Diversity of Bacterial Isolates in Water Mangrove Forest Wonorejo, Surabaya

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Abstract. Plastic waste can be seen in several areas in the Wonorejo mangrove forest, Surabaya, which is also a habitat for various types of microbes. Research on bacterial isolates from water samples in mangrove forests needs to be conducted to determine their diversity. This research was conducted to determine the diversity and dominance of bacterial isolates in mangrove forest waters. Each water sample was taken from a site that is regularly irrigated (station 1) and a site that is flooded (station 2). The number of bacteria in mangrove forest water samples was determined using the Total Plate Count (TPC) method and bacterial isolation was performed using the pour plate method. Subsequently, the bacterial isolates were characterized based on their colonies and observed for their dominance. The number of bacteria at station 1 was 4.85x102 CFU/mL and 2.55x105 CFU/mL at station 2. Both stations showed bacterial diversity. However, several bacterial isolates with the same character can be found in both stations. Further research should be conducted to determine the potential of these bacterial isolates as plastic-degrading agents.

Keywords. Mangroves, Diversity, Bacteria

1 Introduction

Indonesia has extensive mangrove forests that have a high potential to be exploited. Mangrove forest is a biodiversity resource that should be conserved and further explored for their potential. Mangrove forests are habitats for vegetation and animals. In addition, mangrove forests can also be used as natural tourist attractions.

Mangrove forests can be exploited for the concept of natural tourism (ecotourism) which contains elements of education and conservation [1]. Ecotourism attraction in Wonorejo Mangrove Surabaya has natural resources, as a place to live various kinds of flora and fauna [2], a potential source of microbes including Actinomycetes which have antibacterial abilities [3], probiotic candidate bacteria [4], and bacteria capable of degrading crystal violet dye [5]. Littering around rivers in mangrove forests can harm the survival of mangrove forests and marine biota [2]. Based on previous research, the tides of the Madura Strait have a significant effect on the amount of plastic waste at the estuary of the Wonorejo river in Surabaya [6]. A solution to handling waste in the mangrove forest is needed to manage the waste properly, especially waste that is difficult to degrade.

Mangrove forests are also a source of microbes that have a lot of potentials. Several bacterial isolates have been isolated from mangrove forests, including an endophytic bacterial isolate from Sonneratia alba which is capable of producing gelatinase enzymes [7], an endophytic bacterial isolate Avicennia marina, of which two isolates have antibacterial properties against Escherichia coli [8]. Cellulolytic bacteria isolates were also isolated from decayed wood in mangrove forests in South Bangka [9].

Research on the diversity of bacteria in the waters of mangrove and proboscis monkeys has been carried out and successfully isolated 9 bacteria, namely Bacillus spp., Corynebacterium spp., Listeria spp., Enterobacteria spp., Staphylococcus spp., Pseudomonas spp., Micrococcus spp., Aeromonas spp., and Actinobacillus spp. [10]. Analysis of the diversity of cellulose-degrading bacteria from the mangrove forest of Pasar Banggi Village, Rembang Regency, found 5 bacteria, namely Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Bacillus sp., and Proteus mirabilis with low levels of bacterial diversity [11]. Isolation of bacteria from the rhizosphere of the Avicennia mangrove forest was successful in obtaining 5 genera of bacteria [12].

Based on several studies that have been carried out, they have succeeded in isolating bacteria from various areas of mangrove forests. However, it is still important to study bacterial diversity from water in Wonorejo mangrove forests and then exploit their potential. This research purposed to isolate bacteria from mangrove forest water to determine their diversity and dominance. This research is a preliminary study exploring the

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diversity of bacterial isolates from water in Wonorejo mangrove forests.

2 Materials and Methods

This research was an exploration study to isolate bacteria from Wonorejo mangrove forest water. The research was conducted at the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya. The research was conducted in several stages as follows.

2.1 Sample Collection

This study uses water samples from 2 different stations in the Wonorejo mangrove forest area, Surabaya. The first water sample was taken at station 1 (TA1) which is an area in the mangrove forest that is always exposed to running water, while the second water sample was taken from station 2 (TA2) which is an area where the water was stagnant. Water samples were taken using a sterile glass beaker and then put into sterile plastic for further storage in a cool box to be taken to the laboratory. The location of the mangrove water sampling is as shown in Figure 1.

Fig.1 Location of mangrove water sampling

2.2 Bacterial Isolation and Enumeration

Bacterial isolation was carried out using the pour plate method after serial dilution of the water sample was carried out. Pour plate was carried out using PCA [13]. A total of 1 mL of water sample from each station was diluted in 9 mL of sterile distilled water to obtain a sample with a dilution of 10-1, then the same activity was carried out for the next dilution until a dilution of 10-6. Pour plate was done by inserting 1 mL of sample in each dilution into a Petri dish, then added with PCA in a liquid state into a Petri dish. The sample and PCA were then homogenized and allowed to solidify for further incubation at 37oC for 24 hours. Bacterial colonies that grew after incubation were calculated based on the Total Plate Count (TPC) using a colony counter to determine the total number of bacteria in each sample.

2.3 Bacterial Colony Characterization

The bacterial colonies that grew were then characterized based on several characteristics of the bacterial colonies. Colony character which was characterized included colony shape, elevation, edge, surface, and pigmentation. Calculation of the number of each bacterial isolate was calculated to observe its dominance.

2.4 Bacterial Isolate Purification

The bacterial isolates obtained were purified using the streak plate method on Nutrient Agar (NA) (Merrck). The purified bacterial isolates were then transferred to slant NA and incubated at 37oC for 24 hours. The isolates were then stored in the refrigerator for further analysis. The dominant bacterial isolates were then stained with Gram stain.

3 Results and Discussion

Research on the isolation of bacteria from samples taken from mangrove forests has been carried out including from samples of mangrove roots [14][7], mangrove stems [7][15], leaves of mangrove plants [7][8], weathered wood [9], rhizosphere [12], leaf litter [16][17], and soil samples [18]. Mangrove forests are a habitat for various types of bacteria, so it is important to make efforts to determine the diversity of bacteria from mangrove forests. In this study, bacteria were isolated from water samples taken in the Wonorejo mangrove forest, Surabaya.

In this study, bacterial enumeration was carried out in each mangrove forest water sample using the TPC method. Enumeration was carried out to determine the number of bacterial populations in the samples studied. The results of the enumeration of bacteria were shown in Table 1. The results of the enumeration showed that the number of bacteria was higher in the water sample from station 2, this was presumably because station 1 is an area that is always exposed to running water so that the bacterial population can be carried away by the flow of water. This condition is different from the water at station 2 which is in a flooded condition and is not exposed to continuous water flow. The results of the pour plate of Wonorejo mangrove water samples were shown in Figure 2.

Table 1. Enumeration of the number of bacteria in mangrove forest water.

Water Sample	Number of bacteria (CFU/mL)
Station 1	4.8×10^{5}
Station 2	2.55×10^{5}

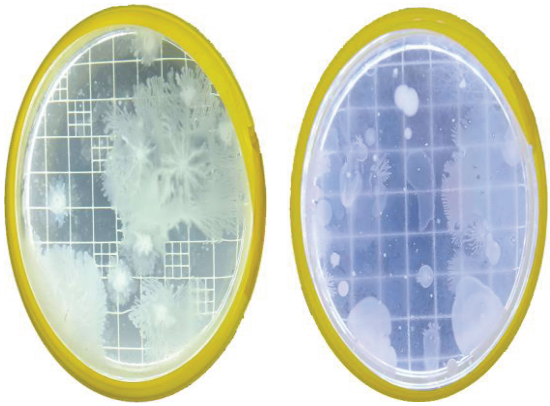


Fig.2 Bacterial colonies: (a) station 1 water sample at a dilution of 10-1; (b) station 2 water sample at a dilution of 10-3

Based on the results of pour plate water samples that have been carried out, bacterial isolates with different colony characters were obtained in each sample. Based on the differences in the character of the growing bacterial colonies, the total number of bacterial isolates obtained in this study were 19 bacterial isolates of which 7 bacteria were obtained from the station (T1A.1-T1A.7) and 12 bacteria (T2A.1-T2A.12) were obtained from station 2. The results of characterization of each bacterial isolate from water samples at station 1 were shown in Table 2.

Table 2. Characteristics of bacterial isolate colonies from mangrove forest water at station 1

Isolate	Form	Elevation	Edge	Surface	Color
T1A.1	rhizoid	raised	lobate	rough	yellowish white
T1A.2	punctiform	convex	entire	smooth	yellowish white
T1A.3	irregular	flat	entire	smooth	yellowish
T1A.4	circular	convex	entire	smooth	yellowish
T1A.5	irregular	flat	undulate	smooth	transparent
T1A.6	circular	convex	entire	smooth	yellowish white
T1A.7	irregular	raised	undulate	rough	white

Characteristics of bacterial isolates obtained from water samples from mangrove forests at station 2 were shown in Table 3. Several bacterial isolates obtained from water samples at stations 1 and 2 have the same colony characteristics. Isolate T1A.1 had the same colony character as isolate T2A.4, which had a rhizoid shape, raised elevation, lobate edges, rough surface, and yellowish white in color. Isolate T1A.6 had the same colony character as isolate T2A.1, which was circular in shape, convex elevation, entire edge, smooth surface, and yellowish white in color. Isolate T1A.2 had the same colony character as T2A.5, which was punctiform shape, convex elevation, entire edge, smooth surface, and yellowish white. Likewise, isolate T1A.5 had the same colony character as T2A.8, which was irregular in shape, raised elevation, undulate edges, rough surface, and white in color.

Table 3. Characteristics of bacterial isolate colonies from mangrove forest water at station 2

Isolate	Form	Elevation	Edge	Surface	Color
T2A.1	circular	convex	entire	smooth	yellowish white
T2A.2	circular	convex	curled	smooth	white
T2A.3	irregular	flat	erose	smooth	transparent
T2A.4	rhizoid	raised	lobate	rough	yellowish white
T2A.5	punctiform	convex	entire	smooth	yellowish white
T2A.6	filamentous	raised	filamentous	rough	yellowish white
T2A.7	circular	convex	entire	smooth	yellowish white (shiny)
T2A.8	irregular	raised	undulate	rough	White
T2A.9	irregular	raised	lobate	smooth	yellowish white
T2A.10	irregular	convex	lobate	rough	White
T2A.11	circular	convex	entire	smooth	yellowish white (shiny edges)
T2A.12	circular	raised	entire	smooth	yellowish white

Several studies on the potential of bacteria originating from mangrove forests have been carried out including research on bacterial isolates that produce extracellular enzymes [19][20], degrading crude oil and polycyclic aromatic hydrocarbons [21], producer of cellulase enzymes [22], pectinolytic bacteria [23]. The mangrove ecosystem has the potential as a source of microbes that produce proteases, amylase, and cellulases as well as other extracellular enzymes [24]. Research on the potential of bacteria originating from mangrove forests is still wide open to be carried out, so it is important to do research to explore the potential of bacterial isolates from mangrove forests.

Table 4. The number of colonies of each bacterial isolate in the water sample at station 1

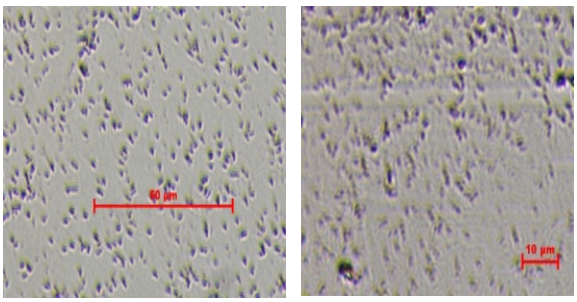
Isolate	Number of Colonies
T1A.1	19
T1A.2	104
T1A.3	5
T1A.4	3
T1A.5	7
T1A.6	1
T1A.7	3

Table 5. Number of colonies of each bacterial isolate in water samples at station 1

Isolate	Number of Colonies
T2A.1	57
T2A.2	59
T2A.3	33
T2A.4	5
T2A.5	342
T2A.6	23
T2A.7	5
T2A.8	67
T2A.9	21
T2A.10	7
T2A.11	1
T2A.12	4

Many microbes are found in mangrove forests, but studies are still needed to link microbial diversity and function as well as an assessment of how environmental changes can affect microbes [25]. Microbial diversity is an important aspect to study because of its potential to produce biomolecules that can be utilized by humans [26]. Bhitarkanika mangrove soil has a diversity of bacteria which is dominated by the genera Bacillus, Pseudomonas, Desulfotomaculum, Desulfovibrio, Desulfomonas, Methylococcus, Vibrio, Micrococcus, Klebsiella and Azotobacter [27].

In this study, based on the calculation of the number of colonies of each bacterial isolate obtained from the mangrove forest water sample at station 1, it was found that the most dominant bacterial isolate was isolate T1A.2. Based on the observation of the pour plate sample, the bacterial isolate T1A.2 had a total number of 104 colonies (Table 4). In the water sample at station 2, the dominant bacterial isolate was T2A.5. Based on the observation of the pour plate sample, isolate T2A.5 had a total number of colonies of 342 colonies (Table 5). Bacterial isolates T1A.2 and T2A.5 were Gram positive bacteria. The results of staining on bacterial isolates T1A.2 and T2A.5 were shown in Figures 3. Bacillus spp. is the dominant bacterium found in the waters of the Mangrove and Proboscis Monkey Conservation Area of Tarakan City [10].



(a) (b)

Fig.3 Gram stain results : (a) isolate T1A.2; (b) isolate T2A.5

4 Conclusion

This research has succeeded in isolating 19 bacterial isolates from mangrove forest water and the colonies have been characterized. Based on the analysis of the number of colonies of each bacterial isolate, isolate T1A.2 was the dominant isolate in the water sample at station 1 while isolate T2A.5 was dominant in the water sample from station 2. Further study is needed to explore the potential of these dominant isolates.

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