Preliminary study of nitrogenous fixation bacteria exploration under palm tree vegetation on peatland ecosystem

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Abstract. The peat ecosystem area has the potential for bacteria that have the potential to act as nitrogen fixers. These bacteria can be applied in various human activities. In fishery activities, nitrogen is an essential component for plankton growth as a live feed. Besides, the ideal N and P components will affect the abundance of plankton in the waters. In this study, the Isolation of candidate bacteria from the peat ecosystem area under palm tree vegetation was carried out in Trumon District, South Aceh. Different depths in the sample soil are an essential parameter, namely the depths of 0-15 cm, 15-30 cm, 30-50 cm. The isolation method was carried out with a scatter plate using Jensen media and obtained 25 isolates which were bio fertilizer candidates. Biodiversity analysis was also carried out to test the three soil depths of the isolated source. A depth of 15 cm shows an isolate diversity pattern that is almost the same as a sample depth of 30 cm.

1. Introduction
The peat ecosystem that is distributed in Indonesia has the potential to store microbial biodiversity. This area is spread across Sumatra (4,778 million hectares), Kalimantan 4,778 million hectares), and Papua (3.69 million hectares) \[1\]. The main component of peat in the form of carbon (60\%) is an essential source of carbon for microbes, besides phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) which are macronutrients also found in this soil type. Meanwhile, micronutrient elements are also complementary, such as Copper (Cu), Zinc (Zn), Manganese (Mn), and Iron (Fe) is quite good amounts \[2\].

The presence of nutrients in peat soils strongly supports microbial biodiversity, which supports flora and fauna biodiversity as well. Microbes in the soil are an essential part of the decomposition process and at the same time, become an indicator of the quality of an ecosystem \[3\]. One of the crucial microbes in peat soil is nitrogen-fixing bacteria \[4\]. Also, to helping with the breakdown of organic matter, microbes in peat soils play an essential role in providing nutrients for plants and producing enzymes that allow plants to grow well \[5\].
Nitrogen-Binding Bacteria (BPN) are a group of bacteria that have nitrogenase enzymes that are able to bind nitrogen (mainly N₂) free from the atmosphere and then reduce them to ammonia compounds (NH₄) and nitrate ions (NO₃⁻) [4]. BPN is classified into two types of microbes that make up biological fertilizers, namely symbiosis with root systems and non-symbiotic (free living in the environment). Symbiotic the BPNs include Rhizobium, while non-symbiotic nitrogen-fixing bacteria are Azotobacter and Azospirillum. Other types are Streptomyces and Lactobacillus sp., which contains enzymes that breakdown cellulose, thereby accelerating the breakdown of organic matter and increasing soil nutrients (Karina, 2016). The BPNs is distributed in fertile soil habitats and marginal soils with different diversity and population [6]. Peat soils are marginal soils that have low fertility characterized by low pH (3.0 - 5.0), and high water content. However, peatlands can still be used as potentially productive land [7].

Tropical peatlands play an important role in maintaining the stability of various ecosystems. Fast conversion of function on peatlands, as agricultural land and human settlements, has further reduced the area of peatlands. This activity has an impact on changes in the structure of the microbial community and bacteria of the peat soil. Besides, the potential for the nitrogen transformation process in the soil also happened [8]. So that research on the study of bacteria on peatlands needs to be done to identify bacteria that are potential to be used in agriculture and fisheries activities.

2. Material and Methods

2.1 Sampling site

The sampling location was carried out in an area of open peatland palm plantations in Gampong Teupin Tinggi, Trumon District, South Aceh (Figure 1). No specific permission was required for this study. Sampling was carried out at 4 different stations, namely Station I, land planted with oil palm with loose soil type (N 02o 40' 06. 70 "E 097o 39' 16. 29"), Station II, land planted with oil but with clay type (N 02o 40' 06. 49 "E 097o 39' 17. 17"), Station III land that is not planted with oil palm with loose soil type (N 02o 40' 06. 75 "E 097o 39' 15. 48"), and IV land that is not planted with oil palms with clay type (N 02o 40' 07. 12 "E 097o 39' 14. 67"). All sampling sites were replicated at three depths (0-15 cm; 15-30 cm; and 30-50 cm). At the sampling location, pH, soil temperature and soil moisture were measured. Analysis and culture of bacteria were carried out at the Microbiology Laboratory of the Department of Biology, Ar-Raniry State Islamic University on November 2019 to January 2020.

![Figure 1. Sampling site in Gampong Teupin Tinggi, Trumon District, South Aceh](image-url)
2.2 Bacterial culture

Biochemical testing and bacterial culture were carried out at the Microbiology Laboratory of the Biology Department of Ar-Raniry State Islamic University from November 2019 to January 2020. The isolation method was carried out with a scatter plate using Jensen media [9]. The growth of BPN can be observed by the presence of colony growth in Jensen medium. Colony purification was carried out by streak plate method and morphological observations, including shape, border colour and elevation were carried out based on previous studies [10]. About 0.1 ml was spread on Jensen medium, after that incubated for 72 hours, and then to get the entire colony using a 10-5 dilution. Colony counts using colony counters.

2.3. Characterization of bacterial, biochemical test and gram stain

In general, the biochemical testing of bacteria uses standard methods such as the gram test for bacteria, the motility test, the citrate test and the Urea test [11]. In the gram test, the pure isolate obtained was carried out with gram staining by scratching one ose of bacterial isolates on the glass preparation so that it did not clot. Then drop one drop of Gram A (Crystal violet) paint for 1 minute, rinse with running water until the colour fades, then fix on a spirit fire. The next step was added one drop of Gram B (Iugol) to the preparation and let it stand for 1 minute. After 1 minute, rinse with Gram C (96% alcohol) until all the dye is gone until crystal violet can no longer be rinsed. Then wash again with running water and fix it on a spirit fire. Next, add one drop of Gram D (Safranin) solution to the preparation and wait for 45 seconds. The final stage is to rinse the preparation under running water and dry the surface. After that, observe the changes in the colour and shape of cells on the microscope.

Motility Test (Sulfide Indole Motility) was conducted using one ose needle of BPN isolates. It was taken and then inoculated by stabbing it on the Sulfate Indole Motility (SIM) medium and then incubated at 37oC for 2 x 24 hours. The catalase test for the BPN isolate was taken as much as one loop using a loop needle then scratched on the glass preparation then dropped the H2O2 reagent. If gas bubbles form, the result is positive, and negative results if no gas bubbles are formed.

Citrate Test (Simmons Citrate Agar) on BPN isolates were taken as much as one loop using a loop needle then scratched on the tilted SCA media. It was incubated for 28-48 hours. If there is a change in the colour of the media from green to blue, it shows a positive reaction, but if it does not change, it shows an adverse reaction. In the last test, the Urea test, BPN isolate was dipped by a loop needle into a test tube containing urea base agar medium and then incubated for 24 hours. If there is a red-orange colour change, this indicates the presence of the urease enzyme, which is owned by the isolate bacteria.

2.4. Statistical analysis in biodiversity indices

Similarity analysis based on microbial at different depth has been generated by PRIMER-e® software v7 [12]. Another data was summaries using Microsoft excel [13].

3. Results and Discussion

3.1. Colony counting

On average, the number of bacterial colonies cultured in the Jensen medium showed a similar pattern. However, at a depth of 15-30 cm, it was able to reach the largest number of colonies (88.3 x 10^6 CFU / gr) found in loamy soils under the oil palm vegetation. Meanwhile, the lowest number of colonies was also at a depth of 15-30 cm in loam soils without oil palm vegetation (Table 1). Thus, the presence of oil palm vegetation and soil types may contribute to the number of bacteria that can be isolated.
Table 1. Average total colony in each peatland type

| No. | Land types                          | ID  | land depth (cm)                  |
|-----|-------------------------------------|-----|----------------------------------|
|     |                                     |     | 0-15                            |
|     |                                     |     | 15-30                           |
|     |                                     |     | 30-50                           |
| 1   | Loose land with Palm Vegetation     | LV  | 79.2 x 10^6 CFU/gr              |
| 2   | Loose land without palm vegetation | LW  | 77.1 x 10^6 CFU/gr              |
| 3   | Clay with Palm Vegetation          | CV  | 67.1 x 10^6 CFU/gr              |
| 4   | Clay with without Palm Vegetation  | CW  | 68.4 x 10^6 CFU/gr              |

| No. | Land types                          | ID  | land depth (cm)                  |
|-----|-------------------------------------|-----|----------------------------------|
|     |                                     |     | 0-15                            |
|     |                                     |     | 15-30                           |
|     |                                     |     | 30-50                           |
| 1   | Loose land with Palm Vegetation     | LV  | 77.1 x 10^6 CFU/gr              |
| 2   | Loose land without palm vegetation | LW  | 76.6 x 10^6 CFU/gr              |
| 3   | Clay with Palm Vegetation          | CV  | 88.3 x 10^6 CFU/gr              |
| 4   | Clay with without Palm Vegetation  | CW  | 70.2 x 10^6 CFU/gr              |

3.2. Biochemistry and morphological characterization

Biochemical testing is one of the basic parameters that can show the characteristics of bacteria (Table 2). Of the 14 isolates in this study, all isolates had the potential to produce enzyme urease with an indication of colour change. Also, most of the 12 isolates had enzyme catalase, while only the H₂S test showed negative properties which meant they were unable to turn the media black.

Table 2. Biochemistry testing for 14 isolates from peatland

| No. | Isolate | Glucose | Lactose | Sucrose | H₂S | Citrate | Catalase | Motility | Urea |
|-----|---------|---------|---------|---------|-----|---------|----------|----------|------|
| 1   | LV11    | +       | -       | -       | -   | +       | +        | +        | +    |
| 2   | LV12    | +       | -       | -       | -   | +       | +        | +        | +    |
| 3   | LV22    | -       | -       | -       | -   | +       | +        | +        | +    |
| 4   | LV23    | +       | +       | -       | -   | -       | -        | -        | +    |
| 5   | LV32    | +       | +       | +       | -   | -       | +        | -        | +    |
| 6   | LW11    | +       | +       | +       | -   | +       | +        | +        | +    |
| 7   | CV11    | +       | +       | +       | -   | +       | +        | +        | +    |
| 8   | CW11    | -       | -       | -       | -   | +       | +        | +        | +    |
| 9   | CW12    | +       | +       | +       | -   | +       | +        | +        | +    |
| 10  | CW13    | +       | +       | +       | -   | -       | -        | +        | +    |
| 11  | CW21    | -       | -       | -       | -   | +       | +        | -        | +    |
| 12  | CW22    | +       | +       | +       | -   | +       | +        | -        | +    |
| 13  | CW31    | +       | +       | +       | -   | +       | +        | -        | +    |
| 14  | CW32    | +       | +       | +       | -   | +       | +        | -        | +    |

In testing the morphological characteristics of cells and colonies, most of them were coccus (12 isolates) and bacilli (2 isolates), meanwhile, in colony morphology, where almost all isolates colony colour was transparent white, except for isolate CW21 on clay media without oil palm vegetation which showed a pink colour (Table 3).

Table 3. Colony and cell morphological from 14 isolates of BPN

| No. | Isolate | Configuration | Margin | Elevation | Colour | Cell Shape | Gram Type |
|-----|---------|---------------|--------|-----------|--------|------------|-----------|
| 1   | LV11    | Round         | Entire | Convex    | White transparent | Coccus | Negative  |
| 2   | LV12    | Round         | Undulate | Flat       | White transparent | Coccus | Positive  |
| 3   | LV22    | Irregular     | Undulate | Flat       | White transparent | Bacilli | Negative  |
| 4   | LV23    | Round         | Entire | Convex    | White transparent | Coccus | Positive  |
| 5   | LV32    | Irregular     | Undulate | Flat       | White transparent | Coccus | Positive  |
| 6   | LW11    | Round         | Entire | Flat       | White transparent | Coccus | Positive  |
| 7   | CV11    | Round         | Undulate | Flat       | White transparent | Coccus | Positive  |
| 8   | CW11    | Round         | Entire | Flat       | White transparent | Coccus | Positive  |
| 9   | CW12    | Irregular     | Undulate | Flat       | White transparent | Bacilli | Negative  |
| 10  | CW13    | Round         | Entire | Convex    | White transparent | Coccus | Positive  |
| 11  | CW21    | Round         | Entire | Convex    | Pink | Coccus | Positive  |
| 12  | CW22    | Round         | Undulate | Flat       | White transparent | Coccus | Positive  |
| 13  | CW31    | Round         | Undulate | Flat       | White transparent | Coccus | Positive  |
| 14  | CW32    | Round         | Undulate | Flat       | White transparent | Coccus | Negative  |
### 3.3 Clustering analysis

The results on the clustering analysis classify an abundance of the number of colonies both peatland depth almost same. However, at a depth of 15-30 cm, it had the highest and most diverse abundance of bacteria. The clustering analysis separated from this from the other two depth groups (Figure 2).

![Figure 2. Clustering analysis based on number colony at different peatland depth.](image)

Deforestation of peatlands for various purposes affects the dynamics of decomposition and emissions of greenhouse gases. In peat that has changed function, there is a change in the quality of the peat substrate which is directly related to the composition and properties of its biotic components and has lower microbial biomass and enzyme activity [14]. Tropical peatlands have an essential role in maintaining the sustainability of various ecosystems around them. However, the area of peat is decreasing due to various human activities. Such as the conversion of peatland for agricultural, plantations and even settlements. There are differences in the community structure of soil bacteria and Archae, which play a role in nitrogen transformation in natural and open peat areas. In general, the two peat areas can be found *Proteobacteria, Actinobacteria, Acidobacteria* and *Firmicutes*, with different numbers [8].

The results of this study indicate that there is a change in the function of oil palm land, showing differences in the number of colonies in each sampling area. The highest number of nitrogen-fixing bacterial colonies was found at depths of 15-30 cm in clay type with oil palm vegetation ranging from $88.3 \times 10^6$ CFU/gram. On peatlands that have been planted with oil palms with a depth of 0-15 cm and 1-30 cm, the results of this study found that the number of nitrogen-fixing bacteria colonies ranged from $79.2 \times 10^6$ CFU/gram and $107.1 \times 10^6$ CFU/gram (Table 1). Both of these locations are areas of the rhizosphere system under oil palm vegetation which provide a lot of root exudate. In the rhizosphere, the bacterial population is more than the population in other parts of the soil, because microbial development is influenced by the metabolic activity of the roots of plants around it. The number of bacteria in the soil varies because bacterial development is highly dependent on soil conditions. In general, the number of bacteria is found on the top layer. The amount that can be found in the soil ranges from 3-4 billion per gram of dry soil and changes with the seasons [15].

The highest microbial population in peatlands is found in the rhizosphere of oil palm plants with a plant age of fewer than six years than in the rhizosphere of older plants [16]. This condition is that the activity of fertilization causes non-polar compounds and low volatility to be absorbed by the roots difficult and more decomposed in the rhizosphere. The results of the decomposition are then used by the microbes present in the root system. The fertilization process carried out in oil palm plantation activities, aeration in
peat areas planted with oil palm also contributes to improving soil nutrition, thereby encouraging microbial growth and activity in changing organic matter [3]. However, on peatlands that have been cleared or have changed the function to agriculture purposes, there is a decrease in microbial activity when compared to unspoiled peat forests [14].

The depth of sampling also affects the total number of colonies; the more profound the number of colonies decreases in number. This decrease was due to increased standing water at the sampling location, causing anaerobic conditions. However, the results of the cluster analysis show that the number of colonies on the surface (0-15 cm) and the depth of 30-50 cm are similar, but at a depth of 15-30 cm, it is estimated that it is the optimal depth with characteristics of the number of colonies that differ from surface and depth (Figure 2). Analysis of the number of populations of soil microorganisms can show the relationship between microorganisms and the root system, the presence of organic compounds and into the soil profile. The number of high soil microbial population is possible to be an indicator of soil fertility, availability of adequate food and energy supplies, water availability, appropriate environmental temperature and other factors required by microbes [15].

In this study, the urease test results showed that all isolates reacted positively (Table 3). These results indicate that the isolates obtained from the results of this study are potential isolates to be developed and applied as biofertilizers [17]. Due to all isolates are not only able to fix nitrogen but also convert nitrogen into ammonia which will be used directly by plants. In biofertilizer activities, the agricultural sector uses a lot [18], but also does not rule out the possibility of fisheries activities that also use biofertilizers in cultivating plankton in the early stages of shrimp farming and natural feed production [19]. The use of biofertilizers and the use of bacteria together are expected to become an environmentally friendly aquaculture management pattern while maintaining a sound ecological system.

4. Conclusion
The initial isolation results obtained 25 nitrogen-fixing bacterial isolates with different colony characteristics. The highest number of bacterial colonies was found at depths of 0-15 cm and 15-30 cm with the condition of peatlands that had been planted with oil palm vegetation. This results occurs in the process of adding elements from fighting activities, namely fertilization and aeration, carried out in the oil palm plantations, which provide much nutrition for the bacteria around the rhizosphere. Based on the results of urease testing on 14 isolates, all positive isolates were able to degrade urea into ammonia, so that it has the potential to be developed into biofertilizers. It is necessary to test the potential of isolates in fixing nitrogen so that it can play an essential role in maintaining nitrogen balance in other ecosystems.

5. References
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