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ANALYSIS OF THE ABILITY OF DIFFERENT TYPES OF MICROALGAE TO INHIBIT THE GROWTH OF FLUORESCENT VIBRIO BACTERIA

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Abstract

Alternative disease prevention that is environmentally friendly in tiger shrimp cultivation has now begun to be carried out a lot, one of which is by using microalgae that play an important role in aquaculture activities. In addition to functioning as natural feed, microalgae also could control microbiological communities in the aquatic environment through various mechanisms, both by inhibiting and supporting the growth of other microorganisms. This study aims to determine the ability of microalgae Porphyridium sp, Vulgaris sp, Phaeodactylum sp, and Nannochloropsis sp in inhibiting the growth of fluorescent Vibrio bacteria which is known to be one of the causes of vibriosis in shrimp farming. The results of the study showed that all microalgae tested had the ability to be antibacterial, especially from the type of Nannochloropsis sp which was able to significantly reduce the number of bacteria in a longer observation period, so it has great potential to be used as an environmentally friendly natural material for the prevention of diseases caused by bacteria in shrimp farming.

Keywords: *ability; Growth; microalgae; vibrio bacteria*

Abstrak

Alternatif pencegahan penyakit yang bersifat ramah lingkungan pada budidaya udang windu saat ini telah mulai banyak dilakukan, salah satu diantaranya adalah dengan menggunakan mikroalga yang memainkan peran penting dalam aktivitas akuakultur. Selain berfungsi sebagai pakan alami, mikroalga juga memiliki kemampuan untuk mengontrol komunitas mikrobiologi dalam lingkungan perairan melalui berbagai mekanisme, baik dengan cara menghambat maupun mendukung pertumbuhan mikro organisme lainnya. Penelitian ini bertujuan untuk mengetahui kemampuan mikroalga *Porphyridium sp*, *Vulgaris sp*, *Phaeodactylum sp*, dan *Nannochloropsis sp* dalam menghambat pertumbuhan bakteri Vibrio berpendar yang diketahui sebagai salah satu penyebab vibriosis pada budidaya udang. Hasil penelitian menunjukkan bahwa semua mikroalga yang dicobakan memiliki kemampuan sebagai antibakteri terutama dari jenis *Nannochloropsis sp* yang secara signifikan mampu menurunkan jumlah bakteri dalam kurun waktu pengamatan yang lebih lama, sehingga sangat potensial untuk dapat digunakan sebagai bahan alami yang bersifat ramah lingkungan untuk pencegahan penyakit yang disebabkan oleh bakteri pada budidaya udang.

Kata Kunci: *Bakteri Vibrio; kemampuan; mikroalga; pertumbuhan*

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INTRODUCTION

Shrimp is one of the main commodities in the fisheries sector, in addition to lobster, crab, seaweed, and tilapia. This commodity has enormous development prospects. As a long-known fishery product, shrimp has high popularity and stable demand in the community. Shrimp species are widespread in a variety of natural habitats, ranging from marine waters, brackish waters, to freshwater, and most of them have been successfully cultivated intensively. In the 2020–2024 Strategic Plan, the Ministry of Maritime Affairs and Fisheries sets a shrimp export target of 4.25 billion US dollars or an increase of 250 percent, with a production volume of 2 million tons. To achieve this target, it is necessary to grow export volume by 15 percent and increase the value of exports by 20 percent every year. Although the target for shrimp farming development has been set, its implementation in the field still faces various obstacles, especially related to sustainability aspects. One of the main challenges in shrimp farming is the emergence of disease attacks, both in ponds and in hatchery units (Kerdmusik et al., 2018). The disease is generally caused by bacterial and viral infections, with vibriosis being one of the most found diseases. Vibriosis is caused by bacteria of the genus Vibrio. According to Chatterjee and Haldar (2012), Vibrio species that are often pathogenic agents include V. harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarum, and V. vulnificus. Surreshvarr et al. (2011) reported that Vibrio infection can affect shrimp from the larval to post-larval stages. Infection by V. harveyi is known to cause a mortality rate of up to 100%, and until now, the treatment of these infections is still not optimal (Flegel, 2012; Suwoyo & Sahabuddin, 2017).

Many efforts have been made to prevent vibriosis attacks, one of which is by continuously administering antibiotics to inhibit the growth of pathogenic bacteria. However, the continuous use of antibiotics has increased the resistance of pathogenic bacteria and negatively impacts the growth of friendly shrimp, either directly or indirectly, through a decrease in water quality (Nanin, 2011 in Nurhayati, et al., 2021). Therefore, the use of antibiotics in shrimp farming activities has currently been severely restricted, even in some cases it has been completely banned with the issuance of Ministerial Decree No. KEP.02/MEN/2007. In addition to the use of antibiotics, the use of chemicals and pesticides to overcome shrimp diseases is also very detrimental (Suryati et al., 2006). This is because these materials cause the accumulation of harmful chemical compounds and environmental pollution that cause cultivation activities to be unenvironmentally friendly and cause residues that are harmful to consumers.

Disease prevention in shrimp farming is currently carried out through an environmentally friendly approach, one of which is by using natural materials. The natural material that is currently starting to be widely used is microalgae. Microalgae or microphytes are unicellular photosynthetic microorganisms, which live in saline or freshwater environments, which convert sunlight, water, and carbon dioxide into algae biomass (Ruane et al., 2010). Microalgae play an important role in aquaculture activities. In addition to serving as natural feed, microalgae can also act as water quality controllers, which play a role in maintaining the stability of water quality as well as the microbalance of communities within it. In addition, several studies have shown that microalgae can control microbiological communities in aquatic environments through various mechanisms, either by inhibiting or supporting the growth of other microorganisms (Fallahi et al., 2021; Wiyoto & Ekasari, 2010).

Research on microalgae as an alternative to the prevention and control of vibriosis in shrimp farming has been carried out extensively, including research conducted by the Research Institute for Brackish Water Aquaculture and Fisheries Extension (BRPBAPPP) Maros (Kadriah et al., 2016-2021). From the exploration and studies that have been carried out, several types of microalgae have the potential to be used as anti-bacterial, either in whole cells or from the extracellular metabolites they produce so that they can be used as an alternative material for the prevention of environmentally friendly vibriosis in shrimp farming.

METHOD

This research was carried out at the Research Center for Brackish Water Aquaculture and Fisheries Extension Maros, South Sulawesi by going through several stages:

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1. Microalgae Culture The microalgae cultures used in this study are Porphyridium sp, Vulgaris sp, Phaeodactylum triconortum, and Nannochloropsis sp, cultured at a volume of 500 mL. The culture media uses sterile seawater with the addition of Conway fertilizer and Vitamin mix. The dose of Conway fertilizer for a 1L culture volume is 2 mL and a vitamin mix of 1 mL. The ratio between microalgae culture and sterile seawater used is 1:4. Culture was carried out for 3-4 days with continuous aeration with a light intensity of 7,000 Lux. After 4 days of culture, the density of microalgae was calculated under a microscope using a haemocytometer;
2. Vibrio bacteria culture fluorescent. Bacterial culture was carried out in a 2,000 mL volume erlenmeyer container. Using Nutrient broth as a medium for bacteria growth. The bacteria used were Vibrio harveyi bacteria which were cultured by taking as many as 10 inoculum of bacterial culture and put them into a pre-prepared NB medium, then incubated in a swaying incubator for 24 hours. After 24 hours, the bacterial density was then calculated using multi-stage dilution using Saline Solution solution (SS);
3. Testing the ability of microalgae inhibition against bacterial growth by cohabitation method (co-culture of microalgae and pathogenic Vibrio bacteria. Cohabitation cultures were carried out using 8 erlenmeyers with a volume of 250 mL for cohabitation treatment and 2 pieces for control treatment, The volume of cohabitation cultures to be used was 100mL. The density of Vibrio bacteria has been calculated using the plating method with multi-stage dilution. Next is to put pure microalgae cultures into cultures of pathogenic Vibrio bacteria that have been aged for 24 hours. The volume of microalgae introduced into the culture of fluorescent Vibrio bacteria is set to obtain a density of 105 cells/mL; and
4. Observation of Vibrio Bacteria Growth Luminous. Observation of bacterial growth was carried out using a specific medium, TCBSA (Thiosulfate Citrate Bile salt Sucrose Agar) with gradual dilution using a physiological saline solution as a diluent. The ability of microalgae to inhibit the growth of fluorescent Vibrio bacteria was carried out by observing bacterial growth from cohabitation sample media. Observations were made at the 0th, 24th, 48th, 36th, 72th, 96th hour and at the 120th hour. Each growing bacterial colony is marked using a bath marker, marked using a permanent marker, and manually counted.

   Bacterial density calculation was carried out using the formula of Lay (1994):

$$\mathbf{N_{tot}\ CFU/mL = T/Q \times 1/S \times 1/V...}$$

Where :

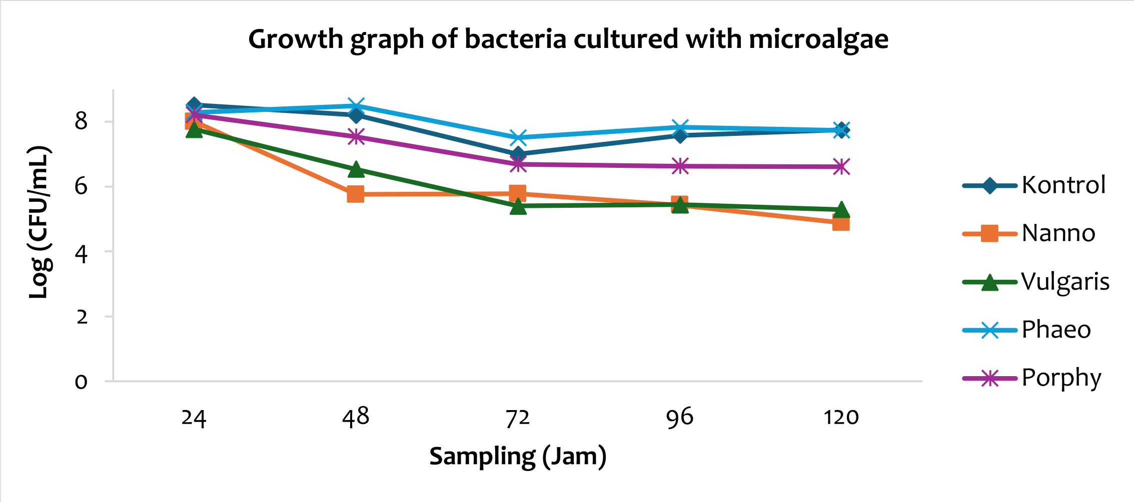
N_{tot}	:	total number of bacterial colonies in milliliters
T	:	Total bacteria in the petri dish
Q	:	Number of Petri cups used
S	:	dilution used
V	:	Inoculated volume

Source: Lay (1994)

RESULT AND DISCUSSION

The ability of different types of microalgae to inhibit the growth of fluorescent vibrio bacteria per hour of observation time can be seen in the following graph:

Figure 1:
Growth graph of bacteria cultured with microalgae



Source: research results

The graph above illustrates the growth of fluorescent Vibrio bacteria cultured with different types of microalgae and controls over a time span of 24 to 120 hours. From the graph, there is a significant difference in inhibition of bacterial growth between the control treatment and the treatment with the addition of microalgae. The control treatment, which did not give microalgae only fluorescent vibrio bacteria, showed a slight decrease in the number of bacteria from the beginning to the end of the observation that was relatively small over time. Despite the decline, the number of bacteria remains quite high, which indicates that the bacteria are still growing well under these conditions.

Treatment with the addition of the microalgae Nannochloropsis sp, showed a significant decrease in the number of bacteria. This decrease was more pronounced than the control treatment, which indicated that the microalgae Nannochloropsis sp had the potential to inhibit the growth of pathogenic bacteria. Chlorella vulgaris: The microalgae Chlorella vulgaris also exhibits inhibition of bacterial growth, although the inhibition rate is not as strong as that of Nannochloropsis sp. A decrease in the number of bacteria was seen but less compared to Nannochloropsis sp. The treatment of Porphyridium sp, appeared to be effective in reducing the number of bacteria when compared to Phaeodactylum tricornutum, but still not more potent when compared to Nannochloropsis sp. The microalgae Phaeodactylum tricornutum showed lower inhibition compared to other algae. There was less bacterial decline, which indicates that Phaeodactylum tricornutum microalgae may not be as effective as other microalgae in inhibiting the growth of fluorescent vibrio bacteria.

In general, it was seen that all types of microalgae (Nannochloropsis sp, vulgaris sp, Phaeodactylum tricornutum, and Porphyridium sp) showed inhibition of bacterial growth when compared to control treatments. Notably, Nannochloropsis sp was most significant in reducing the number of bacteria over a longer period of observation. The control treatment (without microalgae) showed a slight decrease in the number of bacteria, but the number of bacteria remained quite high even after 120 hours of observation. Overall, this graph shows that microalgae have the potential to inhibit the growth of pathogenic bacteria, with Nannochloropsis sp being the most effective microalgae among those tested. These results indicate that the use of microalgae, especially the Nannochloropsis sp type, can be an effective natural solution in controlling pathogenic bacteria.

One of the ways that these microalgae inhibit the growth of fluorescent vibrio bacteria is by disrupting the communication system or quorum sensing (KS) in bacteria. Bacteria that live in a community do not behave individually but can interact with each other to inform other bacteria about their existence and existence. These interactions allow them to act collectively, as if as a multicellular organism. One of the important mechanisms that bacteria use to communicate is known as quorum sensing.

Through quorum sensing, bacteria can detect the number of their population by emitting and responding to chemical signal molecules. When the concentration of signaling molecules reaches a certain threshold, this triggers simultaneous changes in genetic and physiological behavior in the population, such as biofilm formation, virulence production, or bioluminescence. Quorum sensing (KS) is a communication system between cells of the same or different types of bacteria that aims to activate the expression of a certain gene by the bacteria concerned. In this mechanism, bacteria will secrete a molecule into its environment which will then become a signal for the bacteria themselves and other bacteria (Wiyoto and Julie Ekasari, 2010). Gene expression produced by quorum sensing mechanisms include pathogenesis, symbiosis, antibiotic production, motility, biofilm formation (Miller & Bassler, 2001), DNA transfer, and biopendar (Chhabra et al., 2005).

Intervention or inhibition of the quorum sensing process, especially pathogenic bacteria, can be one of the effective strategies in controlling bacterial diseases in aquaculture. According to Defoirdt et al. (2004), the quorum sensing intervention techniques that have been developed include: (1) inhibition of signal molecule biosynthesis, (2) application of antagonistic sensing quorum (both natural and synthetic), (3) chemical inactivation of quorum sensing signals, (4) biodegradation of signal molecules, and (5) application of agonist quorum sensing. Of the five intervention techniques, it is known that microalgae and macroalgae (seaweed) can have a fairly important role in inhibiting the quorum sensing mechanism of pathogenic bacteria.

Research conducted by Kadriah et al (2016) has observed the ability of microalgae as anti-vibriosis and found that some microalgae are known to have the ability to be antivibriosis. The virulence process in Vibrio harveyi involves the production of various virulence factors, including caseinase, gelatinase, lipase, hemolysin, and phospholipase. The regulation of the expression of genes encoding these factors is controlled by the quorum sensing (QS) mechanism, which is a communication system between bacterial cells that depends on population density. QS in V. harveyi involves a multi-channel receptor regulatory pathway with three different types of signaling molecules, which collectively regulate the expression of specific genes (Henke and Bassler, 2004). One of the main signaling molecules used by V. harveyi is N-(3-hydroxy) butanoil-L-homocysteine lactone (Natrah et al., 2011). When cell density reaches a certain threshold, the autoinducer molecule binds to specific regulatory proteins, forming a complex that induces the expression of a few genes involved in the production of bioluminescent enzymes as well as a variety of other pathogenic factors.

In addition to inhibiting cell-to-bacterial communication possessed by microalgae to inhibit the growth of pathogenic bacteria, secondary metabolites produced by microalgae are also known to play an important role in antimicrobial activity. Nannochloropsis sp shows the most significant inhibition of bacterial growth, which is most likely due to the content of **astaxanthin** and **polysaccharides (**Karpinski, et al. 2021). Astaksantin, as one of the most effective carotenoids, is known to have strong antimicrobial activity (Lafarga et al,, 2020) while polysaccharides can function to disrupt bacterial cell membranes. Meanwhile, Chlorella vulgaris contains **chlorophyll** and **carotenoids** that can reduce oxidative stress in bacteria, but its ability to inhibit bacterial growth is more limited compared to Nannochloropsis sp. Overall, these differences in bacterial growth inhibition suggest that the type and concentration of bioactive metabolites in individual microalgae have an important role in their effectiveness as bacterial inhibitory agents.

CONCLUSION

From the results of the research that has been carried out, it can be concluded that the microalgae Nannochloropsis sp has the ability to inhibit the growth of fluorescent Vibrio bacteria better when compared to other microalgae (Vulgaris sp, Porphyridium sp and Phaeodactylum tricornutum) so that it has the potential to be used as an environmentally friendly natural material for the prevention of diseases caused by bacteria (vibriosis).

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