Production Of Secondary Metabolites From Heterotrophic Bacteria As Anti-Pathogenic Bacteria

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Abstract: Aquaculture provides a large business opportunity along with the large demand for fish. One problem is the presence of pathogenic bacteria that attack cultivated fish. The use of synthetic antibiotics has been increasingly reduced and returned to natural antimicrobials. The use of natural anti-microbes has great potential to be developed. One of the things that can be used is secondary metabolism of heterotrophic bacteria which is widely spread in the waters. This study aims to determine the ability of secondary metabolite production of heterotrophic bacteria to inhibit the activity of pathogenic bacteria (Vibrio alginolyticus, Aeromonas hydrophila and Pseudomonas sp).The method used is the experimental method. 10 Heterotrophic bacterial isolates obtained from the collection of marine microbiology laboratories were cultured on Nutrient broth media for 7 days and penned during stationary times, then extracted from secondary media from the media and shells using Etyl Acetate. The secondary metabolite extract was tested against 3 pathogenic bacteria that usually attack fish.Based on the results of the antagonism test conducted, 10 heterotrophic bacterial isolates were able to inhibit the growth of all three pathogenic bacteria. The strength of the inhibition in Vibrio alginolyticus varies between 6.8 - 11.76mm, The strength of the inhibition in Aeromonas hydropila bacteria ranges from 6.8 to 9.5 mm, the strength of the inhibitory strength in Pseudomonas aeroginosa bacteria varies between 6.7-11.1 mm. Overall the inhibition of the three strongest pathogenic bacteria was N isolates (Bacillus cereus code access KM489154.1). and the weakest is Q isolate (Bacillus cereus, code access KY750689.1. Conclusion. Heterotrotic bacteria produce secondary metabolites that can inhibit the growth of pathogenic bacteria in fish. Secondary metabolism of heterotrophic bacteria has the potential to be developed as an anti microbial in pathogenic bacteria in fish..

Index Terms: anti microbes, heterotrophic bacteria, pathogens, Secondary metabolism

1 INTRODUCTION

Heterotrophic bacteria have an important role as decomposers of organic compounds (mineralization) originating from industrial waste, decomposition of feed that is not consumed, and have the ability to inhibit the growth of pathogenic bacteria[1] (Feliatra et al., 2017). Heterotrophic bacteria are a group of bacteria in marine ecosystems that do not only act as decomposers but have the potential to produce probiotics for aquaculture [2] (Feliatra et al., 2016). In addition, heterotrophic bacteria are important decomposers from pollutants [3] (Kong and Ye, 2014). In particular, heterotrophic bacteria have a significant and effective contribution condition for the degradation of petroleum hydrocarbon pollutants in the sea [4]. Marine bacteria including heterotrophic bacteria are capable of producing secondary metabolites. Unlike the primary metabolites, secondary metabolites are not needed for the survival of bacteria, but are important for the adaptation process, besides bacteria mainly produce secondary metabolites with antibiotic activity for survival purposes, more recent studies show that these secondary metabolites also play a key role as molecules signaling [5] [6]

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Secondary metabolites are not produced during rapid cell growth (logarithmic phase), but are usually synthesized at the end of the cell growth cycle, ie in the stationary phase, where the cell population remains because the number of cells that grow is the same as the number of dead cells. Bacteria have great potential to produce carotene. In heterotrophic bacteria, carotenoids are secondary metabolites that play a fundamental role in cell adaptability. Carotene protects cells from UV radiation and oxidative damage [7]. The production of microbial secondary metabolites is highly dependent on fermentation conditions [8]. Bioactive compounds are present in all types of life forms and are produced by organism's secondary metabolism. Secondary metabolites include terpenoids, alkaloids, polyketides, peptides, shikimic acid derivatives, sugars, steroids, and a large mixture of metabolite biogenesis [9]. Secondary metabolites have complex structures and involve unusual biochemical processes. Two families of enzymes, polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS), are very important in the production of various secondary metabolites, many of which are drugs or probiotics [10] . some bacteria have benefits and some cause harm to living things. The secondary metabolite test performed on heterotrophic bacteria will produce antibiotic compounds that can inhibit the growth of pathogenic bacteria. Secondary metabolites of heterotrophic bacteria can be used as probiotics, where disease control strategies in fisheries are always carried out by using probiotics to give better results [11]. This study aims to determine the ability of inhibitory power of secondary metabolites of heterotrophic bacteria against pathogenic bacteria pathogenic bacteria (Vibrio alginolyticus, Aeromonas hydrophila and Pseudomonas sp).

2.RESEARCH METHODS

This study uses an experimental method that is 10 heterotrophic bacterial isolates obtained from the collection of Marine Microbiology laboratory, Faculty of Fisheries and

Marine with species as in Table 1. cultured for ± 7 days at 8 grams of media Nutrient Broth dissolved in 1000 ml of different salinity seawater according to source of heterotrophic bacteria, then the content of secondary metabolites is extracted using Etyl Acetate, the extraction results are separated using a Rotary Evaporator, then air dried. The metabolite extract was dissolved using Methanol to test secondary metabolites. The secondary metabolites of heterotrophic bacteria as a trial unit of 10 isolates were tested in 3 treatments of pathogenic bacteria namely Aeromonas hydrophila, Pseudomonas aeruginosa, Vibrio alginolyticus and each isolate was repeated three times, so that 90 trials were obtained, Inhibitory test of secondary metabolites of pathogenic bacteria using the disc method by dripping 100 µL of secondary metabolites on disc paper measuring 6 mm in diameter, then left to dry, then placed on the surface of the petri dish containing NB media mixed with pathogenic bacteria [12]. Microbial activity occurs when a clear zone is formed around the disc paper. The amount of inhibitory activity is indicated by the diameter of the clear zone formed, the wider the clear zone, the greater the inhibitory power.

Data analysis

The secondary metabolite test activity is defined as the AU (Activity Unit). One AU is the area of resistance per unit volume of the sample antibiotic solution tested (mm2 / ml) [13] .

$$AU\ (mm^2/ml) = \frac{LZ^2 - Lc^2}{\text{[illegible]}} \quad (1)$$

LZ = wide clear zone
Lc= disc area

The data obtained will be analyzed descriptively by comparing the average clear zone formed by the criteria for antibacterial strength.

3.RESULTS AND DISCUSSION

10 Isolates used in this study which have been isolated by previous researchers [14] [15] [16], all species of isolates have been identified and morphology has been identified. The BLAST (Basic Local Alignment Search Tool) system is a system for finding species names, DNA homology percentages as a result of existing database bases in Bank Gen through the site http://www.ncbi.nlm.nih.gov/. BLAST results can be seen in Table 1.

***Table 1**. BLAST Results (Basic Local Alignment Search Tool)*

Isolate	Spesies	Strain	Acces code	Salinity
K	*Uncultured Kerstersia* sp	CLONE OTU-13-ABB	JQ-624321.1	5 ppt
L	*Kerstersia gyiorum*	S7	KM884887.1	5 ppt
M	*Kerstersia gyiorum*	S7	KM884887.1	5 ppt
N	*Bacillus cereus*	SN7	KM489154.1	10 ppt
O	*Bacillus cereus*	LOCK 1002	KT728833.1	10 ppt
P	*Bacillus cereus*	SBABrB5	LC189361.1	10 ppt
Q	*Bacillus cereus*	DFT-5	KY750689.1	10 ppt
R	*Bacillus safensis*	MMD02	*KY495152.1*	15 ppt
S	*Alcaligenes faecalis*	EBD	EF011115.1	15 ppt
T	*Uncultured bacterium*	CLONE HCA14	EU723865	15 ppt

Of the 10 isolates analyzed, 8 of them were isolates that had homologs that reached 95% with those in the bank genes, so it was believed to be the same species. Two of the isolates did not have high homologies with those in bank genes and were believed to be new species and native to Indonesia table 1.

Antimicrobial Activity Test Results

This study used the disc method with a pouring technique showing that there was an effect of giving a secondary metabolite solution to the paotogen bacteria after incubation for 24 hours. The effect of giving a solution of secondary metabolites can be seen if there is a clear zone around the disc paper.

***Table 2**. The inhibition and standard deviation of metabolites secondary to pathogenic bacteri*

Isolat	Bacteria *V.alginolyticus*	Bacteria *A.hydrophilla*	Bacteria *P.aerogenosa*
K	8,66±0,3819	7,86±0,2082	8,7±1,4799
L	8,6±0,3606	8,53±1,2021	9,6±2,0952
M	9,71±0,8312	7,23±0,4509	8,06±0,3786
N	11,76±0,6807	8,43±1,1471	10,06±0,8485
O	8,75±1,3650	6,8±0,3000	8,13±1,0797
P	7,76±0,2517	8,23±0,9292	7,1±0,3606
Q	6,8±0,3606	7,7±1,2530	7,56±0,4041
R	7,33±1,0408	6,93±0,7506	6,76±0,2517
S	9,8±1,8621	8,2±0,3000	11,15±0,8352
T	10,9±1,1449	9,5±0,3000	8,56±0,6658

Based on the results of the antagonism test conducted, heterotrophic bacterial isolates isolated from the Muak River Siak waters were able to produce antimicrobial compounds with a clear zone around the disc paper with different diameter sizes. The clear zone formed is a sign that the inability of pathogenic bacteria to grow around the disc. Clear zones formed generally have a size with a strong category. Based on Table 2, it can be seen that 10 heterotrophic bacterial isolates are able to inhibit the growth of three pathogenic bacteria (Vibrio sp, Aeromonas sp and Pseudomonas sp) with varying clear zone sizes inhibitory response to the growth of V. algynolyticus bacteria, showed that 10 isolates were classified as having a strong inhibitory response. The highest inhibitory value is found in the Bacillus cereus species (KM489154.1) with an average diameter of around 11.76 mm. whereas for the lowest or categorized medium inhibitory, Bacillus safensis (KY495152.1) with an average diameter of about 7.33 mm. The inhibitory response to the growth of A. hydrophila bacteria showed that all bacterial isolates had a moderate response inhibitory response. The uncultured bacterium species (EU723865) has the highest inhibitory response with an average diameter of about 9.5 mm and is a medium category. Whereas for the smallest inhibition power found in the species Bacillus cereus (KT728833.1) with an average diameter of about 6.8 mm and including the medium category. The inhibitory response to the growth of Pseudomonas sp. showed that the highest inhibitory value was found in the species Alcaligenes faecalis (EF011115.1) with an average diameter of about 11.15 mm. and for the smallest inhibition power found in Bacillus safensis isolates (KY495152.1) with an average diameter of about 6.76 mm. Graph of measurement results of the activity of test units of secondary metabolites of heterotrophic bacteria against pathogenic bacteria (Vibrio algynolyticus, Aeromonas hydrophila and Pseudomonas sp). N isolates of Bacillus cereus species (KM489154.1) had the highest inhibitory activity of 1,073.34 mm2 / ml and the lowest inhibitory activity by R isolates of Bacillus safensis

(KY495152.1) species, namely 213,386 mm2 / ml.figure 1. The ability of bacterial isolates to inhibit the growth of pathogenic bacteria is a form of antagonistic activity that is thought to be carried out by producing antimicrobial compounds. [17] Biosynthesis of antimicrobial compounds plays an important role in the attachment process, target colonization to competition in obtaining space and nutrients with microbes. Different result are caused by the ability of each bacterium to fight antibacterial activity differently depending on the thickness and composition of the cell walls. Gram negative bacteria contain lipids in presentations higher than those contained in gram positive bacteria. The structure of gram negative bacteria has an outer membrane covering the thin layer of peptidoglycan, this outer structure of is peptidoglycan is a double layer containing phospholid, protein and lipopolysaccharide Lipopolusaccharide is located in outer layer and is characteristic of gram negative bacteria.

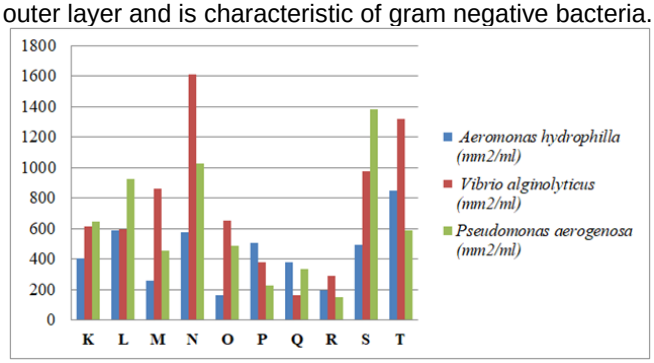

FIGURE 1. *ACTIVITY DIAGRAM OF A TEST UNIT FOR SECONDARY METABOLITES OF HETEROTROPHIC BACTERIA AGAINST PATHOGENIC BACTERIA*

The difference in the ability of inhibitory power in each isolate is caused by differences in the content of secondary metabolites possessed by each isolate that has diffused first into agar, so that the growth of pathogenic bacteria becomes inhibited. In general, the ability to inhibit the growth of other bacteria is caused by the production of probiotic bacteria which can produce bacteriocins which work selectively on several strengths of pathogenic bacteria, lactic acid, acetic acid, hydrogen peroxide, lactoperoxide, lipopolysaccharide, and some antimicrobials [18]. Some factors include: production of antibiotics, bacteriocins, ciderphores, lysosomes, proteases and hydrogen peroxide or affect the pH of the media by producing certain organic acids. This is in line with the study of [19] that bacterial agents such as lactic acid possessed by probiotic bacteria are able to inhibit the growth of pathogenic bacteria because antibacterial agents are able to reduce pH to low so that pathogenic bacteria find it difficult to survive.

4.CONCLUSION

Based on research carried out 10 heterotrophic isolates isolated from the Siak estuary waters were able to inhibit the growth of pathogenic bacteria (Vibrio algynolyticus, Aeromonas hydrophila and Pseudomonas sp) indicated by the presence of clear zones around disc paper with different diameter sizes. The clear zone formed is a sign that the inability of pathogenic bacteria to grow around the disc. Based on the antagonism test, the highest inhibitory value is Bacillus cereus (KM489154.1) with an average diameter of about 10.08 mm, while the inhibition capacity which is classified as the lowest or categorized as moderate is found in Bacillus safensis species (KY495152.1) with an average diameter of around 7.33 mm. The ability to inhibit pathogenic bacteria is suspected because these heterotrophic bacterial isolates produce antibiotic compounds. This compound is a collection of chemicals produced by microorganisms including fungi and bacteria which have the function of inhibiting growth other microorganisms.

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