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Isolation and properties of ammonia-oxidizing bacteria from mangrove sediment, hanura lampung beach

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Abstract. Mangrove forest area is an area that has good water quality. In the sediments of mangrove forests, there are many types of microbes capable of bioremediating inorganic contaminants such as ammonia and the heavy metal mercury. Isolation of bacteria was carried out using specific nitrification media to obtain ammonia-oxidizing bacteria. The sensitivity test for heavy metal mercury was carried out in the laboratory with 9 combinations of concentrations. The results of bacterial isolation from mangrove forest sediments at Hanura Beach, Lampung, obtained four isolates of ammonia-oxidizing bacteria (A1, A1P, A3, and A3P). The sensitivity test for heavy metal mercury showed that the four isolates obtained were able to survive the addition of heavy metal mercury by 0.003 g / L.

Keyword: isolation, ammonia-oxidizing bacteria, mangroves, heavy metal mercury

1. Introduction

Mangrove forest area is an area that has good water quality. Mangrove forest sediments have various types of ammonia-oxidizing bacteria that are able to bioremediate inorganic contaminant compounds such as ammonia [1] and heavy metal mercury [2]. Ammonia is a compound produced from the accumulation of fish waste on the bottom of the waters. The accumulation of ammonia compounds can inhibit the growth of fish or even be toxic which can lead to mass death in fish populations [3]. Mercury enters the waters due to human activities such as pollution by factory waste and aquaculture activities. So that it is found that fish originating from aquaculture ponds or waters around aquaculture ponds contain mercury compounds which accumulate in fish body tissues [4].

Ammonia oxidizing bacteria play a role in maintaining environmental quality by helping the decomposition process in nature. Ammonia oxidizing bacteria use ammonia compounds as the main source of energy and carbon dioxide as a carbon source. These bacteria can survive in environments with low oxygen levels and are polluted [5]. These bacteria can reduce ammonia levels in the environment very well through the nitrification process to convert ammonia to nitrite [6]. Ammonia-oxidizing bacteria are very easy to find in soil layers and



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sediments in water [7]. Bacteria in general can adapt to environments contaminated with heavy metals. The bacterial cell membrane can bind heavy metal ions naturally or is called biosorption. This biosorption process enables bacteria to accumulate heavy metals on their cell membranes so that heavy metal contaminants in the environment where bacteria live are reduced and bacteria can survive [8]

To improve the quality of waters and fisheries, biological solutions are needed to tackle ammonia and mercury pollution. Therefore, in this study, the isolation of ammonia-oxidizing bacteria from mangrove forest sediments was carried out at the Center for Marine Cultivation Fisheries (BBPBL) in Lampung, Hanura Village, Teluk Pandan District, Pesawaran District, Lampung Province, and test their sensitivity to mercury.

2. Materials and Methods

2.1 Sampling

A sampling of mangrove forest sediment was carried out in the mangrove area of Balai Besar Perikanan Budidaya Laut (BBPBL), Hanura Village, Teluk Pandan District, Pesawaran Regency, Lampung Province. Sampling was conducted at three different locations, namely mangrove areas near residential areas, mangrove areas near shrimp ponds and isolated mangrove areas. At the three locations, enough mangrove sediment samples were taken and put into heat-resistant plastic. The sample was then stored in a cooler box which was then stored in a room with a temperature of $\pm 20^{\circ}\text{C}$ in the Microbiology Laboratory of the University of Lampung.

2.2 Isolation of Bacteria

Bacterial isolation was carried out using a modified method [9]. A total of 1.65 grams of sediment from each location was suspended in 1.65 ml of sterile distilled water. The suspensions were made into two groups, namely suspension groups of mangrove forest sediment samples from three locations that were not heated at 85°C and suspension groups of mangrove forest sediment samples from the three locations heated at 85°C . A total of 1 ml of the suspension was then put in 50 ml of specific nitrifying liquid media [0.7 g/L KH_2PO_4 ; 13.5 g/L Na_2HPO_4 ; 2.5 g/L $(\text{NH}_4)_2\text{SO}_4$; 0.5 g/L NaHCO_3 ; 0.005 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 0.01 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$] In this specific nitrifying liquid medium, only bacteria capable of oxidizing ammonia can survive. Incubation on specific nitrifying media was carried out for 5 days on an orbital shaker. Isolation of ammonia-oxidizing bacteria was carried out by using the spread plate method on agar medium and incubated for 24-48 hours.

2.3 Characterization of Bacterial Isolates

Macroscopic observations are carried out by observing the shape and color of a bacterial colony. Microscopic observations were made by staining the bacterial colonies. The staining is divided into two, namely gram staining, spore painting. This staining is done to determine the gram group of bacteria and the availability of endospores in bacterial cells.

2.4 Mercury Sensitivity Test

The mercury sensitivity test was carried out by using Kirby Bauer's disc diffusion method [10]. Bacterial isolates were grown using the pour plater method on SWC agar media. The sensitivity of the bacterial isolates was measured at the diameter of the clear zone that appeared around the filter paper with a diameter of 0.9 cm which was placed on the surface of the agar medium after incubation for 24 hours. The filter paper was pre-immersed in a solution of heavy metal HgCl_2 with 9 combinations of concentrations (0.76, 0.38, 0.19, 0.098, 0.048, 0.024, 0.012, 0.006, 0.003 g/L).

3. Results

Based on the results of the study, it was obtained four ammonia-oxidizing isolates (A1, A3, A1P, and A3P) from mangrove sediments at Balai Besar Perikanan Budidaya Laut (BBPBL) in Lampung, Hanura Village, Teluk Pandan District, Pesawaran Regency, Lampung Province. A1 is a bacterial isolate from mangrove forest sediments near shrimp ponds that are not heated at 85 °C. A1P is a bacterial isolate from mangrove forest sediments near shrimp ponds that are heated at 85 °C. A3 is a bacterial isolate from isolated mangrove forest sediments that is not heated at 85 °C and A3P is a bacterial isolate from mangrove forest sediments heated at 85 °C. From the mangrove area near residential areas, ammonia-oxidizing bacteria isolates were not found.

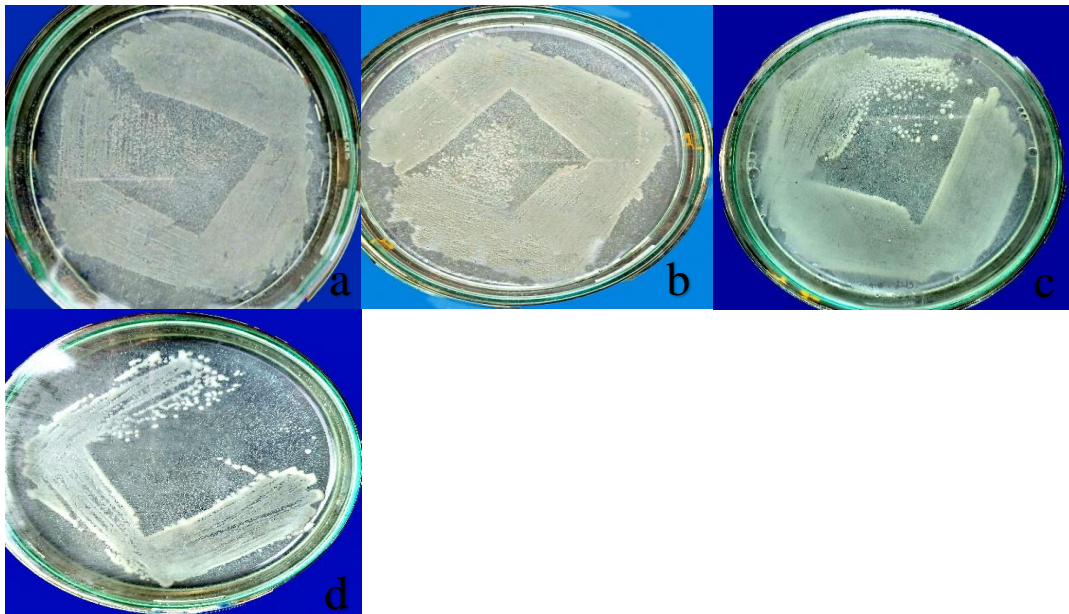


Figure 1. the results of bacterial isolation on agar media, a: A1, b: A3, c: A1P, d: A3P

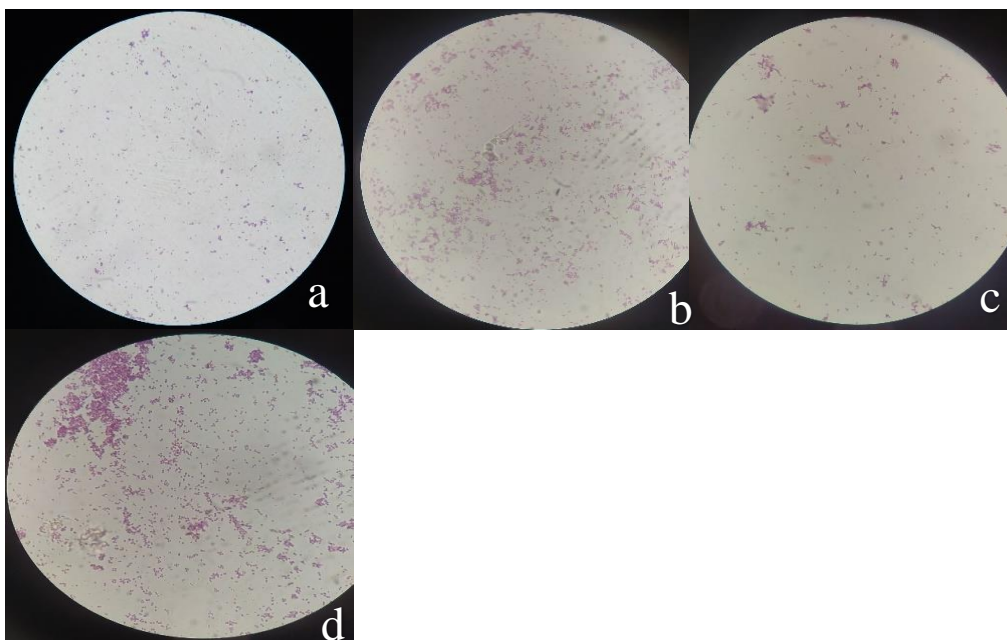


Figure 2. Microscope observation results, a: A1, b: A3, c: A1P, d: A3P

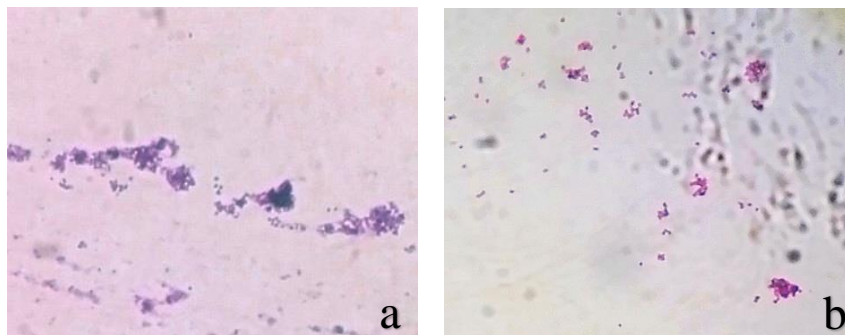


Figure 3. Endospores observation results, a: A1P, b: A3P

Table 1. Colony Characteristics and Morphological Characteristics of bacterial isolates

Isolate	Colony Features	Morphological Characteristics
A1 and A3	Creamy white color Flat Edges Convex Elevation Angle Slimy consistency Smooth Surface Small round shape	Gram-Positive Do not have endospores Small cocci shape
A1P and A3P	Creamy white color Flat Edges Convex Elevation Angle Slimy consistency Smooth Surface Small round shape	Gram-positive Has endospores Small cocci shape

3.1 Isolation of Bacteria

The colony characteristics and morphology were observed for the bacterial isolates that were isolated. The four isolates had almost the same colony shape and almost the same other characteristics, and from the results of the gram staining, the four bacterial isolates were gram-positive bacteria colored purple (Figure 2.). From the results of the spore staining, it was found that A1P and A3P isolates were bacteria that had endospores (Figure 3.), while the A1 and A3 isolates did not have endospores (Table 1.)

Table 2. Sensitivity test of bacterial isolates to Mercury

Isolate	Mercury Concentration (g / L)								
	0.003	0.006	0.012	0.024	0.048	0.098	0.19	0.38	0.76
	Clear Zone Diameter (cm)								
A1	-	0.6	1.4	1.8	1.6	1.7	2.4	2.3	1.6
A1P	-	-	1.5	2.1	2	2.4	2.4	2.4	1.7
A3	-	0.8	1.3	2	1.6	2.4	2.4	2.3	1.7
A3P	-	-	1.3	1.8	1.5	1.6	2.2	2.1	1.6

Note: (-) = no inhibition zone

3.2 Mercury Sensitivity Test

From the test data, it shows that the bacterial isolates that have been isolated have the ability to survive the presence of mercury compounds in the growing media. The four isolates were able to grow and survive against mercury compounds with a concentration of 0.003 g/L, in addition to those isolates labeled A1P and A3P were able to survive in the presence of mercury compounds with a concentration of 0.006 g/L (Table 2).

4. Discussion

4.1 Bacteria isolation

The sediments of mangrove forests are rich in organic matter so that they are a place that has many types of ammonia-oxidizing bacteria. The presence of ammonia-oxidizing bacteria is influenced by the presence of mangrove trees, this is because ammonia-oxidizing bacteria play an important role in the nitrification process in mangrove forest sediments [11]. So that from the research results obtained four isolates of ammonia-oxidizing bacteria, namely A1, A3, A1P and A3P. The four isolates were ammonia-oxidizing bacteria that able to grow on specific nitrifying media containing ammonia as the main composition of the media.

The results of bacterial isolation and their characteristics are presented in Table 1. Based on Table 1. it is known that overall bacterial isolates have similar characteristics. The previous research stated that the results of isolated bacteria from mangrove forest sediments in their study showed the same morphological characteristics with variations in the characteristics of gram-positive or negative bacteria, round or stem cell shape, cream or pale colony color, round or oval colony shape and convex or flat colony elevation [12]. Isolates A1, A3, A1P and A3P are gram-positive spherical bacteria that are commonly found in mangrove forest sediments.

In this study, the mangrove forest sediments were heated at a temperature of 85 °C to look for bacterial isolates that were able to form endospores. Bacteria that can form endospores generally can survive and grow well in limited and dangerous environmental conditions such as high temperatures [13]. Isolates from mangrove forest sediments near shrimp ponds (A1P) and isolates from isolated mangrove forest sediments (A3P) after being incubated for three days were observed with spore staining. From the observations, the two isolates appeared to have endospores which were thought to have formed after the source of the two isolates was heated at 85 °C.

4.2 Sensitivity of bacteria to mercury

From the results of the sensitivity test, it was found that the four isolates were able to survive against mercury compounds at 0.003 g / l. The absence of clear zones on the agar media with disc paper containing 0.003 g / L of heavy metal mercury indicated that the four isolates were able to absorb heavy metal mercury by the biosorption process. Biosorption is the process of absorption of chemicals by bacteria directly from contaminated media or the consumption of food containing chemicals [14]. Biosorption is the ability of the surface of the bacterial cell membrane to absorb metals through Physico-chemical interactions between metals and functional groups on the surface of microbial cells [8]. The previous research stated that ammonia-oxidizing bacteria respond to heavy metals by biosorption on the cell membrane so that ammonia-oxidizing bacteria are potential candidates for bioremediation to remove heavy metal contaminants from the environment [15].

In this research, A1P isolates were incubated for 48 hours showing the ability to grow bacterial colonies in the clear zone around disc paper containing heavy metal mercury concentrate. A1P isolate can re-grow in the clear zone at nine concentrations of heavy metal mercury tested. This made the A1P isolate able to live well without a hitch at the nine concentrations of heavy metal mercury tested. This result is presumably because A1P isolates have exocellular polysaccharides that can absorb heavy metal Hg. Bacteria themselves generally can absorb heavy metals with the ability of exocellular polysaccharides. Bacterial exocellular can absorb heavy metal Hg.

Exocellular bacterial polysaccharides are sugar polymers with large molecular weights that can be completely removed from the cell or remain attached to the cell surface to protect bacteria from extreme conditions [16].

5. Conclusion

It can be concluded from the research that isolates A1, A3, A1P, and A3P were isolated from mangrove sediments at Balai Besar Perikanan Budidaya Laut (BBPBL), Hanura Village, Teluk Pandan District, Pesawaran Regency, Lampung Province, which are ammonia-oxidizing bacteria. The four isolates also could survive at 0.003 g / L heavy metal mercury and A1P was able to grow well at nine concentrations of heavy metal mercury tested.

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