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Effect of Bacillus firmus Bacteria on Total Vibrio harveyi Bacteria and Vanname Shrimp Survival (Litopenaeus vannamei)

Yuli Anis Safitri
Fakultas Perikanan dan Ilmu Kelautan, Universitas Brawijaya, Malang, Indonesia

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ABSTRACT

The purpose of this study was to determine the effect of Bacillus firmus on the number of V. harveyi bacteria and the survival of the vanname shrimp (Litopenaeus vannamei). The main parameters in this study were the number of V. harveyi bacteria and the survival of the vanname shrimp (L. vanname), while the supporting parameters used were water quality including temperature, DO, pH and salinity The results showed the number of V. harveyi bacteria in the media maintenance of vanname shrimp after administration of B. firmus decreased from the first to the third day and increased on the fourth day of the infection period. When compared to the control without giving B. firmus, the number of V. harveyi bacteria actually increased compared to the treatment with B. firmus bacteria. Based on the results of the analysis of variance, it showed that giving B. firmus at different doses did not have a significantly different effect on the growth of V. harveyi but when compared to controls, it showed that giving B. firmus gave better results than without giving B. .firmus. Giving B. firmus bacteria with a density of 102 cfu/ml, 104 cfu/ml and 106 cfu/ml was able to reduce the number of V. harveyi compared to without B. firmus administration. cfu/ml because with a density of 102 cfu/ml it has been able to reduce the number of V. harveyi bacteria. The survival of vanname shrimp in the treatment of firmus bacteria obtained quite high results when compared to the control. giving B. firmus at a density of 106 was 87.5%, without giving B. firmus at 58.5%. Based on the results of analysis of variance on the vanname shrimp survival rate, it was found that there was no significant difference between treatments.

Corresponding Author:

Yuli Anis Safitri
Fakultas Perikanan dan Ilmu Kelautan, Universitas Brawijaya
Jl. Veteran, Ketawanggede, Kec. Lowokwaru, Kota Malang, Jawa Timur 65145
Email: yulianissafitri@gmail.com

1. INTRODUCTION

Shrimp is one of the leading fishery commodities in the fisheries revitalization program, in addition to seaweed and tuna. One type of shrimp which is the main commodity is the white shrimp (Litopenaeus vannamaei) which lives in American waters and is one of the white shrimp which is quite commercial and widely cultivated. in America and then spread to Southeast Asia (Subaidah et al., 2006). The vannamei shrimp has a body covered with a thin hard shell of yellowish-white chitin material with white legs. When compared to tiger prawns or jrebung prawns, the body shape of vannamei prawns is much smaller.

The vanname shrimp body is divided into two major parts, namely the cephalothorax which consists of the head and chest and the abdominal part which consists of the stomach and tail. Cephalotorax is protected by a thick chitin skin or also called carapace.

Vaname shrimp is quite popular in public consumption and is followed by several other types of shrimp, so shrimp cultivation is carried out in order to meet the demand for shrimp consumption. In addition, in general the business opportunities for vanname shrimp farming are not much different from other types of shrimp business opportunities. Because basically shrimp is the mainstay of the government's export commodity in attracting foreign exchange, so that export development is a major concern (Amri and Kanna, 2008). In the natural environment, shrimp can be attacked by various diseases.

Likewise in its cultivation, this disease can even attack shrimp in large numbers and can cause the death of shrimp, so that the losses it causes are very large. One of the diseases that attack shrimp is caused by the genus Vibrio, so this disease is called vibriosis. Vibriosis is often referred to as flaming shrimp disease because infected shrimp appear to glow at night. Many efforts have been made to control several diseases, both using chemicals and antibiotics.

However, the continuous use of these materials will cause problems, namely in the form of environmental pollution and the emergence of organisms that are resistant or resistant to drugs (Supriyadi, 2008). The use of antibiotics should be limited to the types recommended by the government, and as far as possible avoid the use of antibiotics that are included in the prohibited category, such as chloramphenicol, nitrofuran, furazolide, etc.

The use of these antibiotics requires the application of probiotics which have the ability to maintain water quality, inhibit the growth of pathogenic microorganisms, and increase the digestibility of feed (digestibility) in fish or shrimp that are reared. Therefore, to inhibit the growth of pathogenic microorganisms that can attack shrimp so that can cause mass death of shrimp requires the application of bacteria as a biocontrol agent which has the ability to suppress the growth of pathogenic bacteria, especially Vibrio harveyi so as to provide a good environment for shrimp life. One of the bacteria that has the potential to inhibit the growth of these pathogenic microorganisms is Bacillus firmus.

2. METHOD

2.1 Types of research

The research method used is the experimental method. According to Nazir (2005), experimental research is research conducted by manipulating the object of research and the existence of controls. The purpose of experimental research is to investigate whether there is a causal relationship and how big the causal relationship is by giving certain treatments to several experimental groups and providing controls for research. .

2.2 Research variable.

The main variables in this study were the number of V. harveyi bacteria and the survival of the vanname shrimp (L. vanname), while the supporting parameters used were water quality including temperature, DO, pH and salinity.

2.3 Research design

The experimental design used in this study was a Completely Randomized Design (CRD), which is a design used for experiments that have uniform media or experimental sites.

2.4 Sampling location

This research was carried out with samples of B. firmus bacteria through vanname shrimp rearing media to reduce the number of V. harveyi bacteria and the stimulation between the two research test materials.

2.5 Time and Place of Research.

This research was conducted at the Laboratory of Disease and Environmental Health, Situbondo Brackish Water Aquaculture Center, from June to August 2009.

2.6 Tools and materials

The tools used during the research are as follows: Laminar Air Flow (LAF); Vortex; One set of autoclave sterilizer; Effendorf tube; Bunsen; Electric heater; Petridisk; Refrigerator; Dropper pipette; Measuring cup; Micropipette; Erlenmeyer; Tri angel; Digital scale; Colony Counter; Incubator; Test tube; Tank capacity of 15 liters; Test tube rack; Loop needle; Aerator, aeration stone, hose; Water quality test equipment: pH meter, DO meter, refractometer, thermometer.

The materials used during the research are as follows: Vanname shrimp (L. Vannamei) PL 4, obtained from vanname shrimp hatcheries around the Situbondo Brackish Water Cultivation Center (BBAP); B. firmus isolate, obtained from vannamei shrimp ponds in Gresik area; Isolate of V. harveyi, which was obtained from the Laboratory of Fish and Environmental Health BBAP Situbondo; TCBSA Media (Thiosulphate Citrate Bile Sucrose Agar); TSA Media (Triptic Soy Agar); NB Media (Nutrient Broth); Alcohol; KCl; MgSO4; NaCl; Aquades; Aluminum foil; Vanname shrimp rearing media water.

2.7 Research procedure

The tools to be sterilized are wrapped using parchment paper or newsprint, then tied with thread, while media such as TSA and TCBSA are put into the Erlenmeyer and covered with cotton and aluminum foil. Then it is put into the autoclave and filled with sufficient water. It is continued with the preparation of trisalt solution and other media and the calculation of bacteria to meet the applicable requirements and finally the shrimp maintenance and observation.

2.8 Data analysis.

The data obtained from the results of the treatment in the study were analyzed to determine the effect of the treatment on the response of the parameters being measured. The analysis uses analysis of variance or the F test. If the F value shows a significantly different (significant) effect then proceed with the BNT test (Small Significant Difference). The final results of BNT can be followed by regression analysis which provides information about the effect of the best treatment on the response.

3. RESULTS AND DISCUSSION

3.1 Research result

3.1.1 Total Amount of V. harveyi Bacteria in Rearing Media of Vannanme Shrimp (L. vannamei) Post Larvae for 4 x 24 Hours of Channel Infection Period

The table below explains that the total number of V. harveyi bacteria in vanname shrimp post larvae rearing media decreased from the first day to the third day and increased on the fourth day of the infection period. This means that the administration of B. firmus bacteria is effective up to 3 x 24 hours, so it is necessary to add B. firmus bacteria after 3 x 24 hours.

B. firmus bacteria were able to reduce the number of V. harveyi bacteria up to 103 cfu/ml. When compared to the control without B. firmus administration, the number of V. harveyi bacteria actually increased compared to the treatment with B. firmus bacteria. Based on the results of the analysis of variance, it showed that the administration of B. firmus at different densities did not have a significant effect on growth. V. harveyi but when compared to controls, it showed that giving B. firmus gave better results compared to without giving B. firmus. Administration of B. firmus bacteria at densities of 102, 104 and 106 cfu/ml was able to reduce the number of V. harveyi compared to without administration of B. firmus. So for efficiency, it is recommended to use B. firmus with a density of 102 cfu/ml because a density of 102 cfu/ml has been able to reduce the number of V. harveyi bacteria. This shows that B. firmus is antagonistic to V. harveyi.

Table 1. Total Number of V. harveyi Bacteria in Rearing Media of Vanname Shrimp Post Larvae for 4 days of Infection Period.

Day	Treatment			
	102 (cfu/ml) (A)	104 (cfu/ml) (B)	106 (cfu/ml) (C)	K (cfu/ml)
0	7.108	7.108	7.108	7.108
1	7,3.105	9,7.105	10,7.105	8,6.108
2	14,7.104	19,3.104	24,3.104	3.107
3	5,2.104	5,6.104	6.104	5.106
4	9,9.104	10,9.104	11,3.103	2,4.106

3.1.2 Vannamei Shrimp Graduation by Giving B. firmus Bacteria

Vanname shrimp survival is the main parameter observed in this study. Passing is calculated after 4 x 24 hours from the time of bacterial infection. Based on observations during the study, data on the survival of vannamei shrimp (Litopenaeus vannamei) were obtained.

Table 2. Post larval survival of vanname shrimp (L. vannamei) during rearing (%) by administering B. firmus bacteria

	Test			
treatment	1	2	3	Average

102 cfu/ml	85.8 %	98.3 %	95.8 %	93.3 %
104 cfu/ml	87.5 %	88.3 %	95.8 %	90.5 %
106 cfu/ml	85 %	81.7 %	95.8 %	87.5 %
Control	60 %	49.1 %	66.6 %	58.6 %

The survival of vanname shrimp in the treatment with B. firmus bacteria obtained quite high results when compared to the control. Thus it can be concluded that the administration of B. firmus bacteria can increase the survival rate of vanname shrimp that live on media infected with V. harveyi bacteria. giving B. firmus. It is suspected that B. firmus is able to suppress the growth of V. harveyi bacteria so that it can increase the survival of vannamei shrimp when compared to controls.

3.1.3 Water Quality Analysis

The results of observations on the quality of the media water during the study still gave a value in the range desired by the vannamei shrimp to form a defense against disease, especially infection by pathogenic bacteria such as V. harveyi. The quality of the water media plays a very important role, because the emergence of disease or infection by bacteria, one of which is caused by unbalanced aquatic environmental conditions for the shrimp being cultivated. Water quality parameters measured were temperature, pH, salinity and dissolved oxygen levels.

According to Subaidah et al., (2006) states that the optimum temperature for vannamei shrimp is between 23 - 30°C, the fastest growth in vannamei shrimp occurs at a temperature of 27°C rather than 30°C. Shrimp will die if the temperature is below 15°C or above 33°C within 24 hours or more. So that the temperature of 27.5 – 290C during the study is still included in the optimum temperature range for vanname shrimp maintenance. The pH range values obtained during the study were 7.74 – 8.3. The optimum pH range for vanname shrimp maintenance is 7 – 9 (Kordi, 2007). According to Amri and Kanna (2008), a pH value above 10 can kill shrimp, while a pH below 5 results in slow shrimp growth. Likewise Irianto (2005), states that an increase in pH beyond the tolerable limit will cause illness or stress. Dissolved oxygen in vannamei shrimp rearing media during the study ranged from 5 – 5.7 mg/liter. Dissolved oxygen content that is good for the life of vanname shrimp is more than 3 ppm and should be kept in the range of 4 - 8 ppm (mg/liter).

3.2 Discussion

These organic acids include citric acid, glutamate, succinic, lactic, oxalic, glyoxalic, malic, fumaric, tartaric, alpha ketobutyric, simple aliphatic acids, amino acids and phenolic acids (Buntan, 1999 in Anonymous, 2009). B firmus produces citrate, lactate, acetate, oxalate, propionic acid, phenol, and ρ-hydroxyl benzoate (Zlotnikov et al., 2001 in Shanaz, 2008).

In B. firmus, the bioactive ingredients that are suspected as anti-microbial are phenolic compounds. Phenol is an acidic alcohol, so it is also called carbolic acid (Jawetz et al., 1996 in Susanti, 2009). Phenol compounds and their derivatives (flavonoids) are antibacterials that work by interfering with the function of the cytoplasmic membrane, H+ ions in phenolic compounds attack the phosphate groups in the bacterial cytoplasmic membrane so that the phospholipids in the bacterial cell wall break down into glycerol, carboxylic acids and phosphoric acid. In such circumstances, phospholipids cannot maintain the shape of the membrane, the cytoplasmic membrane leaks, causing the bacteria to inhibit or die (Lullman et al., 2000 in Batoran, 2003).

Phenol has the ability to denature proteins and damage cell membranes. Phenol binds to proteins through hydrogen bonds, causing the protein structure to be damaged. Most cell structures and cell membranes contain proteins and fats. Instability in the cell wall and cytoplasmic membrane of bacteria causes the function of selective permeability, active transport function, control of the protein arrangement of the bacterial cell to be disrupted. Disruption of the integrity of the cytoplasm results in the escape of macromolecules and ions from the cell. The results of measurements of salinity in vannamei shrimp rearing media during the study ranged from 33 - 34 ppt. In SNI (2006), it is stated that the salinity for fry maintenance is between 29 – 34 ppt. Compared to other types of shrimp, vanname shrimp prefers culture media water with lower salinity or salt content, which is 10 - 35 ppt (Amri and Kanna, 2008).

4. CONCLUSION

From the results of the study, it was concluded that administration of B. firmus at densities of 102, 104, 106 cfu/ml through vanname shrimp (L. vanname) rearing media can reduce the number of V. harveyi bacteria in vanname shrimp (L. vannamei) post larvae rearing media. compared to the

control (without administration of B. firmus) and B firmus can be used as a biocontrol agent because it can suppress the number of V. harveyi bacteria in vanname shrimp (L. vannamei) rearing media. B firmus with a density of 102 cfu/ml can be used as a biocontrol agent in maintenance of vanname shrimp for cost efficiency Administration of B. firmus with densities of 102 cfu/ml, 104 cfu/ml, 106 cfu/ml can increase the post larval survival value of vanname shrimp with successive values as follows 93.3%, 90.5 %, 87,5% of the water quality measurements of the rearing medium were within the normal range for vanname shrimp life, namely temperatures ranging from 27.5 – 290C, D0 5 – 5.7 ppm, pH 7.74 – 8.3, salinity 33 – 34 ppt.

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From the research, it is suggested to use B. firmus bacteria as a biocontrol agent in rearing vanname shrimp especially post larvae in an effort to prevent V. harveyi attack and it is advisable to use B. firmus with a density of 102 cfu/ml because a density of 102 cfu/ml has been able to reduce the number V. harveyi bacteria.

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