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Research Article

Study of Rhizosphere Bacteria on the Coast of Mempawah Mangrove as Bioremediation Agents

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Abstract

The production and accumulation of organic matter in the mangrove ecosystem allow this area to be rich in microbes and potentially develop as a source of various extracellular enzymes. This research aims to create effective microorganisms as bioremediation agents and determine the composition of the types of bacteria found in mangrove areas, sediments, leaf litter, stems, and mangrove plants. Sediment and vegetation were obtained as samples for being diluted and isolated, then spread onto media to get a single pure colony using Zobell 2216 method. The virgin territory formed is distinguished by characterization using macroscopic observations. It was found that the composition of bacteria isolated from the mangrove area contained 59 isolates with various visible characteristics consisting of 20 isolates of sedimentary bacteria, 27 isolates of bacteria on stems, and 12 isolates of bacteria derived from mangrove leaves. Bacterial isolates S08, B27, and D04 have the potential for the bioremediation of Hg metal. Bacterial isolates S58, B35, and D13 have potential for Pb bioremediation, while bacterial isolates S27, B35, and D12 have potential as Fe bioremediation agents.

Keywords: Mangrove vegetation; mangrove bacteria; bioremediation; mangrove potential

1. Introduction

The mangrove ecosystem has a high diversity and is very productive for various animals, plants, and microorganisms. The variety of these microorganisms causes high nutrient requirements, so organisms are responsible for the degradation process and the formation of the essential compound in carbon flow in mangrove forest sediments (Holguin et al., 2001). A mangrove environment is also a place for developing microorganism communities which are part of the essential components of this environmental function. The roots of mangrove plants form an oxygenated rhizosphere and contain a variety of substrates. It is an excellent zone for the activity of microorganisms, mainly bacteria (Macek et al., 2000).

One of the mangrove ecosystem locations in West Kalimantan Province is the Mempawah mangrove area. The soil types in this mangrove forest are alluvial and muddy soil (glei humus). One of the heavy metal pollution currently a problem in Mempawah Regency is mercury heavy metal pollution due to Unlicensed Gold Mining (PETI) activities (Adijaya and Yamashita, 2004). In addition, the Mempawah mangrove area is located or adjacent to settlements and other productive activities such as aquaculture and industry. Therefore, this mangrove area is under high anthropogenic pressure. Mangrove forests are in a transitional zone between land and sea; thus, the site has a high potential for accumulating heavy metals because it is directly adjacent to the ground. Moreover, the mangrove forest became a place where waters and land meet through rivers and sea waters, where river flows can be a waste carrier from industry and households (Setyawan and Winarno, 2006).

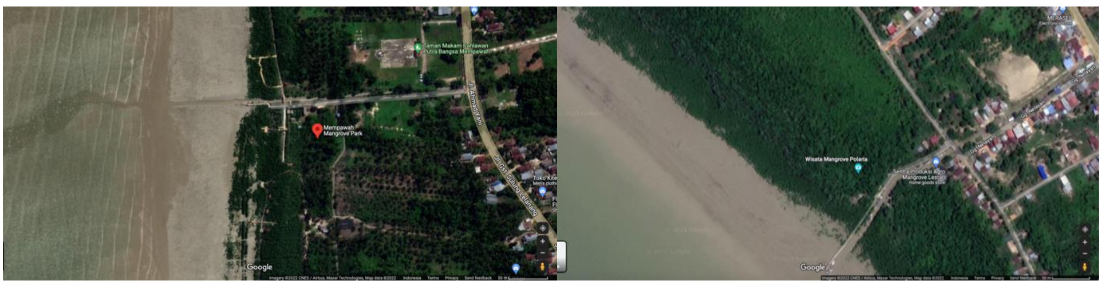
Bioremediation techniques are promising methods for cleaning up oil-contaminated mangrove sediments because they have been successfully used in environmental oil mitigation around the world, including in the oil spills in the Mexican Gulf in 2010 and Prince William Sound, Alaska, in 1989 (Bragg et al., 1994; Duke, 2016; Santos et al., 2011). Proteobacteria, Firmicutes, and Bacteroidetes, along with Gammaproteobacteria, Flavobacteriales, and Clostridiales, were studied in Rio de Janeiro, Brazil. The results revealed the potential of this strategy for environmental decontamination and suggested that environmental and ecological factors may select bacterial populations in distinct niches (Machado et al., 2019). Another study from Tamil Nadu, India, has demonstrated that the mangrove environment is crucial for the reduction and biosorption of cadmium since it is home to a diverse range of microorganisms (Priyalaxmi et al., 2014).

To reduce levels of accumulation of heavy metals in mangrove ecosystems, bioremediation techniques that utilize microorganisms, especially bacteria, can be carried out. Research conducted by de Fretes et al. (2019) found ten strains of Pb, Cu, and Cd metal tolerant bacteria from mangrove sediments in Pengudang and Tanjung Uban, Bintan Island, Indonesia. The results showed that bacterial isolates isolated from mangrove sediments had MIC values of 800-900 ppm for Pb, 100-800 ppm for Cu, and 100-200 ppm for Cd. Three bacterial isolates have high resistance to three types of heavy metals: Bacillus oceanisediminis PGD1A, Vibrio alginolyticus PGD5A, and Halobacillus kuroshimensis PGD9B. These bacteria indicate that isolates from mangrove sediments can be used efficiently for heavy metal removal from the ecosystem. The isolates of Bacillus pumilus bacteria isolated from the Karnataka Indian mangrove area could survive at Pb concentrations up to 900 ppm and remove nearly 96% Pb at neutral pH (Sahoo and Goli, 2020). Research by Irianto and Husein Sastranegara (2013) states that biological remediation techniques (bioremediation) use microorganisms, especially indigenous bacteria, which are the solution to controlling petroleum contamination. Using indigenous bacteria in bioremediation applications will be more effective because the bacteria are well adapted to the environment and able to develop well. This research is vital for developing indigenous bacteria to become effective microorganisms as bioremediation agents.

2. Location and Material

2.1. Time and Location

The study was conducted in July 2021 in the Mangrove Ecosystem Region of Mempawah, West Borneo. The survey site was run in two different locations behind the industrial and residential areas of Mempawah Mangrove Park (MMP) Tourist Area (0.3970303856224846, 108.94627157720714) and Mendalok Village Area (0.46129224193486235, 108.93089211695698).



(a) **(b)**

Figure 1. (a) Mempawah Mangrove Park (MMP) tourist area (0.3970303856224846, 108.94627157720714) and (b) Mendalok Village Area (0.46129224193486235, 108.93089211695698)

2.2. Materials

The materials and tools used in this research are sediments from mangrove vegetation stems and leaves in the rhizosphere, hand soon, plastic, label, cool box, camera, GPS, camera, lead solution, glucose, ammonium sulfate, calcium phosphate, titriplex, bromothymol blue, alcohol 70%, reagent test, aluminum foil, petri dish, identification book, stationary, and meter.

3. Methods

3.1. Sample Collection

Sediments in the mangrove vegetation, mangrove stem, and leaves in Menpawah coastal mangrove ecosystem were obtained in two different areas, the Mempawah Mangrove Park (MMP) Tourist Area (0.397030856224846, 108.94627157720714) and the Mendalok Village Area (0.46129224193489235, 915.9235). Each sample was taken from three major plant species in each region. Sediment samples, mangrove stems, and leaves were stored in sterile plastic bags in a cool box. One hundred grams of sediment samples were taken from the rhizosphere and placed in sterile plastic bags. The sample was then brought into the laboratory for bacterial isolation.

3.2. Sample Preparation and Serial Dilution

Ten grams of decomposition sediment sample were dissolved in 90 ml of 0.9% saline, diluted in 5-fold serial, then isolated to get the colony. Next, the number of bacteria obtained was calculated and purified to obtain a single colony. The foliage treatment was started by weighing 10 g of the sample, sterilizing it with 70% ethanol for 30 seconds, and then rinsing it twice with distilled water. The sterile specimen was cut into small pieces and soaked in 0.9% saline, and then the suspension was diluted in a 3-fold serial.

3.3. Isolation and Purification of Bacteria

The sample and the dilutions were plated onto Zobell 2216 medium (Setyati and Subagiyo, 2012). The composition of the medium is 15 g bacteriological agar, 5 g bacteriological peptone, and 1 g yeast extract. All components were dissolved in distilled water to prepare a medium, the volume was adjusted to 1000 ml, and the mixture was stirred with a magnetic stirrer to adjust the pH from 7.5 to 7.6.

The isolation of bacteria using the poured plate method. For each dilution series, 0.1 ml samples were taken and evenly distributed in Petri dishes containing Zobell 2216 solid selection medium. Bacteria grew uniformly and were cultured at 30 °C for 48 hours. Next, the former colonies were observed. Based on the difference in colony appearance, the purification step was continued by scraping the growing colonies with a solid Zobell 2216 selective medium to give a pure isolate.

3.4. Morphological Characterization

The morphology of the isolates was recorded in tabular form. The colonies' shape, edge, surface, elevation, size, and color were assessed.

3.5. Selection of Bacterial Isolates

The isolated bacterial isolates that have been purified are taken with ose wire, grown on nutrient broth media, and then incubated for 24 hours. Disc paper with a diameter of 1 cm is added and left for a while. Meanwhile, growth media were prepared, each containing Hg, Pb, and Fe with a concentration of 1 ppm. Bacterial isolates were selected as remediation agents by testing the ability to grow bacteria on media-containing metals (Hg, Pb, and Fe). Paper discs overgrown with bacteria are placed on them and incubated for 2 x 24 hours. Then the growth of bacteria was observed by measuring the area overgrown with bacteria. The wider the space for bacteria to grow, the greater their ability to grow on media containing heavy metals.

4. Results and Conclusions

4.1. Method Diversity of Native Bacteria from Mangrove Plant Sediments, Stems, and Leaves on the Menpawah Coast

4.1.1. Bacterial Isolates from Mangrove Sediments

Mangroves provide a unique ecological environment for diverse bacterial communities (Selvam et al., 2010). The diversity of bacteria found in mangrove forests on the coast of West Kalimantan is not widely disclosed. However, given the characteristics of West Kalimantan mangroves, each bacterium has its advantages in different areas, and its potential needs to be identified and further investigated (Taketani et al., 2010). Separation was performed on medium using streak and pore plate techniques with serial dilutions from 10-3 to 10-7 times and then characterized using macroscopic observation. A study on mangrove deposits revealed 20 purified isolates, as shown in Table 1 below.

Table 1. Bacterial isolates from mangrove sediments

No	Code	Characteristics					
		Shape	**Edge**	**Surface**	**Elevation**	**Site**	**Colour**
1.	S01	round	smooth	smooth	convex	medium	cloudy white
2.	S02	irregular	lobate	smooth	flat	big	milky white
3.	S03	filamentous	lobate	smooth	convex	big	cloudy white
4.	S04	round	curled	concentric	flat	medium	yellowish
5.	S08	irregular	smooth	smooth	flat	medium	clear
6.	S13	round	smooth	smooth	flat	small	light yellow
7.	S14	irregular	wany	smooth	smooth	big	cloudy white
8.	S15	round	smooth	concentric	flat	big	cloudy white
9.	S16	round	smooth	smooth	convex	small	orange
10.	S22	filamentous	filamentous	smooth	flat	big	cloudy white
11.	S23	round	smooth	smooth	flat	small	cream
12.	S26	filamentous	filamentous	smooth	flat	big	milky white
13.	S27	round	smooth	smooth	convex	small	milky white
14.	S49	round	smooth	smooth	convex	medium	cream
15.	S54	irregular	smooth	smooth	flat	big	cloudy white
16.	S55	filamentous	filamentous	smooth	flat	big	milky white
17.	S57	actino					
18.	S58	irregular	filamentous	smooth	flat	big	light yellow
19.	S61	round	smooth	smooth	flat	big	white
20.	S67	actino					

Previous studies have shown that exposed mangrove bacterial communities to hydrocarbons in their deposits, such as preselecting a potential minority population, can respond quickly in an oil-stressed environment. It has been shown that previous exposure may have stimulated it (Taketani et al., 2010). Comparative analysis of metal resistors from different geographic regions revealed a high distribution of cobalt-zinc-cadmium resistivity functions. The taxonomic classification of the readings showed the ubiquitous distribution of proteobacterium in the dataset examined. This data may indicate a strong resilience of the mangrove microflora to metal contamination (Puthusseri et al., 2021).

4.1.2. Bacterial Isolates from Mangrove Plant Stems

The production and accumulation of organic matter at the bottom of mangrove ecosystems can enrich the region with microorganisms and evolve as a source of various extracellular enzymes [16]. In this study, bacteria were isolated from the mangrove trunk. Isolation resulted in 27 bacterial isolates observed with the naked eye for colonies with different properties, as shown in Table 2.

Table 2. Bacterial isolates from mangrove plant stems

No	Code	Characteristics					
		Shape	**Edge**	**Surface**	**Elevation**	**Site**	**Colour**
1.	P01	round	smooth	smooth	convex	medium	cloudy white
2.	P02	lobate	lobate	smooth	flat	big	transparent
3.	P03	round	smooth	smooth	convex	big	pale yellow
4.	P04	dots	smooth	smooth	flat	small	white
5.	P06	round	smooth	smooth	flat	medium	cloudy white
6.	P07	lobate	curled	smooth	flat	medium	cloudy white
7.	P08	round	curled	smooth	convex	small	cloudy white
8.	P09	lobate	filamentous	smooth	flat	medium	cloudy white
9.	P10	round	curled	smooth	flat	big	white
10.	P11	lobate	waved	smooth	flat	medium	cloudy white
11.	P12	dots	smooth	smooth	flat	big	white
12.	P13	lobate	filamentous	smooth	flat	big	cloudy white
13.	P14	round	waved	smooth	flat	big	cloudy white
14.	P15	round	lobate	cracked	convex	small	creme
15.	P16	round	smooth	smooth	convex	big	white
16.	P23	lobate	lobate	smooth	flat	big	transparent
17.	P24	round	smooth	smooth	convex	medium	cloudy yellow
18.	P26	round	smooth	smooth	convex	small	pale yellow
19.	P27	lobate	lobate	smooth	flat	big	cloudy yellow
20.	P28	round	smooth	smooth	convex	medium	white
21.	P29	round	smooth	smooth	convex	medium	orange
22.	P30	round	smooth	smooth	convex	big	yellow
23.	P33	lobate	filamentous	smooth	flat	small	white
24.	P34	lobate	filamentous	smooth	flat	small	cloudy white
25.	P35	round	smooth	smooth	flat	medium	orange
26.	P46	round	curved	smooth	flat	big	cloudy white
27.	P51	round	smooth	smooth	convex	big	white

The previous study revealed essential insights into the bacterial diversity and activity in mangrove ecosystems, with the isolation and characterization of many microorganisms associated with the plant Bruguiera cylindrica. These isolates displayed various adaptation features that could be useful for biotechnological applications, such as enzymatic and phosphate solubilizing activities (Mamangkey et al., 2021). Another study found that bacterial isolates have a high tolerance to heavy metals Pb, Cu, and Cd, such as Bacillus oceanisediminis PGD1A, Vibrio alginolyticus PGD5A, and Halobacillus kuroshimensis PGD9B. The heavy metal tolerant bacterial isolates can be tested as biological agents in the heavy metal bioremediation process from polluted ecosystems (de Fretes et al., 2019).

4.1.3. Bacterial Isolates from Mangrove Leaves

Bacteria that inhabit mangrove leaves are indigenous bacteria that inhabit plant tissues. The method used in this study was a study and experiment on the isolation of bacteria from the leaves of mangrove plants characterized by morphology-descriptive quantitative data analysis. Based on research data, isolates of 26 bacteria with different morphological characteristics were obtained from the leaves of mangrove plants, as shown in Table 3.

Table 3. Bacterial isolates from mangrove leaves

No	Code	Characteristics					
		Shape	Edge	Surface	Elevation	Site	Colour
1.	L02	oval	smooth	concentric	flat	medium	milky white
2.	L03	dot	punctiform	flat	flat	small	milky white
3.	L04	round	smooth	smooth	flat	small	transparent
4.	L08	round	smooth	concentric	flat	medium	white
5.	L09	round	smooth	smooth	flat	medium	milky white
6.	L12	round	lobate	concentric	flat	medium	milky white
7.	L13	round	rata	smooth	convex	small	crème
8.	L15	round	curled	wrinkled	flat	small	transparent white
9.	L17	round	smooth	concentric	flat	medium	creme
10.	L18	round	filamentous	concentric	flat	medium	white
11.	L22	round	wavy	concentric	flat	medium	cloudy white
12.	L28	filamentous	filamentous	smooth	flat	big	cloudy white

Based on research that has been conducted regarding the identification of decomposed leaf litter decomposers of A. lanata in the Mangrove Forest of Sungai Bakau Kecil Village, it was found that the bacterial genera were Bacillus, Kurthia, Sporosarcina, Listeria, Corynebacterium, Azotobacter, Alcaligenes, Pseudomonas, Serratia, Vibrio, and Actinobacillus (Junaidi et al., 2019).

4.2. Mempawah Coast Potential of Mangrove Bacteria as Bioremediation Agent

Mangrove forest is a biome in a transitional area between land and sea. The existence of tides in the mangrove area can cause salt levels and nutrient content to fluctuate, so aquatic and terrestrial microorganisms favor this area (Yan et al., 2015). Thus, mangrove forests have a high potential for accumulation of heavy metals because they are directly adjacent to the mainland and are a meeting place for waters from the land through rivers and sea waters, where river flows can be carriers of waste from industry and households (Kannan et al., 2016).

Heavy metal pollution can be caused by the presence of iron (Fe), manganese (Mn), zinc (Zn), cadmium (Cd), chromium (Cr), copper (Cu), and lead (Pb), nickel (Ni) and mercury (Hg). These heavy metals can be divided into two types based on the point of view of toxicology. The first type is essential heavy metals, where living organisms need their presence in specific amounts, but excessive amounts can cause toxic effects. These heavy metals are Zn, Cu, Fe, Co, Mn, Ni, etc. The second type is non-essential or toxic heavy metals, whose presence in the body is unknown or harmful, such as Hg, Cd, Pb, Cr, and others (Tchounwou et al., 2012). In this study, 59 bacterial isolates were successfully isolated from sediment, mangrove stems, and leaves. There are 20 isolates from sediment, 27 isolates from branches, and 12 isolates from mangrove leaves. Then all isolates were grown in media containing heavy metals. The types of heavy metals tested were Hg, Pb, and Fe.

4.2.1. Bacterial Potential of Mangrove Sediments in Metal Bioremediation

Twenty bacterial isolates obtained from mangrove sediment samples were tested for their growth ability on media containing one ppm of Hg. The results showed that all isolates could grow on this medium, although it only showed a small area. Mercury (Hg) is a limiting factor for bacterial growth in the medium; only mercury-tolerant bacteria can grow. The three isolates with the most significant growth ability on media containing Hg were isolated S08 with a growth area of 3.44 cm, followed by isolated S23 with a size of 3.05 cm, and isolated S02 with a growth area of 2.59 cm. S15 isolate could grow large in media containing Pb (4.92 cm) and Fe (4.98 cm). Meanwhile, isolate S08 also had a high growth ability in media containing Hg and Pb of 1 ppm, respectively 3.44 cm and 4.47 cm, as shown in Table 4.

Table 4. Bacterial potential of mangrove sediments in metal bioremediation

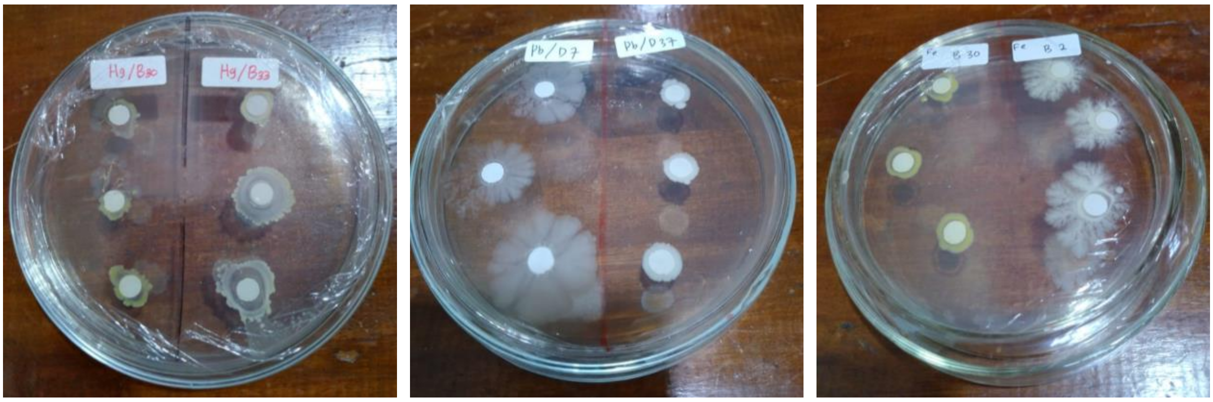
No	Code	Metal bioremediation test results		
		Hg	**Pb**	**Fe**
1.	S01	2.01	0.63	3.79
2.	S02	2.59	4.01	1.94
3.	S03	0.69	2.35	3.17
4.	S04	1.99	3.96	4.98
5.	S08	3.44	4.47	1.50
6.	S13	0.48	0.56	0.88
7.	S14	1.15	3.64	0.58
8.	S15	1.41	4.92	4.98
9.	S16	0.85	0.39	0.22
10.	S22	0.59	2.03	3.15
11.	S23	3.05	2.74	2.26
12.	S26	0.32	2.92	1.87
13.	S27	0.59	0.87	13.0
14.	S49	0.73	0.00	0.20
15.	S54	1.69	1.75	0.28
16.	S55	0.61	3.51	0.37
17.	S57	0.21	0.69	0.30
18.	S58	0.57	6.99	0.63
19.	S61	1.61	1.63	0.61
20.	S67	0.69	0.60	0.42

Mercury (Hg) is a heavy metal classified as the most dangerous pollutant because it is a neurotoxin for organisms and humans (Gochfeld, 2003). Mercury can accumulate in the aquatic and sediment environments and acts as a mercury transporter which causes the spread of mercury. Most heavy metals contaminating the marine environment will be deposited in sediments. Mercury properties allow them to easily bind organic matter and settle to the bottom of the water, making the mercury concentration in the sediment high (Amin et al., 2009). Besides accumulating in sediments, heavy metals can also get into the mangrove structure (Parvaresh et al., 2011).

The potential test of the ability to grow mangrove sediment bacteria on media containing Pb heavy metal of 1 ppm showed that not all bacterial isolates were able to grow under these conditions. One type of bacteria that cannot grow is S49. The bacterial isolate that could boost the best was isolated S58 with a growth area of 6.99 cm, followed by S15 (4.92 cm) and S08 (4.47 cm). The accumulation of Pb by bacteria based on the position of the heavy metal was divided into extracellular accumulation, intracellular accumulation, and absorption by the cell surface. Extracellular accumulation can occur due to the binding of metal ions by polymers or extracellular polysaccharides produced by microbial cells and the interaction between positively charged metal ions and the reactive site on the negatively charged cell surface. In contrast, intracellular accumulation occurs due to a diffusion process that does not require direct microbial activity, where the genes in the plasmid control these metabolic processes (Wulandari et al., 2005).

The growth area of bacterial isolates isolated from Mempawah mangrove sediments shows the ability of these bacteria to grow on media containing heavy metals (Hg, Pb, and Fe). Similar studies on bacterial isolates from mangrove sediments in Pengudang and Tanjung Uban, Bintan Island, showed that these isolates could be used in the remediation of heavy metals in ecosystems, such as isolates of Bacillus oceanis edminis, Vibrio alginolyticus and Halobacillus kuroshimensis (de Fretes et al., 2019). Research by (Aminullah et al., 2009) also obtained three rhizobacteria isolates on the roots of Rhizopora mucronate exposed to heavy metal lead (Pb). The mangrove sediments harbor unique microbial communities which have developed novel metal resistance mechanisms. (Debasmita et al., 2022).

The test results of the potential growth ability of mangrove sediment bacterial isolates on media containing Fe showed that most of the isolates were able to grow. Iron (Fe) is a metal needed by organisms for their metabolism, but iron can harm organisms and the environment in high concentrations. Iron is essential as a component of cytochrome pigments in cellular respiration (M. T. Madigan et al., 2012) and as a cofactor for enzymes (Prescott et al., 2008) on microorganisms. However, high concentrations of iron that pollute the environment can pose a danger to organisms. According to the Regulation of Kementerian Lingkungan Hidup dan Kehutanan Number 5 of 2014 concerning Wastewater Quality Standards, the quality standard for dissolved iron metal (Fe) is 7 mg/L.



(a) (b) (c)

Figure 2. Bacterial potential test in bioremediation of: (a) mercury (Hg); (b) lead (Pb); (c) iron (Fe)

In this study, the highest growth ability of mangrove sediment bacterial isolates on media containing one ppm Fe was the S27 isolate, 12.99 cm wide. This resistance test was conducted to determine the ability of isolates in a medium containing Fe metal. In general, all microorganisms require Fe for metabolic activity and as a significant structural component in several enzymes. A study stated that some Bacillus species were resistant to Fe metal up to 6000 mg/L on a nutrient agar medium. However, some Bacillus species could only withstand up to 100 mg/L (Baby et al., 2014). One of the resistance mechanisms in bacteria to metals is the presence of RND (Resistance, Nodulation, Cell Division) proteins that regulate metal transport through cell membranes. In Bacillus subtilis, Fe uptake is controlled by Fur protein. Under an environment rich in Fe, Fur protein suppresses the Fe uptake system (Nithya et al., 2011).

4.2.2. Bacterial Potential of Mangrove Plant Stems in Metal Bioremediation

The bacteria isolated from the stems of mangrove plants amounted to 27 isolates. The results of the potential test of 27 bacterial isolates against heavy metals Hg, Pb, and Fe can be seen in Table 5.

Table 5. The bacterial potential of mangrove plant stems in metal bioremediation

No	Code	Metal bioremediation test results		
		Hg	Pb	Fe
1.	P01	1.98	2.20	0.53
2.	P02	0.89	3.37	2.81
3.	P03	1.23	5.93	0.38

No	Code	Metal bioremediation test results		
		Hg	Pb	Fe
4.	P04	1.27	8.08	4.89
5.	P06	0.53	0.65	0.57
6.	P07	1.15	4.64	1.01
7.	P08	0.78	0.53	0.62
8.	P09	2.36	0.45	0.58
9.	P10	1.47	0.85	4.25
10.	P11	1.08	3.74	1.09
11.	P12	1.05	0.75	0.52
12.	P13	2.19	0.47	0.51
13.	P14	0.64	0.00	3.37
14.	P15	0.99	0.79	0.69
15.	P16	0.52	2.20	2.20
16.	P23	1.22	5.52	4.24
17.	P24	0.48	0.49	0.83
18.	P26	1.18	0.57	0.42
19.	P27	7.42	0.76	9.68
20.	P28	0.55	1.08	1.42
21.	P29	2.48	0.92	1.15
22.	P30	0.71	0.62	0.74
23.	P33	1.39	4.62	4.99
24.	P34	0.64	2.88	0.38
25.	P35	0.78	14.01	11.9
26.	P46	1.42	4.98	4.61
27.	P51	0.85	0.55	5.31

Bacteria isolated from mangrove stems were endophytic bacteria. Endophytic bacteria can be found in almost all plants on this earth and are bacteria that grow in plant tissues. Endophytic bacteria can be isolated from a plant's roots, stems, and leaves (Dourado et al., 2012). The results of the selection test for bacterial isolates of mangrove stems that were able to grow on media with a Hg content of 1 ppm showed that isolate B27 increased vastly with an area of 7.42 cm. Meanwhile, media containing Pb 1 ppm heavy metal showed bacterial isolate B35 grow with a size of 14.01 cm and isolate B04 with an area of 8.08 cm. In media with Fe content of 1 ppm, isolates that were able to grow with the most significant size were B35, with an area of 11.90 cm, and isolated B27, with an area of 9.68 cm. Bacterial isolate B35 had a high growth ability in media with the heavy metal content of Pb and Fe, with colony diameters of 14.01 cm and 11.90 cm, respectively. In addition, isolate B27 also had a high growth ability in media with Hg and Fe content of 7.42 cm and 9.68 cm, respectively. This result shows that one type of bacterial isolate is tolerant to one heavy metal and can also be susceptible to various kinds.

The results of this study indicate that each microorganism has a different resistance to heavy metals. Microorganisms can survive at high concentrations of heavy metals depending on the activity of these bacteria, capable of eliminating the toxic effects of heavy metals in contaminated areas (Sá-Pereira et al., 2009). Former research showed that heterotrophic bacteria could tolerate mercury, zinc, and nickel at 300-500 g/g in soil (Anyanwu, 2011). This result indicates that the growth of bacteria showed different responses influenced by the type and concentration of heavy metals.

The accumulation of heavy metals by bacteria is also based on the absorption of heavy metals. The absorption of heavy metals by microorganisms can be divided into passive and active uptake (Suhendrayatna, 2001). Passive uptake occurs when heavy metal ions are bound to the cell wall of the bio-sorbent, known as biosorption. The biosorption process is more effective in a specific pH and the other ion presence in the media that can be precipitated as insoluble salts. The passive uptake mechanism is carried out by ion exchange and the formation of complex compounds. The ion exchange process occurs because heavy metal ions replace the ions in the cell wall (Demir and Ahunbay, 2013). In contrast, the formation of complex compounds occurs between heavy metal ions and functional groups such as carbonyl, amino, thiol, phosphate, and hydroxy carboxyl in alternating and fast ways.

Bacteria isolated from mangrove stems also showed their ability to grow on media containing heavy metals (Hg, Pb, and Fe); thus, this indicates the area where bacteria thrive. Bacteria isolated from mangrove stems are endophytic bacteria. Mangrove plants can also store heavy metals in their bodies, known as bioaccumulation. Research conducted by Natsir and Y. Hanike (2019) shows that the average heavy metal content of lead (Pb) in roots is 1.748911 mg/kg, stems is 2.996012 mg/kg, and leaves are 0.812896 mg/kg.

4.2.2. Bacterial Potential of Mangrove Leaves in Metal Bioremediation

Bacteria isolated from mangrove leaves are also endophytic bacteria because they grow in plant tissues. Twenty-six isolates were separated, then tested for their growth potential on media containing heavy metals Hg, Pb, and Fe with a concentration of 1 ppm. The potential test of bacteria on mangrove leaves against heavy metals can be seen in Table 6.

Table 6. The bacterial potential of mangrove leaves in metal bioremediation

No	Code	Metal bioremediation test results		
		Hg	Pb	Fe
1.	L02	1.17	0.85	0.32
2.	L03	1.25	1.93	0.91
3.	L04	2.30	2.75	0.78
4.	L08	0.00	2.92	0.98
5.	L09	1.86	0.79	0.77
6.	L12	1.08	2.79	1.15
7.	L13	0.00	6.83	1.08
8.	L15	0.00	0.00	0.53
9.	L17	0.41	3.05	1.12
10.	L18	1.17	6.42	0.60
11.	L22	0.00	0.36	0.00
12.	L28	0.40	0.35	0.31

The isolates of bacteria derived from mangrove leaves were tested on media containing one ppm of heavy metals, such as Hg, Pb, and Fe. Four isolates were unable to grow on media containing Hg. Isolate D04 had the most significant growth with a size of 2.30 cm on media with one ppm Hg, while only an isolate could not grow in media with one ppm of Pb, while isolate D13 can grow well on the media, which is 6.83 cm. At the same time, the media with Fe content of 1 ppm showed isolated D12, which can grow as large as 1.15 cm. However, there was one isolate that was unable to grow. Isolates D13 and D17 were also tolerant to media containing one ppm Pb and Fe, each having an area of 6.83 cm and 1.08 cm in isolate D13, while isolate D17 had a size of 3.05 cm in media containing Pb 1 ppm. Furthermore, 1.12 cm wide on media containing one one ppm Fe.

The mechanism for cleaning heavy metals by microorganisms is mostly an ion exchange process (Suhendrayatna, 2001). This mechanism can be divided into three mechanisms which are divided into;

processes related to metabolism and functions that are not associated with cell metabolism, while based on the position of heavy metals, can be divided into extracellular accumulation (precipitation), intracellular accumulation, and metal uptake by the cell surface. The last mechanism is based on how bacteria adsorb heavy metals. The mechanism of heavy metal accumulation through ion exchange processes based on cell metabolism can be divided into binding and active transport phases. The binding process is not related to the process of cell metabolism, then the absorption through the cell wall, followed by active transport related to cell metabolism. Heavy metals accumulate in the cell membrane (extracellular) and the cytoplasm (intracellular) in the metabolic process.

The study by (Subagiyo et al., 2017), who isolated microorganisms from mangrove leaves, showed that the mangrove ecosystem is a potential source of microorganisms to produce protease, amylase, and cellulase enzymes and is also possible for various other extracellular enzymes. Another study showed that the diversity of decomposed R. apiculata leaf litter bacteria was six bacteria, such as Bacillus sp., Listeria sp., Enterobacteria sp., Aeromonas sp., Actinobacilus sp., and Bacteroides sp. The most dominant bacteria found were Bacillus sp. (Yulma et al., 2017).

5. Conclusions

In this study, it can be concluded that the composition of bacteria isolated from the mangrove area contained 59 isolates with various macroscopic characteristics consisting of 20 isolates of sedimentary bacteria, 27 isolates of bacteria on stems, and 12 isolates of bacteria derived from mangrove leaves. Bacterial isolates S08, B27, and D04 have the potential for the bioremediation of Hg metal. Bacterial isolates S58, B35, and D13 have potential for Pb bioremediation, while bacterial isolates S27, B35, and D12 have potential as Fe bioremediation agents. For further research, it is necessary to identify the type of isolate that has the potential as a metal bioremediation agent for Hg, Pb, and Fe, and it is essential to test the potential of bacteria as a bioremediation agent with a higher metal concentration.

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