The effect of yeast and lactic acid bacteria as probiotic on the total of Vibrio spp. in rearing water of post larvae tiger shrimp (Penaeus monodon)

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Abstract. Probiotics are an alternative that can be used to prevent the incidence of vibriosis in the cultivation of tiger shrimp (Penaeus monodon). Yeast can be used as a probiotic and immunostimulant because it can increase the total of beneficial microbes. Lactic Acid Bacteria (LAB) are chosen to be developed as a probiotic because it is able to produce organic acids, hydrogen peroxide and bacteriocin which act as antagonistic agent to Vibrio spp. This study aimed to determine the effect of RABAL probiotics on the total Vibrio spp. in medium of post larvae tiger shrimp (Penaeus monodon). The study was conducted with an experimental method using a completely randomized design (CRD) with 4 treatment groups and 3 repetitions. Calculate of the total amount of Vibrio spp. and LAB on rearing water was carried out using Total Plate Total (TPC) method. Based on the results of the study, it was found that administering RABAL probiotics with a dose range of 2.5 to 7.6 ml / L was proven to reduce the amount of Vibrio spp. in rearing water of post larvae tiger shrimp (Penaeus monodon).

1. Introduction

Tiger shrimp cultivation is still a potential economic source for shrimp farmers in various coastal areas of Indonesia. This is inseparable from the increasing demand for tiger shrimp seeds by shrimp farmers [1]. However, since the 1980s, problems have arisen that can threaten the sustainability of tiger shrimp cultivation, namely the problem of shrimp disease and a decrease in the quality of the culture environment [2]. Vibriosis is a disease that frequently infects cultured shrimp [3]. Vibriosis is a bacterial disease caused by infection with Vibrio spp. [4]-[5]. Vibriosis in shrimp can arise due to an imbalance between the environment, shrimp and disease agents, that cause shrimp to become stressed so that the shrimp’s immunity weakens and eventually becomes susceptible to disease [6].
Increased population of *Vibrio* spp. in the rearing water during hatchery and rearing can result in a decrease in shrimp survival rate [7]-[8]-[9].

The use of antibiotics is a common choice in shrimp hatchery centers in an effort to prevent bacterial infection that causes vibriosis [10]. So far the use of disinfectants and antibiotics has limited success in preventing or curing aquatic diseases. The massive use of antibiotics for disease control in animals can increase and encourage the emergence of natural bacterial resistance. Bacteria that are already resistant can transfer their resistance genes to other bacteria that have never been exposed to antibiotics [11]-[12]-[13]. Other eco-friendly alternatives to control shrimp disease can be used in the form of probiotics, vaccines, immunostimulants, prebiotics, phage therapy and bioactive compounds from plant extracts [14]. To date, most probiotics proposed as biocontrollers and bioremediation agents for aquaculture belong to the LAB group (mainly to the genera Lactobacillus, Lactococcus, Leuconostoc, Enterococcus and Carnobacterium), to the genera Vibrio, Bacillus, and Pseudomonas or to the species *Saccharomyces cerevisiae* [15]-[16]. Lactic Acid Bacteria have been tested on warm-blooded animals for use as probiotics and efforts have also been made to apply Lactic Acid Bacteria as probiotics in shrimp farming [17]. Yeast can be used as a probiotic and immunostimulant because it can increase the total of beneficial microbes [18]. Yeast has the ability to increase the total of hemocytes (in crustaceans), macrophages, T cells and B cells in fish [19].

Research on the effect of RABAL probiotics on the total amount of *Vibrio* spp. is still very minimal to be done, therefore it is necessary to conduct research to evaluate the use of RABAL probiotics in reducing the total amount of *Vibrio* spp. in post-rearing water tiger shrimp larvae (*Penaeus monodon*). This study aims to see the effect of RABAL probiotic on the total amount of *Vibrio* spp. in rearing water of post-larvae tiger shrimp (*Penaeus monodon*) and the benefits obtained, which can help shrimp farmers in responding to the problem of vibriosis disease and environmental quality degradation in tiger prawn cultivation. The benefits of this research can also be used as reference material and information for Academics, Researchers, Government and Stakeholders.

### 2. Material and Method

#### 2.1 Places and Time Research

This research was conducted at Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh. The research was carried out from January 2020 to May 2020.

#### 2.2 Research Tools and Materials

The tools used in this study were measuring cups, container boxes, aerators, aquarium hoses, sample bottles, diluent bottles, petri dishes, stirring rod L, single channel, micro-pipette, incubator and colony counter. The materials used in this study were fruit waste (pineapple and papaya fruit), *Lactobacillus casei* starter, *Lactobacillus plantarum* starter, molasses, yeast tape, 360 PL30 tiger shrimps, 15 liters of seawater, 2% NaCl, 0.9% NaCl, *De Man Rogosa Sharpe Agar* (MRSA) media and *Thiosulfate Citrate Bile Salt Sucrose Agar* (TCBSA) media.

#### 2.3 Research Method

This research was conducted with an experimental method using a completely randomized design (CRD) with 4 treatment groups and 3 replications. The examination and counting of the total amount of *Vibrio* spp. in the tiger shrimp rearing water is carried out at the beginning and the end of maintenance period.
Table 1. Research Design

| Treatment Group | TPC Vibrio spp. | Average (Σ) |
|-----------------|-----------------|-------------|
|                 | U1              | U2          | U3          |
| P0              | P0(1)           | P0(2)       | P0(3)       |
| P1              | P1(1)           | P1(2)       | P1(3)       |
| P2              | P2(1)           | P2(2)       | P2(3)       |
| P3              | P3(1)           | P3(2)       | P3(3)       |

Note: U1-U3 = Replication

2.4 Research Procedure

2.4.1 Preparation of Containers and Experimental Animals. This research was conducted on a laboratory scale using a container box as a treatment area. Each box was filled with pond soil with a thickness of 10 cm and dried, then given dolomite lime evenly on the surface and added as much as 15 liters of seawater. The rearing animals used were PL30 tiger shrimps with a stocking density of 2 heads / liter obtained from BPBAP Ujung Batee, Aceh Besar District, Aceh. The tiger shrimp maintenance was carried out in seawater with the salinity of 30 ppt [20]. Before adding the tiger shrimps, the total amount of Vibrio spp. must had been checked in rearing water by using the Total Plate Total (TPC) method. The tiger shrimps used in this study were acclimatized for 7 days in the water with an aerator. During the maintenance of tiger shrimps, it was given a commercial feed twice a day as much as five percent of the biomass. Shift pond and water change were carried out every ± 7 days as much as 30% of the total initial volume [21].

2.4.2 Making Yeast Probiotics and Lactic Acid Bacteria (RABAL). Yeast probiotics and lactic acid bacteria (RABAL) were made based on research by Nurliana et al. [22].

2.4.3 Treatment of Maintenance Water. In this study, post larvae of tiger shrimps were randomly divided into 4 groups and then given the RABAL probiotic with different doses. In group 1 (P0) the shrimp was stored in a container box without the provision of RABAL probiotics, in groups 2 (P1), 3 (P2) and 4 (P3) the shrimp were stored in a container box added with the RABAL probiotic with each a dose of 2.5 each. ml / liter of seawater, 5.02 ml / liter of seawater and 7.6 ml / liter of seawater.

2.4.4 Analysis of Vibrio spp. In calculating the total of Vibrio spp. previously, serial dilutions were carried out with a 1: 9 dilution ratio. 1 ml of the water samples were added to 9 ml of 2% NaCl diluent solution. The sample is homogenized. This dilution is considered a 10⁻¹ dilution. A total of 1 ml from the 10⁻¹ dilution was added to the 10⁻² dilution, and then it was homogenized again. After dilution, using the spread method, pour each water sample that has been diluted as much as 0.1 ml on Thiosulfate Citrate Bile Salt Sucrose Agar (TCBSA) media, then the plates were incubated in an incubator at 37 °C for 24 hours.

2.4.5 Analysis of Lactic Acid bacteria (LAB). LAB was isolated from the water sample, then as much as 1 ml of the water sample was diluted 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ on 0.9% NaCl medium aseptically. The results of the dilution were grown using the pour plate method in MRSA media, and then incubated in an incubator at 37°C for 24 hours.

2.4.6 Research Parameters. The parameter in this study was the total amount of Vibrio spp. in post-rearing water tiger shrimp larvae (Penaeus monodon).

2.4.7 Data Analysis. The research data were analyzed using variance analysis (ANOVA) unidirectional patterns with the help of the SPSS program. If the results were significantly different, it would continue with the Duncan test.
3. Results and Discussion

Examination and counting the total amount of *Vibrio* spp. in the rearing water of post larvae tiger shrimp was carried out at the beginning and the end of the maintenance period. Based on the research results, the growth of the *Vibrio* spp. can be seen in Figure 1.

![Figure 1. Colony growth of Vibrio spp. on TCBSA media](image)

*Thiosulphate Citrate Bile Salt Agar* (TCBSA) is a selective medium commonly used to grow *Vibrio* spp [23]. TCBSA media are generally used to isolate Vibrio spp. that has facultative anaerobic properties and can grow well under relatively high alkaline and bile salt conditions [24]. In this study, there were 2 types of *Vibrio* spp colonies that were observed in TCBSA media, namely the yellow and greenish colonies. According to Mailoa and Setha [25], the colonies of *Vibrio* spp. that can utilize the sucrose tend to be yellowish in color while the colonies that cannot to utilize the sucrose tend to be greenish in color. Ilmiah *et al.* [26], reported that the species *Vibrio* spp. which is able to utilize the sucrose is *Vibrio metschnikovii* with colonies that grow yellowish. Meanwhile, the *Vibrio* spp. which is unable to utilize the sucrose is *Vibrio parahaemolitycus* with colonies that grew greenish.

The colony growth of *Vibrio* spp. on TCBSA media was then calculated based on the TPC method. Total *Vibrio* spp. in the water medium before inserting the tiger shrimp post larvae was 2.8 log10 CFU / ml. These data indicate the density of the total amount of *Vibrio* spp. was within the normal allowable threshold. This was consistent with the statement of Roza and Zafran [27], stated that the safe limit for the total amount of *Vibrio* spp. which can be tolerated in shrimp rearing water is 4.9 log10 CFU / ml. The calculation of the total amount of *Vibrio* spp in water medium was also carried out at the end of the post-rearing for tiger shrimp larvae period. The results of the statistical analysis of the total amount of *Vibrio* spp and LAB colonies are presented in Table 2.

| Treatment Group | Total Amount of Bacterial Colonies (log10 CFU / ml) |
|-----------------|-----------------------------------------------------|
|                 | *Vibrio* spp. | Lactic Acid Bacteria (LAB)          |
| P0              | 2.5 ± 0.05d   | 4.5 ± 0.03a                        |
| P1              | 2.3 ± 0.06c   | 5.9 ± 0.04b                        |
| P2              | 2.0 ± 0.08b   | 6.2 ± 0.05c                        |
| P3              | 1.9 ± 0.03a   | 6.4 ± 0.04d                        |

Note: a,b,c,d Different superscripts of letters in the same column showed significant differences (P < 0.05).

P0: Untreated,
P1: Probiotic RABAL with a dose of 2.5 ml / L
P2: Probiotic RABAL with a dose of 5.02 ml / L
P3: Probiotic RABAL with a dose of 7.6 ml / L
Table 2 showed the total average (± SD) of *Vibrio* spp. The highest amount in post larvae rearing water for tiger shrimps was found in P0 treatment with the total amount of bacterial colonies was 2.5 ± 0.05 log10 CFU / ml and the lowest in P3 treatment with the total amount of bacterial colonies was 1.9 ± 0.03 log10 CFU / ml. Based on these results, it was found that the total amount of *Vibrio* spp. was lower in treatment of P1, P2 and P3 (giving RABAL probiotic) and significantly different (P < 0.05) compared to treatment of P0 (control). Meanwhile, the highest average (± SD) of LAB was in P3 treatment with the total amount of bacterial colonies was 6.4 ± 0.04 log10 CFU / ml and the lowest was in P0 treatment with the total amount of bacterial colonies was 4.5 ± 0.03 log10 CFU / ml. The results of the variance analysis showed that giving RABAL probiotics with different doses (P1, P2, and P3) could significantly increase the amount of LAB (P < 0.05) compared to the control group (P0). The results also indicated that the total colonies of *Vibrio* spp. and LAB in post larvae tiger shrimp rearing water depending on the RABAL dose entered.

**Figure 2.** Colony growth of Lactic Acid Bacteria (LAB) on MRSA media.

MRSA (*De Man Rogosa and Sharpe Agar*) is a selective medium commonly used to grow Lactic Acid Bacteria [28]. Lactic Acid Bacteria are used to characterize a broad group of Gram-positive, namely nonmotile, non-sporulatory bacteria, which are generally catalase-negative, deficient in cytochromes, utilize carbohydrates by fermentation and produce lactic acid as the main or only product of sugar fermentation. This group of bacteria is widely distributed in nature and consists of a stem form (*Carnobacterium, Lactobacillus, Weissella*) and a coccus form (*Aerococcus, Enterococcus, Lactococcus, Leuconostoc, Pediococcus* and *Streptococcus*) as single cells, pairs or chains [29]. In the P0 treatment, LAB growth was found on MRSA media. This was presumably because the post-maintenance media of tiger shrimps there is LAB naturally. This is supported by [30] which stated that Lactic Acid Bacteria are normally found in seawater and freshwater. The existence of Lactic Acid Bacteria derived from the probiotic RABAL was thought to have a tendency to reduce the *Vibrio* spp. population in this research. This was confirmed by the opinion of [31], which stated that the Lactic Acid Bacteria have the ability to produce organic acids (lactic and acetic acids), hydrogen peroxide and bacteriocins which have antagonistic properties against *Vibrio* spp. [32] reported that LAB isolated from fish and applied to rearing water was clearly able to eliminate *Vibrio* spp.

**4. Conclusion**

Based on the research results, it can be concluded that giving RABAL probiotics with a dosage range of 2.5 – 7.6 ml / L has been proven to reduce the total amount of *Vibrio* spp. in rearing water post larva tiger shrimp (*Penaeus monodon*).

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