ANALYSIS OF SQUID (LOLIGO EDULIS) INK LIQUID EXTRACT AS AN ANTI –VIBRIOSIS SUBSTANCE AGAINST VIBRIO ALGYNOLITICUS

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Abstract: In shrimp and fish culture V. alginolyticus is more frequently isolated from vibriosis sick grouper The high mortality in Tigers grouper hatchery as vibriosis cases need a way to overcome the other antibacterial products. Squid ink, as a squid waste product, which contains a variety of bioactive components in the form of a simple amine and paralyzing protein which is environmentally friendly was used as an anti vibriosis substance. The methods used in this research were: Phase I: Extraction of squid ink; Phase 2 Minimum Inhibitory Concentration Test (MIC) , Scanning Electronic Microscope (SEM) observation, and GC-MS chromatography test for the active ingredient; Phase 3 : in-vivo trials on squid ink extract against Vibrio in fish seeds. An experimental method with completely randomized design using three treatment doses of squid ink extract (365,5; 312,5; and 265,5 ppm) to treat the invected juvenile with V. algynoliticus, each with three replications was used.
The conclusion of the research results were squid ink extract can be served as bactericidal for V. algynolyticus at a dose of 265.5 ppm. Squid ink contains 9-octadecenoic acids / oleic acids as an antibacterial agent. The Tiger grouper juvenile which has invected with V. algynoliticus reached 100 % survival rate after squid ink extraction treatment.

Key words: Squid ink, V. alginoliticus, E. fascogutatus, survival rate.

I. INTRODUCTION

Tiger grouper (E. fascogutatus) fish is one fish that has a very high economic value in Indonesia. Therefore, it is widely cultivated and especially important for export to the country of Singapore, Taiwan, and Hong Kong (Handayani et al., 2004). Tiger grouper fish which have the potential and market opportunities is very promising for aquaculture product. But it is still a major problem for a limited seed supply, both in terms of quantity and quality.

One of the obstacles encountered in grouper aquaculture is primarily pathogenic bacteria attack the larval stadia and seeds. The occurrence of these pathogens attack causes loss of quality and production efforts on grouper hatchery, harvest failure and even death. (Nuchsin, 2004).

Vibrio illness called vibriosis, penaeid bacterial septicemia, penaeid vibriosis, luminescent vibriosis or red leg disease (Aguirre-Guzma et al., 2004). This disease is a major bacterial disease, especially at the seesd can lead to mortality up to 100% within 2 weeks. Several species of Vibrio bacteria are frequently isolated from diseased groupers, i.e. , E. coioides (Huang, 2005), Polkadot Grouper, Cromileptes altivelis (Nitimulyo et al., 2005) and Tiger grouper, E. fuscoguttatus (Desrina et al., 2006); are Vibrio alginolyticus, V. anguillarum, V. vulnivicus (Kanchanakhan, 1996; Nitimulyo et al., 2005). Signs of the disease include fatigue, tissue death, slow growth and metamorphosis, disability, bolitas negricans, bioluminansi, muscle opacity and melanization. In many cases, opportunistic Vibrio, which only cause disease when the host organism immunity declining or experiencing physical stress, the frequency of infection is common in intensive cultivation and poor environmental conditions. (Alderman and Hastings, 1998).

Vibriosis often leads to a critical problem for the life of shrimp and fish. In Vibrio anguilarum (Norgvist et al., 1990), V. vulnificus (Kothary and Kreger., 1987), V. proteolyticus (David et al., 1992), a zinc metalloprotease is an important virulence factor, which causes tissue damage in fish and shrimp. V. alginoliticus virulence factors that play a role is the phospholipase, hemolysin, protease (cysteine proteases, metalloproteases, serine proteases), lipopolysaccharide, a bacteriophage, bacteriocin-like substance (BLIS) and chitinase (Arachchige, 2010).
To control the disease, particularly bacterial disease, has been using different types of antibiotics. But it turns out the use of antibiotics can lead to new strains of resistant bacteria, so it is not effective in the prevention of disease.

One way of controlling bacterial diseases is to use squid ink, which is considered as fishery waste product. Various amine bioactive components simple and paralyzing protein was found in chepalopoda. These bioactive components can be used for the prevention of pathogenic bacteria (Cariello and Zantli, 1977; Walker and Masuda, 1990).
By utilizing squid ink as an anti-vibriosis will be able to utilize fishery waste products, produce an anti vibriosis substance while improving outcomes of Tiger grouper aquaculture product.

II. METHODS

2.1 Squid Ink Extract

The squid, Loligo edulis, was obtained from the fish landing place in Lamongan, East Java. Squid ink was taken from squid ink sac and sentrifuged on 15.000 g for 15 min, the supernatant was collected and stored at -20 ° C for further utilized.

2.2 V. alginoliticus

V. alginoliticus was obtained from a pure culture of Fish Quarantine Office, Surabaya, cultured in NA before used.

2.3 Minimum Inhibitory Concentration (MIC) Test

Mc Farland standard test was used to get the exact dose of squid ink liquid extract extract to inhibit the Vibriosis infection.

MIC Test was done using a circular paper (6 cm diameter) which was depth in different doses of squid ink liquid extract, 265.5, 312, and 362.5 ppm. The thin paper then laid on the plates that the V. alginoliticus grown up and scratched on solid medium of TCBSA with density of 108 cfu/ml.

To know the effect of squid ink extract to V alginoliticus, the reaction was observed using Scanning Electronic Microscope (SEM).

2.4 GC-MS

Supernatant of squid ink extract for the best dose of test results was sent to the laboratory for chromatography analysis using GC-MS.

2.3 Statistical Analysis

Completely randomized design was used for MIC test and analyzed using SPSS 16 series for ANOVA.

III. RESULTS AND DISSCUSION

3.1 MIC test

Squid ink liquid extract can inhibit the activity of V. alginoliticus bacteria. It is characterized by the formation of local constraints (clear zone) around the hole sinks bacterial culture media, where regional barriers at the edge of the pale yellow, while the central area of whitish paper discs with a diameter of 6 mm. Size of the zone of inhibition produced by the power of the antibacterial substance to inhibit bacterial assay as described by Agung et al. (2008), that any antibacterial substance has the ability to inhibit bacteria in a concentration

The average value of the inhibition zone located on 265.5 ppm treatment squid ink liquid extract of 10.85 mm, 312.5 ppm squid liquid extract was11 mm while 362.5 ppm squid liquid extract was 11.27 mm. This was consistent with the distribution of power category inhibitory activity by Liana (2010), that the strength of the inhibition of bacterial categories are as follows:

a. Bottleneck area of 5 mm diameter or less then categorized weak inhibitory activity.
b. 5-10 mm diameter were categorized.
c. Diameter of 10-19 mm categorized strong.
d. 20 mm or more was considered very strong.

Statistical result of the challenging test can be concluded that the squid ink liquid extract at different doses provide greater inhibitory power when compared to controls (0%), but nosignificant different among the different doses of treatment. Using 312 ppm of squid ink liquid extract can be used as anti-vibriosis against V. algynoliticus. The higher the concentration of squid ink liquid extract given the power resistor formed more broadly. This was due to the higher concentration of treatment, the higher number of the more antibacterial compounds. Jawetz et al., (1982), explained that the higher the concentration of antibacterial is used, then the ability to kill bacteria will go faster.

The images of Scanning Electronic Microscopy (SEM) showed that after V. alginoliticus was given exposure of squid ink extract, the bacterial cell was brokenafter being fed squid ink extract, which resulted in the death of the bacteria (Figure 1).

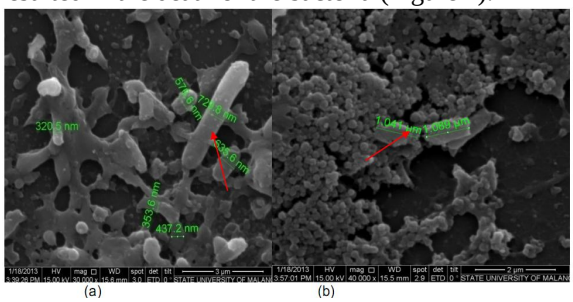


Figure 1. V. alginoliticus before and after treatment in SEM

Description: (a) V. alginoliticus before treatment, (b) V. alginoliticus after treatment with squid ink extract.

3.2 GC-MS

The GC-MS results showed that squid ink extracts has the highest abundance of 9-octadecenoic acids/oleic acids (Figure 2) in 18.242 retention time.

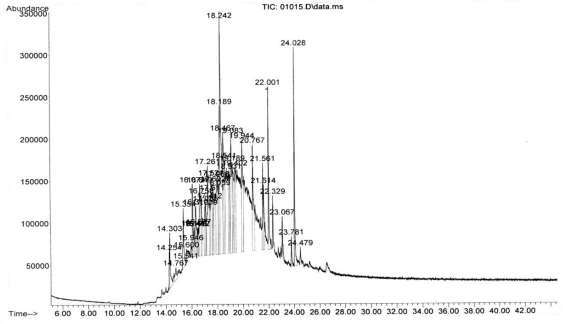


Figure 2. GC-MS result

Abou-elela et al. (2009), shows the results of GC-MS of marine natural products Cytosoria brown algae and sponge Spongia compressa oficinalis, which showed

antimicrobial effect derived from amino acids and esters (acids hexadecanoic and octadecanoic acids). The ink secretion of mollusk and some of which are proteins in the range from 62 to 249 kDa which are all playing specific roles in the chemical defense mechanism of inking mollusk (Venilla et al., 2011)

CONCLUSIONS

The conclusion of the research was squid ink extract can be served as bactericidal against V. algynolyticus at a dose of 265,5 ppm. Squid ink contains 9-octadecenoic acids / oleic acids as antibacterial agent.

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