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MONITORING THE ABUNDANCE OF *Vibrio* sp. BACTERIA IN THE WATER OF VANAME SHRIMP (*Litopanaeus vannamei*) BREEDING POND AT JEPARA BRACKISH WATER AQUACULTURE CENTER

Laela Trianingtyas[1]*, Ufianah[1], NavitaDesy Ratnaningsih[1], Kasprijo[1], Reza Muhammad Riady[1], Ita Rizkiyanti[2], Muhammad Zainuddin[3]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, JenderalSoedirman University, Purwokerto 53122, Central Java, Indonesia
[2]Jepara Brackish Water Aquaculture Center (BBPBAP) 59418,Jepara, Central Java, Indonesia
[3]NahdlatulUlama Islamic University (UNISNU), 59451, Jepara, Central Java, Indonesia

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Correspondence
laela.trianingtyas11@gmail.com

Abstract

The presence of *Vibrio* sp. in the breeding pond is one of the important factors that affect the activities of *L. Vannamei* breeding. *Vibrio* sp. in waters can cause losses if the number is above the threshold that should be. The high abundance of *Vibrio* sp. bacteria in the cultivation environment is an indicator that shows the potential for disease that can reduce the growth rate and survival of shrimp. The purpose of the study was to determine the abundance and dominance of *Vibrio* sp.in *L. vannamei* breeding ponds using the TPC (Total Plate Count) calculation technique. The methods used include bacterial sampling, preparation of tools and materials, making bacterial culture media (NA and TCBS), dilution, bacterial inoculation, and calculating the number of bacterial colonies. The results of the calculation of bacterial abundance showed that there were 9 breeding ponds with different abundances. The highest abundance was found in pond BV1 with a total bacterial value of 9.6 x 10^5 CFU/ml. Meanwhile, the highest vibrio dominance was found in pond BV4 with a percentage of 43.69%.

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Introduction

L. vannamei is one of the most commonly consumed fisheries commodities in Indonesia due to its variety of advantages. There are several advantages of this shrimp species, including the highest growth rate and survival rate, its ability to respond to feed, its relatively high stocking density rates and low susceptibility to disease and stress (Anam*et al*., 2016). Based on this, *L. vannamei* is one of the leading fisheries export commodities (Hadiroseyani*et al*., 2023), where it was recorded that in 2021, the *L. vannamei* cultivation industry managed to export 5.33 million kg (Ministry of Maritime Affairs and Fisheries, 2022). This has driven an increase in the need for quality L. *vannamei*, so to meet it, seeding activities are needed (Lestari *et al*., 2018).

L. vannamei seeding is a series of

processes for caring for newly hatched shrimp seeds until they reach the right size for distribution (Anwar, 2021). Seeding activities are one of the determining factors for the success of *L. vannamei* cultivation activities in supporting maximum productivity (Budi and Aqmal, 2021). In addition, success in implementing the seeding process will also support cultivation efforts in providing quality shrimp seeds (Anita *et al*., 2017). According to Panjaitan*et al*., (2015), three main factors influence seeding activities, including feed, environmental conditions for maintenance, and biota. The presence of biota, especially microorganisms such as bacteria in the maintenance media, is one of the most important factors that need to be considered (Pariakan and Rahim, 2021).

Bacteria are microscopic organisms that can interact with other microorganisms both beneficially and detrimentally (Amrullah*et al*., 2023). According to Hatmanti (2003), one of the most common species of pathogenic bacteria found and causing disease in *L. vannamei* cultivation is *Vibrio* sp. bacteria. The presence of *Vibrio* sp. bacteria in waters can cause losses if the number is above the threshold that should be. The high abundance of *Vibrio* sp. bacteria in the cultivation environment is an indicator that shows the potential for disease that can reduce the growth rate and survival of shrimp (Kharisma and Manan, 2012). Therefore, it is necessary to understand the high abundance and population dynamics of *Vibrio* sp. in the cultivation environment in order to prevent and control disease outbreaks.

Monitoring the abundance of *Vibrio* sp. bacteria in the water of *L. vannamei* hatchery ponds needs to be done to ensure the health and productivity of shrimp seeds. Additionally, monitoring the abundance of *Vibrio* sp. bacteria is essential for maintaining the health of *L. vannamei* in hatcheries. This study aims to determine the abundance and dominance of *Vibrio* sp. bacteria in *L. vannamei*hatchery ponds as one of the monitoring steps for preventing disease in *L. vannamei* seeds.

Materials and methods

Time and Place

This research was conducted from August 2nd – August 23th, 2023 at the Jepara Brackish Water Aquaculture Center (BBPBAP). Inoculation and culture of bacterial samples were carried out in the Microbiology Laboratory of the Center for Brackish Water Fisheries Cultivation of Jepara.

Tools and Material

The tools used include conic, bucket,

scales, test tubes, test tube racks, micropipettes, bunsen, measuring cups, petri dishes, Erlenmeyer flasks, magnetic stirrers, hot plate magnetic stirrers, autoclaves, measuring pipettes, vortexes, incubators, and laminar air flow (LAF).

The materials used include tips, Nutrient Agar (NA), Thiosulfate Citrate-Bile Salts Sucrose (TCBS), NaCl, Magnesium sulfate ($MgSO_4$), Potassium chloride (KCl), aquades, tissue, bacterial samples (water), 96% alcohol.

Methods

Bacterial Sampling

Bacterial sampling was carried out at the vaname shrimp hatchery located in the Bandengan area of BBPBAP Jepara. The samples taken were water samples from several vaname shrimp hatchery ponds with post-larvae stages. Water samples were taken using a bucket inserted into the *L. vannamei* hatchery pond. Then the water sample was put into a 30 ml conical tube, the sample was then taken to the BBPBAP Jeparamicrobiology testing laboratory.

Bacterial Culture Media Production

The preparation of bacterial culture media consists of making media for all types of bacteria, namely Nutrient Agar (NA) and Thiosulfate Citrate Bile Salts Sucrose (TCBS) as selective media for *Vibrio* sp. The process of making media begins with weighing according to the concentration which is then added with trisalt (a solution of 3 salts), namely NaCl, $MgSO_4$, and KCl, and distilled water. After that, heat and homogenize the media on a hot plate to be heated until dissolved and boiling. Then the media is sterilized using an autoclave at a temperature of 121^oC and a pressure of 2 atm for 15 minutes. After being sterilized, the culture media is poured into a petri dish as needed and stored until ready to be used to grow bacteria.

Bacteria Dilution

The bacteria dilution process was carried out using a trisalt solution with a volume of 9 ml. The dilution process was carried out using a multi-stage dilution method. For each sample, a diluent solution of trisalt was prepared in 2 reaction tubes. The bacterial sample was homogenized and then taken as much as 1 ml and added to the first reaction tube containing trisalt (10^{-1} dilution). Then, 1 ml was taken again from the first reaction tube (10^{-1}) and added to the second reaction tube (10^{-2}).

Bacterial Inoculation

The bacterial inoculation procedure was carried out using the spread plate method. Bacterial samples at the dilutions that had been carried out were taken as much

as 0.1 ml each and then added using a micropipette into 2 different media. Bacterial samples at a dilution of 10^{-1} were planted on TCBS media and bacterial samples at a dilution of 10^{-2} were planted on NA media. Furthermore, the bacterial samples on the media were spread evenly using a sterilized holistic using a Bunsen burner. The results of the bacterial culture were then incubated in an incubator for 24 hours to then observe their abundance.

Bacteria Abundance

The abundance of bacterial colonies growing on TCBS media is calculated using a tool called a colony counter. Making a quadrant is needed to calculate the total bacteria in very large numbers to facilitate calculations. The number of bacteria is entered into the TPC formula to determine their density and abundance. After the calculation is carried out, the total bacteria that have been obtained are recorded as data and then entered into the following modification formula by (Madigan and Martinko, 2006):

$$\text{Number of bacteria} = \text{Number of colonies} \times \frac{1}{\text{Dilution}} \times \frac{1}{\text{Culture volume}} \text{ CFU/ml}$$

Calculation of *Vibrio* sp. Dominance

A calculation of *Vibrio* sp. dominance is performed using the data obtained from the total abundance of bacteria and the total number of *Vibrio* sp. The dominance calculated is the percentage of the total dominance value of *Vibrio* sp. to the total bacteria. The *Vibrio* sp. dominance value is calculated in each breeding pond using the following dominance formula:

$$\text{Dominance (\%)} = \frac{\text{Total number of vibrios}}{\text{Total number of bacteria}} \times 100$$

Data Analysis

Data collection was carried out directly in the field, namely in the *L. vannamei* shrimp hatchery pond, using primary data. Then an analysis was carried out on the primary data and compared with secondary data in scientific articles such as journals and books. Data processing was done by calculating the total abundance of bacteria and vibrio obtained based on the TPC formula. The abundance data obtained was then averaged by processing it using Microsoft Excel software. The results of the data analysis were in the form of the average abundance of bacteria and *Vibrio* sp. in each breeding pond. Meanwhile, the dominance value was calculated after obtaining the results of the total abundance of bacteria and total of *Vibrio* sp.

Results

Vibrio Abundance

The calculation of the abundance of *Vibrio* sp.acteria was carried out using the

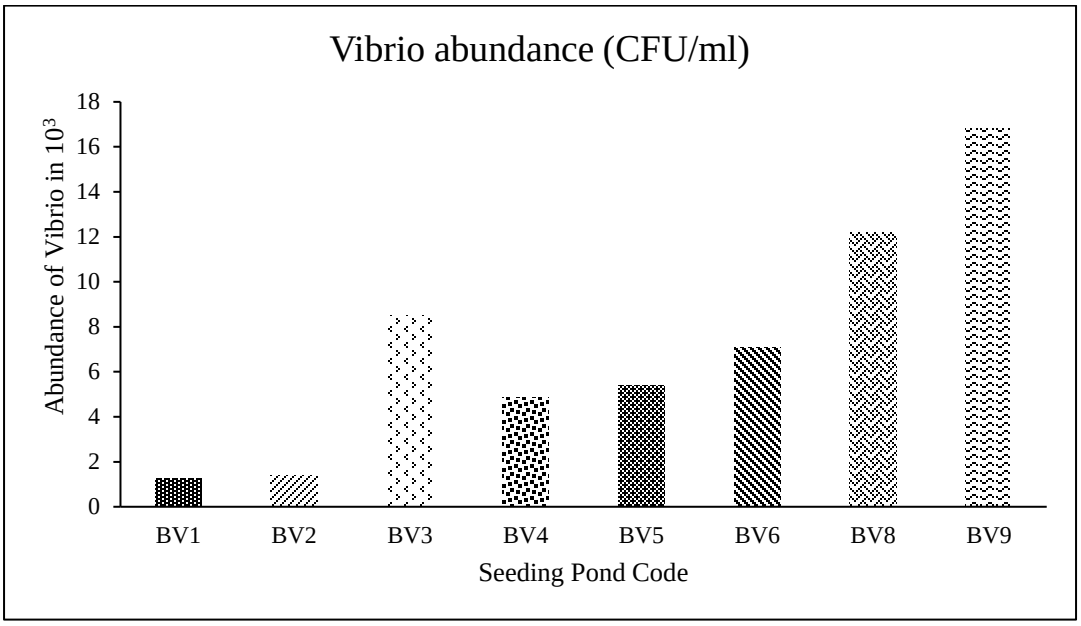

Figure 1. Vibrio Abundance Data

Total Plate Count (TPC) method. This method is used to breed living microorganism into the media so that they can grow and form colonies that can be observed visually and counted without using a microscope (Wati, 2018). The calculation of bacteria with this method was carried out using a colony counter tool. Based on the calculations that have been carried out, data on the abundance of *Vibrio sp.* were obtained in eight different vaname shrimp breeding ponds show in table and figure 1.

Water Quality

Water quality include ammonia (TAN), nitrite (NO_2), nitrate (NO_3), and Total Organic Matter (TOM). The following is water quality data in the maintenance pond during bacterial sampling. Water quality data during observation can be presented in Table 2.

Table 1. *Vibrio* sp. Abundance Data in *L. vannamei* Shrimp Breeding Pond Water

Pond	Total Vibrio CFU/ml
BV1	$1{,}3 \times 10^3$
BV2	$1{,}4 \times 10^3$
BV3	$8{,}5 \times 10^3$
BV4	$4{,}9 \times 10^3$
BV5	$5{,}4 \times 10^3$
BV6	$7{,}1 \times 10^3$
BV8	$1{,}2 \times 10^4$
BV9	$1{,}7 \times 10^4$

Table 2. Water Quality Data of Seeding Ponds

Code	Test Parameters			
	TAN mg/l	**NO_2 mg/l**	**NO_3 mg/l**	**TOM mg/l**
BV1	0.173	0.038	0.074	173.580
BV2	0.459	0.109	0.182	171.630
BV3	0.078	0.002	0.010	64.370
BV4	1.358	0.181	0.311	165.323
BV5	0.690	0.033	0.073	143.090
BV6	0.489	0.017	0.039	179.380
BV8	1.523	0.049	0.000	81.120
BV9	1.152	0.041	0.074	140.700

Vibrio Dominance

Vibrio sp. in *L. vannamei* hatchery ponds have Bacterial abundance calculations that were carried out to compare the abundance of *Vibrio* sp. and general bacteria in hatchery ponds. In addition, total calculations and general bacterial abundance also need to be carried out to determine the dominance of *Vibrio* sp. over bacteria as a whole. Bacterial abundance data are presented in the following Table 3.

Based on the results of the calculation of the abundance of bacteria total and *Vibrio* sp. the value of the dominance of *Vibrio* sp. over the total bacteria can then be determined. V*ibrio* sp. dominance is calculated to determine how much *Vibrio sp.* dominates the total bacteria in the *L. vannamei* breeding pond. Data on the dominance of vibrio over the total bacteria are presented in the table 4.

Discussions

The highest abundance of *Vibrio* sp. was found in pond BV9 with 1.7 x 10^4 CFU/ml determined using the Total Plate Count (TPC) method. Meanwhile, the lowest abundance was in pond BV1 with a total abundance of 1.3 x 10^3 CFU/ml. The

Table 3. Total Abundance Data of Bacteria

Pond	Total Bacteria CFU/ml
BV1	9,6 x 10^5
BV2	9,0 x 10^3
BV3	3,4 x 10^5
BV4	1,1 x 10^4
BV5	3,5 x 10^4
BV6	5,9 x 10^4
BV8	7,9 x 10^4
BV9	3,6 x 10^5

Table 4. Dominance of Vibrio Bacteria Over Total Bacteria

Pond	Vibrio dominance
BV1	0,13%
BV2	15,56%
BV3	2,50%
BV4	43,96
BV5	15,43%
BV6	12,03%
BV8	15,44%
BV9	4,67%

abundance of *Vibrio* sp. in the water samples of the vaname shrimp hatchery pond is still within the normal threshold, where according to Taslihan*et al*., (2004) in Anjasmara*et al*., (2018) that the maximum limit of vibrio abundance in shrimp cultivation water is 10^4 CFU/ml. The high abundance of *Vibrio* sp.in *L. vannamei* hatchery ponds can occur due to several influencing factors. One of the influencing factors is changes in salinity, temperature, and water quality conditions in the pond. Additionally, cultivation management practices such as feeding, different types of feed, shrimp density, and water circulation systems also influence *Vibrio* sp. abundance.

Meanwhile, based on the abundance results obtained, it can be seen that each pond has a different abundance. The difference in abundance can occur due to different pond conditions, where each pond has different water quality conditions and environmental characteristics such as different physical, chemical, and biological conditions. The difference in high abundance values in *L. vannamei* breeding ponds is thought to be caused by the accumulation of substances from the remaining shrimp seed feed that settles in the breeding pond. This is supported by Ariadi*et al*., (2023), who that the presence of *Vibrio* sp. in shrimp breeding ponds correlates with the accumulation of organic matter where the higher the accumulation of organic matter in the pond, the higher the abundance of vibrio bacteria in it.

Generally, the growth of *Vibrio* sp. in an aquatic environment is influenced by two factors, namely internal and external factors (Jesus *et al*., 2013). Some internal factors that influence include the degree of acidity (pH), water activity, oxidation-reduction ability, nutrient content, and structure of food materials in the water area. Meanwhile, external factors that influence include the condition of the aquatic environment, temperature, humidity level, oxygen pressure

(O2), light intensity, and exposure to ultraviolet rays in the area (Hikmawati*et al*., 2019).

Water quality conditions such as changes in temperature, salinity, and ammonia content in ponds affect the presence and abundance of *Vibrio* sp. High salinity and ammonia concentrations in water are directly proportional to the increase in the number of *Vibrio* sp. (Heenatigala& Fernando, 2016). Ammonia concentration tends to increase when water salinity is low and vice versa (Kim *et al*., 2019). Low salinity will increase the concentration of ammonia, nitrite, and nitrate so that which can cause shrimp to be more susceptible to stress and at risk of *Vibrio* sp. bacterial infection (Kathyayani*et al*., 2019).

Based on the results of the calculation of total bacteria in NA media using the Total Plate Count (TPC) method, the highest abundance was in pond BV1 with a total bacterial value of 9.6 x 10^5 CFU/ml. The lowest abundance was in pond BV2 with a total bacterial value of 9.0 x 10^3 CFU/ml. The results of the calculation of bacterial abundance showed that the total bacterial value in all breeding ponds was greater than the total *Vibrio* sp. value. Based on the results obtained, the abundance of vibrio in breeding ponds was smaller than the abundance of bacteria. This is because the bacteria found in breeding ponds are not only *Vibrio* sp. but there are also other bacteria in them. According to Zulfikar (2023), the maximum percentage of the comparison between total bacteria and *Vibrio* sp. in the aquatic environment is 5%. If the percentage exceeds 5%, then it is included in the vulnerable conditions for *L. vannamei* cultivation activities. Consequently, to prevent disease outbreaks and mass deaths caused by*Vibrio* sp., the percentage of vibrio in the cultivation environment should not exceed the total number of bacteria.

Based on the table 4, it can be seen that the total vibrio value is lower than the total bacteria value. Therefore, vibrio has a modest dominance value against the total population of bacteria. The highest vibrio dominance value is at 43.96%, namely in the BV4 pond. Environmental factors such as water salinity conditions, ammonia, temperature, and pH are some of the factors that can cause high dominance of *Vibrio* sp. in the BV4 seed pond. The life stage or phase of *L. vannamei* can also affect the dominance of vibrio bacteria. Changes in environmental conditions such as different diets, temperatures, and salinities at each phase of shrimp growth can affect the availability of nutrients and waste in the pond.

The high dominance of *Vibrio* sp. in the breeding pond will cause several risks related to the health of*L. vannamei* . If the vibrio dominance value is high, then it will be at risk of causing disease. This is in accordance with the opinion put forward by Supito*et al*., (2008), that the high dominance and abundance of V*ibrio* sp. in shrimp ponds or ponds is one indication of conditions that can cause health problems in shrimp.

Conclusion

The results showed that *Vibrio* sp. in the breeding pond were still within the normal range of vibrio abundance, namely in the range of 10^4 CFU/ml, with the highest abundance occurring in BV4 with 1.7 x 10^4 CFU/ml. Meanwhile, the highest dominance of vibrio was in the BV4 pond with a percentage of 43.96% of the total bacteria.

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