

Application of Commercial Probiotics to Control *Vibrio* sp. in Whiteleg Shrimp (*Litopenaeus vannamei*) Culture at PT Sumber Agromina Jaya

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ABSTRACT

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Whiteleg shrimp (*Litopenaeus vannamei*) is a leading aquaculture commodity with high economic value and can be farmed intensively with high stocking density. Intensive farming systems have the potential to increase the accumulation of organic matter and metabolic waste, which can trigger the growth of pathogenic bacteria such as *Vibrio* sp. The presence of these bacteria can cause health disturbances in shrimp and reduce survival rates. The purpose of this study is to determine and test the effectiveness of probiotics in suppressing the population of *Vibrio* sp. in whiteleg shrimp (*Litopenaeus vannamei*) farming. Data collection methods were carried out primarily and secondarily through direct observation, interviews, active participation in the field, documentation, and literature studies. Control techniques were performed through several stages, namely pond preparation, probiotic culture using molasses and skim milk, mixing probiotics into feed, application of probiotics to ponds. as well as routine monitoring of water quality. The observed parameters include nitrate (NO₃), nitrite (NO₂), ammonium (NH₄), phosphate (PO₄), alkalinity, Total Organic Matter (TOM), and the total population of *Vibrio* sp. The results of the study showed that the application of commercial probiotics was effective in suppressing the growth of *Vibrio* sp. with a population density of 10³-10⁴ CFU/ml during cultivation through the mechanisms of nutrient competition and the production of antibacterial compounds, as well as helping to maintain the stability of pond water quality within the optimal range for shrimp growth.

INTRODUCTION

Whiteleg shrimp (*Litopenaeus vannamei*) is a fishery commodity that has high economic value in both domestic and international markets, due to its fast growth rate, relatively high survival rate, and efficient feed utilization. According to Hadi *et al.* (2018), which states that this species has the ability to live in coastal and marine areas throughout its life cycle. In

addition to its wide distribution area, this species is also able to survive in areas with exposure to rainfall and evaporation cycles throughout the year which will affect fluctuations in salinity and water temperature. The challenge or problem often faced by farmers is the disease that often attacks the whiteleg shrimp population which can cause mass mortality and drastically reduce harvest yields (Sekar *et al.*, 2019).

One of the causes of unsuccessful cultivation is the attack of a disease that attacks shrimp, namely vibrio. *Vibrio* attacks on whiteleg shrimp cultivation are generally triggered by poor water quality, high stocking density, accumulation of organic waste from feed, and decreased shrimp immunity due to stress or double infection with other viruses (Suprakto *et al.*, 2024). According to Rusadi *et al.* (2019), which states that this bacterium is opportunistic and highly virulent so that it can cause mass mortality of up to 80% in a short time. Management efforts that can be done include maintaining environmental quality through water management, siphoning the bottom of the pond, and the use of probiotics such as (*Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp.) are more effective in suppressing the growth of pathogenic bacteria.

The use of probiotics in shrimp cultivation, particularly whiteleg shrimp, can improve growth through improved fermentation. Probiotics are live microorganisms that, when consumed in sufficient quantities, function to produce lactic acid, digestive enzymes, and antimicrobial compounds to balance the intestinal microbiota or water of whiteleg i shrimp ponds and suppress the pathogen *Vibrio* sp. Probiotics in fermented feed improve nutrient digestibility, suppress pathogens, and feed conversion efficiency, thus supporting optimal growth of whiteleg shrimp. The use of probiotics in aquaculture aims to improve the quality of the aquatic environment through the biodegradation process and control microbes in the water and digestive tract.

Probiotics are used in shrimp cultivation, especially vannamei, to improve growth, immunity, and water quality. Their use involves activation and administration through feed or pond water at specific doses. According to Dewi *et al.* (2023), which shows that probiotics are effective in increasing growth by improving water quality and suppressing pathogens. Meanwhile, the use of antibiotics should be avoided because they have the potential to cause resistance, pollute the environment, and endanger consumers (Suprakto *et al.*, 2024). Therefore, it is important to carry out this reseach so that students can be able to carry out *Vibrio* sp. population control techniques using commercial probiotics in whiteleg shrimp (*Litopenaeus vannamei*) cultivation at PT Sumber Agromina Jaya.

METHODS

This research was conducted from October 10 to November 7, 2025, at PT Sumber Agromina Jaya, North Lombok Regency. The methods used in compiling this report include direct observation, active participation in cultivation activities, interviews with field technicians, and literature studies as supporting data. The data obtained were then analyzed descriptively and comparatively to compare the dynamics of water quality and bacterial populations between the two ponds. The stages of cultivation activities observed include pond preparation, sterilization and initial fertilization, feed management, probiotic application, and monitoring of water quality and shrimp health. *Vibrio* sp. population control was carried out through the application of commercial probiotics fermented using molasses.

Water quality observations were carried out routinely on DOC 56, 63, 70, 77, and 84 in two different ponds, namely F8 and F9. The parameters analyzed included Total Organic

Matter (TOM), total alkalinity, nitrite (NO₂), nitrate (NO₃), ammonium (NH₄), phosphate (PO₄). Air quality parameters were measured periodically in each research pond. TOM parameters were determined using the permanganometry method, alkalinity using the acid-base titration method, nitrite (NO₂) using the diazotization method, nitrate (NO₃) using the cadmium reduction method, ammonium (NH₄) using the Nessler method, and phosphate (PO₄) using the ascorbic acid method. Total bacterial population (TBC) and total *Vibrio* sp. (yellow *Vibrio* and green *Vibrio*). The bacterial measurement method that has been carried out is by counting *Vibrio* bacteria starting from taking pond water samples and then planting them using TCBS (Thiosulfate Citrate Bile Salts Sucrose Agar) media.

RESULTS

The results of this study include pond preparation, probiotic application, and monitoring of water quality and shrimp health. *Vibrio* sp. population control is carried out through the application of commercial probiotics fermented using molasses and skim milk before being applied to the pond and mixed into the feed. Water quality observations are carried out routinely on DOC 56, 63, 70, 77, and 84 in two different ponds, namely F8 and F9. The parameters analyzed include Total Organic Matter (TOM), total alkalinity, nitrite (NO₂), nitrate (NO₃), ammonium (NH₄), phosphate (PO₄), as well as total bacterial population (TBC) and total *Vibrio* sp. (yellow *Vibrio* and green *Vibrio*).

a. Initial Preparation

Based on the activities carried out in whiteleg shrimp cultivation at PT Sumber Agromina Jaya, the total area is 50 m × 50 m, so the total pond area is: pond area = 50 m × 50 m = 2,500 m². The pond is lined with High Density Polyethylene (HDPE). Initial preparation begins with probiotic culture. The probiotic used is a commercial probiotic, Biomin GH.



Figure 1. Biomin GH

b. Water Quality Parameters

The results of the water quality parameters in this study can be seen in Table 1.

Table 1. Water Quality Parameters for Ponds F8 and F9

DOC	Ponds	TOM (mg/L)	Alkalinity (mg/L)	NO ₂ (mg/L)	NO ₃ (mg/L)	NH ₄ (mg/L)	PO ₄ (mg/L)
56	F8	65	136	0	4	1	0
56	F9	65	136	0	4	0	0
63	F8	74	140	0	5	1	1

DOC	Ponds	TOM (mg/L)	Alkalinity (mg/L)	NO ₂ (mg/L)	NO ₃ (mg/L)	NH ₄ (mg/L)	PO ₄ (mg/L)
63	F9	65	140	0	5	0	1
70	F8	72	144	0	8	0	0
70	F9	70	140	0	7	0	1
77	F8	57	144	0	8	2	1
77	F9	51	144	0	13	1	1
84	F8	66	140	0	3	1	1
84	F9	79	136	0	5	1	1

Based on the results of table 1 nitrate, nitrite, NH₄, and PO₄ in sample ponds F8 & F9, the NO₂ value is very low (approaching 0 mg/L), the highest NO₃, in pond F9 DOC 77 = 13 mg/L, followed by NH₄ with a range of 0-1 mg/L and relatively low PO₄ in DOC 56–84, with F9 generally having a slightly higher NO₃ and NH₄ peak than F8 so that it tends to be more at risk of causing stress if not controlled.

c. *Vibrio* sp. Population Calculation

Vibrio bacterial counts begin with pond water sampling and are then cultured on TCBS (Thiosulfate Citrate Bile Salts Sucrose Agar) media. The method used to calculate the abundance of *Vibrio* sp. bacteria is by observing bacterial colonies in petri dishes, using the TVC (Total *Vibrio* Count) method. Samples taken from aquaculture ponds or ponds for shrimp and other organisms are cultured on agar media. TCBS (Thiosulfate Citrate Bile Salt Sucrose Agar). *Vibrio* and Total Bacteria (TB) populations can be seen in Table 2.

Table 2. *Vibrio* and Total Bacteria (TB) Populations

DOC	Ponds	Yellow <i>Vibrio</i> (CFU/mL)	Green <i>Vibrio</i> (CFU/mL)	TBC (CFU/mL)
56	F8	830	0	230,000
56	F9	0	0	1,690,000
63	F8	60	0	290,000
63	F9	0	0	10,000
70	F8	420	370	1,020,000
70	F9	2040	10	1,640,000
77	F8	80	60	1,420,000
77	F9	250	240	70,000
84	F8	550	90	1,020,000
84	F9	1,860	0	1,120,000

DISCUSSION

Probiotics are microorganisms that have a positive effect on shrimp cultivation. Probiotics are used to overcome pathogenic bacteria including *Vibrio* sp. and improve water quality. The probiotic used is a commercial probiotic, namely Biomin GH. Biomin GH, contains a combination of beneficial bacteria such as (*Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., and *Pediococcus* sp.) This strain works synergistically to inhibit pathogens such as *Vibrio* sp., increase survival, and improve digestion of aquatic animals.

Biomin GH is a probiotic that helps increase shrimp growth and endurance by improving water quality, reducing the accumulation of organic waste, and inhibiting the growth of pathogenic microorganisms. This is in line with the opinion of Firmansyah (2024), who stated

that the use of Biomin GH as a probiotic in shrimp cultivation can improve the quality of the rearing environment by reducing toxic ammonia and nitrite levels, thereby creating healthy pond conditions and supporting optimal shrimp growth.

Bacillus sp. in the Biomin GH probiotic content produces digestive enzymes such as amylase, protease, and lipase to break down nutrients and ammonia/nitrite while controlling the pathogen *Vibrio* sp., while *Lactobacillus* sp. functions as a biocontrol agent that helps stimulate the natural immunity of shrimp so that shrimp become more resistant to disease, *Enterococcus* sp. inhibits gram-negative pathogens and increases immunity and stress resistance, and *Pediococcus* sp. produces lactic acid for space competition, stabilizes intestinal pH, and supports optimal growth. This probiotic also plays a role in maintaining the balance of microbes in the cultivation media that compete with pathogenic bacteria. According to research from Samadan *et al.* (2023), which states the positive effects of probiotic use in aquaculture include boosting the immune system by improving water quality and controlling pathogenic microbes. This supports the growth of shrimp raised with regular probiotic use.

The bacterial culture process at PT Sumber Agromina Jaya uses Biomin GH probiotics through a fermentation process. The fermentation process is carried out using a dynamo and a mixer that functions as a mechanical stirrer to ensure homogeneity of the mixing of nutrients and probiotics evenly, which is very important for the growth of bacteria such as (*Bacillus* sp., *Pediococcus* sp., *Enterococcus* sp., and *Lactobacillus* sp.) contained in Biomin GH. Research from Imanan *et al.* (2025), which states that the stirring tool (molen) and pH meter are the most influential in bacterial culture because they ensure the media remains homogeneous and the environmental conditions are optimal for the activity of probiotic bacteria. Internal pumps and water hoses facilitate water circulation and oxygenation, which support the metabolism of probiotic microbes. According to Maulida (2022), it shows that the use of internal pumps and water hoses to stir the fermentation media so that nutrients are evenly distributed and particles do not settle, increasing the efficiency of probiotic floc formation.

Biomin GH as a bacterial starter such as (*Bacillus* sp., *Pediococcus* sp., *Enterococcus* sp., and *Lactobacillus* sp.) is mixed with molasses, skim milk, and brackish water as culture material. Brackish water is a mixture of freshwater and seawater with a salinity range of 5-7 ppt, which is often used in Biomin GH probiotic cultures. The salinity and pH of brackish water are within the appropriate range for probiotic activity and the growth of whiteleg shrimp, namely a pH of around 7.5-8.7 and a salinity of 5-7 ppt, which are supporting factors for the growth of bacteria as probiotic agents. Brackish water is chosen for bacterial culture because its salinity and mineral composition support the growth of probiotic microorganisms that improve water quality and directly support shrimp health and production. This is in line with research by Dewi *et al.* (2023), which states that probiotics in brackish water media can reduce levels of toxic substances such as ammonia and help form stable biofilms that are beneficial for the microbial ecosystem of cultivation.

Based on the results of table 1, nitrate, nitrite, NH_4 , and PO_4 in sample ponds F8 & F9 show a similar pattern, namely very low NO_2 values (approaching 0 mg/L), the highest NO_3 , in pond F9 DOC 77 = 13 mg/L, followed by NH_4 with a range of 0-1 mg/L and relatively low-moderate PO_4 in DOC 56–84, with F9 generally having slightly higher NO_3 and NH_4 peaks than F8 so that it tends to be more at risk of causing stress if not controlled. This is in line with the opinion of Lusiana *et al.* (2021) who stated that the optimal value of ammonium is 0.5 mg/L. According to Prastiwi *et al.* (2025), it was determined that the ideal ammonium value for whiteleg shrimp is less than 0.1 mg/L, which indicates that higher ammonium levels can potentially harm shrimp health.

The NO₂ value in this data is still in the safe range because it is far below 1 mg/L, the graph results show 0 mg/L. According to Prastiwi *et al.* (2025), the optimal nitrite value for whiteleg shrimp is less than 0.5 mg/L. Nitrite (NO₂) is a breakdown product in the nitrification process, namely the conversion of ammonia (NH₃) to nitrate (NO₃), which is toxic to shrimp. The safe nitrite threshold in waters is no more than 1 mg/L.

The measurement results showed that the NO₃ value in F9 reached 13 mg/L in DOC 77, still below the range of 20–30 mg/L reported to be tolerable for whiteleg shrimp. This is in line with the opinion of Makmue *et al.* (2019), who stated that the optimal nitrite value for whiteleg shrimp is 50 mg/L. According to the opinion of Agrianti *et al.* (2022), who stated that the increase in nitrate levels was caused by the entry of domestic or agricultural waste (fertilization) which generally contains a lot of nitrates. The explosion of plankton and other microorganisms in whiteleg shrimp ponds was triggered by high nitrate levels.

The PO₄ values in F8 and F9 data are below approximately 1 mg/L, still considered normal but need to be maintained so as not to increase, as explained by the opinion of Claude & Boyd (2020), that excess phosphate can trigger eutrophication and oxygen instability. The composition of dissolved nutrients such as the values in the graph, the use of probiotics as probiotics remains important to suppress the accumulation of organic matter, reduce ammonia and nitrite, and inhibit *Vibrio* through competition and production of antimicrobial compounds, as reported by Thompson *et al.* (2022 and Lakshmi *et al.* (2013), stating that the antagonistic activity of lactic acid bacteria against shrimp pathogenic *Vibrio*.

Alkalinity in table 1 of ponds F8 and F9 shows a range of values of 136–144 mg/L with the lowest value of 136 mg/L. In DOC 77 with the highest value of 144 mg/L, so both are still within the normal ideal range of alkalinity of vannamei ponds. According to the opinion of Suwoyo *et al.* (2018), which states that the optimal alkalinity value for shrimp ponds is recommended to be 100 mg/L or in the range of 120-160 mg/L. According to Hossain *et al.* (2013), which states that ponds given probiotics show a more stable pH (around 7.5–8.8) with alkalinity ranging from around 130 mg/L.

Total Organic Matter gradually increases from the initial DOC of 56-84 with varying values, the highest value in pond F9 at DOC = 79, the optimal TOM range in shrimp ponds is in the range of 40-80 which means organic matter such as leftover feed, feces, and biofloc is increasingly accumulating and becoming an excellent food substrate for opportunistic bacteria such as *Vibrio* sp. This is in line with the opinion of Supriatna *et al.* (2020), which states that the maximum limit of organic matter in whiteleg shrimp cultivation is 88.4 mg/l.

During the vaname shrimp cultivation process, TOM values continue to increase due to the accumulation of leftover feed, feces, and dead plankton. Renitasari *et al.* (2021) stated that leftover feed will increase TOM levels. In the F9 TOM graph, the TOM value was initially around 51 and increased to nearly 80 mg/L in DOC 84, then fluctuated but tended to remain high until the end of the cultivation. This pattern is similar to F8, but the TOM peak in F9 is slightly higher and lasts longer.

Regular application of probiotics to ponds or through feed, these beneficial bacteria will compete to utilize the organic matter, producing organic acids and antimicrobial compounds, so that even though the TOM increases, the proportion of dominant bacteria shifts to probiotic microbes and the opportunity for *Vibrio* sp. to reproduce excessively can be suppressed. This is in line with the opinion of Yunarty *et al.* (2024), which states that increasing TOM levels in whiteleg shrimp cultivation media are positively and significantly correlated with the abundance of *Vibrio* sp., meaning that the higher the dissolved and suspended organic matter, the *Vibrio* population also tends to increase. Research by

Amalliyah *et al.* (2025), also confirms that the management of organic matter and the use of probiotics are recommended to suppress the risk of vibriosis because these probiotics are able to reduce the organic load and inhibit the growth of *Vibrio* through competition and the production of antibacterial metabolites.

Based on the results of table 2 of pond F8 and pond F9 above, the presence of vibrio is always present during cultivation, while green vibrio begins to appear in F8 DOC 70 with a value of 360 CFU/mL and F7 DOC 77 = 60 CFU/mL. Probiotic bacteria have a greater number than the number of vibrio so they can suppress the pathogenic effects of vibrio. The application of probiotics in pond F8 and pond F9 appears to be able to inhibit the growth of pathogenic bacteria such as *Vibrio* sp. According to the opinion of Jannah *et al.* (2018), which states that probiotics are able to increase immune parameters (THC, hyaline cells, granule cells) and simultaneously suppress the growth of bacteria or vibrio pathogens so that the density of vibrio in the shrimp intestine and maintenance media becomes lower. These results are the basis that probiotics are effective in suppressing the population of *Vibrio* sp. through a combination of increasing shrimp immunity and microbial competition.

The values in Table 2 of *Vibrio* and bacteria show that in ponds F8 and F9, the density of yellow *Vibrio* (yellow colonies in TCBS) and green *Vibrio* (green colonies) varies from very low (close to 0 colonies/mL at the beginning of DOC) to a peak of around above 103 colonies/mL in the middle-late DOC with the general sequence of F8 having a high *Vibrio* peak starting at DOC 70 with a total of 370 CFU/mL of green vibrio, 420 CFU/mL of yellow V with a TBC value of 1,020,000 CFU/mL, the highest value of yellow vibrio in pond F9 DOC 70 = 2040 CFU/mL. compared to F8 it tends to be lower in DOC 77 with a V Yellow value of 80 CFU/mL. V Green 60 with a TBC value of 1,420,000 CFU/mL, which shows that F9 tends to show less frequent and lower *Vibrio* peaks, while F8 has several high spikes, when compared to the Total Bacteria Count (TBC), it is seen that TBC is always much larger than *Vibrio* so that most of the bacteria in the water are not *Vibrio*, and conditions are considered safer when TBC is high but *Vibrio* is relatively low. Then the application of probiotics to the cultivation pond to reduce the vibrio population.

Vibrio values up to around 104 colonies/mL are still close to the upper limit considered tolerable, but if the peak approaches or exceeds 104 colonies/mL, it is abnormal and carries a risk of vibriosis, especially in ponds such as F9 which have sharper spikes; these spikes are usually related to the accumulation of organic matter, poor feed management, minimal water changes, or water quality (pH, DO, ammonia) that favors *Vibrio* growth. According to the opinion of Tamrin *et al.* (2024) and Winna *et al.* (2024), which stated that some ponds with *Vibrio* 1.0×10^3 cfu/mL have a high risk of vibriosis and mass mortality, while below this threshold is relatively safer. Thus, when the F8 or F9 graph shows a yellow/green *Vibrio* value approaching or exceeding the 10^3 cfu/mL, these conditions are no longer normal and indicate problems such as organic matter buildup, poor feed management, or fluctuations in water quality that favor the growth of pathogenic *Vibrio*. This is in line with Tamrin *et al.* (2024) that high organic loads and declining water quality increase the density of *Vibrio* sp. beyond quality standards and trigger disease outbreaks.

CONCLUSION

Based on the results of research conducted at PT Sumber Agromina Jaya, it can be concluded that the use of Biomin GH probiotics effective in suppressing the population of

Vibrio sp. The population density of *Vibrio* sp. during the study was 10^3 - 10^4 CFU/ml. The water quality in this study was still within normal limits.

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