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Assessment of the *Holothuria atra* (Echinodermata: Holothurians) habitat based on the antibacterial effectiveness approach at Karimunjawa, Central Java Province, Indonesia

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Abstract. *Sulardiono B, Anggoro S, Rudiyanti S, Rahman A. 2019. Assessment of the Holothuria atra (Echinodermata: Holothurians) habitat based on the antibacterial effectiveness approach at Karimunjawa, Central Java Province, Indonesia. Ocean Life 3: 11-17.* Sea cucumber *Holothuria atra* lives in shallow marine benthic habitats and in its life cycle depends on the quality of its environment. Bacteria in the body of sea cucumbers come from the bioaccumulation of bacteria in the sediment that they exposed to through the process of absorption of sediments as a deposit feeder. To inhibit bacterial growth in the body, sea cucumbers release bioactive compounds as antibacterial. The purpose of the study is to determine the effectiveness of sea cucumber inhibition ability of sea cucumber *H. atra* as an antibacterial in the waters of Karimunjawa. A total of 15 individual sea cucumbers were taken from the waters of Menjangan Besar and 20 individuals from the Alang-alang waters, then cleaned and dissected to take the part of the body wall. The body wall samples obtained from each observation station was as much as 1 kg, which was then sliced into small pieces and preserved in 96% ethanol, and and the bioactive compound was extracted by maceration method. Antibacterial activity test was carried out by disc diffusion method on paper discs (10mm) and using the media of EMBA (Ethylene Methyl Blue Agar) (Merck) for *E. coli* bacteria and BPA (Baird Parker Agar) for *S. aureus* bacteria. The obtained antibacterial test results were based on the measurements of the formed clear zone diameter, so that it can be known the effectiveness status of the antibacterial sea cucumbers of each observation station. The results of the study show that the antibacterial effectiveness of sea cucumber *H. atra* in the Menjangan Besar waters is higher than in the Alang-alang waters. The increase of sea cucumber bioactive compounds in the waters of Menjangan Besar shows that the waters are suitable for the development zone of sea cucumber cultivation

Keywords: Bacteria, bioactive compound, *Holothuria atra*, Karimunjawa

INTRODUCTION

Menjangan Besar waters are included in the Karimunjawa National Park, Central Java Province, Indonesia. Based on the decision of the General Directorate of Forest Protection and Nature Conservation, No. SK.79/IV/Set-3/2005, the Menjangan Besar waters are included in the tourism and cultivation development. The Menjangan Besar waters have coral reef ecosystems and their associations, and as a suitable habitat for the survival of sea cucumbers (Sulardiono et al. 2017). In addition, the Menjangan Besar Island area is a tourist destination with various activities, including floating hotels, fish cage and sea transportation supporting tourism activities. The increasing intensity of activities in the Menjangan Besar waters has caused an increase in domestic waste so that the aquatic environment becomes vulnerable to pollution. On the other hand, from 2013 to 2016, the waters of Menjangan Besar have experienced a decline in coral reef ecosystems as a habitat of 7.92 ha (Januardi et al. 2016). Furthermore, Sulardiono et al. (2018), informs that the status of the Menjangan Besar water is included in the oligotropic category. Based on this, it is thought to influence the pattern of dynamics of sea cucumber life in the habitat of coral reef ecosystems. Furthermore, Sulardiono et al. (2017) informs that the fertility status of the Menjangan Besar waters is oligotropic category, which means that the water fertility conditions are low to medium. Based on this, it is alleged that it will influence the pattern of the dynamics of sea cucumber life in the habitat of coral reef ecosystems in the waters of Menjangan Besar. Sea cucumber *H. atra* which is then termed *H. atra* is a type of sea cucumber (Echinodermata: Holothurians) that is able to cope with extreme shallow aquatic environments. *H atra* sea cucumber likes habitats with various types of substrates and can adapt to sand and fine sandy habitats to maintain heat by coating themselves with fine sand on their entire body (Azis 1995). Dissanayake and Stefansson (2012) state that *H. atra* sea cucumbers live predominantly in shallow seagrass beds with a depth of 10 m, and the more towards the deeper sea, the *H. atra* density decreases.

Marine biota is a source of bioactive compounds having diverse functions in nature, including anti-bacterial activity (Farjami et al, 2015) and one type of marine biota containing bioactive compounds is *H. atra* sea cucumber (Lawrence et al. 2009). The same thing is said by Mayer et al., (2013) that marine biota (including sea cucumber) contains bioactive compounds which have a variety of functions, one of which is as an anti-bacterial. The study of bioactive compounds from sea cucumbers is usually widely

used for the development of the pharmaceutical field, but not much for development in the ecological dimension. Sea cucumber *H. atra* as a deposit feeder that lives in shallow coastal habitats has the potential to capture pathogenic bacteria that accumulate in sediments through its food chain system, so that sediments are very good agents for microbes in the environment that enter the body of sea cucumbers. The concept is sea cucumber originating from potential waters against contamination of pathogenic bacteria is thought to affect biological activity in producing bioactive compounds, such as antibacterial, as a form of adaptation. The ecological function of bacterial-invertebrate interactions has not been widely studied, that microbial metabolites can play an important role in chemical defense associated with hosts and in forming symbiotic community structures. The metabolic and physiological changes experienced by these organisms in response to adaptation in extreme environmental conditions produce structural and functional production of bioactive compounds (Rizzo and Giudice 2018). Increased pollution and microbial effects of an environment are followed by increased biological activity of sea cucumbers to produce bioactive compounds as antibacterial. Based on this, the condition of the environmental quality of benthic ecosystems can be assessed from the antimicrobial properties of sea cucumbers that live in that environment. The purpose of the study is to determine the effectiveness of inhibition ability of sea cucumber *H. atra* as an antibacterial in the waters of Karimunjawa. The principle of the antibacterial test is the formation of a clear inhibitory zone diameter, which measured the diameter of the formation of clear circles after reducing the diameter of the circle of disc paper.

Escherichia coli and *Staphylococcus aureus* which is then termed *E. coli* and *S. aureus* are bacteria that can be used as indicators of the marine environment, through the effectiveness test of anti-bacterial abilities of bioactive compounds produced from sea cucumber *H. atra*. *E. coli* bacteria is a normal flora in human intestines that plays a role in health and lives normally. These bacteria can cause disease under certain conditions (Brooks et al., 2001). *E. coli* bacteria are included in the group of Gram negative bacteria with thinner cell walls. Gram negative bacteria are bacteria that cannot maintain the crystal violet dye during the Gram staining process so it will be red under a microscope observation. *E. coli* bacteria have bacillary form with a length of about 2 micrometer and a diameter of 0.5 micrometer, are facultative anaerobes, move and live in the range temperature of 20-40 °C with optimum 37 °C (Dwidjoseputro 1985). Under a condition of less clean environmental sanitation, these bacteria can transmit the diseases through direct contact (Radji 2011). Bacteria *S. eureus* are included in the group of gram-positive bacteria with thicker cell walls. Gram-positive bacteria are bacteria that can maintain methyl purple dyes during the gram coloring process. *S. aureus* bacteria have a round shape with a diameter of 0.7-1.2 micrometers, are facultative anaerobes, form no spores, and stay still most of the time (McCaig et al., 2006), growing optimally at temperature of 37 ° C, with room temperature of 20-25 °C. *S. aureus* bacteria can cause infectious wound in humans (Welsh et al, 2010). Contamination is through direct contact with *S. aureus* in a wound or infection.

Bioactive compound as secondary metabolites were previously regarded as waste or useless thing by experts, but the advancing technology can turn secondary metabolism into something with many high benefits, such as antibacterial, antiviral, anti-oxidant, anti-inflammatory, and other benefits specifically related to pharmaceutical products. However, secondary metabolites in relation to organisms and their environment have not been widely known, especially the effects of organisms in extreme environments related to their biological activities, which are exposed to extreme environmental effects, such as heavy metals, pathogenic bacteria, Harmful Algae Blooms (HABs) and disturbing substances others that can have an effect on the metabolic compounds produced by an organism as an antibacterial to inhibit disturbing bacteria. Secondary metabolites that refer to microbial metabolites are defined by Bennett and Bentley (1989) as an intermediate or product that can be found in most living systems synthesized by a number of bio-chemical pathways.

Ecologically, benthic ecosystems play an important role in the life cycle of the organisms. Various forms of association between microbes (including pathogenic bacteria) with other biotic and abiotic elements in one ecosystem will provide a form of natural balance that allows the life cycle of organisms to grow and develop naturally, but when the environment has begun to be contaminated by pollutants from anthropogenic sources, the organism life cycle becomes disrupted, including the effect of sea cucumber biological activity. The principle of the antibacterial test is the formation of a clear inhibitory zone diameter, which measured the diameter of the formation of clear circles after reducing the diameter of the circle of disc paper. Usually the study of sea cucumber biological resources was developed for pharmaceutical and health purposes, but its use for environmental aspects has not been done much, so in this study a study of sea cucumber biological resources was developed for the use of sea cucumber habitat assessment, specifically about the effectiveness of sea cucumber antibacterial activity against *E. coli* and *S. aureus* in the Menjangan Besar waters.

MATERIALS AND METHODS

Study area

The research was conducted at Karimunjawa, Central Java Province (Figure 1), which consists of two stations, namely Menjangan Besar waters and Alang-alang waters, which is then denoted as station A and station B.. The consideration of determining the two stations is based on the division of land use zones according to the Decree of the Directorate General of Forest Protection and Nature Conservation No. 79/IV/Set-3/2005 dated June 30, 2005. Analysis of the test sample was carried out by the Fisheries Product Technology laboratory and the Central Laboratory of Diponegoro University, Semarang, Indonesia.

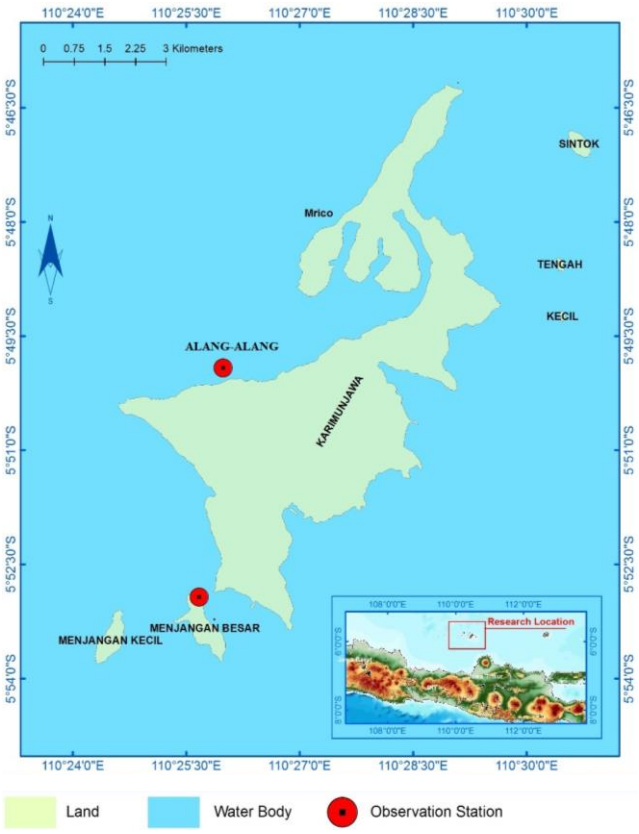

Figure 1. The observations stasion in Karimunjawa, Central Java, Indonesia

The materials were samples of sea cucumber *H. atra* and chemical stuffs, including aquades, ethanol 96%, and 10% ammonia (Merck), chloroform (Merck), 2 N hydrochloric acid (Merck), Mayer reagents, 1 N Sodium hydroxide (Merck), Physiological Na Cl 0.9% sterile, distilled water distillate, Lieberman Buchard reagent, and spirit. The test bacteria were isolates of *Escherichia coli* and *Staphyllococcus aureus* which were then referred to as *E. coli* and *S. aureus.* For bacterial growth media and the antibacterial activity test, Merck EMBA (Ethylene Methyl Blue Agar) (Merck) for *E. coli* bacteria and BPA (Baird Parker Agar) (Merck) for *S. aureus*, and Nutrient Broth are used. As a positive control, synthetic amoxicillin 500 mg antibacterial is used.

Procedures

Sea cucumber collection

The sea cucumber *H. atra* samples were obtained from 15 sea cucumbers of Menjangan Besar waters with a length of about 15-25 cm and from 20 sea cucumbers of Alang-Alang waters with a length of about 20-35 cm, which were then cleaned and inserted in a coolbox container. The samplings of sea cucumbers were by boat fishing and by diving. Habitat conditions in the waters of Menjangan Besar and Alang-alang at the time of sampling were coral reefs and their associations.

Extraction materials

Sea cucumber extraction used maceration method with immersion in 96% ethanol solution. The use of this ethanol solution is based on the results of a study by Dwicahyani, et al. (2018) informing that ethanol solvent is the best solvent for extracting bioactive compounds in sea cucumber *H. atra.* Total of 15 individuals of sea cucumber were taken from the waters of Menjangan Besar and 20 individuals from the Alang-alang waters, then they were cleaned and dissected to take part of the body wall. From the body wall, samples were obtained. From each observation stations 1 kg of the samples were taken, and sliced into small pieces and then they were blended, and stored in 300 mL of ethanol for 72 hours. After being filtered with filter paper, they were evaporated with evaporator at 40°C to separate the solution, so that a crude extract is obtained.

Test of antibacterial activity

Antibacterial activity testing method uses the disc diffusion method, with 10 mm disc paper. The media used is modified EMBA (Ethylene Methyl Blue Agar) and BPA (Baird Parker Agar), taking into account that EMBA and BPA are solid media, where EMBA is selective for the type of *E. coli* bacteria by giving positive results, and BPA is more selective for *S. aureus.* Extract samples from each observation location were dissolved in distilled water with a ratio of 50 mg, 75 mg, 100 mg, and 150 mg/mL of extract material fractions, and then they were used for testing antibacterial activity against *E. coli* and *S. aureus.* Some parts were taken and put in a petri dish. In the ethanol solvent fraction, it was dripped in 25 µL into paper discs for each sea cucumber extract formula. The use of ethanol as a solvent was as a negative control, each concentration was applied on *E. coli* and *S. aureus* bacterial replications, and sporicidal extract control against *E. coli* and *S. aureus* bacteria used synthetic antibacterial amoxicillin 500 mg with extract concentration.

Paper discs with a diameter of 10 mm were applied by immersing them into a solution of ethanol extract and pure isolates for ± 30 minutes. Then 50 ml of prepared solution was put into a sterile petri dish. Then the media is taken and poured into a petri dish which has been filled with a pure culture solution of the test bacteria. Using tweezers, the disc paper is placed on the surface of the dish and pressed slowly. A number of papers are required according to prescribed treatments and replications. Next, they were incubated at 37°C for ± 24 hours. Then, the inhibitory zone was measured using the calipers. The area of the inhibitor zone is reduced by the diameter of the disc and the diameter of the solvent inhibition zone. Based on observations, the inhibitory zone is a clear distribution area of the inhibition. The data from the three replications were used as indicators of antibacterial effectiveness that shows the strength of bacterial inhibition and refers to the criteria of Greenwood (1995), namely: Ø <5 mm = weak, Ø 5-10 mm = moderate, Ø 10-20 ml = strong, and Ø> 20 = very strong against antibacterial. As a comparison, the control of the positive extract against *E. coli* and *S. aureus* bacteria

used synthetic antibacterial amoxicillin 500 mg with the same extract concentration.

The measurement results of the effectiveness of sea cucumber inhibitions between the observation stations of the Menjangan Besar waters and Alang-alang waters were compared, and the results of the measurement of inhibitory effectiveness between the Menjangan Besar waters and Alang-alang waters against positive control using synthetic antibiotics amoxicillin 500 mg were also compared, thus it represented habitat conditions in Menjangan waters compared to other waters based on the criteria for environmental characteristics based on secondary data at the related location.

Data analysis

Data is compiled using Microsoft SPSS 17.0 software. ANOVA test was used to find out the difference in the effectiveness of antibacterial inhibitory of *H. atra* sea cucumbers at station A and B, by comparing the diameter of the disc paper used in each concentration in both *E coli* and *S. aureus* test bacteria on both observation stations A and B.

RESULTS AND DISCUSSION

Antibacterial activity

The results of the inhibitory test based on the measurement of the diameter of the inhibitory zone or clear zone on the disc paper can be seen in Table 1. Table 1 shows that the test for the extract of sea cucumber *H. atra* against *E. coli* and *S. aureus* in various concentration at the Station A showed the same inhibitory pattern, meaning that an increase in extract concentration tends to cause an increase in the inhibitory zone value. Likewise, treatment of *E. coli*, *S. aureus*, and positive controls with 500 mg synthetic antibacterial amoxicillin showed the same pattern of inhibitory ability.

Qualitatively, the value of the measurement of the inhibitory zone diameter of sea cucumber extract *H. atra* against *S. aureus* in all treatments was higher than the treatment against *E. coli* bacteria, but the diameter of the sea cucumber inhibition zone in Menjangan waters was greater than the diameter inhibitory zone in Alang-alang waters. Based on the criteria of Greenwood (1995), the measurement result at both stations shows the same category of inhibitory effectiveness, namely the moderate. If the treatment of sea cucumber *H. atra* extract at the two observation stations was compared with the treatment of positive control of amoxicillin 500 mg, the value of the diameter of the inhibitory zone in sea cucumbers from the Menjangan Besar waters and the Alang-alang waters appears to be lower than that in the positive control. The control treatment was used as a comparison with the optimal antibacterial effectiveness in accordance with the standards for pharmaceutical needs, where the results obtained in the inhibitory effectiveness category were strong to very strong against E. coli bacteria and very strong against S. aureus.

Based on the two-way ANOVA test at 99% confidence level (α = 0,001), and if the results of the test are less than 0.001, it can be concluded that there are very significant differences between treatments, and vice versa if the P value < 0.001 is obtained between treatments, then there was no significant difference. Between the inhibition zone diameter values at station A and station B at various concentrations, a very significant difference with the *P-value* = 8.1E-20 <0.001 was showed (Table 2).

Table 2. ANOVA test results for inhibition of *H. atra* sea cucumbers on *E. coli* and *S. eureus* bacteria in Menjangan Besar waters (Station A) and Alang-alang waters. (Station B), Karimunjawa, Indonesia

Treatment	*P value* (α = 0.001)
Station A against station B	8.1E-20
Station A against positive control	6.8E-27
Station B against positive control	6.3E-33

Table 1. Data on the measurement of the antibacterial activity of sea cucumber *H. atra* against *E. coli* and *S. eureus* at the Menjangan Besar waters (station A) and Alang-alang waters (station B).

Concentration (mg/mL)	The average diameter of the inhibitory zone extract and positive control					
	Station A		Station B		Positive control (amoxcillin 500 mg)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
50	4.28 ± 0.12	5.22 ± 0.14	3.75 ± 0.10	4.2 ± 0.80	16.33 ± 0.25	19.42 ± 0.21
75	5.82 ± 0.15	7.28 ± 0.15	4,40 ± 0.13	6,15 ± 0.1	17.55 ± 0.22	21.23 ± 0.20
100	8.22 ± 0.15	10.20 ± 0.13	7.13 ± 0.10	8.85 ± 0.1	19.28 ± 0.22	22.68 ± 0.32
125	10.22 ± 0.15	12.19 ± 0.15	8.81 ± 0.15	10.78 ± 0.15	21.51 ± 0.21	24.98 ± 0.20
150	11.83 ± 0.13	13.85 ± 0.10	10.9 ± 0.13	12.26 ± 0.17	23.26 ±v0.22	27.20 ± 0.21
Inhibitory effectiveness (Greenwood 1995)	Moderate	Moderate	Moderate	Moderate	Strong to very strong	Very strong

Discussion

Although the antibacterial activity of sea cucumber *H. atra* against *E. coli* and *S. aureus* in both the Menjangan Besar waters and Alang-alang waters was classified as moderate in its inhibiting ability, statistically there were significant differences in both locations, where the potential inhibition ability of sea cucumber from the Menjangan Besar waters is greater than the potential inhibition ability of sea cucumber from Alang-alang waters. Environmental conditions in which sea cucumbers live in are important aspects that must be known. Ecologically, sea cucumbers are soft-bodied echinoderms that live in the waters as their habitat. In the life cycle of sea cucumbers as sedimentary animals, they use benthic biomass as a source of food and play an important role in recycling nutrient sediments (Schneider et al. 2011), which is known as a "bioturbation" process. According to Uthicke (1999), sea cucumber *H. atra* can recycle as many as 4,600 kg of dry sediment/year. Sediments absorbed by sea cucumbers as deposit feeder provide potential as agents of pathogenic bacteria originating from polluted environments. The accumulation of bacteria entering the body of the sea cucumber provides physiological effects through its biological activity**,** by releasing the bioactive compounds produced by them

The differences in the ability of sea cucumber *H. atra* in inhibiting the growth of *E. coli* and *S. aureus* in the Menjangan Besar waters and Alang-alang waters can be explained from several aspects, including the aspects of the aquatic environment and the biophysical-chemical processes that occured in extreme and/or polluted water environment conditions, sea cucumber *H. atra* tends to carry out biological activities to produce bioactive compounds such as antibacterial. Bioactive compounds are a form of secondary metabolic compounds that function to defend themselves from unfavorable environmental conditions (Verpoorte and Alfermann 2000). Furthermore it is said that secondary metabolites are characterized by very large chemical diversity, and each organism has its own set of secondary metabolites, some of which they can share with related or totally unrelated organisms. For example, the bioactive compounds produced by sea cucumber organisms are a form of secondary compounds that are related to the physiological processes of sea cucumbers whose responses are related to the effects to the environment. According to Giudice and Rizzo (2018), metabolic and physiological changes by organisms is an adaptation response to extreme conditions resulting in structural and functional bioactive compounds. According to Harper et al. (2001), secondary metabolites are adaptive and function as host defenses against pathogens, parasites, predators, competitors, and epibiota.

The results showed that the sea cucumber *H. atra* inhibition zone against *S. aureus* bacteria from both Menjangan Besar waters and Alang-alang waters was higher than the sea cucumber inhibition zone against *E. coli* bacteria. This gives an indication that the antibacterial ability of sea cucumber *H. atra* to *S. aureus bacteria* is stronger than that of *E. coli* bacteria. Bacteria *S. aureus* as Gram positive bacteria are more powerful at attacking fish including sea cucumbers in the benthic ecosystem. *S. aureus* bacteria as Gram positive pathogenic bacteria (Woodford and Livermore 2009), in which the bacteria have the potential to attack sea cucumbers. This is because the *S. aureus* bacteria are Gram positive bacteria which are more dangerous than *E. coli* as Gram negative. The outer membrane on the cell wall of the *S. eureus* bacteria can protect the bacteria and host defense system so that it inhibits the bioactive compounds produced by sea cucumbers.

However, when viewed based on the observation station, the diameter size produced on sea cucumbers originating from the Menjangan Besar waters of sea cucumber *H. atra* extract against *E. coli* and *S. aureus* gave higher than sea cucumber from Alang-alang waters. The high average value of sea cucumbers originating from the Menjangan Besar waters provides an indication that the bioactive compounds produced by sea cucumbers from the Menjangan Besar waters higher than sea cucumber from Alang-alang waters. The interesting thing from the results of this study is the antibacterial enhancement of sea cucumber *H. atra* in the Menjangan Besar marine habitat as a tourism development zone and this cultivation is thought to be due to the presence of pathogenic bacteria accumulating in the body is more dominant than sea cucumbers from Alang-alang waters as a rehabilitation zone.

The effectiveness of antibacterial sea cucumber *H. atra* from Menjangan Besar waters relates to the environmental conditions of sea cucumber habitat as a unit in the benthic ecosystem in which the sea cucumber habitat is located. The decrease in environmental quality in the Menjangan Besar waters is thought to be the effective cause of sea cucumbers to produce antibacterial bioactive compounds as a form of sea cucumber adaptation in accepting these changes in environmental quality. As it is known that the Menjangan Besar waters is tourism and marine culture development zone with various activities, so that these waters are thought to have deterioration in the quality of the aquatic environment. This is supported by a previous study by Sulardiono et al (2018) on the location in Menjangan Besar waters in which the status of water quality tends to be oligotrophic or in the low quality category. Different things happen to sea cucumbers originating from the waters of Alang-alang, in which the waters are included in the rehabilitation zone. This is supported by the results of statistical tests that there is a very significant difference in the effectiveness of the antibacterial inhibition of sea cucumber *H. atra* against the test bacteria in Menjangan Besar waters and Alang-alang waters. This very significant difference also occurs in the Menjangan Besar and Alang-alam waters against the positive control of amoxicillin 500 mg.

The onset of the disease can be caused by emergence of new pathogens or changes in the environment (Burge et al. 2014; Harvell et al., 2004). Sea cucumber *H. atra* living in the benthic ecosystem will be certainly very affected by a natural balance system (Tjokrokusumo, 2006). Natural balance will be formed if all the elements involved are mutually supportive of one another. Microbes are one of

the biotic elements in an ecosystem that can play a role in such a system, in which the type and number of microbes are highly dependent on their environmental conditions and microbes can play a positive or negative role. Decreasing environmental quality as a benthic ecosystem will change the life order of organisms including changes in the pattern of biological activity of sea cucumber *H. atra* in their habitat, in which sea cucumbers make physiological adaptations to defend themselves against attack of pathogenic bacteria that accumulate in their bodies by producing bioactive compounds as antibacterial against pathogenic bacteria. It is known that pathogenic bacteria can appear in unhealthy or polluted aquatic environments. According to Slater and Jeffs (2010), the characteristics of the basic sediment are one of the important components that influence sea cucumber habitat preferences. Bioaccumulation of pollutants and/or pathogenic bacteria in the body of sea cucumbers takes place through a consuming process as a deposit feeder. Sea cucumbers as benthic deposit feeder animals (Uthieke, 2001) capture sediments as bacterial agents entering and accumulating in their intestines to grow and develop, so that they can interfere with sea cucumber's health. This increase in pathogenic bacteria was followed by an increase in the production of bioactive compounds by sea cucumber *H. atra*. Related to the relationship between bacteria and the production of this bioactive compound, Kamarudin and Rehan (2018) compared the pathogenic bacteria in the intestine of sea cucumber *S. horrens* and *H. leucospilota*, and the result showed that pathogenic bacteria in *S. horrens* were higher than that in sea cucumber *H. leucospilota*, and the high pathogenic bacteria in sea cucumber *S. horrens* pushed the potential for production of bioactive compounds by sea cucumbers into higher level.

In conclusion, based on the results of this study, it can be concluded that at the two observation sites, the antibacterial activity of sea cucumber *H. atra* against *S. eureus* bacteria was higher than that against *E. coli* bacteria. Statistically, there is a very significant difference in sea cucumber antibacterial activity between the one in the waters of Menjangan Besar and Alang-alang waters, in which antibacterial activity of sea cucumbers originating from the waters of Menjangan Besar are higher than sea cucumbers from the Alang-alang waters. It is also showed that sea cucumber *H. atra* from the Menjangan Besar waters gave more effective response to produce bioactive compounds to inhibit *E. coli* and *S. aureus* bacteria. This condition is different from sea cucumbers originating from the Alang-alang aquatic environment. Given the potential of sea cucumber bioactive compounds from the Menjangan Besar waters is higher than that of sea cucumbers from Alang-alang waters, so, the potential of sea cucumber *H. atra* in the Menjangan Besar waters can be used as an alternative in developing commercial sea cucumbers.

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REFERENCES

Aziz A. 1995. Beberapa catatan tentang teripang bangsa Aspidochirotida. Oseana 20 (4): 11-23. [Indonesian]

Burge CA, Eakin M, Friedman CS, Froelich B, Hershberger PK, Hofmann EE, Petes LE. 2014. Climate change influences on marine infectious diseases: implications for management and society. Ann Rev Mar Sci 6: 249-277.

Dwicahyani T, Sumardiyanto, Rianingsih L. 2018. Uji bioaktivitas ekstrak teripang keling *Holothuria atra* sebagai antibakteri *Staphyllococcus aureus* dan *Escherichia coli*. J Pengolahan & Bioteknologi Hasil Perikanan 7 (1): 15-24. [Indonesian]

Farjami B, Muhammad AN, Yazdan M, GholamReza I, Melika N, Abdollah A, Abazar P. 2013. Antibacterial activity of the sea cucumber *Holothuria leucospilota*. Intl J Mol Chem Microbiol 1: 225-230.

Januardi R, Hartoko A, Purnomo PW. 2016. Analisis habitat dan perubahan luasan terumbu karang di Pulau Menjangan Besar, Kepulauan Karimunjawa menggunakan citra satelit. Manag Aquat Resour J 5 (4): 302-310. [Indonesian]

Kamarudin KR, Rehan MM. 2018. Gram-positive bacteria with commercial potential from the gastrointestines of *Holothuria (Mertensiothuria) leucospilota* (Timun Laut) and *Stichopus horrens* (Gamat) from Malaysian Waters. Pertanika J Trop Agric Sci 41 (2): 605-620.

Lawrence AJ, Afifi R, Ahmed M, Khalifa, Paget T. 2010. Bioactivity as an options value of sea cucumber in the Egyptian Red Sea. Conserv Biol 24 (1): 217-225.

McCaig LF, McDonald LC, Mandal S, Jernigan DB. 2006. *Staphylococcus aureus*-associated skin and soft tissue infections in ambulatory care. Emerg Infect Dis 12 (11): 1715-1723.

Rizzo C, Giudice AL. 2018. Marine invertebrates: Underexplored sources of bacteria producing biologically active molecules. Diversity 10 (3): 52. DOI:10.3390/d10030052

Roihanah S, Sukoso, dan Andayani, 2012. Aktivitas antibakteri ekstrak teripang *Holothuria* sp terhadap bakteri *Vibrio harveyi* secara in vitro. J Exp Life Sci 2 (1): 1-5. DOI: 10.21776/ub.jels.2012.002.01.01

Schneider K, Silverman J, Woolsey E, Eriksson H, Byrne M, Caldeira K. 2011. Potential influence of sea cucumbers on coral reef $CaCO_3$ budget: A case study at One Tree Reef. J Geophys Res Biogeosci 116: G04032. DOI: 10.1029/2011JG001755

Sulardiono B, Purnomo PW, Haerudin. 2017. Tingkat kesesuaian lingkungan perairan habitat teripang (Echinodermata: Holothuroidae) di Karimunjawa. Saintek Perikanan 12 (2): 93-97. [Indonesian]

Sulardiono B, A'in C, Muskananfola MR. 2018. Profiles of water quality at Menjangan Besar Island, Karimunjawa, Central Java Province, Indonesia. Biodiversitas 19 (6): 2308-2315.

Tjokrokusumo SW. 2006. Bentik makroinvertebrata sebagai bioindikator polusi lahan perairan. J Hidrosfir 1 (1): 8-20. [Indonesian]

Woodford N, Livermore DM. 2009. Infections caused by Gram-positive bacteria: a review of the global challenge. J Infect 59 (1): 4-16.

Yudo S. 2010. Kondisi kualitas air sungai Ciliwung di wilayah DKI Jakarta ditinjau dari parameter organik, amoniak, fosfat, deterjen, dan bakteri coli. Jurnal Air Indonesia 6 (1): 34-42. [Indonesian]

Bakus GJ. 1973. The Biology and Ecology of Tropical Holothurians. In: Jones OA, Endean R (eds.). Biology and Geology of Coral Reefs. Volume II, Biology 1. Academic Press, New York.

Brooks GF, Butel JS, Morse SA. 2001. Mikrobiologi Kedokteran. Buku 1. Penerbit EGC, Jakarta. [Indonesian]

Dwidjoseputro 1998. Dasar-Dasar Mikrobiologi. Penerbit Djambatan, Jakarta. [Indonesian]

Greenwood. 1995. Antibiotika Susceptibility (Sensitivity) Test, Antimicrobial and Chemotherapy. McGraw Hill Company, USA.

Harper MK, Bugni TS, Copp BR, James RD, Lindsay BS, Richardson AD, Schnabel PC, Tasdemir D, Vanwag-oner RM, Verbitski SM, Ireland CM. 2001. Introduction to the chemical ecology of marine natural products. In: McClintock JB, Baker BJ (eds.). Marine Chemical Ecology, Marine Biology. CRC Press, Boca Raton, FL, USA

Harvell CD, Aronson R, Baron N, Connell J, Dobson A, Ellner S, Gerber L, Kim K, Kuris A, McCallum H, Lafferty K, McKay B, Porter J, Pascual M, Smith G, Sutherland K, Ward J. 2004. The rising tide of ocean diseases: unsolved problems and research priorities. Front Ecol Environ 2 (7): 375-382.

Kurniawan A. 2012. Penyakit Akuatik. Buku Ajar. UBB Press, Bangka. [Indonesian]

Slamet JS. 1996. Kesehatan Lingkungan. Gajah Mada Univerity Press, Yogyakarta. [Indonesian]

Verpoorte R, Alfermann AW. 2000. Metabolic Engineering of Plant Secondary Metabolism. Kluwer Academic Publishers, Dordrecht, Netherlands.

Bennett JW, Bentley R. 1989. What's in a name? Microbial secondary metabolism. Adv Appl Microbiol 34: l-28.

Mayer AMS, Rodriguez AD, Taglialatela-Scafati O, Fusetani N. 2013. Marine pharmacology in 2009-2011: Marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous, mechanism of action. Mar Drugs 11 (7): 2510-2573.

Uthicke S. 1999. Sediment bioturbation and impact of feeding activity of *Holothuria (Halodeima) atra* and *Stichopus chloronotus*, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef. Bull Mar Sci 64 (1): 129-141.

Slater MJ, Jeffs AG. 2010. Do benthic sediment characteristics explain the distribution of juveniles of the deposit-feeding sea cucumber *Australostichopus mollis*?. J Sea Res 64 (3): 241-249.