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

Ambeng¹,², H Zubair³, NP Oka³ and A Tonggiruh⁴

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Jl. PerintisKemerdekaan Km 10, Makassar, South Sulawesi, Indonesia.
²Soil and Water Conservation, Faculty of Agriculture, Hasanuddin University
³Conservation Biology, Faculty of Forestry, Hasanuddin University
⁴Department of Geological Engineering, Faculty of Technic, Hasanuddin University
⁵Graduate Student of Geoscience and Environmental Technology, Department of Geological Engineering, Hasanuddin University

Correspondence: ambeng@unhas.ac.id

Abstract. Mangrove ecosystem is an area with high potential of natural resources which nowadays remains unexplored. One of them is microorganismsthathidden 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, Macrococcus, 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 x 10⁴ 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 the 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
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$_2$SO$_4$, H$_2$O$_2$ 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 $10^1$ was obtained. After that, a dilution of $10^2$ to $10^7$ were made. Then, each dilutions of $10^5$, $10^6$, and $10^7$ 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/ factor of 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           | -                | +        | H₂S        | +            | +         | +          | +          | 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           | -                | +        | H₂S        | -            | -         | -          | +          | Basil       | Positive    |
| C32           | -                | +        | -          | -            | -         | +          | -          | Basil       | Positive    |
| C41           | -                | +        | -          | -            | -         | +          | -          | Basil       | Positive    |
| 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
TSIA test results also obtained 2 isolates that produce H₂S, 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 isolate 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 O₂ 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   | Alteromonas| A11, A12, A21, A22, A51, B11, B31, B42, C41, C62 |
| 2   | Staphylococcus | A32, A41, A62, B41, C11 |
| 3   | Bacillus   | A42, A52, B51, B52, B62, C12, C22, C31, C32, C42 |
| 4   | Vibrio     | B21, B22, B61, C21, C51, C52, C61 |
| 5   | Macrooccus | A31                    |
| 6   | Listeria   | A61                    |
| 7   | Escherichia| 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 Macrooccus 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 10 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 10³ - 8.73 x 10⁴ CFU. At a depth of B (15-30 cm) results obtained ranged from 7.08 x 10⁴ - 8.76 x 10⁴ CFU. While for the depth of C (30-45 cm) results obtained ranged from 7.51 x 10³ - 9.48 x 10⁴ CFU.
Figure 1. Abundance of bacterial colonies (Total Plate Count / TPC) for each sampling point (sediment depth: A= 0-15 cm; 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.

References
[1] Fatiqin A 2015 Exploration of akrinomiset as a producer of antibiotics from mangrove soil Sonneratia caseolaris in Tanjung Api-Api Jurnal biota 1.
[2] Lankau RA and Strauss SY 2007 Mutual feedbacks maintain both genetic and species diversity in a plant community Science 317: 1561-1563.
[3] Dias, ACF, Andreote FD, Rigonato J, Fiore MF, Melo IS, Araujo, WI 2010 The bacterial diversity in Brazilian non-disturbed sediment Anto. van Leuwe. 98: 541-551.
[4] Lane DJ, PaceB, Olsen GJ, Sthal DA, Sogin ML, Pace NR 1985 Rapid determination of 16S Ribosomal RNA sequences for phylogenetic analyses Proceeding of the National Academic of Sciences of the United States of America 82: 6955-6959.
[5] McGuire KL, Fierer N, Bateman C, Treseder KK and Turner BL 2012 Fungal community composition in neotropical rain forests: the influence of tree diversity and precipitation Microb Ecol. 63: 804-812.
[6] Alongi DM 1994 The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystem Hydrobiology 285: 19-23.

[7] Austin B, 1993 Marine Microbiology Cambridge University Press.

[8] Leung JYS 2015 Habitat heterogeneity affects ecological functions of macrobenthic communities in a mangrove: implication for the impact of restoration and afforestation Global Ecology and Conservation 4: 423-433.

[9] Dewi AK, Lisna M, Rolan R 2017 Isolation of bacteria from mangrove soil Rhizopora sp. in the city of Bontang Mulawarman Pharmaceuticals Conferences.

[10] Junior FLS, Armando CFD, Cristiane CF, Rodrigo GT, Andre OSL, Itamar SM, Fernando, DA 2008 Endo- and exoglucanase activities in bacteria from mangrove sediment Brazilian Journal of Microbiology 44(3): 969-976.

[11] Triyanto, Alim I, Irfan D, Priyambada, Jaka Wand Duranta DK 2008 Isolation and characterization of denitrification bacteria isolated from the mud of the mangrove area Journal of Fisheries Science X(1): 1-10.

[12] Islamiah DN, Rahmawati, Riza L 2017 Types of Rizosphere Bacteria Avicennia Mangrove Region in Terusan Village Mempawah Hilir District West Kalimantan Journal Protobiont 6(3): 165-172.

[13] Waluyo L 2008 Basic microbiology method techniques University of Muhammadiyah Malang Press Malang.

[14] Marista E, Khotimah Sand Linda R 2013 Phosphate solvent bacteria isolated from three types of rhizosphere soil from nipah banana plants (Musa paradisica var. nipah) in the city of Singkawang Journal Protobiont 2(2): 93-101.

[15] Fardiaz S 2004 Food microbiology analysis Raja Grafindo Persada. Jakarta.

[16] Hasyimi HM 2010 Microbiology and Parasitology for Nursing Students CV Trans Info Media. Jakarta.

[17] Marchesi JR, Sakuichi S, Andrew JW, Tracey AM, John CF, Sarah JH and William GW, 1998. Design and Evaluation of Useful Bacterium-Specific PCR Primers that Amplify Genes Coding for Bacterial 16S rRNA. Appl Environ Microbiol. 64(2): 795-799.

[18] Pelczar MJ dan Chan ECS 2007 Basics of Microbiology Volume 1 UI Press Jakarta.

[19] Hutchings and Saenger 1987 Ecology of Mangrove University of Queensland Press Queensland.

[20] Rojas A, Holguin G, Glick BR, Bashan Y 2001 Synergism between Phyllobacterium sp. (N2 – fixer) and Bacillus licheniformis (P solubilizer), both from a semiarid mangrove rhizosphere FEMS Microbiol Ecol. 35: 181-187.

[21] Behera BC, Singdevsachan SK, Mishra RR, Sethi BK, Dutta SK and Thatoi HN 2016. Phosphate solubilising bacteria from mangrove soils of Mahanadi River Delta India World Journal of Agricultural Research 4(1): 18-23.

[22] D’Costa PM, Kalekar S, Bhosle S 2004 Diversity of free living and adhered bacteria from mangrove swamps Indian Journal of Microbiology 44: 247-250.

[23] Kolm, HE, Schoenenberger MFB, Piemont MR, Souza PSA, Schnell E, Scuhl G, Mucciato, MB and Mazzuco R 2002 Temporal variation of bacteria in superficial waters of Paranagua and Antonina Bays Parana Brazil Braz. Arch. Biol. Technol. 45: 27-34.

[24] Pandey, R, Muller A, Napoli CA, Selinger DA, Pikaard CS, Richards EJ, Bender J, Mount DW, Jorgensen RA 2002. Analysis of histone acetyltransferase and histone deacetylase families of Arabidopsis thaliana suggests functional diversification of chromatin modification among multicellular eukaryotes Nucleic Acids Res. 30(23): 5036-55.

[25] Sutiknowati LI 2010 Abundance of Phosphate Bacteria in Seagrass Padang Banten Bay Oceanoology and Limnology in Indonesia 36 (1): 31.

[26] Hanafiah KA 2007 Basics of Soil Science PT Raja Grafindo Persada Jakarta.