumulation of organic matter is influenced not only by shrimp feeding behavior but also by environmental conditions that promote decomposition. For instance, stable temperatures and pH levels during the period enhanced microbial activity, accelerating organic matter decomposition and raising TOM levels **(Rahmi *et al.*, 2023)**. Additionally, water exchange played a role in affecting TOM levels by diluting organic matter concentrations in the ponds.

The ammonia levels in pond P averaged 0.02 ± 0.03ppm, whereas pond K had an average of 0.06 ± 0.17ppm. Pond P exhibited lower ammonia levels compared to pond K. During the first week of observation, pond K recorded an exceptionally high ammonia level of 0.78 ppm, exceeding the permissible limit of ≤ 0.1ppm for *vannamei* shrimp ponds **(Ministry of Marine Affairs and Fisheries Regulation No. 75 of 2016)**. High ammonia levels in pond K suggest a potential accumulation of nitrogenous waste, which, if unmanaged, can be toxic to aquatic organisms **(Sorower *et al.*, 2020; Nagaraju *et al.*, 2022)**. Factors contributing to nitrogen compound accumulation, including ammonia, include overfeeding, organic matter decomposition, and excessive phytoplankton blooms, which collectively increase ammonia concentrations in the water **(Wijesekara *et al.*, 2005; Nkuba *et al.*, 2021)**.

The average nitrite levels in ponds P and K were 0.05 ± 0.03 and 0.05 ± 0.02ppm, respectively, with no significant differences between the test ponds. These nitrite levels remain well below the maximum permissible limit of ≤ 1 ppm **(Ministry of Marine Affairs and Fisheries Regulation No. 75 of 2016)**. This indicates that nitrification processes, which convert ammonia into nitrite and subsequently into nitrate, were effective in this aquatic environment **(Miazga-Rodriguez *et al.*, 2012)**. Nitrification, a biological process involving the oxidation of ammonia to nitrite and then to nitrate, is crucial for maintaining water quality in ponds. The increase in nitrite-nitrogen concentrations in the pond water reflects active nitrification facilitated by nitrifying bacteria such as Nitrosomonas, which convert ammonia-nitrogen to nitrite-nitrogen **(Atmomarsono & Nurbaya, 2014)**.

The alkalinity values observed in ponds P and K ranged from 131.24 to 134mg/ L. According to the Ministry of Marine Affairs and Fisheries Regulation No. 75 of 2016, the recommended alkalinity range for semi-intensive shrimp farming is 100–150mg/ L. Similarly, **Hossain and Haque, (2013)** suggested that bicarbonate alkalinity levels between 86.31 and 143.84mg/ L are suitable for shrimp farming. These findings indicate that the alkalinity levels measured in this study fall within the optimal range for aquaculture. Alkalinity is associated with the water's ability to neutralize acidic conditions without causing a significant decrease in pH. Moreover, alkalinity serves as a buffer against acidity **(Listriyana *et al.*, 2023)**. Significant fluctuations in alkalinity concentrations suggest the influence of various environmental factors and differing farming activities in each pond. High alkalinity levels can result from the increase of inorganic chemicals, such as bicarbonates, which may occur due to the decomposition of organic matter in the ponds. During this phase, the decomposition of organic matter from residual feed and shrimp waste accumulated in the control pond can release bicarbonate ions into the water, thus increasing alkalinity. The breakdown of organic materials such as feed and waste can directly influence pond water alkalinity by elevating bicarbonate concentrations, which are crucial for maintaining a stable pH environment that supports aquatic life **(Boyd *et al.*, 2016)**. Stable environmental conditions, such as consistent pH and temperature, can further accelerate decomposition processes, indirectly contributing to rising alkalinity levels. Additionally, ponds with infrequent water exchange may experience increased alkalinity due to the accumulation of ions from various sources.

The water hardness levels recorded in this study ranged from 2,059 to 2,123mg/ L in both ponds P and K. These values reflect fluctuations in hardness levels in the ponds, influenced by various factors such as mineral supplementation, water exchange, and biological activities within the ponds. The dynamics of water hardness in aquaculture systems are crucial as they directly affect the health and growth of aquatic species, particularly shrimp, which are sensitive to environmental changes **(Truong *et al.*, 2023)**. High hardness levels are attributed to the addition of calcium and magnesium minerals to the ponds. Calcium is essential for the growth and development of crustaceans, including shrimp, as it plays a critical role in forming their exoskeletons **(Nesapriyam *et al.*, 2022)**. An increase in water hardness can also result from the accumulation of minerals from water sources or from specific mineral supplements added to support shrimp growth during early development stages. Moreover, stable environmental conditions, such as balanced pH, can maintain these minerals in more soluble forms, further contributing to increased water hardness. In ponds where water exchange is minimal, mineral concentrations tend to rise due to the lack of dilution, which can also lead to elevated hardness levels. While this accumulation can be beneficial under certain conditions, excessive hardness may pose risks, such as mineral precipitation, which can negatively impact water quality and shrimp health **(Nesapriyam *et al.*, 2022)**.

The total population of *Vibrio* bacteria during the cultivation period showed significant differences between the treatment and control ponds. The highest total *Vibrio* density, 6.73×10^2 CFU/mL, was recorded in pond K during week 5, while the lowest density, 1.13×10^2 CFU/mL, was observed in pond P during the same period. This indicates that the presence of *T. reesei* in the treatment pond significantly suppressed the growth of *Vibrio* bacteria. In week 5, pond P had a *T. reesei* population of 0.72×10^2 CFU/mL, whereas no *T. reesei* was detected in pond K throughout the study. These findings align with the results of PCA, which revealed a negative correlation between *Vibrio* sp. and *T. reesei* populations; as the population of *T. reesei* increased, the population of *Vibrio* sp. decreased. A study by **Bahry *et al.* (2021)** supports this observation, confirming that *T. reesei* exhibits anti-*Vibrio* activity. The highest anti-*Vibrio* activity was observed at a concentration of 500 µg/disk, with an inhibition zone of 3.83 ± 0.2mm. The anti-*Vibrio* mechanism is attributed to the release of extracellular enzymes by *T. reesei*, targeting the bacterial cell wall, specifically breaking down peptidoglycan lipopolysaccharides through cellulase and protease activity. Furthermore, **Zhang *et al.* (2016)** highlighted genetic enhancements in *T. reesei* that increase cellulase production. Their study demonstrated a significant upregulation of the cellulase-coding genes cbh1 and cbh2 in the engineered strain *T. reesei* U3 compared to the wild-type strain *T. reesei* Rut-C30, resulting in a 1.4-fold increase in cellulase transcription. Similarly, research by **Dashtban and Qin (2012)** showed that overexpression of thermotolerant β-glucosidase in *T. reesei* significantly enhanced its cellulolytic activity. These transformants exhibited exceptionally high total cellulase activity, underscoring the potential of genetic modifications to enhance the enzymatic capabilities of *T. reesei*. The increased cellulolytic activity is likely a key factor in the suppression of *Vibrio* bacteria observed in the treatment pond containing *T. reesei*.

The total abundance of *Vibrio* sp. in all ponds during the maintenance period was within normal levels, remaining below the quality standard limit of 1×10^3 CFU/mL. This may be attributed to the low salinity levels across all maintenance ponds. The optimal salinity range for *Vibrio* growth is 10–30ppt, whereas the salinity in the ponds was much lower, ranging from 7.18 to 7.45ppt. This low salinity also led to a higher prevalence of *Vibrio cholerae* compared to *Vibrio parahaemolyticus*, as *V. cholerae* is more tolerant of low salinity conditions than *V. parahaemolyticus*. Studies have shown that *V. cholerae* can thrive in salinities as high as 45% in the presence of sufficient dissolved organic matter, demonstrating its adaptability to varying salinity levels **(Magny *et al.,* 2009)**. Conversely, *V. parahaemolyticus* has been found to be less tolerant of low salinity, which may explain its reduced growth rate compared to *V. cholerae* under the same conditions **(Arfao *et al.,* 2021)**.

Research findings indicate that the abundance of *T. reesei* in the treatment pond ranged from 0.25×10^2 to 0.85×10^2 CFU/mL, while no *T. reesei* was detected in the control pond throughout the observation period. The significant difference between the treatment and control ponds can be attributed to the experimental treatment applied. In the treatment pond, *T. reesei* was actively introduced as part of the trial, resulting in its detectable abundance. Meanwhile, no *T. reesei* was added to the control pond, explaining the consistent value of 0 CFU/mL. Although *T. reesei* is naturally present in the environment, its presence in the control pond was likely very limited, making it difficult to detect without additional intervention. This aligns with findings that highlight the need for external inoculation to establish *T. reesei* populations in specific environments, particularly when competing with other microorganisms **(Meng *et al.,* 2020)**. Fluctuations in *T. reesei* abundance in the treatment pond may be influenced by several factors. During the first week, the low abundance might have been due to the initial adaptation phase of *T. reesei* to the new pond environment. In this phase, *T. reesei* may require time to acclimate to physicochemical conditions such as temperature, pH, and DO levels, which could affect its metabolic activity and growth. The increase in *T. reesei* abundance in subsequent weeks, particularly in the sixth week, could be associated with increasingly favorable environmental conditions. Additionally, the stability of physicochemical parameters, such as optimal temperature and pH levels during the trial, may have promoted higher enzymatic activity, enhancing *T. reesei* ability to grow and proliferate.

Based on the research findings, bacterial abundance was recorded within the range of 0.0708×10^4 to 24.917×10^4 CFU/mL. In Pond P, bacterial abundance ranged from 0.1002×10^4 to 22.283×10^4 CFU/mL, while in Pond K, it ranged from 0.0708×10^4 to 24.917×10^4 CFU/mL. The significant differences in bacterial abundance could be attributed to several factors, including nutrient availability, water physicochemical conditions, and the dominant bacterial species at each observation phase. Physicochemical conditions such as temperature, pH, and DO levels play critical roles in shaping bacterial communities. Optimal conditions may lead to higher bacterial density, while suboptimal conditions can inhibit growth **(Saha *et al.*, 2017; Akani *et al.*, 2021)**. In the first week, the low bacterial abundance was due to the initial adaptation of bacteria to the new environment and the limited availability of substrates as a nutrient source. During this early phase, bacterial communities often experience an acclimation period, resulting in lower abundance as they adjust to new environmental conditions **(Mishra *et al.*, 2010)**. Additionally, at the beginning of the observation, DO levels and nutrient availability in the water might not have been optimal to support high bacterial growth. Low DO levels can restrict the metabolic activity of aerobic bacteria, thereby reducing their abundance **(Morghad *et al.*, 2020; Igoni, 2023)**. Conversely, the high bacterial abundance observed in week seven in treatment pond U2 and week five in the control pond may be associated with improved environmental conditions that supported bacterial growth. As the experiment progressed, nutrient concentrations likely increased due to the accumulation of organic matter from feed residues and decomposed plant material, providing substrates for bacterial growth **(Hasan *et al.*, 2015)**. The stability of physicochemical parameters such as temperature, pH, and DO, along with bacterial interactions, likely created more favorable conditions for bacterial proliferation. For instance, consistent temperature and optimal pH levels can enhance bacterial enzymatic activity and metabolic rates, leading to higher growth rates **(Osińska *et al.*, 2019; Adeoyo & Omaku, 2022)**. Furthermore, synergistic interactions among different bacterial species can contribute to a more robust microbial community, facilitating higher overall abundance **(Jang *et al.*, 2011; Harpeni *et al.*, 2019)**.

Monitoring results of the abundance of *Vibrio* sp., *T. reesei*, and bacteria revealed growth and decline ratios. The application of the probiotic *T. reesei* in the treatment pond proved effective in suppressing the *Vibrio* sp. population. This was evident from the 19.23% reduction in the TVC/TTC ratio compared to the control pond. In Pond P, the TVC/TTC ratio was 80.77%, while in the control pond, this ratio reached 100%. Additionally, the TVC/TBC ratio in the treatment pond was lower, at 1.83%, compared to 3.96% in the control pond. This indicates that in the treatment pond, *Vibrio* sp. constituted only a small portion of the total bacterial community. Conversely, in the control pond, *Vibrio* sp. dominated the bacterial community, which could negatively affect the health of cultured organisms. These findings demonstrate that the use of *T. reesei* can enhance microbial diversity in ponds, replacing the dominance of *Vibrio* sp. with more beneficial bacteria. The reduction in *Vibrio* sp. populations was due to the production of secondary metabolites by *T. reesei*, such as enzymes and antimicrobial compounds, which inhibit *Vibrio* sp. growth **(Bahry *et al.*, 2021)**. Additionally, probiotics compete with *Vibrio* sp. for nutrients and living space, effectively controlling pathogenic bacterial populations **(Kurniaji *et al.*, 2023)**. These results align with the understanding that probiotics can enhance microbial diversity and suppress pathogens, thereby supporting a healthier aquatic environment **(Meng *et al.*, 2020; Samirana *et al.*, 2023)**.

The growth performance of shrimp is one of the key indicators of success in this study. The results demonstrated that the administration of the probiotic *T. reesei* in the treatment pond (P) had a positive impact on the survival rate of the whiteleg shrimp (*Litopenaeus vannamei*) compared to the control pond (K) without treatment. Although the initial population in Pond P was lower at 128,500 individuals compared to 138,417 individuals in Pond K, the final population in Pond P was higher, with 37,728 individuals compared to 28,513 individuals in Pond K. This resulted in a higher survival rate (SR) in Pond P at 29.91% compared to 21.09% in Pond K, indicating that probiotics enhanced the resilience of the whiteleg shrimp. However, the significant decline in population and low SR were primarily due to mass mortality during the stocking period. This mortality was attributed to failed acclimatization caused by a substantial difference in salinity between the shrimp's initial environment (22 ppt) and the rearing pond (7 ppt). According to **Muin *et al.* (2018)**, salinity is an exogenous factor that can significantly affect shrimp growth performance. Optimal salinity levels are critical for larval survival, as supported by **Rakhfid *et al.* (2018)**, who reported that extreme salinity fluctuations could result in high mortality rates in shrimp larvae.

The success of shrimp farming is not solely assessed by SR but also by productivity metrics. The specific growth rate (SGR) and weight gain rate (WGR) in Pond P were slightly lower than in Pond K, at 2.79% and 48.33%, compared to 2.93% and 50.86%, respectively. This difference could be attributed to factors such as food competition or stress caused by the higher population density at the end of the trial. Nevertheless, the productivity of Pond P was significantly higher at 0.20 kg/m² compared to 0.15 kg/m² in Pond K, indicating that probiotic supplementation effectively enhanced overall harvest yields. However, the productivity levels observed were relatively low for semi-intensive farming. This limitation was influenced by the short cultivation period in this study, which lasted only 44 days, compared to the three-month cultivation period reported by **Cahyanurani and Edy (2022)**. Short cultivation periods may not provide sufficient time for shrimp to reach optimal harvest size, even when SGR and WGR are within normal ranges, ultimately resulting in lower overall productivity **(Tarunamulia *et al.*, 2023).**

CONCLUSION

This study demonstrated that the addition of *T. reesei* probiotics in the treatment ponds effectively controlled the growth of *Vibrio* sp., with an abundance of 2.33×10^2 CFU/mL compared to 4.71×10^2 CFU/mL in the control ponds, as evidenced by the Mann-Whitney test (p-value < 0.05) and a reduction in *Vibrio* sp. populations by 19.23%. While environmental parameters such as temperature, salinity, pH, DO, TOM, ammonia, nitrite, alkalinity, and hardness showed no significant differences between treatments (p-value > 0.05), there was potential for reduced ammonia levels in the treatment ponds (0.001–0.078mg/ L) compared to the control ponds (0.002–0.78mg/ L). Additionally, *T. reesei* probiotics improved the SR of *vannamei* shrimp in the treatment ponds, despite mass mortality caused by salinity differences during seed stocking. Although the SGR and WGR in the treatment ponds were slightly lower, their productivity was higher than that of the control ponds, highlighting the potential of probiotics in enhancing aquaculture success.

ACKNOWLEDGEMENTS

Special thanks to all the staff at PT. Bagja Barokah Sarerea for their dedication and hard work in supporting this research, ensuring its smooth execution.

FUNDING INFORMATION

This study was funded by The Ministry Education, Culture, Research and Technology through the Matching-Fund Kedaireka 2023 program with grant number 282/E1/HK.02.02/2023. Partly funded by PMDSU grant number 3596/E4/DT.04.02/2023.

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