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Identification of bacteria in *Rhizophora mucronata* leaf litter and sediment

YULMA[1], ASIHING KUSTANTI[2], SOEMARNO[3], MOHAMMAD MAHMUDI[4], BURHANUDDIN IHSAN[5,♥]

[1]Graduate Program, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[2]Department of Socio-Economic, Faculty Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[3]Department of Soil Science, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[4]Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[5]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Borneo Tarakan. Jl. Amal Lama No. 1, North Kalimantan, Indonesia. Tel.: +62-811-53011775, Fax.: +62-551-2052558 ♥email: ihsan.muslim1924@gmail.com

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Abstract. *Yulma, Kustanti A, Soemarno, Mahmudi M, Ihsan B. 2025. Identification of bacteria in* Rhizophora mucronata *leaf litter and sediment. Biodiversitas 26: 3054-3060.* The mangrove ecosystem is an area that has high productivity, with litter production that contributes to increasing nutrients. This study aimed to determine the bacterial community in mangrove vegetation, especially in sediment and decomposed *Rhizophora mucronata* leaf litter. This study was conducted in the Mangrove and Crab Conservation Area. *R. mucronata* leaf litter was collected with a 2 × 2 m container. Furthermore, the leaf litter was dried at 105°C until its weight was constant. The decomposition rate and bacterial identification were measured at intervals of 14, 28, 42 and 56 days. Meanwhile, sediment was taken at different depths, namely 10, 20, and 30 cm, and as much as 250 g for each depth using a biopore drill. Identification of bacteria in litter and sediment was carried out with three tests, namely: Gram staining test (Gram-negative, Gram-positive and bacterial forms), primary test (3% KOH, 3% H_2O_2 and Oxidase) and further test (O/f, Glucose and motility). The results showed that the bacterial community that plays a role in decomposing organic matter and accelerating the rate of decomposition in *R. mucronata* leaf litter is diverse with different characteristics, including *Bacillus* sp., *Aeromonas* sp., *Pseudomonas* sp., *Listeria* sp., *Actinobacillus* sp., *Micrococcus* sp., and *Acinetobacter* sp. At the same time, the bacterial community found in the sediment includes *Pseudomonas* sp., *Aeromonas* sp., *Micrococcus* sp., *Bacillus* sp., *Flavobacterium* sp., *Enterobacteria* sp., and *Listeria* sp. The most dominant bacteria found is *Bacillus* sp. because one of the endophytic bacteria found in plant tissue can increase plant growth through mechanisms such as biological nitrogen fixation, phosphate and potassium solubilization.

Keywords: Bacteria, leaf litter, mangrove, *Rhizophora mucronata*, sediment

INTRODUCTION

The mangrove ecosystem is an area that has a very high level of productivity, with litter production that contributes ecologically, starting from a place to find food, growth, spawning and protection. The mangrove ecosystem in Tarakan City, Indonesia, which was designated as a Mangrove and Crab Conservation Area based on Regional Regulation No. 04 of 2002 covering an area of 35 ha, experienced area degradation due to the conversion of mangrove land into several uses such as fish farms, settlements, transportation routes, and expansion of traditional markets. The impact of the Mangrove and Crab Conservation Area degradation is a change in the structure of the mangrove community from the *Avicennia marina* (Forssk.) Vierh. species to *Rhizophora mucronata* Lam. (Yulma and Wijayanti 2024). Degradation also affects the function of the conservation area as a protected area for flora and fauna and the stability of the coast of Tarakan city which results in a decrease in the contribution of nutrients from the mangrove ecosystem to the waters. The decrease in nutrient contribution is partly due to mangrove degradation, which results in reduced litter production.

Reduced litter production can slow down the decomposition process and inhibit the nutrient cycle, thus disrupting the ecological balance (Chollet et al. 2021; Zhang et al. 2023). The decrease in litter production also has an impact on the existence of the bacterial community; when litter production decreases, the available organic matter also decreases, which can inhibit the growth and diversity of bacterial population (Oliveira et al. 2019; Clores et al. 2020; Gao et al. 2024). Bacteria are one of the microorganisms that play a role in the decomposition process of mangrove litter and waters and sediments. So, changes in the diversity of the bacterial community affect the rate of litter decomposition and jeopardize the potential for accumulation of organic matter, which will have an impact on the growth of the mangrove ecosystem (Muwawa et al. 2021; Lu et al. 2022; Li et al. 2024).

Therefore, the presence of bacteria is significant in the mangrove ecosystem because it has an ecological role in the decomposition process and produces protein-rich detritus. The litter decomposition process also functions as a nutrient cycle carried out by bacteria to support primary productivity (Rannavre and Donde 2023; Zhou et al. 2023; Khomutovska et al. 2024). Bacteria play a role in converting complex organic matter in litter into simpler forms so that it can be utilized by various other organisms in the ecosystem (Yulma et al. 2017; Arfarita et al. 2024; Li et al. 2024; Zhang et al. 2024). The release of nutrients from mangrove leaf litter also plays a role in biogeochemical processes in the aquatic environment, directly or indirectly affecting water quality

and food availability for fish and shrimp (Alam et al. 2021). In addition, the benefits of the decomposition process by bacteria will improve sediment quality (Farid and Gobel 2023). Good sediment can be a place to live for sessile organisms, thus forming a balanced food chain in the mangrove ecosystem.

Bacteria that live in sediment play an essential role in increasing plant growth and production because of their ability to bind Nitrogen (N_2) from the air and convert ammonium into nitrate. In addition, sediment contains more organic matter that supports balance in the mangrove ecosystem (Sarker et al. 2021). Leaf litter can enrich the sediment bacterial community to increase metabolic activity and biodiversity (Guo et al. 2020; Liu et al. 2021; Liu et al. 2022). The sediment and litter bacterial communities in the nutrient cycle have a continuous role in the decomposition process. Meanwhile, biota in the mangrove ecosystem, such as crabs, can help to break down and shred leaf litter, facilitating microbial colonization and nutrient release, thereby increasing the litter decomposition rate (Chen et al. 2020; Masagca 2024). Another strategy carried out by crabs is that they directly consume mangrove leaf litter and complement their diet with the microbial community found in the sediment (Lu et al. 2024). Thus, this study determined the bacterial community in mangrove vegetation, especially in sediment and decomposed *R. mucronata* leaf litter.

MATERIALS AND METHODS

Study area

The *Rhizophora mucronata* leaf litter and sediment were sampled in the Mangrove and Crab Conservation Area, and bacterial identification was carried out in the Fish Nutrition and Feed Laboratory, Faculty of Fisheries and Marine Sciences, University of Borneo Tarakan, North Kalimantan, Indonesia (coordinate point 3°17'45.54"N, 117°36'39.30"E). Confirmation of bacterial identification by the Laboratory of the Fish Quarantine Station, Quality Control and Safety of Class II Fishery Products Tarakan. This study was conducted from March to May 2024 with three sampling points: Point 1, located at the mouth of the Pamusian River, point 2 located near the watchtower; and Point 3, located close to the settlement (Figure 1).

Litter and sediment collection

The leaf litter of *R. mucronata* was collected using a 2 × 2 m litter container and dried further at a temperature of 105°C until its weight was constant and put into 30 × 30 cm litter bags, 72 pieces weighing 20 g per bag. Litter bags were installed at each point, 24 bags each, and tied to the roots or stems of *R. mucronata*. Measurement of decomposition rates and bacterial identification were observed at intervals of 14, 28, 42 and 56 days. Meanwhile, sediment sampling was also done at a depth of 10, 20, and 30 cm, with three repetitions at each station point, and the sampling was carried out at low tide. Each station point was arranged at a distance of 10 meters between points, and each station point was 10 × 10 m in size. Sediment samples were taken as much as 250 g at each point, put into a sterile sample bottle, and then stored in a refrigerator at a temperature of 5-10°C (Yulma et al. 2017).

Bacterial isolation

Bacterial isolation was carried out by weighing 10 g of finely ground *R. mucronata* leaf litter and then put into an Erlenmeyer flask containing sterile water from the mangrove environment for dilution. The dilution process was carried out up to 10^{-7}, then cultured on Tryptic Soy Agar (TSA) media. The bacterial culture was incubated for 24-48 hours, and then the bacterial colonies that grew were purified by making a subculture on TSA media. Different colonies were taken and then incubated for 24 hours. Meanwhile, bacterial isolation from sediment was carried out by weighing 5 g of sediment and then ground using a mortar and pestle. Furthermore, the finely ground sediment was put into a 250 mL Erlenmeyer flask. The dilution process is carried out in the same way as the litter (Yulma et al. 2017).

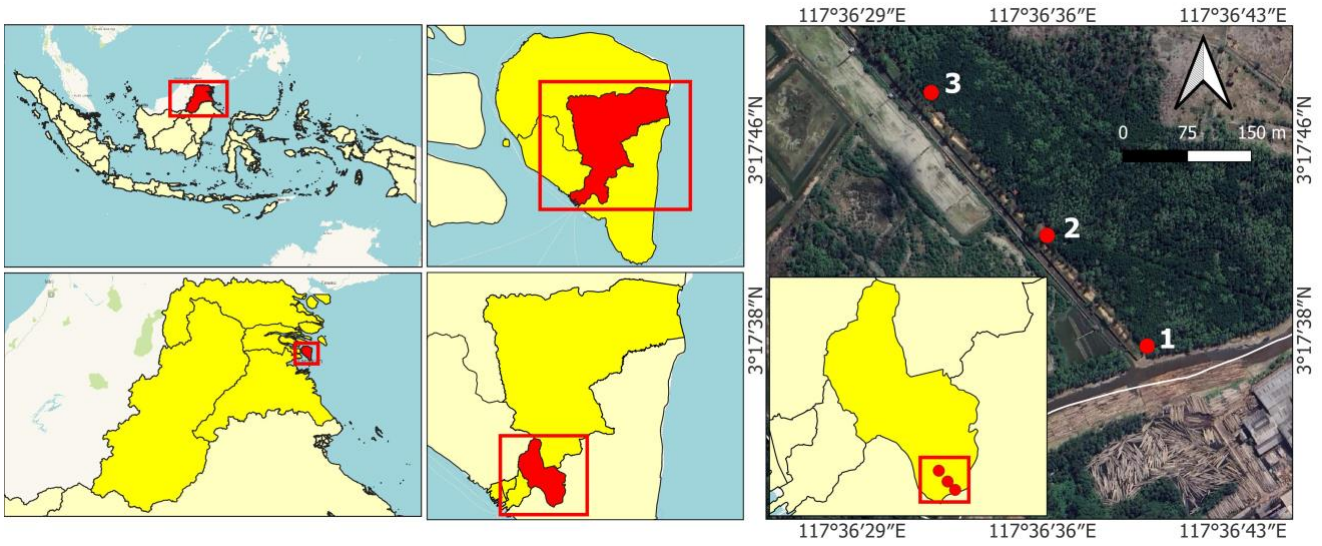

Figure 1. Map of research location in Tarakan, North Kalimantan Province, Indonesia

Table 1. Redox potential of soil substrates

Redox status	Range E_h (mV)
Aerated soil	+400 mV
Slightly reduced soil	+100 s/d +400 mV
Reduced soil	-100 s/d +100 mV
Highly reduced soil	-100 s/d -300 mV

Identification of bacteria

Bacterial identification is carried out morphologically and biochemically. Pure bacterial cultures were grown on TSA media in two petri dishes for each isolate and then incubated at 30°C for 24 hours. Identification of bacteria in litter and sediment is carried out with three tests, namely: Gram staining test (Gram-negative, Gram-positive and bacterial forms), primary test (3% KOH, 3% H_2O_2 and Oxidase) and further test (O/f, Glucose and motility) (Cowan and Steel's 1974; Yulma et al. 2017).

Decomposition rate calculation

Data on changes in litter mass were observed for 2 months with intervals of 14, 28, 42, and 56 days undergoing decomposition. This method is used to determine the value of the decomposition rate calculated using the formula by Yulma et al. (2017):

$$R = \frac{Wo - Wt}{T}$$

Where :

R : Decomposition rate g/day
Wo : Initial weight of dry litter sample (g)
Wt : Weight of dry litter sample after observation time t (g)
T : Time (day)

Redox potential (E_h)

Redox potential is used to describe the overall reduction or oxidation capacity of a system. Redox potential is measured in millivolts (mV) relative to a standard hydrogen electrode and is generally measured using a platinum electrode with a saturated calomel electrode as a reference. The activity of microorganisms affects the transformation process of organic and inorganic compounds and the soil's redox potential. The main status of conditions in the substrate is based on redox potential (Table 1), according to Kaurichev and Shishova (1967).

Physico-chemical parameters

Physico-chemical parameters were measured to determine their effect on the bacterial community that carries out the decomposition process. The parameters measured include temperature, salinity pH and Dissolved Oxygen (DO).

RESULTS AND DISCUSSION

Litter decomposition rate

The dry weight of *Rhizophora mucronata* leaf litter was observed every 14 days. The results of observations of the dry weight of litter that had been decomposed during observations up to 56 days (Table 2). The results of the observations of dry weight were different between times. The earlier the observation time, the greater the remaining dry weight of the litter. The remaining litter at each location differed, although not significantly. The most considerable dry weight was located at location 1, which has the characteristics of a river estuary where fishing boats are transported (Figure 2). While calculating the decomposition rate at each time and location (Figure 3). The highest decomposition rate occurred on the 14th day of observation, while the lowest was on day 56. The longer the observation time, the lower the decomposition rate.

Identification of bacteria in litter and sediment

The results of bacterial identification in *R. mucronata* leaf litter contained seven bacteria with different characteristics, including *Bacillus* sp., *Aeromonas* sp., *Pseudomonas* sp., *Listeria* sp., *Actinobacillus* sp., *Micrococcus* sp., and *Acinetobacter* sp. The bacteria identified in each observation and location have similarities, including *Bacillus* sp. (Table 3). At the same time, the bacteria found in the sediment consisted of seven bacteria, including *Pseudomonas* sp., *Aeromonas* sp., *Micrococcus* sp., *Bacillus* sp., *Flavobacterium* sp., *Enterobacteria* sp., and *Listeria* sp. (Table 4). The bacteria found in the litter and sediment have similarities. At a depth of 0-10 cm, the bacteria found are aerobic bacteria, such as *Bacillus* sp., *Enterobacteria* sp., *Pseudomonas* sp. and *Micrococcus* sp., while at a depth of 10-30 cm, anaerobic bacteria were found, such as *Aeromonas* sp., *Flavobacterium* sp., *Enterobacteria* sp., and *Listeria* sp.

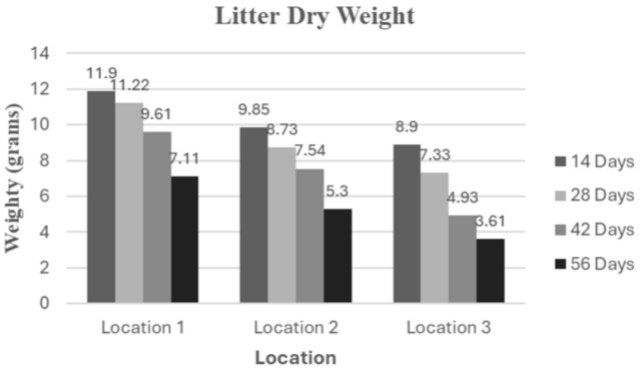

Figure 2. Dry weight of litter at each location

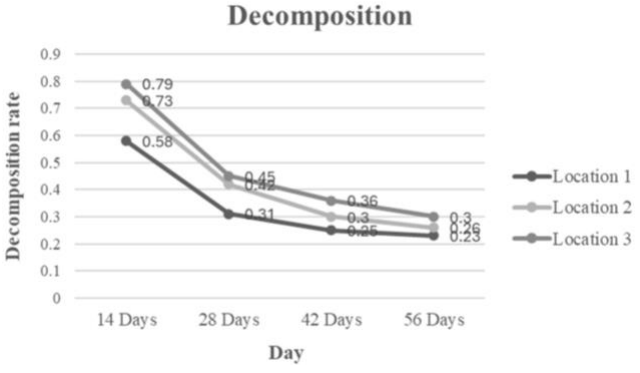

Figure 3. Rate of decomposition of *Rhizophora mucronata* litter

Table 2. Results of dry weight decomposition rate measurements

Time	Location 1			Location 2			Location 3		
	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3
14 days	11.36	12.67	11.68	9.56	10.30	9.70	8.61	9.68	8.40
28 days	10.86	11.89	10.91	8.08	9.66	8.45	6.38	8.16	7.44
42 days	9.35	10.33	9.16	6.60	8.12	7.90	4.41	5.03	5.36
56 days	6.59	7.31	7.44	5.53	6.15	4.23	2.08	3.03	4.36

Table 3. Identification of litter bacteria from different locations

Time	Bacterial identification		
	Location 1	Location 2	Location 3
14 days	*Bacillus* sp. *Actinobacillus* sp.	*Bacillus* sp. *Listeria* sp.	*Bacillus* sp. *Aeromonas* sp. *Pseudomonas* sp.
28 days	*Bacillus* sp. *Actinobacillus* sp.	*Bacillus* sp. *Aeromonas* sp.	*Bacillus* sp. *Aeromonas* sp. *Listeria* sp.
42 days	*Aeromonas* sp. *Actinobacillus* sp. *Micrococcus* sp.	*Bacillus* sp. *Pseudomonas* sp	*Bacillus* sp. *Aeromonas* sp.
56 days	*Bacillus* sp. *Aeromonas* sp.	*Bacillus* sp. *Listeria* sp.	*Bacillus* sp. *Pseudomonas* sp. *Acinetobacter* sp.

Table 4. Identification of sediment bacteria from different locations

Depth	Bacterial identification		
	Location 1	Location 2	Location 3
10 cm	*Bacillus* sp. *Pseudomonas* sp. *Micrococcus* sp.	*Aeromonas* sp. *Enterobacteria* sp.	*Aeromonas* sp.
20 cm	*Pseudomonas* sp. *Micrococcus* sp. *Aeromonas* sp.	*Pseudomonas* sp. *Aeromonas* sp.	*Bacillus* sp. *Pseudomonas* sp. *Aeromonas* sp.
30 cm	*Aeromonas* sp. *Flavobacterium* sp.	*Aeromonas* sp.	*Bacillus* sp. *Aeromonas* sp. *Listeria* sp.

Table 5. Redox potential (E_h) measurements

Depth	Place		
	Location 1	Location 2	Location 3
10 cm	-264 mV	-274 mV	-193 mV
20 cm	-325 mV	-68 mV	-310 mV
30 cm	-198 mV	-52 mV	-299 mV

Table 6. Physical and chemical parameters of water

Parameter	Place		
	Location 1	Location 2	Location 3
Temperature	27-29°C	27-29°C	26-27°C
Salinity	20-32 ppm	19-29 ppm	25-27 ppm
pH	6.87-7.73	6.64-7.80	6.32-7.84
Dissolved oxygen	6.4-9.3 mg/L	2.4-87 mg/L	2.0-5.5 mg/L

Redox potential (E_h)

Redox potential measurements are carried out to determine the category of a substrate that is influenced by the organic matter content in the substrate. The results of the redox potential quality measurements show differences between each location (Table 5), location 1, at a depth of 10 and 20 cm, has the best value due to the fine substrate structure and richness in organic matter. While at a depth of 30 cm, location 3 has the best value. This allows for reduced organic matter deposits obtained from organic waste from the community, but all locations show that the substrate is highly reducing. Positive E_h values are generally characteristic of well-oxygenated bottom deposits, coarse sediments, or poor organic matter. Negative E_h values are characteristic of bottom deposits rich in organic matter and mostly fine sediments.

Water quality parameters

The aquatic environment plays a significant role in the growth of mangroves and microorganisms. The results of water quality measurements are generally good and still by the development of mangroves and bacteria found in litter and substrates (Table 6). The highest salinity is located at location 1 because it is situated in the estuary of the river, which is close to the sea and has dissolved oxygen. While the temperature and pH still have almost the same value. Each location has different water quality, but it is still within an acceptable range.

Discussion

The reduction in dry weight of *R. mucronata* leaf litter at any time is greatly influenced by the role of bacteria in decomposition. The presence of bacteria not only degrades the main elements of litter but can also change the results of degradation into nutrients that can be utilized by mangroves and other organisms so that the dry weight of the litter decreases over time. According to Purahong et al. (2016), the reduction in litter weight is caused by the quality of the litter being permanently changed by microorganisms during the decomposition process, A significant reduction in dry weight is also caused by other species of organisms that live in the location, including worms (*Nereis* sp.) (Yunasfi et al. 2024).

The decomposition rate of *R. mucronata* leaf litter can be influenced by bacteria that play a role in decomposing organic matter. According to Perangin-angin et al. (2014), the decomposition rate carried out by bacteria and fungi as decomposers can break down organic particles by releasing chemical compounds to form proteins. These products are not only used as a source of nutrition for mangroves but are

also an important food source for other organisms. In addition, litter quality also plays an important role in the decomposition process (Maeda 2016). The litter decomposition process can be accelerated by adjusting the particle size (Goodman et al. 2019). The distribution and size of litter particles also affect the growth of decomposing microorganisms and maintain sufficient porosity for the aeration process (Du et al. 2020). The larger the litter particle size, the more difficult it is for microorganisms to reach the centre, causing slow decomposition. As stated by Cristiano and Di Sabatino (2023), the decomposition rate is influenced by the rate of litter fragmentation. Several studies have shown that contrasting litter types can positively and significantly affect litter decomposition rates (Li et al. 2009; Pereira et al. 2019).

The decomposition rate at each location is different; location 3 has the highest decomposition process of 0.49 g/day. Location 3 is a location that has a muddy substrate, is overgrown with mangrove plants, and is close to residential areas. This shows that the substrate, mangrove vegetation and human activities can affect the decomposition rate. Human waste, such as food waste, can provide nutrients for bacteria to decompose. In earlier research it has been stated that discarded food waste can increase bacterial activity by releasing enzymes and metabolites that help disintegrate organic matter (Mugivhisa and Manganyi 2025). Bacteria also encourage the nitrogen cycle, which produces growth regulators and optimizes decomposition efficiency. In addition, at location 3, substrate factors also affect fine substrate fractions with high organic matter so that substrate microorganisms can utilize organic matter to accelerate the rate of decomposition (Yulma et al. 2020).

The bacterial community that plays a role in decomposing organic matter and accelerating the decomposition rate in *R. mucronata* leaf litter is diverse. The most dominant bacteria found in *Bacillus* sp. *Bacillus* sp. bacteria are one of the endophytic bacteria in plant tissue that can increase plant growth through mechanisms such as biological nitrogen fixation, phosphate and potassium solubilization, and siderophore production (Santoyo et al. 2016; Zhang et al. 2019a), so these bacteria can be found in all mangrove vegetation. *Pseudomonas* bacteria are also found in litter; these bacteria are a group of proteolytic bacteria that play a role in the process of protein decomposition (Marathe et al. 2018; Zhang et al. 2019b) because bacteria will decompose litter enzymatically, one of which is through the active role of proteolytic enzymes. However, some bacteria are found the least, only found at location 1 in 42 days of observation, namely *Micrococcus* sp. bacteria. *Micrococcus* bacteria were found the least because they live on the substrate but can be found in the litter. They are caused by being carried by ocean currents so that they settle on the litter. According to Marin et al. (2021), *Micrococcus* live on the substrate. In addition, *Micrococcus* bacteria have also been isolated from sediments and marine invertebrates, including sponges and corals (Wang et al. 2021). *Micrococcus* is a non-spore-forming *Actinomycetes* (family Micrococcaceae) widespread throughout terrestrial, aquatic, and marine environments (Nuñez 2014).

In addition, the bacterial community found in the sediment has similarities to the *R. mucronata* leaf litter bacteria, including *Pseudomonas* sp., *Aeromonas* sp., *Micrococcus* sp., *Bacillus* sp., and *Listeria* sp. These cosmopolitan bacteria are widely distributed and are found in several habitats, such as water, sediment, leaves and land. However, *Flavobacterium* sp. and *Enterobacteria* sp. were not found in *R. mucronata* leaf litter. *Flavobacterium* bacteria (phylum Bacteroidota, family Flavobacteriaceae) are often isolated from environmental sources. These bacteria are commonly found in freshwater environments and soil, especially in the rhizosphere. Therefore, these bacteria are the least found even though this strain has been isolated from brackish water or seawater (McBride 2014). In addition, *Enterobacteria* sp. bacteria were found the least at all locations, although these bacteria are anaerobic and can live in conditions lacking oxygen. Other factors may cause these bacteria to be found very rarely. According to (Chan et al. 1979), the presence of sediment containing higher concentrations of organic nutrients than seawater can affect the survival of bacteria. The presence of oxygen greatly influences the depth of the substrate, so bacteria found on the surface require oxygen.

In contrast, in deeper substrates, bacteria can live without oxygen. Microorganisms, including bacteria, play an important role in the decomposition of litter and substrates by utilizing organic matter and producing reduction so that the abundance of organic matter will positively impact substrate reduction. This shows that the substrate can support the decomposition rate and bacterial growth. According to Pepper et al. (2015), anaerobic substrates, which are reduction environments, have a negative redox potential that can reach -300 mV. In addition, a positive E_h value is obtained in yellowish sandy sediments, while in mud sediments rich in organic matter with a grey to black layer, a negative E_h value is obtained (Godson et al. 2022). Thus, a positive value indicates oxidized sediment, while a negative value indicates reduced sediment.

In this study, the effect of physicochemical parameters on the growth of the bacterial community was insignificant. This is because the physicochemical parameters are still by the range of bacterial life found, so the decomposition rate is faster. According to Yunasfi et al. (2024), aquatic environments such as temperature, salinity, and pH can affect microorganisms to accelerate the decomposition rate. Thus, the bacterial community in the mangrove ecosystem can accelerate the decomposition rate of *R. mucronata* leaf litter and sediment with the support of the environment and biological factors.

In conclusion, the bacterial community isolated in present study like *Bacillus* sp., *Aeromonas* sp., *Pseudomonas* sp., *Listeria* sp., *Actinobacillus* sp., *Micrococcus* sp., and *Acinetobacter* sp. plays a diverse role with different characteristics in decomposing organic matter and accelerating the rate of decomposition in *R. mucronata* leaf litter. At the same time, the bacterial community found in the sediment includes *Pseudomonas* sp., *Aeromonas* sp., *Micrococcus* sp., *Bacillus* sp., *Flavobacterium* sp., *Enterobacteria* sp., and *Listeria* sp. Therefore, further studies are needed regarding the development of conservation areas in the

future as mangrove protection areas, considering the existence of mangroves as nutrient producers in the biogeochemical cycle that support aquatic productivity.

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