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Identification of bacteria from mangrove forest in Mamburungan, Tarakan City

Yulma¹, Burhanuddin Ihsan², Awaludin², Zainuddin², Stephanie Bija³, Maisyah Rani¹, Ayu Andira¹, Fitri Ramadani¹, and Sunarti¹

¹Department of Waters Resources Management, Faculty of Fisheries and Marine Science, Borneo Tarakan University, Tarakan, Indonesia.

²Department of Aquaculture, Faculty of Fisheries and Marine Science, Borneo Tarakan University, Tarakan, Indonesia

³Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Borneo Tarakan University, Tarakan, Indonesia

E-mail: yulma.yuki@gmail.com

Abstract. Bacteria has a main role in the food chain and waters productivity. Bacteria in the mangrove ecosystem mostly are from litter, sediment, and sea waters. The research objective is to determine the genus of bacteria that was found in *Avicennia marina* and *Sonneratia alba* leaf litter as well as bacteria in waters and sediment from a mangrove forest in Mamburungan. The method was designed by using explorative descriptive, and the analysis parameter was the genus. This study was done in the field experimentally with two phases; they were collected of mangrove leaf litter, waters, and sediment, followed by identification of bacteria in the Fish quarantine station, quality control, and safety of Tarakan Class II Fisheries Laboratory. The results showed that seven genus bacteria of *Sonneratia alba* was found, such as *Bacillus*, *Aeromonas*, *Listeria*, *Staphylococcus*, *Enterobacteria*, *Bacteroides*, *Actinobacillus* and nine genus of *Avicennia marina*, they were *Bacillus*, *Listeria*, *Aeromonas*, *Pseudomonas*, *Enterobacteria*, *Corynebacterium*, *Actinobacillus*, *Plesiomonas*, *Flavobacterium*. In sediment, there were ten genus, i.e., *Bacillus*, *Plesiomonas*, *Enterobacteria*, *Corynebacterium*, *Listeria*, *Aeromonas*, *Micrococcus*, *Actinobacillus*, *Bordetella parapertussis*, *Clostridium*. In sea waters, there were eight genus like *Bacillus*, *Listeria*, *Aeromonas*, *Corynebacterium*, *Micrococcus*, *Actinobacillus*, *Pseudomonas*, and *Enterobacteria*. The dominant bacteria that were found in litter, sediment, and sea waters were *Bacillus*.

1. Introduction

Mamburungan mangrove forest has an area of about 32 hectares with dominant mangrove species, namely *Sonneratia alba*, *Avicennia marina*, *Avicennia alba*, *Rhizophora apiculata*, and *Nypa fruticans* [1]. Mangrove litter production from *Avicennia marina* and *Sonneratia alba* species in Mamburungan mangrove forest ranges from 0.432 - 1.308 g/m²/ day and decomposition rate of 0.22 - 0.35 g/day [2]. There are indirect benefits of mangroves on fisheries, such as food supply for marine communities through the detritus food chain, which starts from the whole mangrove leaf litter [3]. The decomposition process in mangrove litter is an activity or process of decomposition and destruction of organic material derived from animals or fallen plant leaves into simple organic compounds. The decomposition of



organic matter is also often called the process of mineralization, where this process is a microbial process (decomposer) in obtaining energy for propagation.

Bacteria are one of the important components that play a role in the process of litter decomposition in mangrove ecosystems. The decomposition process carried out by bacteria is able to increase nutrients through the process of carbon mineralization and nitrogen assimilation [4–6]. Mangrove litter that is decomposed by microorganisms will produce organic material that is absorbed by plants, and some of it will be dissolved and carried by the tide to the surrounding waters. Bacteria found in mangrove litter are bacteria originating from the land and sea waters.

Sediment microorganisms play an important role in the degradation of organic matter both through the biogeochemical cycle and the use of ammonia, nitrites, nitrates, including pollution control. The organic material cycle that occurs in waters is the same as the organic cycle that occurs in sediments. Carbon, together with other elements such as phosphorus (P) and nitrogen (N) through the process of photosynthesis, produces a network of plants that become animal food. Bacteria in mangrove ecosystems such as litter, water, and sediments are closely related; this is due to the cooperation in utilizing and producing organic material so that the level of productivity of mangrove forest ecosystems is very high. The purpose of this study was to determine the bacteria contained in *Avicennia marina* and *Sonneratia alba* mangrove leaf litter as well as bacteria in the water and sediments in Mamburungan mangrove forest.

2. Research Methodology

This research was carried out experimentally in the field with two stages, including the collection of mangrove leaf litter, water, and sediment in the Mamburungan mangrove forest area, followed by the identification of bacteria in the Fish Quarantine Station Laboratory, Quality Control and Safety of Class II Tarakan Fisheries. The tools used in the study include a litter trap, a litter bag, a rope, a plastic bag, aluminum foil, an oven, analytical scales, glassware. Materials used in the study included mangrove leaf litter (*Bruguiera parviflora*, *Rhizophora apiculata*, *Sonneratia alba*, and *Avicennia alba*), H₂O₂ solution, Trypticase soy agar (TSA), KOH 3%, paraffin, and oxidase strips paper.

2.1. Mangrove Leaf Litter Collection

The general method used to collect mangrove leaf litter-fall was by litter-trap (litter catching nets) [7]. The litter-trap is a 2 m x 2 m container, made of nylon with a mesh size of about 1 mm, and the bottom is weighted. 3 Litter-traps were installed at each observation station, which was placed on mangrove vegetation with height above the highest tide line. Mangrove litter collected in the form of leaves were then dried and put into a litter bag (Litter-bag) of 20 g measuring 30 cm x 30 cm made of nylon with a mesh size of 1 mm. 8 litter-bags were installed at each observation station; 4 bags to measure the rate of decomposition and four bags to identify bacteria. The litter-bag was then tied at each station to the mangrove roots so that it was not carried away by the tide. Furthermore, the litter-bag was taken from each observation location at intervals of 14, 28, 42, 56 days, and then cleaned and dried and then brought to the laboratory for analysis.

2.2. Isolation of Mangrove Leaf Litter Bacteria

Bacterial isolation was done by weighing the litter of mangrove leaves that had been crushed as much as 10 grams, then put into the Erlenmeyer flask containing water from the mangrove environment that had been sterilized for dilution. The dilution process was carried out up to level 10⁻⁷, then cultured on TSA media. The bacterial culture was incubated for 24 - 48 hours, then the bacterial colonies that grew were purified by making a subculture to TSA media and taken in different colonies, then incubated for 24 hours.

2.3. Isolation of Sea Water Bacteria

Bacterial isolation was done by taking a sample of water as much as 10 ml and then put in an Erlenmeyer flask for dilution. The dilution process was carried out up to level 10⁻⁷, then cultured on TSA media.

The bacterial culture is incubated for 24 - 48 hours, then the bacterial colonies that grew were purified by making a subculture to TSA media and taken different colonies, then incubated for 24 hours.

2.4. Isolation of Sediment

Isolation of sediment bacteria was carried out by weighing 5 grams of sediment, then mashed using mortar and pestle. Then, the fine sediment was put into the Erlenmeyer flask for dilution. The dilution process was carried out up to level 10^{-7} , then cultured on TSA media. The bacterial culture was incubated for 24 - 48 hours, then the bacterial colonies that grew were purified by making a subculture to TSA media, and different colonies were taken, then incubated for 24 hours.

2.5. Bacteria Identification

Bacterial identification was carried out by three tests, namely: gram staining (gram-negative, gram-positive, and bacterial form), main test (KOH 3%, H_2O_2 3%, and Oxidase) and further tests (O / f, Glucose, and motility) [8].

2.6. Decomposition Rate Analysis

Litter mass change data were observed for two months at intervals of 14, 28, 42, 56 days that experienced decomposition; this method is used to determine the value of the decomposition rate calculated using the following formula [9].

$$R = \frac{W_0 - W_1}{T} \quad (1)$$

Note:

R = Decomposition rate (g/day)

T = Time (day)

W_0 = Initial dry litter sample weight (g)

W_1 = Dry litter sample weight after the t observation time (g)

3. Results and Discussion

The results of isolation and identification of bacteria in the Mamburungan Mangrove Forest Area from mangrove leaf litter contained seven bacteria from *Sonneratia alba* and nine bacteria from *Avicennia marina* (Table 1), mangrove water contained eight bacteria (Table 2), and mangrove sediment contained ten bacteria (Table 3). In general, bacteria found in the Mamburungan mangrove forest area diverse, either gram-positive or negative or biotic and abiotic. It indicates that the fertility or primary productivity of the Mamburungan mangrove ecosystem was very high.

3.1. Mangrove Leaf Litter

Bacteria found in mangrove leaf litter *A. marina* and *S. alba* did not have significant differences, but in general, had similarities in both diversity and number of individual bacteria. This shows that there were similar characteristics of bacteria produced from mangrove litter because they came from leaf tissue called endophytic bacteria.

Bacteria found in *A. marina* and *S. alba* mangrove leaf litter in the Mamburungan mangrove forest area had similarities with bacteria found in Mangrove and Bekantan Conservation Areas in the research of Yulma *et al.*, [10] including *Bacillus*, *Aeromonas*, *Listeria*, *Stapylococcus*, *Enterobacteria*, *Bacteroides* and *Actinobacillus*. The similarity of this bacterial genus was caused by endophytic bacteria found in leaf tissue and organic matter in high mangrove areas such as nitrogen and phosphorus.

Table 1. Types of bacteria that played a role in the process of decomposition of mangrove leaf litter in Mamburungan mangrove forest area

No.	Type of Mangrove	Type of Bacteria	Number of Bacteria (colonies)
1.	<i>Sonneratia alba</i>	<i>Bacillus</i>	18
		<i>Aeromonas</i>	15
		<i>Listeria</i>	8
		<i>Stapylococcus</i>	2
		<i>Enterobacteria</i>	1
		<i>Bacteroides</i>	1
		<i>Actinobacillus</i>	1
2.	<i>Avicennia marina</i>	<i>Bacillus</i>	25
		<i>Listeria</i>	13
		<i>Aeromonas</i>	11
		<i>Pseudomonas</i>	10
		<i>Enterobacteria</i>	10
		<i>Corynebacterium</i>	4
		<i>Actinobacillus</i>	3
		<i>Plesiomonas</i>	2
		<i>Flavobacterium</i>	1

The decomposition rate of *A. marina* and *S. alba* mangrove leaf litter (Figure 1) was not much different both at the beginning and at the end of the observation. The rate of decomposition was higher at the beginning of the observation; this was presumably due to the high organic matter contained in litter and the presence of microorganisms, while at the 56th day, there was a decrease in the rate of decomposition. This was caused by a decrease in organic matter and nitrogen content contained in the residual leaf litter. This was in line with Hodgkiss and Leung [11]; Yulma *et al.*, [9]; Yulma *et al.*, [10] that the highest activity of fungal cellulolytic enzymes occurs at the beginning of decomposition.

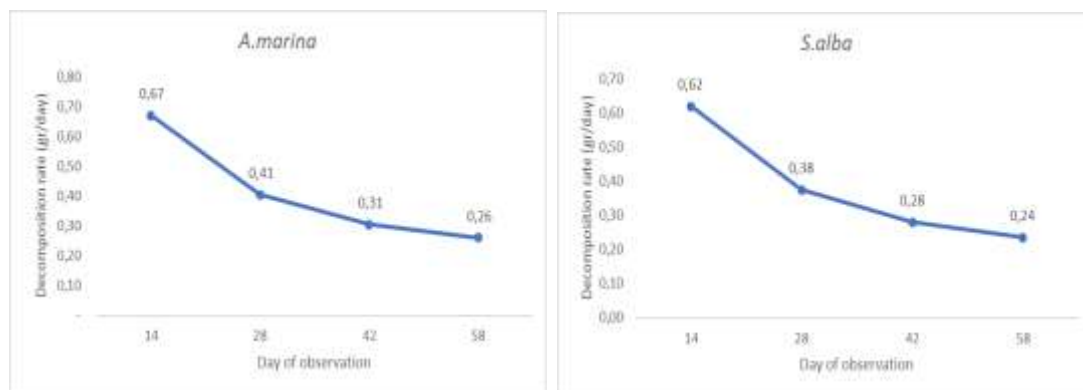


Figure 1. The decomposition rate of *A. marina* and *S. alba* mangrove leaf litter

Other factors that influence the rate of decomposition are water environmental factors (temperature, salinity, and pH) and substrate environmental factors (substrate fraction and substrate/decomposer microorganisms). Manan [12] states that the environment is always wet and humid and the temperature is always high throughout the year, causing the decomposition process of mangrove forest litter to proceed very quickly so that the process of humification (humus formation) immediately continued with the mineralization process. The optimum temperature limit for bacteria ranges from 27-36°C, which is very influential for the decomposition of mangrove litter with the assumption that mangrove leaves are the basis for metabolism. Another factor affecting the rate of decomposition is the density and effect of tidal currents [13].

Decomposition or simplification of the organic content of mangrove leaves easily occurs when litter falls and is trapped in the mangrove ecosystem. Organic materials contained in the litter will be consumed by the decomposer. This is supported by research by Yulma *et al.*, [10] that the rate of decomposition of mangrove leaf litter will contribute to the organic matter in the mangrove forest ecosystem which plays a role in the growth and development of organisms such as crabs, shrimp, fish, and other organisms. In addition, according to Polunin [14], the high rate of decomposition of mangrove leaf litter is caused by the presence of microorganisms, especially bacteria and fungi.

3.2. Bacteria in Mangrove Water

In the water of the Mamburungan mangrove forest, eight genus of bacteria were found. Bacteria that were found had similarities with bacteria found in mangrove leaf litter. It showed that one of the decomposition processes that occurred was influenced by aquatic bacteria with the tidal movement of seawater. The results of this study were not much different from the research of Yulma *et al.*, [15] that showed the diversity of bacteria in the water of Mangrove and Bekantan Conservation Areas (KKMB) as many as nine bacteria (Genus) namely *Bacillus spp.*, *Corynebacterium spp.*, *Listeria spp.*, *Enterobacteria spp.*, *Pseudomonas spp.*, *Aeromonas spp.*, *Micrococcus spp.*, *Staphylococcus spp.*, and *Actinobacillus spp.*, And the most dominant found was *Bacillus spp.*

Aquatic bacteria in mangrove forests cannot be separated from organic material either carried by the flow of seawater to the mangrove area or organic material contained from mangrove leaf litter. According to Romimohtarto and Juwana [10], the cycle of organic matter in the sea is the same as the organic cycle in freshwater and on land environments. Carbon, together with other elements such as phosphorus (P) and nitrogen (N) through the process of photosynthesis, produces a network of plants that become animal food. Besides, mangrove litter production will produce high organic matter in the water, so the growth rate and bacterial development are higher because one of the factors that cause bacterial growth is an organic matter [10].

Table 2. Bacteria in Mangrove Water

No.	Types of Bacteria	Number of Bacteria (colonies)
1.	<i>Bacillus</i>	21
2.	<i>Listeria</i>	11
3.	<i>Aeromonas</i>	8
4.	<i>Corynebacterium</i>	7
5.	<i>Micrococcus</i>	3
6.	<i>Actinobacillus</i>	3
7.	<i>Pseudomonas Enterobacteria</i>	1
8.	<i>Enterobacteria</i>	1

3.3. Bacteria in Sediments

The result of the research showed that the presence of bacteria in sediments in the Mamburungan mangrove area was quite varied with the discovery of various types of bacteria. Bacteria in sediments were more often found when compared to bacteria in waters and leaf litter, this occurred because the process of overhauling organic material was more common in sediments through the provision of oxidizing agents, especially oxygen and other organic materials such as phosphorus and nitrogen as a source of organic material in the mangrove area. This was in accordance with the research of Yulma *et al.*, [16] that there were 16 bacteria genus found in sediments in the Mangrove and Bekantan Conservation Area (KKMB), namely *Enterobacteria*, *Eubacterium*, *Listeria*, *Actinobacillus*, *Bacteriodes*, *Streptococcus*, *Plesiomonas*, *Corynebacterium*, *Pseudomonas*, *Pseudomonas*, *Pseudomonas*, *Pseudomonas*, *Pseudomonas*, *Pseudomonas*, *Aeromonas*, *Bordetella paraptussis*, *Micrococcus*, *Staphylococcus*, *Clostridium*, *Neisseria*.

Bisset *et al.* [17] stated that the diversity of sedimentary microorganism communities was very high. This is due to the content of sediments supporting and assisting in the formation of aerobic and anaerobic microenvironment cooperation. For example, a decrease in oxygen levels due to microorganism activity in a room rich in organic matter will form an anaerobic microenvironment that supports facultative and obligate anaerobic microorganism activities. This causes the emergence of groups of microorganisms with certain specific physiological properties that are in accordance with the conditions of the microenvironment [18].

Table 3. Bacteria in Mangrove Sediments

No.	Types of Bacteria	Number of Bacteria (colonies)
1.	<i>Bacillus</i>	16
2.	<i>Plesiomonas</i>	13
3.	<i>Enterobacteria</i>	7
4.	<i>Corynebacterium</i>	7
5.	<i>Listeria</i>	6
6.	<i>Aeromonas</i>	5
7.	<i>Micrococcus</i>	4
8.	<i>Actinobacillus</i>	4
9.	<i>Bordetella parapertussis</i>	2
10.	<i>Clostridium</i>	1

The bacteria found in the most dominant mangrove forest area was the *Bacillus* bacteria. *Bacillus* was one of the endophytic bacteria found in plant tissue so that this bacterium could be found in all mangrove vegetation. Endophytic bacteria naturally occur in healthy plant organs and soil sediments such as *Pseudomonas* and *Bacillus* [19]. In addition, *Bacillus* has a fast growth cycle and is a bacterial decomposition of phosphorus organic matter. Yulma *et al.*, [20] stated that the content of organic phosphorus contained in the *S. alba* mangrove species is 0.08%. This phosphorus content affected its growth in the decomposition process.

Bacillus spp. is naturally found in sediments because of its ability to produce endospores. Besides, *Bacillus* can also face various environmental changes, such as changes in nutrient levels, water, and temperature [21]. Many other studies mention that *Bacillus* bacteria are more dominant in mangrove ecosystem areas as in the study of Shome *et al.*, [22] that isolated bacteria from sediments found in South Andaman mangroves by isolating 38 bacteria and found that the most dominant bacterial isolate was *Bacillus spp.* for up to 50%.

4. Conclusion

The result of bacteria isolation and identification in Mamburungan Mangrove Forest from *Sonnerratia alba* leaf litter obtained seven bacteria, *Avicennia marina* nine bacteria, mangrove water eight bacteria, and mangrove sediment ten bacteria.

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