

Potential of *Trichoderma* to improve probiotic performance in Vannamei shrimp cultivation

Sutarman^{1,*}, Dony Prasetyo², Lukman Hudi^{3,4}, Syarif R. Nurbaya⁴, Sriyono⁴ and Mulyadi⁴

¹ Department of Agrotechnology, Faculty of Science and Technology, Universitas Muhammadiyah Sidoarjo, Indonesia

² Department of Aquaculture, Faculty of Agriculture-Animal Science, Universitas Muhammadiyah Malang, Indonesia

³ Departement of Food Technology, Faculty of Science and Technology, Universitas Muhammadiyah Sidoarjo, Indonesia

⁴ Center for Agriculture and Fishery Studies, Directorate of Research and Community Service, Universitas Muhammadiyah Sidoarjo, Indonesia

Abstract. The purpose of this study was to determine the effect of microbe consortium on the physical characteristics of pools water and the growth of vannamei shrimp. The experiment was arranged in a Completely Randomized Design consisting of five types of microbe consortium treatments including *Trichoderma*, *Lactobacillus*, probiotic bacteria, *Trichoderma-Lactobacillus*, and *Trichoderma-probiotic bacteria* with a population density of 0.5×10^4 CFU.mL⁻¹ for fungi and 10^4 CFU. mL⁻¹ for bacteria. A total of 5,000 shrimp seeds were planted in each of the 15 circular pools with a volume of 12.56 m³. The parameters observed were pH, temperature, salinity, dissolved oxygen, water brightness, as well as Average Daily Growth (ADG), and Feed Conversion Rate (FCR). The data were analyzed through ANOVA followed by a 5% LSD test, and correlation analysis was also conducted. The results showed that *Trichoderma* either alone or in combination with the probiotic bacteria and *Lactobacillus* spp., increased the pH, temperature, salinity, dissolved oxygen, and water brightness as well as shrimp's daily growth and feed utilization efficiency. ADG value has a positive correlation with the average increase in pH and water clarity. Meanwhile, FCR correlated with an elevation in ADG and pH, as well as a decrease in water brightness.

1 Introduction

Ensuring food security and promoting export potential have become essential factors in today's global market. These factors have led to an increased focus on quality food production, with shrimp being one of the commodities relied upon to achieve these goals. However, shrimp cultivation practices require careful attention to achieve optimal yield; therefore, it is necessary to implement technology that can increase productivity in cultivation but is efficient and environmentally friendly. One of the opportunities for this is the use of effective microbes that can increase the productivity and health of cultivated shrimp.

* Corresponding author : sutarman@umsida.ac.id

The use of probiotics in shrimp cultivation has emerged as a promising approach to enhancing harvest achievement. Certain probiotic microbes, particularly *Lactobacillus* and *Bacillus* spp., have been found to be effective in controlling diseases caused by *Vibrio* bacteria and other pathogenic microbes. Moreover, probiotics can encourage the development of indigenous microbes and beneficial plankton [1], ultimately helping to stabilize the pool ecosystem [2]. Probiotic inhibit and reduce the opportunity for pathogens to parasitize the host [3-4], as well as enhance the host's physiological resistance [5], thereby producing peroxidase enzymes and controlling pH in host digestion [6-7]. However, the success of shrimp cultivation is strongly determined by the dynamics of the physical environment of pool water, influenced by factors such as pH, temperature, salinity, dissolved oxygen, and plankton's potential uniqueness [8]. Physical changes often occur due to dynamics in the atmosphere around the pools and activities of organisms, abiotic elements, and their interactions [9].

Probiotic application in vannamei shrimp cultivation poses a challenge in terms of dependence on using effective bacteria types [10-11]. These bacteria types are mainly employed to suppress pathogenic bacteria, facilitate organic matter mineralization, and promote ecosystem stability [12]. However, their effectiveness in ensuring successful shrimp cultivation is not guaranteed [13]. This can be attributed to the complex dynamics within a pool environment capable of hindering probiotic bacteria activity and their ability to overcome all disturbances [14]. Another possible cause includes the ineffectiveness of some probiotic bacteria strains in degrading chitin-rich shrimp molting waste, which can accumulate in pools. To address this issue, Trichoderma fungi have been proposed as an alternative solution.

Trichoderma, a genus of fungi known for the ability to produce chitinase enzymes, has shown effectiveness in controlling various types of pathogens [15] by damaging fungi cell walls composed of chitin. Additionally, it produces antimetabolite compounds that inhibit pathogens [16] and has been used as a biofertilizing agent in agriculture due to being capable of degrading organic matter to provide nutrition for plants [17-18]. There is a need to test Trichoderma's application as a biological agent for enhancing performance of indigenous microbes in a shrimp aquaculture pool and probiotic based on a bacteria consortium and the growth of plankton. This will help gain insight into its ability to improve pool health and shrimp productivity, as well as reduce the use of artificial feed. Therefore, this study aimed to determine the effect of Trichoderma on improving microbial performance in influencing the physical characteristics of pool water as a growth medium. In addition, the response of vannamei shrimps to the activity of infested probiotic microbes also needs to be studied.

2 Research method

2.1 Pool preparation

The first step in this study involved preparing a round tarpaulin pool with a diameter of 4 m and a height of 1.2 m (Figure 1). The pool was equipped with an air aerator that had a voltage of 35 watts, a maximum pressure of 30 kPa, and a maximum air output of 3,000 L.hour⁻¹, along with an air supply hose and a sewage system. Water from the nearby reservoir was then introduced into the experimental pool, filling it up to a height of 100 cm. Next, saponin was added to the pool water, stirred to a concentration of 10 ppm, and incubated for 3x24 hours. This treatment was mainly carried out by pool farmers on the Sidoarjo coast and its surroundings. Factually, saponins do not kill all pool water microbes but can suppress pathogenic bacteria, specifically *Vibrio*, to an allowable population density, namely $\leq 10^3$ CFU.mL⁻¹ for *Vibrio*. In preparing the pool, the water's physical condition was always

maintained at pH 7-8, 10% salinity, and 10 ppm oxygen dissolved. The decrease in pH is balanced by adding 1 g/liter of CaCO₃. Table 1 shows the biological conditions of pathogenic microorganisms and total plankton in the pool water before the release of shrimp seeds and the application of probiotic.

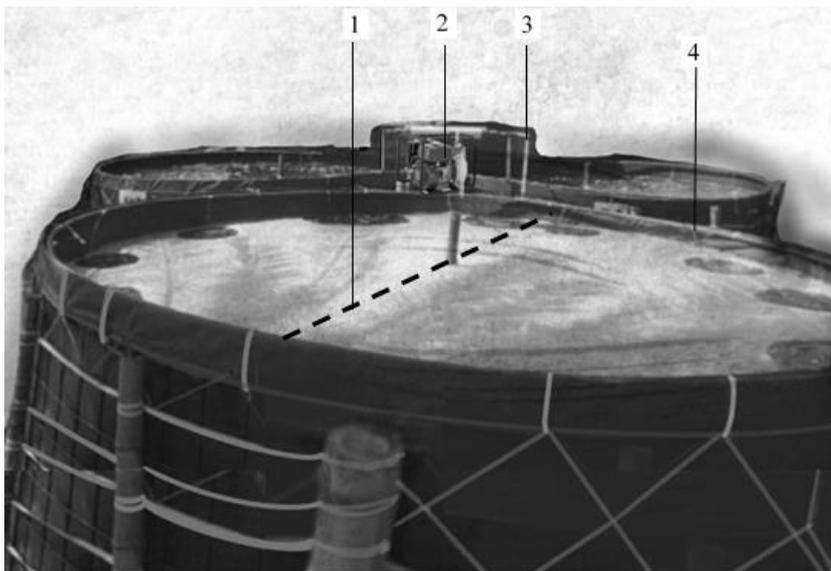


Fig. 1. Tarpaulin pool. 1: 4 m pool diameter, 2: air inflator, 3: air exhaust branch pipe, 4: poolside circular pipe having air outlets spaced 78-80 cm

Table 1. The average density of pathogenic microbes and plankton in pool water before the treatment application and release of shrimp seeds

No.	Parameters	Units	Test Result	Remarks
1	Total <i>Vibrio</i>	CFU.mL ⁻¹	2.0x 10 ¹	Maximum 10 ³ [19]
2	Total <i>E. coli</i> & <i>Coliform</i>	CFU.mL ⁻¹	1.6 x 10 ¹	
3	Total <i>Salmonella</i>	CFU.mL ⁻¹	0	
4	Plankton	Individu.mL ⁻¹	4.3x10 ⁴	5 Types (*)

*Consists of *Anabaena* sp., *Coalestrum* sp., *Gyrosigma* sp., *Tetraedron* sp., and *Nitzschia* sp.

Probiotic required for the experiment were prepared in the form of a liquid formulation containing *Lactobacillus* probiotic and bacteria consortium. Additionally, a prototype probiotic fungus used consisted of *T. asperellum* Tc-07 and *Trichoderma* sp. Tc-25, which is a biological agent in the collection of the UMSIDA Microbiology and Biotechnology Laboratory. Probiotic *Lactobacillus* spp. consisted of two bacteria isolates, while bacteria consortium comprised *Nitrosomonas* sp., *Pseudomonas* sp., *Lactobacillus* sp., *Azotobacter* sp., and *Azotosporulina* isolates.

In the formulation, probiotic *Lactobacillus* spp. with a density of 10⁷ CFU.mL⁻¹ was added on the fourth day until it reached 0.5x10⁵ CFU.mL⁻¹. Three days after the application, shrimp seeds which had been acclimatized for 24 hours to the conditions of the tarpaulin pool water, were stocked. Up to 5,000 shrimp fries with an average size of 1.5 cm were placed in a pool with a water volume of 12.56 m³, while the feed given was gradually adjusted to their weight. The physical conditions of the pool water such as pH, temperature, salinity, dissolved oxygen, and pool water brightness were measured daily, namely in the morning (7.00 AM) and evening (5.00 PM) before feeding.

Six hours after stocking the fry, probiotic was given according to the treatment arranged in a CRD consisting of *Lactobacillus* spp., probiotic bacteria, Trichoderma, Trichoderma-*Lactobacillus* spp., and Trichoderma-probiotic bacteria. All probiotic bacteria were given a single formula of 50 ml or 25 ml, each of which was mixed with other formulas. For Trichoderma fungi, 400 g was given as a single formula and 200 g as a mixture with bacteria. The total amount of bacteria in each pool water was 10^4 CFU.mL⁻¹, while Trichoderma as single and mixed formulas was 0.5×10^4 CFU.mL⁻¹ and 2.5×10^3 CFU.mL⁻¹, respectively. The probiotic was applied with the same cell density every week.

2.2 Observational variables

The variables observed were: (i) the physical characteristics of the pool at 30 and 40 days after stocking (DAS) shrimp seeds, and (ii) the life response of shrimp measured by their Average Daily Growth (ADG) (g.day⁻¹) and Feed Conversion Rate (FCR), each of which was calculated using formulas 1-2 presented below. The physical parameters measured every morning and evening were pH, temperature, salinity, dissolved oxygen, and pool water brightness. Observations were made 30 DAS shrimp seeds which was the first critical phase in vannamei shrimp cultivation. The last observation was conducted at the age of 42 DAS to ensure consistency of changes in the physical environmental conditions of the pool and shrimp's response to the application of various probiotic microbes and Trichoderma consortium.

$$ADG = \frac{w_t - w_o}{t} \quad (1)$$

Supposing w_o represents the weight measured during the initial sampling, w_t was the weight obtained during subsequent sampling at a particular time, and t = the time between the two samplings.

$$FCR = \frac{\text{the total weight of the food given}}{\text{Shrimp biomass}} \quad (2)$$

2.1 Experimental design and statistical analysis

The field experiment was arranged in a Randomized Block Design (RBD) with five treatments repeated three times. All the physical parameters of the pool were carefully observed in each experimental unit. The data collected were subjected to ANOVA at 5% and 1% levels of significance. To determine the difference between the treatments, the analysis was continued with a 5% LSD test. The entire physical parameter data as well as ADG and FCR values were analyzed to investigate the correlation between parameters.

3 Results and discussion

3.1 Physical characteristics of the pool water

The application of Trichoderma in complementing probiotic performance showed a very significant ($p < 0.01$) effect on pH, temperature, salinity, dissolved oxygen, and pool water brightness on observations at 30 and 42 DAS. The average physical component of each treatment is shown in Tables 2-3.

Table 2. Effect of application of probiotic microbes on several physical variables of water in pool at 30 DAS

Treatment (Various Probiotic Microbes)	Pool physical variables				
	pH	Temperature (°C)	Salinity (%)	Temperature (°C)	Dissolved oxygen (ppm)
Trichoderma	7.48±0.01 ^{ab}	29.14±0.04 ^c	9.23±0.21 ^a	51.67±14.92 ^{ab}	7.58±0.03 ^{ab}
<i>Lactobacillus</i> spp.	7.42±0.25 ^b	30.30±0.48 ^a	8.67±0.76 ^{ab}	10.67± 1.15 ^c	7.48±0.12 ^{bc}
Bacteria Probiotic	7.55±0.07 ^{ab}	29.12±0.25 ^c	8.95±0.22 ^{ab}	35.10± 3.41 ^b	7.64±0.08 ^a
Trichoderma- <i>Lactobacillus</i> spp	7.59±0.15 ^a	29.54±0.26 ^b	8.64±0.59 ^b	43.51±15.60 ^b	7.54±0.03 ^b
Trichoderma-Bacteria Probiotic	7.56±0.10 ^{ab}	29.14±0.43 ^c	8.67±0.56 ^{ab}	62.47± 2.48 ^a	7.35±0.02 ^c

Numbers followed by different superscripts in the same column indicate significantly different; LSD values = 0.17, 0.35, 0.58, 12.59, and 0.09

Table 3. Effect of application of probiotic microbes on several physical variables of water in pool at 42 DAS

Treatment (Various Probiotic Microbes)	Pool physical variables				
	pH	Temperature (°C)	pH	Temperature (°C)	pH
Trichoderma	7.31±0.06 ^b	30.42±0.04 ^b	9.36±0.14 ^a	14.05±3.96 ^a	6.71±0.17 ^b
<i>Lactobacillus</i> spp.	7.36±0.02 ^b	30.92±0.54 ^a	8.23±0.94 ^b	10.48±0.23 ^b	6.75±0.11 ^{ab}
Bacteria Probiotic	8.49±1.16 ^a	30.49±0.24 ^b	8.86±0.26 ^{ab}	13.54±3.80 ^{ab}	6.77±0.08 ^{ab}
Trichoderma- <i>Lactobacillus</i> spp	7.29±0.01 ^b	30.68±0.21 ^{ab}	8.72±0.68 ^{ab}	14.17±3.41 ^a	7.09±0.56 ^a
Trichoderma-Bacteria Probiotic	7.28±0.01 ^b	30.64±0.14 ^{ab}	9.27±0.02 ^a	12.76±0.21 ^{ab}	6.43±0.16 ^b

Numbers followed by different superscripts in the same column indicate significantly different; LSD values = 0.67, 0.36, 0.69, 3.72 and 0.35

3.2 Shrimp growth response

The biological response of shrimp in the form of ADG and FCR values at 30 and 42 DAS differed significantly ($p < 0.01$) based on the differences in microbial applications. The combination of Trichoderma and Lactobacillus consortium showed the highest average ADG and FCR as indicated in Table 4-5.

Table 4. Effect of probiotic microbial application on ADG and FCR values of Vanami Shrimp growth at 30 ASD

Treatment (Various probiotic microbes)	Shrimp growth response	
	ADG (g.d ⁻¹)	FCR
Trichoderma	0.063±0.003 ^{ab}	1.21±0.65 ^{ab}
<i>Lactobacillus</i> spp.	0.052±0.013 ^b	1.98±0.99 ^b
Bacteria probiotics	0.072±0.022 ^{ab}	2.35±0.89 ^{ab}
Trichoderma- <i>Lactobacillus</i> spp	0.074±0.012 ^a	1.22±0.20 ^a
Trichoderma- Bacteria probiotics	0.069±0.005 ^{ab}	1.63±0.14 ^{ab}

Numbers followed by different letters in the same pool indicate different effects; LSD values = 0.016 and 0.015

Table 5. Effect of probiotic microbial application on ADG and FCR values of Vanami Shrimp growth at 42 ASD

Treatment (Various probiotic microbes)	Shrimp growth response	
	ADG (g.d ⁻¹)	FCR
Trichoderma	0.115±0.017 ^{ab}	0.68±0.26 ^{ab}
<i>Lactobacillus</i> spp.	0.113±0.010 ^b	0.46±0.05 ^b
Bacteria probiotics	0.118±0.016 ^{ab}	0.60±0.08 ^{ab}
Trichoderma- <i>Lactobacillus</i> spp	0.121±0.010 ^a	0.45±0.20 ^a
Trichoderma- Bacteria probiotics	0.119±0.004 ^{ab}	0.37±0.03 ^{ab}

Numbers followed by different letters in the same pool indicate different effects; LSD values = 0.86 and 0.16

3.3 Correlation between physical and biological components

The analysis results of all physical and biological parameters showed a correlation matrix as presented in Tables 6 and 7, respectively, for the 30 and 42 DAS conditions.

Table 6. Correlation between pool water physical components and vannamei shrimp growth indicators at 30 DAS

	pH	Temperature	Salinity	Dissolved Oxygen	Water Brightness	ADG	FCR
pH	1						
Temperature	-0.215	1					
Salinity	-0.648	-0.091	1				
Dissolved Oxygen	0.055	0.144	0.151	1			
Water Brightness	0.223	-0.683	0.174	-0.114	1		
ADG	0.524	-0.271	-0.224	0.286	0.477	1	
FCR	-0.418	-0.082	0.276	-0.129	-0.324	-0.568	1

Table 7. Correlation between pool water physical components and vannamei shrimp growth indicators at 42 DAS

	pH	Temperature	Salinity	Dissolved Oxygen	Water Brightness	ADG	FCR
pH	1						
Temperature	-0.215	1					
Salinity	-0.648	-0.091	1				
Dissolved Oxygen	0.223	-0.683	0.174	1			
Water Brightness	0.055	0.144	0.151	-0.114	1		
ADG	0.357	-0.051	-0.147	0.318	0.121	1	
FCR	-0.168	-0.226	0.233	-0.096	0.475	-0.589	1

3.4 Discussion

Based on Table 2, the application of Trichoderma either alone or in combination with Lactobacillus consortium and probiotic bacteria generated a higher mean pool pH at 30 DAS compared to treatments without Trichoderma. However, the highest achievement was obtained in probiotic bacteria treatment at the end of the observation period. During the first critical phase of shrimp growth or 30 DAS, Trichoderma activity was found to affect the degradation of organic matter in the pool water. The increase in water pH in the system may be due to the microbial degradation of organic matter [20]. Trichoderma can quickly use its environmental resources to obtain energy and control space [21]. This fungi degrades organic matter to produce nutrients [15] and metabolites that act as growth hormones for plants [22], and also maintain plants' health [23] and resistance against abiotic environmental stress [24].

In this experiment, Trichoderma was used to support the growth of phytoplankton in pool water. Changes in pH were negatively correlated with changes in salinity, namely -0648, as presented in Tables 6-7. After 30 DAS probiotic bacteria consortium indicated increased biological activity in degrading organic matter, leading to a higher elevation in pH. Along with the activity of Trichoderma, various minerals were produced, which contributed to an increase in salinity. Until 42 DAS, Trichoderma treatments, either alone or in combination with Lactobacillus and probiotic bacteria, yielded the highest mean pool water salinity as presented in Table 3. During the decomposition process, each aerobic organism requires more oxygen [25-26], leading to the mineralization of organic matter, which increases dissolved salts. According to Table 2-3, the activity of Trichoderma and Lactobacillus triggered an increase in salinity. However, in the combination of Trichoderma and probiotic bacteria, dissolved oxygen was at the highest level at the end of the observation period [27], leading to an increase in the solubility of this molecule. It seems that the microbes in the combination of both consortia have shown their optimal role in promoting the growth of pool water phytoplankton, contributing to an increase in dissolved oxygen. Photosynthesis in aquatic plants absorbs CO₂, which can capture heat and produce oxygen [28], leading to an increase leads to an increase in the solubility of this molecule in pool water.

Lactobacillus spp. triggered the highest average pool water temperature compared to treatments as indicated in Table 2-3. The combination of these bacteria with Trichoderma consortium potentially contributed to an increase in temperature and salinity, specifically at 30 DAS. Lactobacillus plays a role in the aquatic environment by influencing the breakdown of organic matter to produce substances that absorb higher environmental temperatures, and increasing shrimp weight [29]. Application of probiotic bacteria and Trichoderma showed the highest brightness value in the first critical phase of shrimp life. This is a physical condition representing the activity of both microbes in degrading organic matter, and the increase in overhaul activity will reduce the average condition of organic matter in pool water. Furthermore, the collection of the particles can block light penetration into the pool water depth. This pattern remains consistent across 42 watersheds, indicating that the role of Trichoderma and its combination with other probiotic consortia is constant in the process of decomposing organic matter, including plankton biomass, dead microbes, molted shells, and shrimp feces.

Table 6-7 shows that the daily growth of shrimp is consistent with the changes in pool water resulting from the activity of Trichoderma and other bacteria consortia. The increase in water brightness, which reflects a higher decomposition intensity, is particularly observed in the presence of Trichoderma. Similarly, Application of Trichoderma and its respective combination with Lactobacillus and probiotic bacteria consortia produced a high ADG value and improved feeding efficiency as evidenced by a higher FCR value. However, the correlation between all the pool physical factors and AD/FCR values in response to the activity of probiotic microbes and Trichoderma consortium was partly weak. This suggests

that the response of shrimp to probiotic microbial activity up to the 42 DAS period does not correlate strongly with the physical environment. Based on Table 6, the level of ADG correlation to physical factors sequentially from strongest to weakest was 0.524 (pH), 0.477 (water brightness), 0.286 (dissolved oxygen), -0.271 (temperature), and -0.224 (salinity) at 30 DAS. This similar pattern is found after 42 DAS but at a lower correlation level as presented in Table 7. Besides the direct effects of physical factors on shrimp life, indirect factors contributed by various organisms growing in pool water, in form of plankton that are beneficial or not, may affect shrimp growth. This shows that the biological relationship between organisms appears to be stronger than the relationship between hosts and physical factors [30]. Most plankton types in the experimental pool were of little use to shrimp. Meanwhile, some species such as *Coelastrum* sp., *Anabaena* sp., *Gyrosigma* sp., *Tetraedron* sp., and *Nitzschia* sp. were found to be ecologically important for ecosystem stability [31-32], despite not being beneficial to shrimp growth [33,34].

Trichoderma treatment yielded a high ADG value, meaning that the total activity of this fungi both alone and in combination with the tested bacteria consortium provided essential minerals and suitable conditions for shrimp. Moreover, FCR value indicates the conversion of feed into shrimp biomass [35]. In this case, a high FCR value demonstrates that Trichoderma has facilitated the life activities of probiotic bacteria, leading to an increased lifespan of shrimp natural feed organisms.

4 Conclusion and recommendation

The application of Trichoderma consortium, either alone or in combination with the consortium of probiotic bacteria and *Lactobacillus* spp., showed an increase in pH, temperature, salinity, dissolved oxygen, and pool water brightness, as well as daily growth in vannamei shrimp culture. Fungi and bacteria consortia application led to a reduction in feed consumption per gram of shrimp biomass weight. Shrimp growth as indicated by ADG value was positively correlated with an average increase in pH and water brightness at 30 days after stocking (DAS), while the correlation level decreased at 42 DAS. Furthermore, FCR value showed a decreased feed utilization efficiency for increasing shrimp biomass, correlating with an elevation in ADG and pH, as well as a decrease in water brightness at 30 DAS. This pattern persisted at 42 DAS, but with a lower correlation level.

The results of this research can be one of the considerations in the development of a bacteria-based probiotic consortium by utilizing potential indigenous Trichoderma fungi in field testing and application in Vanamei shrimp cultivation.

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