Application of whole cell microalgae as an alternative to Vibriosis prevention

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Abstract. Increasingly intensive aquaculture activities make the development of the disease increase. One of them is Vibriosis, a disease caused by pathogenic Vibrio bacteria. Several alternative disease prevention have been widely used. One of them is by using microalgae. This study aims to determine the effect of the use of microalgae in whole cells on the growth of pathogenic bacteria. Using 5 types of microalgae, namely Melosira sp, Phaeodactylum sp, Nannochloropsis sp, Porphyridium aerugineum, and Porphyridium sp with 10⁵ cells/mL of density. In vitro, the challenge test was carried out using a Complete Randomized Design with 6 treatments (5 types of microalgae and 1 control treatment without microalgae), which were repeated 3 times. While in vivo, it was carried out using PL 16 tiger shrimp with a density of 20 heads/jar volume of 20 L. The results showed that in vitro can give whole cell microalgae types P.aerugineum, Nannochloropsis sp, and Melosira sp can prevent the growth of luminous pathogenic Vibrio bacteria. While in vivo administration of microalgae Phaeodactylum sp and Porphyridium aerugineum may inhibit the growth of fluorescent pathogenic Vibrio bacteria better than other microalgae and control.

1 Introduction

Vibriosis infection caused by Vibrio bacteria remains an essential problem in shrimp culture. Some methods can be used to prevent vibriosis in shrimp culture. Probiotics, vaccines, and natural products such as microalgae are some of the environmentally friendly alternatives developed to prevent vibriosis infection in shrimp culture.

In the aquaculture industry, microalgae have several important roles, including as a source of feed, as bioremediation to utilize aquaculture waste for the photosynthesis process, help reduce excess CO₂ levels in waters, and can improve the immune system of aquatic organisms. The results of later studies showed that microalgae have very complex interactions with bacteria, one of which is microalgae can inhibit bacterial growth [1]. Based on this, this study aims to determine the ability of microalgae to anti-vibriosis for disease prevention in shrimp culture.

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2 Material and Method

This study was conducted in vitro and in vivo using 5 types of microalgae, each applied in whole cell. The 5 types of microalgae are Melosira sp, Phaeodactylum sp, Nannochloropsis sp, Porphyridium aerugineum, and Porphyridium sp. In vitro, testing was carried out using 250 mL Erlenmeyer filled with sterile seawater with a salinity of 28 ppt as much as 100 mL. Microalgae were given with a density of $10^5$ cells/mL, and the challenge test was carried out using $10^7$ CFU/mL of pathogenic fluorescent vibrio bacteria. Using a complete randomized design with 6 treatments (use of 5 types of microalgae and 1 treatment as a control) repeated 3 times. In vivo testing using the same 5 types of microalgae in vitro testing and 2 control treatments, positive and negative, which were repeated 3 times. Using 20 and PL 16 tiger shrimp/jar (volume size 3 L filled with 2 L of saline sterile seawater 28 ppt) infected with Vibrio pathogen density $10^7$ CFU/mL. The parameters observed include the growth of luminous pathogenic bacteria on the test media every 24 hours for 120 hours and the survival of larvae in the in vivo test. Water samples were cultured on Thiosulphate Citrate Bile Sucrose Agar (TCBSA) medium by stratified dilution. After 24 hours, the growing bacterial colonies were counted using the Buller method [2]

$$P = \frac{Q}{T} \times \frac{1}{S} \times \frac{1}{V}$$

(1)

Where:
P = bacterial population (CFU/mL for water or CFU/g for sediment)
Q = total number of bacteria growing in one dilution rate (colony)
T = number of plates used
S = dilution rate
V = volume of sampel (0.1 mL)

The data obtained are analyzed statistically using graphs. The survival rate of the larvae in the rearing container is observed and calculated every 24 hours for 120 hours.

3 Result and Discussion

The results of the in vitro challenge test using whole cell microalgae can be seen in Figure 1. The graph shows that several types of microalgae given in whole cell form could inhibit the growth of fluorescent pathogenic Vibrio bacteria and had differences in growth for each treatment and control. In treatment with the administration of microalgae Porphyridium aerugineum, pathogenic Vibrio bacteria were not detected at 72 hours after infection. Pathogenic Vibrio bacteria was also not detected in treatment, where Melosira sp at 96 hours after infection and Nannochloropsis sp at 120 hours. At the end of this research, treatment with the microalgae Porphyridium sp. and Phaeodactylum sp resulted in the presence of pathogenic Vibrio bacteria at concentrations of $10^3$ CFU/mL. In Vitro, it can be concluded that whole cell administration of microalgae types P.aerugineum, Nannochloropsis sp, and Melosira sp can inhibit the growth of pathogenic bacteria.
In vitro, it can be seen that the percentage of survival rate in treatment with microalgae administration is not significantly different from control (+) and only significantly different from control (-). These data show that isolates of pathogenic Vibrio bacteria used in this artificial infection are quite pathogenic.

**Graph 2.** The growth of pathogenic Vibrio bacteria in the in vivo challenge test using whole cell microalgae

Graph 2 shows that bacteria have a logarithmic growth pattern. The early phases of growth show an adaptation phase where bacteria need adjustments to grow in the new environment in which they are. This can be seen in sampling 2. There was a decrease in the
bacterial population from the initial population of $10^7$ CFU/mL to $10^3$ CFU/mL. The population of these bacteria increased in the 3rd sampling, where in this phase, the bacteria experienced an exponential growth phase. The next phase of growth is the stationary phase or the phase where bacteria do not experience significant growth because they have experienced peak growth in the exponential phase, and the next phase is the death phase [3]

![Graph showing survival rate percentage of tiger shrimp larvae in a challenge test using whole cell microalgae](image)

**Fig. 3.** The survival rate percentage of tiger shrimp larvae in a challenge test using whole cell microalgae

The ability of microalgae to inhibit the growth of *Vibrio* bacteria is due to the production of anti-bacterial compounds such as alkaloids, peptides, terpenoids, phenolics, and other compounds [4], which are largely yet to be identified. This antimicrobial property can inhibit bacterial growth by various mechanisms, including, as explained by [7], the permeability of the cytoplasmic membrane, which causes food to exit the cell, changes in protein and nucleic acid molecules, inhibition of the activity of enzymes, and inhibition of nucleic acid and protein synthesis are some examples of how anti-bacterial compounds can damage cell walls and inhibit or change them after they have been formed. Competition with microalgae for nutrients like nitrates, phosphates, and heavy metals can also prevent bacterial growth. Microalgae can utilize the resources they require simultaneously and effectively to inhibit bacterial growth.

Another ability that microalgae have to inhibit bacterial growth is by disrupting communication between bacterial cells, known as quorum sensing. Control of *Vibriosis* in shrimp using microalgae as anti-quorum sensing is another alternative that continues to be developed. Quorum sensing is a communication system between cells of similar or different types of bacteria that aims to activate the expression of a particular gene by the bacterium concerned [5][6]. To get mutual agreement, bacteria will excrete a molecule into their environment, which will then be a signal to the bacteria themselves and other bacteria. When the concentration of molecules in their environment has reached a certain level, these molecules will then become feedback for the bacteria concerned to activate and express a gene. The pathogenicity of a microorganism is closely related to the release of virulence factors possessed by the organism [8].

### 4 Conclusion

In the in vitro challenge test, whole cell administration of the microalgae *Porphyridium aerugineum*, *Melosira* sp, and *Nannochloropsis* sp can inhibit the growth of luminous pathogenic Vibrio bacteria, whereas, in the in vivo challenge test, *Porphyridium aerugineum*, and *Phaeodactylum* sp are better able to inhibit the growth of pathogenic *Vibrio* bacteria.
bacterial population from the initial population of 10^7 CFU/mL to 10^3 CFU/mL. The population of these bacteria increased in the 3rd sampling, where in this phase, the bacteria experienced an exponential growth phase. The next phase of growth is the stationary phase or the phase where bacteria do not experience significant growth because they have experienced peak growth in the exponential phase, and the next phase is the death phase [3] Fig. 3.

The survival rate percentage of tiger shrimp larvae in a challenge test using whole cell microalgae. The ability of microalgae to inhibit the growth of Vibrio bacteria is due to the production of antibacterial compounds such as alkaloids, peptides, terpenoids, phenolics, and other compounds [4], which are largely yet to be identified. This antimicrobial property can inhibit bacterial growth by various mechanisms, including, as explained by [7], the permeability of the cytoplasmic membrane, which causes food to exit the cell, changes in protein and nucleic acid molecules, inhibition of the activity of enzymes, and inhibition of nucleic acid and protein synthesis are some examples of how antibacterial compounds can damage cell walls and inhibit or change them after they have been formed. Competition with microalgae for nutrients like nitrates, phosphates, and heavy metals can also prevent bacterial growth. Microalgae can utilize the resources they require simultaneously and effectively to inhibit bacterial growth.

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