Diversity of Soil Bacteria Communities: A Case Study in Wonorejo Mangrove

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Abstract. Mangrove habitats are found in coastal areas and have unique environmental conditions. The mangrove habitat contains abundant and various microbial resources, which play critical roles in mangrove ecosystem maintenance and function. One of the intriguing mangrove habitats to study is the Wonorejo Mangrove, Surabaya. In this current research, the abundance and diversity of bacterial communities in four areas of Wonorejo Mangrove, Surabaya, were determined. The four areas of interest included mangrove by the river with a jogging track (station 1), mangrove by the river without a jogging track (station 2), mangrove in the estuary (station 3), and mangrove in the pond (station 4). Soil samples were collected from the four stations, then the abundance of bacteria was determined by using the Standard Plate Count method. The bacterial colonies that grew from each sample were then listed and characterized based on their colony morphology. The results indicated that the highest abundance was at station 4 with the number of bacteria at 1.83×10^7 CFU/ml, while the lowest abundance was at station 1 with 5.3×10^4 CFU/ml. The structure of bacterial communities of each station was varied. Nevertheless, one of the isolates, namely isolates TS1.1 can be observed in all of the samples. We can conclude that the abundance and the diversity of bacterial communities are affected by environmental factors. This research further deepens our understanding of the bacterial dynamics in Wonorejo mangrove habitats.

Keywords: Diversity, Bacteria, Mangrove, Dynamic.

1 Introduction

Use Mangroves are unique intertidal estuarine ecosystems of the tropical and sub-tropical coasts. Approximately, 60–70% of the coastlines are covered with mangroves. Mangrove ecosystems have various significant ecological and economic roles, including shielding coastlines from storm damage and erosion, degrading environmental pollutants, and providing conservatory habitats for numerous aquatic organisms [1,2].

Mangrove ecosystems are one of the considerable productive ecosystems in the world, which are distinguished by high levels of salinity, high redox potential and organic matter contents, and high rates of nutrient recycling [1]. With such unique environmental circumstances, the mangrove habitat contains abundant and distinct microbial resources, which make mangroves the hotspots for microbial diversity. The mangrove microbiome consists of bacteria and fungi (91%), algae (7%), and protozoa (2%) [3]. These microbes play critical roles in the productivity, conservation, and recovery of mangroves. Microbial diversity in mangrove soil is influenced by biogeographical, ecological, and anthropogenic factors.

One of the bewitching mangrove habitats to study is the Wonorejo Mangrove, Surabaya. Initially, this nature conservation area was created to prevent abrasion in the eastern area of Surabaya City. In an area of approximately 200 hectares, various mangroves are planted. Currently, Mangrove Wonorejo not only functions as a conservation area but also as a tourist attraction that combines recreational and educational tourism.

The expansion of the Wonorejo mangrove function as a tourist attraction will certainly have an impact on its microbial biodiversity. Therefore, it is critical to conduct research to determine soil chemistry, microbial abundance, and microbial diversity at several stations in the Wonorejo mangrove.

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2 Material and Methods

2.1 Site description

Soil samples were taken from four stations in the Wonorejo Mangrove area, Surabaya, East Java, Indonesia. The locations of the four stations were depicted in **fig 1.** Stations 1 and 2 are located in a mangrove area by the river. There is a jogging track in station 1, but not in station 2. Station 3 is located in the mangrove area in the estuary and station 4 is located in the mangrove area in the pond.

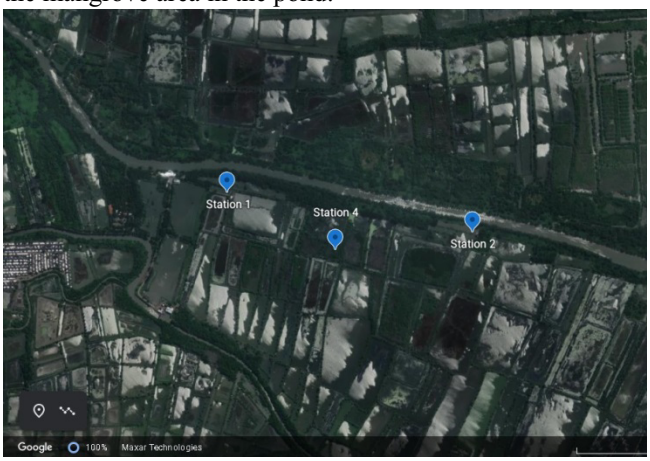

Fig 1. Four sampling site in Wonorejo Mangrove area

2.2 Soil sampling and soil chemistry

Soil samples were collected from five points at each station in September 2021. Samples were taken from a depth of 5 to 10 cm from the surface and placed into sterile plastic to composite. The sample was then stored in a cool box to be brought to the laboratory. Soil temperature, soil moisture, soil pH, light intensity, and salinity of each station were also determined. These parameters were measured at five points at each station with four replicates.

2.3 Abundance of soil bacteria of Wonorejo Mangrove

5 g of soil samples from each station were separately dissolved into 45 ml of sterile distilled water. This solution was considered as a dilution of 10^{0}. Subsequently, 1 ml of dilution 10^{0} was dissolved in 9 ml of sterile distilled water to obtain a dilution of 10^{-1}. This serial dilution was carried out up to a dilution of 10^{-6}. 1 mL of each dilution was then planted in a petri dish with Plate Count Agar (Merck) media by pour plate method. After 24h incubation at 30 °C, the abundance of soil bacteria was determined based on the Total Plate Count.

2.4 Colony characterization

Each colony that grew after incubation for 24 hours was further characterized. Colony characterization includes form, elevation, margin, surface, and pigmentation. Each colony with different characters was named with a certain code. Subsequently, the data were analyzed descriptively.

3 Results and Discussion

3.1 Soil chemistry and environmental factors

The four stations selected as sampling sites have different characteristics. Station 1 is near the river with a jogging track which indicates the relatively high human activities in the area. In comparison, station 2 which is also near the river without a jogging track showed low human activities. Stations 3 and 4 are located in estuary and pond areas, respectively, with different levels of human activities as well. In addition to these characteristics, the soil chemistry at the four stations has several similarities and differences as presented in Table 1.

Table 1. Soil chemistry of each station.

Station	Soil temperature (°C)	Soil moisture (%)	Soil pH	Light intensity (Lux)	Salinity (ppm)
1	27.7	10	7.0	310	1000
2	29.1	10	7.1	350	1000
3	29	10	7.2	617.4	1023
4	30.2	10	7.2	1800	1010

Table 1 showed that the soil temperatures of the four stations ranged from 27.7-30.2 °C. The bacteria that could grow in this temperature range are mesophilic. Mesophilic bacteria grow in a temperature range of 20-45 °C, with the optimal growth temperature in the range of 30-39 °C [4].

Soil moisture and soil pH at the four stations have a similar value of 10% and 7.0-7.2 (neutral), so that it may not affect the abundance or diversity of bacteria at each station. Likewise, the salinity ranges from 1000-1023 ppm which is classified as slightly saline.

The light intensity at stations 1 and 2 was relatively low due to the presence of shade, while at the other two stations it was relatively high due to less or no shade (station 4). This high-light intensity can affect the community structure of soil bacteria [5].

3.2 Abundance and diversity of soil bacteria in Wonorejo Mangrove

The calculation of the total number of bacteria at each station with the Standard Plate Count method showed that the highest abundance was found at station 4, while the lowest abundance was at station 1 (Table 2). However, the diversity of bacteria in these two stations was relatively lower than in the other two stations (stations 2 and 3) (Table 3).

Table 2. The abundance of soil bacteria in each station

Station	Total bacteria num (CFU/ml)
1	$5.3x10^{4}$
2	$1.57x10^{6}$
3	$8.45x10^{4}$
4	$1.83x10^{7}$

The highest number of bacteria found at station 4 can be related to soil temperature and also the availability of nutrients. The soil temperature at station 4 was 30.2 °C which is the optimal temperature range for the growth of mesophilic bacteria. In addition, station 4 is a pond area so the availability of nutrients is higher. However, these conditions can also trigger the dominance or co-dominance of certain species of bacteria so that the diversity of bacteria at the station was low. These results were consistent with previous studies in two mangroves with different levels of human activities that indicate differences in community structure and also the dominance of a particular genus of bacteria [6].

Table 3 showed an isolate that can be found at all stations, namely TS1.1. In addition, there were also isolates TS1.3 which can be found at stations 1, 2, and 4. In this study, further characterization and identification have not been done, so the genus or species of the two isolates remained unknown.

Some studies on bacterial diversity in mangrove areas show that Proteobacteria is the largest phylum found in mangrove ecosystems globally [3]. However, the environmental factors of mangroves as well as the impact of human activities may cause variations in the structure of the bacterial community in each mangrove ecosystem [3]. Several bacterial classes are abundant in mangrove ecosystems, including the Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, and Bacilli [6]. A recent study on bacterial diversity from Mangroves sediment in Klawalu Sorong, West Papua, identified some bacterial species such as *Bacillus safensis* strain C251, *Bacillus amyloliquefaciens* strain NO10, *Clostridium* sp. JC336, and *Bacillus australlimaris* [7].

Table 3. Isolates code and its colony morphology of each station.

Isolates	Colony morphology					Station
	Form	Elevation	Margin	Surface	Pigmentation	
TS1.1	Circular	Convex	Entire	Smooth	yellowish white	1,2,3,4
TS1.3	Punctiform	Flat	Entire	Smooth	white	1,2,4
TS1.8	Irregular	Flat	Entire	Smooth	transparent	1
TS2.2	Circular	Flat	Entire	Smooth	white	2,3
TS2.4	Irregular	Raised	Lobate	Rough	white	2
TS2.5	Rhizoid	Raised	Lobate	Rough	yellowish white	2
TS2.7	Irregular	Flat	Lobate	Smooth	transparent	2,3
TS2.8	Circular	Flat	Entire	Smooth	white	2
TS2.9	Circular	Flat	Entire	Smooth	transparent	2
TS3.3	Circular	Flat	Entire	Smooth	transparent white	3
TS3.5	Filamentous	Flat	filamentous	Rough	yellowish	3
TS3.6	Irregular	Flat	Undulate	Smooth	transparent	3
TS3.7	Irregular	Flat	Lobate	Smooth	yellowish	3
TS3.10	Irregular	Raised	Lobate	Smooth	transparent white	3
TS3.13	Irregular	Flat	Undulate	smooth	transparent	3
TS3.19	Irregular	Raised	Undulate	Rough	yellowish	3
TS4.2	Circular	Convex	Entire	Smooth	glistening	4
TS4.8	Circular	Flat	Lobate	Rough	transparent white	4
TS4.11	Irregular	Raised	Undulate	Smooth	yellowish white	4

This current study can deepen our insight into the abundance and diversity of soil bacteria in the Wonorejo Mangrove area which was affected by various factors. Periodic monitoring of the dynamics of bacterial communities in various areas with diverse characteristics in the Wonorejo Mangrove is necessary to determine the impact of the expansion of mangrove functions as a tourist attraction. The monitoring method can also improve by using the molecular analysis method.

4 Conclusion

The highest abundance of bacteria was found at station 4 with the number of bacteria as much as $1.83x10^7$ CFU/ml, while the lowest abundance was found at station 1 with the number of bacteria $5.3x\ 10^4$ CFU/ml. However, the diversity at the two stations is relatively low compared to the other two stations due to the effects of various factors. The structure of bacterial communities of each station was varied. However, one of the isolates, TS1.1, can be observed in all stations. Constant monitoring needs to be performed to maintain the sustainability of the Wonorejo mangrove ecosystem.

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