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## Isolation and characterization of bacteria from mangrove sediment at coastal area in Pangkep South Sulawesi

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## Isolation and characterization of bacteria from mangrove sediment at coastal area in Pangkep South Sulawesi

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**Abstract.** Mangrove ecosystem is an area with high potential of natural resources which nowadays remains unexplored. One of them is microorganisms that hidden in the depth of the mangrove sediments. Therefore, this study aims to isolate and characterize mangrove's sediment bacteria from estuary coast of Pangkajene River, South Sulawesi, Indonesia. Sampling activities conducted at 6 stations with 3 different depths, those are 0-15 cm, 15-30 cm and 30-45 cm. Microbiological analysis includes microscopic, biochemical and analysis of the abundance of bacterial colonies using Total Plate Count test. From isolation and characterization, it was obtained 35 bacterial isolates consist of 7 different genus, those are Bacillus, Staphylococcus, Vibrio, Micrococcus, Alteromonas, Escherichia, and Listeria. Furthermore, result from Total Plate Count test obtained varied with the highest abundance of bacterial colonies was found at the depth of 30-45 cm with  $9.48 \times 10^4$  CFU.

### 1. Introduction

Coastal ecosystem is an area which provides various natural resources potential, one of them is mangrove ecosystem. Mangrove ecosystem is an area where the estuary of the land and the sea meets. This area is always affected by the sea tides so that there is integration of physical, biological and chemical elements of land and sea [1].

Mangrove ecosystem shows high biological variety ranging from Pisces, Crustaceans, Molluscs, Aves, Reptiles and Mammals [2]. This variety needs the availability of high nutrition to start the food web [3], provided by bacteria which have adapted to the salinity variations and the availability of low oxygen in mangrove sediments [4].

Microorganisms in mangrove ecosystem has an important role in the decomposition and mineralization of organic materials and providing nutrients for plants [5]. In addition, bacteria are also responsible for degrading and recycling essential elements such as carbon, nitrogen and phosphorus used by other living creatures [6]. Information regarding the bacterial community in mangrove sediments on the Pangkajene River estuary coast does not provided yet, therefore this



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research is required in order to become important information in managing biological resources around the mangrove forest area.

## 2. Material and Methods

### 2.1. Sampling Equipment

The tools used include sample bottles, cooler boxes, autoclaves, bunsen, petri dishes, erlenmeyer, test tubes, test tube racks, round ose, glass cups, measuring cups, hotplates, incubators, magnetic stirrers, microscopes, meters, shakers, and scales analytic. The materials used are aquadest, alcohol, crystal violet, iodide solution, safranin, NA media, 1% of H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub> solution, TSIA media, NB media, SIM media, MR-VP media, and denatured alcohol.

### 2.2. Mangrove sediment sampling

The sample used in this study came from the coast of Pangkajene River estuary. Samples taken from six stations, in which three sediment samples were taken from each station at different depths (A= 0-15 cm; B= 15-30 cm; C= 30-45 cm). The sediment samples were taken with a core sampler with a diameter of 8 cm [7]; [8]. Then, the sediment samples are stored aseptically to decrease the bacterial contamination from the air and soil surface [9]. The sample bottles were given the location information and stored in a cool box to be taken to the laboratory [10]. In the laboratory, the samples were stored in the refrigerator with a temperature of 5-10°C until it is used [11].

### 2.3. Bacteria isolation and purification

The bacteria isolation was performed using pour plate method [12];[13]. The sediment samples were weighed as much as 10 grams and then suspended in 90 ml of sterile aquadest and homogenized using a shaker. Next, 1 ml of the suspension was taken and put into 9 ml of sterile aquadest in the test tube so that dilution factor of 10<sup>-1</sup> was obtained. After that, a dilution of 10<sup>-2</sup> to 10<sup>-7</sup> were made. Then, each dilutions of 10<sup>-5</sup>, 10<sup>-6</sup>, and 10<sup>-7</sup> were put into aseptic petri dishes, added by NA media and left until it solidified, and incubated at 30°C for 2x24 hours until a colony was grown on the media[14].

The purification was performed by taking the bacterial colonies that have different characteristics in each petri dish. Bacterial colonies were taken using ose needles and streaked on NA media, and then incubated for 2x24 hours at 30°C [13].

### 2.4. Analysis of the abundance of bacteria colonies

The analysis of bacteria abundance in the sediment used *Total Plate Count* (TPC) test. The dilution result obtained in the isolation and purification process was then continued by analyzing the abundance of bacteria colonies. For the calculation of bacteria colonies using TPC method, the following method was used [15].

**Total Plate Count:** The amount of bacteria colonies x 1/ factorof dilution

### 2.5. Characterization

The bacteria characterization was performed through the observation of microscopic morphological characters and physiological character tests using biochemical tests. The microscopic observation was conducted by observing the shape, size, and color of cells through Gram staining [13]. Characterization and classification of microorganisms such as bacteria can be classified based on enzymatic reactions and biochemical reactions. Bacteria can grow on certain types of metabolites detected by microbial interactions with reagent test agents that produce reagent colors. Cell reactions will occur by carrying out certain tests [16]. Bacterial isolates can be identified by several biochemical reactions tests, namely Triple Sugar Iron Agar (TSIA) test, SulfidIndol Motility (SIM) test, catalase test, citrate test, and Methyl Red-VogesProskauer (MR-VP) [17]; [18].

### 3. Result and Discussion

Isolation and purification of mangrove sediment bacteria from the Pangkajene River estuary at six sampling points with each point having three different sediment depths, obtained 35 isolates of bacteria that were successfully purified. The 35 isolates have different colony morphological types, which prove that mangrove ecosystems are rich in microorganisms.

**Table 1.** The biochemical and microscopic test results of mangrove sediment bacteria

Isolates Code	TSIA		citrate test	SIM test			MR-VP test		Catalase test	Cell shape	Type of gram
	Slant	Butt		Sulfid	Indol	Motility	MR	VP			
A11	+	+	-	-	-	-	+	-	-	Basil	Negative
A12	-	+	-	-	-	-	+	-	-	Basil	Negative
A21	-	+	-	-	-	-	+	-	-	Basil	Negative
A22	-	+	-	-	-	-	+	-	-	Basil	Negative
A31	-	+ H <sub>2</sub> S	+	+	+	+	+	-	+	Coccus	Positive
A32	+	+	-	-	-	-	+	-	+	Coccus	Positive
A41	-	+	-	-	-	-	+	-	+	Coccus	Positive
A42	-	+	-	-	-	-	+	-	+	Basil	Positive
A51	+	+	-	-	-	-	+	-	-	Basil	Negative
A52	+	+	-	-	-	-	+	-	+	Basil	Negative
A61	-	+	-	-	-	-	+	-	+	Basil	Positive
A62	+	+	-	-	-	-	+	-	+	Coccus	Positive
B11	-	-	-	-	-	-	+	-	-	Basil	Negative
B21	-	+	-	-	-	+	+	-	-	Basil	Positive
B22	-	+	-	-	-	+	+	-	-	Basil	Positive
B31	-	-	-	-	-	-	+	+	-	Basil	Negative
B32	-	-	-	-	-	-	-	-	+	Basil	Negative
B41	-	-	-	-	-	-	+	+	-	Coccus	Positive
B42	-	-	-	-	-	-	+	-	-	Basil	Negative
B51	+	+	-	-	-	+	+	+	-	Basil	Positive
B52	+	+	-	-	-	+	+	+	-	Basil	Positive
B61	-	-	-	-	-	+	+	-	-	Basil	Positive
B62	+	+	-	-	-	+	+	-	-	Basil	Positive
C11	+	+	-	-	-	+	+	-	+	Coccus	Positive
C12	-	+	-	-	-	+	+	+	-	Basil	Positive
C21	-	+	-	-	-	+	+	-	+	Basil	Positive
C22	-	+	-	-	-	+	+	-	-	Basil	Positive
C31	-	+ H <sub>2</sub> S	-	-	-	-	+	+	-	Basil	Positive
C32	-	+	-	-	-	+	+	+	-	Basil	Positive
C41	-	+	-	-	-	+	+	-	-	Basil	Negative
C42	-	+	-	-	-	+	+	-	-	Basil	Positive
C51	-	+	-	-	-	+	+	+	-	Basil	Positive
C52	-	+	-	-	-	+	+	-	-	Basil	Positive
C61	-	+	-	-	-	+	+	-	-	Basil	Positive
C62	-	+	-	-	-	-	+	-	-	Basil	Negative

The results of biochemical tests and gram staining according to [18] presented in Table 1. shows that there are 9 isolates that could ferment carbohydrates perfectly, it was characterized by discoloration in the media Triple Sugar Iron Agar from red to yellow and gas formation on the media

[11]. TSIA test results also obtained 2 isolates that produce H<sub>2</sub>S, namely isolates A31 and C31 which are characterized by the formation of black deposits on the basis of the media [11]. The citrate test there was 1 isolate that showed a positive result, namely the A31 isolate, which was marked by changes in the color of the media from green to blue. Sulfid and Indol test results obtained 1 plate which showed a positive result, namely isolate A31. Motility test results found 16 isolates that showed positive results. For the MR test, only 1 isolate showed a negative result, namely B32 isolate. For the VP test there were 8 isolates that showed positive results. The catalase test there were 10 isolates which showed positive results, it was indicated by the formation of air bubbles on the NB media as a sign that the bacteria could produce O<sub>2</sub> by producing the enzyme catalase through degradation of hydrogen peroxide [11]. Microscopic observations of 35 bacterial isolates showed 12 isolates in the form of bacilli (gram) and were positive gram, 17 isolates in the form of bacilli and negative gram, and isolates in the form of a cube (round) and positive gram in 6 isolates. The coccus bacterial isolate is only 17% and this is in accordance with the statement by [19] that coccus bacteria are generally less than bacillus-shaped bacteria.

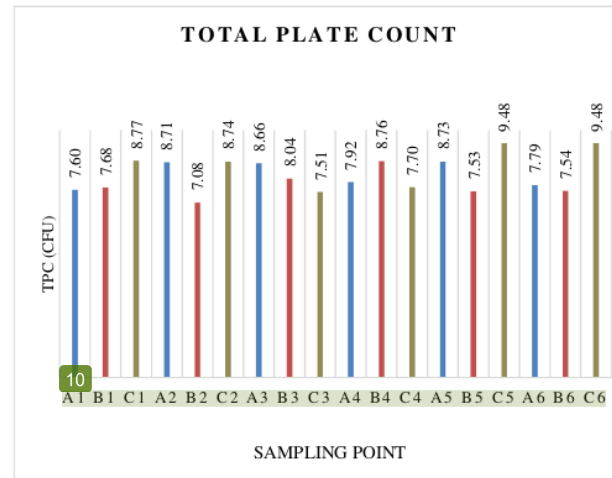
Gram staining results show 66% classified as positive gram, this is different to [19] which explains that almost all marine bacteria are negative gram and the number of positive gram bacteria is only 10% of the total marine bacterial population, and the largest proportion consists of negative gram bacteria in the form of bacilli, and generally move with the help of flagella.

**Table 2.** The results of identification of microscopic observations and biochemical tests.

No.	Genera	Isolates Code
1	<i>Alteromonas</i>	A11, A12, A21, A22, A51, B11, B31, B42, C41, C62
2	<i>Staphylococcus</i>	A32, A41, A62, B41, C11
3	<i>Bacillus</i>	A42, A52, B51, B52, B62, C12, C22, C31, C32, C42
4	<i>Vibrio</i>	B21, B22, B61, C21, C51, C52, C61
5	<i>Micrococcus</i>	A31
6	<i>Listeria</i>	A61
7	<i>Escherichia</i>	B32

Microscopic observations and biochemical tests identified based on the characters in Holt, et.al [18], found 7 genera of a total of 35 isolates, 10 genera of *Alteromonas* isolates, 10 *Bacillus* isolates, 5 *Staphylococcus* isolates, 7 *Vibrio* isolates, 1 isolate of *Micrococcus*, *Listeria*, and *Escherichia* respectively, as presented in Table 2. The bacterial community mentioned above has also been found by previous researchers at different locations: the genus *Vibrio* in the mangrove sediments of *Avicenniagerminans* [20]; the genus *Micrococcus* in mangrove sediments in Odisha, India [21]; *Bacillus*, *Staphylococcus*, *Escherichia*, and *Micrococcus* communities in India [22]; the genus *Listeria* in mangrove sediments from the mangrove forest of Cilacap, Central Java [11]; *Escherichia* [23]; and *Alteromonas* [24].

The abundance of bacterial colonies for each sampling point converted in log<sub>10</sub> can be seen in Figure 1. The total number of bacterial logs per observation point has a variable number. At the depth of A (0-15 cm) results obtained ranged from 7.60 x 10<sup>3</sup> - 8.73 x 10<sup>4</sup> CFU. At a depth of B (15-30 cm) results obtained ranged from 7.08 x 10<sup>4</sup> - 8.76 x 10<sup>4</sup> CFU. While for the depth of C (30-45 cm) results obtained ranged from 7.51 x 10<sup>3</sup> - 9.48 x 10<sup>4</sup> CFU.



**Figure 1.** Abundance of bacterial colonies (Total Plate Count / TPC) for each sampling point (sediment depth: A= 0-15cm; B= 15-30 cm; C=30-45 cm).

The calculation of the abundance of bacterial colonies, the highest number was obtained at the depth of C (30-45 cm), namely at points C5 and C6 with the total number of bacteria as much as  $9.48 \times 10^4$  (cfu / ml). This is in accordance with [25], that bacterial concentration or density is related to the thickness of the substrate or sediment. The higher the sediment thickness, the higher the concentration of bacteria. According to [26], a high bacterial population in sediments illustrates that there is sufficient food or energy supply and is deposited in sediments. However, it can also be influenced by other factors such as the chemical composition of both organic and inorganic sediments which can be used as a reference for further research.

#### 4. Conclusion

The conclusions of this study were 35 isolates, consisting of 7 different genera, namely genus Bacillus, Staphylococcus, Vibrio, Micrococcus, Alteromonas, Escherichia, and Listeria. Different mangrove sediment depths showed different bacterial abundances and the highest at a depth of 30-45 cm with a total bacterial amount of  $9.48 \times 10^4$  CFU.

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