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Antimicrobial agents derived from heterotrophic bacteria against pathogenic bacteria

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Abstract. Pathogenic bacteria often cause problems in fish farming. Prevention efforts using synthetic antibiotics may engender negative impacts on the environment. Accordingly, the use of natural antimicrobial compounds is required to minimize the risk. There are several types of heterotrophic bacteria which can produce antimicrobial agents. This study aims to determine the ability of the secondary metabolites extract isolated from heterotrophic bacteria in inhibiting the growth of pathogenic bacteria. The method used in this study was an experimental method. Four isolates of bacterial heterotrophic originating from waters of Sungai Pakning were cultured on nutrient broth for 10 days which would later be extracted by using ethyl acetate. The secondary metabolites extract of the heterotrophic bacteria was tested on 3 pathogenic bacteria that usually attack fish. The antimicrobial test results indicated that the secondary metabolites extract of the heterotrophic bacteria was able to inhibit the growth of *Aeromonas salmonicida* bacteria from 11.77 to 12.53 mm, *Edwardsiella tarda* bacteria from 10.70 to 12.40 mm and *Edwardsiella ictaluri* bacteria from 10.97 to 12.38 mm. Overall, the inhibition of the strongest pathogenic bacteria was JS11 isolates (*Bacillus* sp. strain SMMA8 code access LN869534.1). Heterotrophic bacteria produced secondary metabolites which can inhibit the growth of pathogenic bacteria in fish. Antimicrobial agents of heterotrophic bacteria are potentially to be developed.

1. Introduction

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Heterotrophic bacteria is an organism that cannot produce its own food, relying instead on the intake of nutrition from other sources of organic carbon, mainly plant or animal matter. These kinds of bacteria have a very important role in marine ecosystems which are able to utilize organic and inorganic materials in the environment where they grow as a source of nutrition. Heterotrophic bacteria have a role as a decoder and are able to remineralize organic materials into simple inorganic components that are returned to the soil and the atmosphere as nutrients [1-3].

Heterotrophic bacteria also play a role in the transformation of energy flows and in the carbon cycle, so as to maintain the continuity of the life cycle of marine biota [4]. In the water, the heterotrophic bacteria can decompose pollutants [5] and in fish farming, heterotrophic bacteria have the potential as probiotics [6].



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Heterotrophic bacteria as part of microorganisms in the sea can produce potential chemical compounds for drugs, nutritional supplements, cosmetics and enzymes. Generally, these potential chemical compounds come from secondary metabolites of microbes. The secondary metabolites are compounds synthesized by microbes through biosynthetic processes that have pharmacological and biological activities. Secondary metabolites can play a role in improving microbial life when competing with other species [7].

Some microorganisms of this type of bacteria can produce secondary metabolites as antimicrobial compounds, which are antagonistic to pathogenic bacteria [8-14]. The use of antimicrobial compounds derived from these bacteria can reduce the use of synthetic antibiotics that can have a negative effect on the environment.

This study aims to determine the ability of the extract of secondary metabolites of heterotrophic bacteria in inhibiting the growth of pathogenic bacteria (*Aeromonas salmonicida*, *Edwardsiella tarda* dan *Edwardsiella ictaluri*).

2. Materials and Methods

2.1 Isolation of secondary metabolites

An experimental method was used in this research. Four isolates of heterotrophic bacteria that investigated were originally from waters of Sungai Pakning of Bengkalis Regency, Riau and have been genetically identified (Table 1). Heterotrophic bacteria isolates were cultured during 10 days at 8 grams of Nutrient Broth media dissolved in 1000 ml salinity of 29 ppt. The secondary metabolite was extracted using Ethyl Acetate. The extraction results were separated using a Rotary Evaporator at a temperature of 50°C at 50 rpm, then dried at room temperature [15].

2.2 Inhibitory test

The secondary metabolites extract was tested on 3 types of pathogenic bacterias: *Aeromonas salmonicida*, *Edwardsiella tarda* and *Edwardsiella ictaluri*. The inhibitory test of extraction of secondary metabolites in pathogenic bacteria used the disc method [16]. The extraction of Secondary metabolites was dissolved using Methanol. Then, 100 µL was dropped on paper disc with a diameter of 6 mm. Hence, let it dry. Then, it were placed on the surface of the petri dish containing nutrient media. Finally, it were incubated at 30°C for 24 hours.

The inhibition of the secondary metabolites extract against the growth of pathogenic bacterias was indicated from the formation of clear zones around the paper disc. The amount of inhibition activity was indicated by the diameter of the clear zone formed, if the clear zone wider, the inhibitory power will bigger.

2.3 Data analysis

The activity of the secondary metabolites test unit is defined as the AU (Activity Unit). One AU is the area of resistance per unit volume of the antibiotic solution sample tested (mm² / ml) [17].

$$AU \text{ (mm}^2\text{/ml)} = \frac{LZ-LC}{\text{Sample volume}} \quad (1)$$

LZ = the width of the clear zone

LC = Disc Area

The data obtained were descriptively analyzed by comparing the magnitude of the clear zone formed as criteria for antibacterial activity.

3. Results and Discussion

The heterotrophic bacteria isolates used in this study were genetically identified using the BLAST system. The BLAST (Basic Local Alignment Search Tool) system is a system for finding species names, percentages of homology of DNA sequence from database bases in Gen Bank through the site <http://www.ncbi.nlm.nih.gov/>. The BLAST results of heterotrophic bacterial isolates are presented in Table 1.

BLAST analysis results showed that all heterotrophic bacterial isolates had homology from 96 to 99%, so it was believed to be the same species as the database in bank genes. JS8 isolates were *Bacillus toyonensis*, JS10 *Bacillus cereus*, JS11 *Bacillus* sp. and JS19 *Pseudoalteromonas* sp. Endorsement to the species level if it has the same 99% DNA sequence and at the genus level, the DNA sequence was 97% [18].

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

Isolate	Species	Strain	ID	Homology
JS8	<i>Bacillus toyonensis</i>	DFT-2	KY750686.1	96%
JS10	<i>Bacillus cereus</i>	DFT-5	KY750689.1	96%
JS11	<i>Bacillus</i> sp.	SMMMA8	LN869534.1	99%
JS19	<i>Pseudoalteromonas</i> sp.	DJ8	MG561859.1	99%

The results showed that the extracts were able to inhibit the growth of pathogenic bacteria; *A. salmonicida*, *E. tarda* and *E. ictaluri* which were indicated by the presence of clear zones formed around the paper disc. The amount of inhibition produced ranging from 10.7 mm to 12.53 mm (Table 2). The inhibitory ability of the extraction of the secondary metabolites was classified as *strong* [19]. The results of this test indicated, the greater diameter of the clear zone produced in the in vitro test, the stronger inhibition activity of the substances isolated from heterotrophic bacteria.

Table 2. Inhibition activity of the secondary metabolites extract against pathogenic bacteria

Isolate	<i>A. salmonicida</i> (mm)	<i>E. tarda</i> (mm)	<i>E. ictaluri</i> (mm)
JS8	11.77±0.231	10.70±0.306	10.97±0.351
JS10	11.47±0.252	11.10±0.058	11.27±0.208
JS11	12.53±0.153	12.30±0.361	12.38±0.115
JS19	12.10±0.321	12.40±0.361	12.26±0.320

The secondary metabolite of JS11 *Bacillus* sp. isolates (LN869534.1) had the highest inhibition activity against bacteria *A. salmonicida* with a diameter of 12.53 mm and the highest isolate of JS19 *Pseudoalteromonas* sp. (MG561859.1) inhibited *E. tarda* bacteria with a diameter of 12.40 mm. The formation of a clear zone in the pathogenic bacteria culture media indicated that the heterotrophic bacteria could produce secondary metabolites in the form of antimicrobial substances such as antibiotics or other compounds.

The results of secondary metabolites extract test against pathogenic bacterias showed different inhibition activity in each isolate. The diameter of the inhibition zone produced is different, due to differences in the ability to produce antimicrobial compounds by heterotrophic bacteria. The ability of antimicrobial compounds to inhibit microorganisms depends on the concentration and the type of antimicrobial substances produced [20].

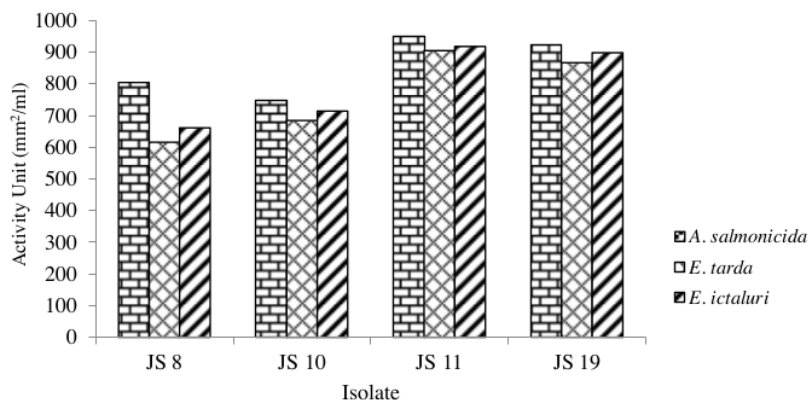


Figure 1. The activity of the extraction test unit of secondary metabolites against pathogenic bacteria

Graph of measurement results of the activity of test units of secondary metabolites of heterotrophic bacteria against pathogenic bacteria. JS11 *Bacillus* sp. isolate had the highest inhibition activity of 950.5 mm² / ml against *A. salmonicida* bacteria and JS19 *Pseudoalteromonas* sp. isolate had the highest inhibition activity of 924.4 mm² / ml against *E. tarda* bacteria (Figure 1). Heterotrophic bacteria are thought to produce bioactive compounds that can damage and degrade the structural components of cell walls of pathogenic bacteria. Microbial populations can release chemical substances that have bactericidal or bacteriostatic abilities that can affect other microbial populations [21].

Bacillus bacteria can produce at least 66 types of antibiotics and certain strains of *Bacillus* are biocontrol agents [22]. *Pseudoalteromonas* bacteria which are symbiotic with *Sarcophyton* sp. (soft coral) are able to produce carotenoid pigments, which have antibacterial activity against the *Staphylococcus aureus* bacteria [23].

4. Conclusion

Test of secondary metabolites extract of the heterotrophic bacteria can inhibit the growth of pathogenic bacteria, *A. salmonicida*, *E. tarda* and *E. ictaluri*. The inhibition zone of secondary metabolites of JS11 *Bacillus* sp. isolate (LN869534.1) against *A. salmonicida* was 12.53 mm and of JS19 *Pseudoalteromonas* sp. isolate (MG561859.1) against *E. tarda* was 12.40 mm. Those inhibition activities of secondary metabolites produced including a strong activity. Antimicrobial agents from secondary metabolites of heterotrophic bacteria have a potential to be developed.

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