Analysis of peat bacterial diversity in oil palm plantations and a logged forest in Jambi, Indonesia, using PCR-DGGE technique

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Abstract. Jambi Province has around 617,000 hectares of peatlands spread across six districts, including Tanjung Jabung Timur. Reports on microbial diversity under different land cover, especially those using molecular techniques, were very limited. The study aimed to evaluate the impact of peatlands conversion on soil bacterial biodiversity, through the analysis of 16S-rDNA gene employing polymerase chain reaction combined with denaturing gradient gel electrophoresis (PCR-DGGE). Peat soil samples were taken from the topsoil (rhizosphere) of 3 sites, i.e. (A) conventional drained (50 to 70 cm) oil palm plantation, (B) shallow drained (30 to 50 cm) oil palm plantation, and (C) logged-over peat forest. Sequential analysis based on the 16S rRNA gene showed that 15 operational taxonomic units (OTUs) could be grouped into 3 phyla, namely Proteobacteria, Acidobacteria, and Actinobacteria. The alignment result of nucleotide shows that 4 of 15 bands of DGGE were identified as the uncultured bacterium (Sulfurospirillum, Acidobacteria, Rhodoplane, and Magnetospirillum). Overall, the Shannon-Weaver biodiversity index showed that the conversion of peatlands to oil palm plantation increased bacterial biodiversity. This could be attributed to drainage, fertilization, and biomass inputs under the oil palm plantations. The higher biodiversity under oil palm plantations also implies a higher rate of peat decomposition under this land use, relative to the forest.

1. Introduction

Indonesia has the most extensive peatlands among tropical countries, which around 13.4 million hectares is distributed mainly on the islands of Sumatra, Kalimantan, and Papua [1]. Jambi in Sumatra has about 617,000 hectares of peatlands spread across six districts, including Tanjung Jabung Timur which has around 300,000 ha of peatlands [2]. Conversion of peat forests into oil palm plantations is believed to influence the environment and ecosystem and affect below ground biodiversity including microbial diversity and their activities [3]. Most of the peatlands in this district has been converted to oil palm plantations, however, reports on their impact on microbial biodiversity, especially those employing molecular techniques, were very limited.

The microbial diversity of an ecosystem is an indicator of soil fertility [4]. However, this could also be an indicator of the rate of peat decomposition. Information on microbial diversity can provide an understanding on how the peatland environment affects microbes and vice versa. Furthermore, this information can be used as a basis for developing an improved management system of the converted land in the future.
Relative to fungi, bacteria are the most abundant group of soil microorganisms and are the first organisms to colonize dead wood and metabolize the easily accessible substrates [5]. Bacteria may also produce metabolites that functions as growth factors for fungi [6]. Some bacteria may equally compete for growth substrates with fungi and could be more active in aerobic decomposition than fungi in peatland [7].

Analysis of bacterial diversity can be done using culture and non-culture approaches [8]. Culturable bacteria on growth media are only a small part of the diversity of bacteria in nature. The non-culture approach using molecular biology methods provides an opportunity to analyze the diversity through the bacterial genome which is obtained directly from nature without going through the culture stage [8]. Biodiversity analysis in the same location using a culture approach was carried out in previous studies [9]. However, this analysis could not show the overall diversity of bacteria in an environment. Culture-based methods to evaluated microbial diversity are usually unsuccessful because only 0.1-1% to 1-10% of microbial species can grow in vitro and different microbiological and molecular strategies have been considered to improve microbial cultivation [10]. In the last years, culture-independent techniques had become the main tools for the evaluation of microbial communities. Amplicon-based metagenome sequencing are commonly performed by analyzing the prokaryotic 16S ribosomal RNA gene. Between traditional and high accurate techniques for microbial diversity assessment, Denaturing Gradient Gel Electrophoresis, (DGGE) is an inexpensive fingerprinting technique and could be employed for several applications, including analysis of complex communities, monitoring of population shifts, and sequence heterogeneities. Through DGGE it is possible to obtain taxonomic information because bands can be excised, re-amplified and sequenced [11].

This study aimed to evaluate the impact of peatlands conversion in Tanjung Jabung Timur, Jambi Province, on soil bacterial biodiversity through the analysis of the 16S-rDNA gene employing PCR-DGGE technique.

2. Materials and methods

2.1. The Study Sites

Soil samples were collected from peat areas in Mendahara Ulu Sub-District, Tanjung Jabung Timur, Jambi Province, Indonesia (figure 1). Peat soil samples were taken from the topsoil or rhizosphere (0-20 cm) of two transects in 3 sites (table 1), i.e. (A) Conventionally drained (50-70 cm) oil palm plantation, (B) Shallow drained (30-50 cm) oil palm plantation, and (C) Logged-over peat forest. The transects were about 40 m apart, while the sampling points were 10, 25, 40, 65 and 80 m, respectively, from the drainage channel. Individual soil samples were homogenized, composited, and treated as a single sample, to gain a representative estimate. Soil samples were transferred in plastic bags and kept in a larger plastic bag in an icebox to maintain the temperature around 2 to 4°C.

2.2. DNA Extraction

DNA was extracted from peat soil samples (0.25 g) using the Power Soil DNA Isolation Kit (MoBio, CA, USA) following the manufacturer’s protocol. The amount and quality of the DNA were quantified using Nanophotometer TM P360 (Implen GmbH, Schatzbogen, Germany). The minimum concentration of DNA for optimal amplification was established at 10 ng μl⁻¹ with OD₂₆₀/₂₈₀ purity of 1.7-1.8. Extracted genomic DNA was stored at -20°C until further analysis.

2.3. Polymerase Chain Reaction (PCR)-Denaturing Gradient Gel Electrophoresis (DGGE) Analysis

The microbial DNA was amplified using universal bacterial 16S rDNA primers: (i) 341-F (5’-CCTAGCGG-GAG-GCA-GCA-G-3’), (ii) 31F-GC (5’-CGC-CCG-GCG-CGC-GGG-GGG-GCC-GCC-GGG-GGG-GCA-GGG-GG-GG-GCA-GGG-G-3’), (iii) 534-R (ATT-ACC-GCG-GCT-GCT-GCT-GCT-G-3’) [12].
### Table 1. Location of sampling points studied in Tanjung Jabung Timur, Jambi, Indonesia

| Sites | Coordinates | Description *) |
|-------|-------------|----------------|
| A     | 103°39.341’ E - 01°13.508’ S | Peatlands under oil palm stands aged 10 years, located in Pandan Lagan Village. At depths >1.0 m in this peat soil there is a hard layer that resembles young coal. This peat has a thickness of > 3.0 m. |
| B     | 103°35.377’ E - 01°14.301’ S | Peatlands under oil palm stands 8 years old, located in Pematang Rahim Village. Peat thickness ranges from 1.5 m to 2.0 m. |
| C     | 103°35.380’ E - 01°14.180’ S | Logged-over peat forest that has become protected forest in Pematang Rahim Village. Peat thickness of about 1.5 m and does not function so that in the rainy season the land is often flooded. |

**Figure 1. Location map of study sites**

The PCR was performed using the following steps: pre-denaturation 94°C for 5 min, followed by 35 cycles of denaturation (at 95°C for 30 sec), annealing (at 58°C for 30 sec), elongation (at 72°C for 30 sec), and a final extension at 72°C for 7 min. PCR products were migrated to agarose 1.5% for 45 min. at 80 V. Visualization of DNA was carried out using G: BOX Gel Documentation (Syngene, Frederick, USA) with staining ethidium bromide (EtBr). The PCR products were subsequently subjected to DGGE analysis.

PCR products were separated on a vertical gel containing 8% (w/v) polyacrylamide (acylamide:bisacrylamide ratio of 37.5:1) with a denaturing gradient of 45% to 65% at the bottom of the gel. 30 μl PCR products with 10 μl loading dye were pipetted into the individual lanes and DGGE was performed at 60°C and 130V with 1X TAE buffer for 6 h. Microbial DNA was excised and purified from DGGE and then re-amplified using 16S rDNA primers, 341F (without GC-clamp), and 534R [12]. The polyacrylamide gel was then stained with EtBr 0.1% dye for 15 min. Visualization was carried out using G: BOX Gel Documentation (Syngene, Frederick, USA). The gel documentation results were then analyzed by LabImage software (Kapelan, Germany). The software is used to estimate the value of the normalization of ribbon volume data that appears. This value shows the proportion of the ribbon (individual) in a community (pi). The pi value is used to calculate the Shannon Weaver diversity index (H’ = −Σpi (ln pi)) and determine the diversity of the microorganism community in a sample [13]. Analysis of clustering is done using Past3 (Norway) software. The
ribbon that appears on the gel is converted to binary data (no bands in the same position). The dendrogram construction was carried out using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm method and the Dice similarity index [14].

2.4. Band Matching Analysis of PCR-DGGE Profiles

The analysis of PCR-DGGE patterns was performed using Phoretix 1D Gel Analysis software (Total Lab Quant Ltd, United Kingdom). Dice similarity coefficient (Dsc) was used to compare the whole image profiles. The unweighted pair group with mathematical averages (UPGMA) at 1% position tolerance was performed to generate the dendrogram tree [14].

2.5. DNA Elution and Sequencing

DNA bands that appear were cut and inserted into a microtube containing 100 µL of sterile Nuclease Free Water. Microtube is then stored at -20°C overnight. One microliter of excision DNA was then re-amplified using the primary pair P341F / 534R (16S rDNA) without GC clamps. The PCR condition used was the same as the previous PCR condition. The DNA that was successfully amplified was then sent to the laboratory for the sequencing process; to read the DNA sequence. Sequencing results were then analyzed using ChromasPro (Technelysium, AU) software for assembly and trimming purposes. Sequence similarity searches were conducted using the nucleotide-nucleotide basic logic alignment search tool (BLASTn) of the National Centre for Biotechnology Information (NCBI) GenBank database to identify the nearest relatives of the partially sequenced 16S rDNA genes of excised bands.

2.6. Statistic for Biodiversity Index

The nucleotide sequences were aligned and the Neighbor-Joining Tree was generated using MEGA version 6.0 (Molecular Evolutionary Genetics Analysis [http://www.megasoftware.net]). The Neighbor-Joining phylogenetic tree was reconstructed based on the position of the 16S rRNA gene by using the Kimura two-parameter substitution model evaluated by 1,000 bootstrap resampling of the data, and nodes with bootstrap values were indicated.

3. Results and discussion

3.1. Diversity and Pattern of Bacterial Communities

The DGGE profile showed that there were 15 bands or Operational Taxonomic Units (OTU) at different positions (figures 2 and 4a). The sample from site A was detected to have the most bands or most diverse bacterial, followed by site B and site C. This was confirmed by the results of Shannon-Weaver's prokaryotic biodiversity (table 2).

Shannon-Weaver's prokaryotic biodiversity index showed that the sampling site A had the most diverse bacterial species, calculated at 2.625 followed by B 2.565 and C 2.353.

| Sites                        | Shannon Diversity Index (H') |
|------------------------------|------------------------------|
| A Conventionally drained oil palm | 2.625 ± 0.064                |
| B Shallow drained oil palm       | 2.565 ± 0.012                |
| C Logged-over forest            | 2.353 ± 0.017                |
In general, the conventionally drained oil palm plantation (site A) had the most bands, compared to shallow drained oil palm plantation (site B) and logged-over peat forest (site C). This is shown by the results of the clustering analysis using binary data which shows that the bacterial community pattern of each sample is grouped based on the origin of the peatland obtained (figure 3). The bacterial community at site A is similar to the bacterial community at site B. In terms of bacterial diversity, it can be seen that the peatland at site A has a higher bacterial diversity. This is indicated by the highest Shannon diversity index \( H' \) on peatlands at site A.

**Figure 2.** DGGE profiles showing bacterial communities of samples; each excised, cloned, and the sequenced band is numbered; (A) Conventionally drained oil palm plantation, (B) Shallow drained oil palm plantation, and (C) Logged-over peat forest.

**Figure 3.** UPGMA Dendrogram Peatland Bacterial Community from DGGE profile based on the 16S rRNA gene. (A) Conventionally drained oil palm plantation, (B) Shallow drained oil palm plantation, and (C) Logged-over peat forest.
3.2. Distribution and Composition of Bacterial Communities

Figure 4a shows the percentage of the abundance of bacteria in the three sites, and figure 4b shows the bacterial phyla percentage of the three sampled sites. The bacterial phyla chart (figure 4b) shows the percentage of bacterial phyla at each site, with the following composition: Alpha-proteobacteria (60.7%, 64.8%, 67.6%), followed by Acidobacteria (19.5%, 14.7%, 13.4%), Actinobacteria (11.4%, 10.8%, 7.3%) and Epsilon-proteobacteria (8.4%, 9.6%, and 11.7%), respectively for sites A, B and C.

The results of sequence analysis based on the 16S rRNA gene showed 15 OTUs were affiliated to Proteobacteria, Acidobacteria dan Actinobacteria (table 3 and figure 5).

Table 3. Sequence analysis of DGGE bands of the Operational Taxonomic Unit (OTU)

| OTU | Database Genebank Species                  | Similarity Ratio | Access     | Phylum                        |
|-----|-------------------------------------------|------------------|------------|-------------------------------|
| 1   | Uncultured *Sulfurospirillum* sp. 077      | 162/168 (96%)    | GU 556293.1| Epsilon-proteobacteria        |
| 2   | *Acidobacterium ailaau* PMMR2             | 166/168 (99%)    | NR 153719.1| Acidobacteria                 |
| 3   | Uncultured *Acidobacteria* D.an-89        | 167/168 (99%)    | JX505156.1 | Acidobacteria                 |
| 4   | *Aquiducibacter paucihalophilus* TH1-2    | 167/170 (98%)    | NR 156862.1| Alphaproteobacteria           |
| 5   | *Occallatibacter savannae* A2-1c          | 166/168 (99%)    | NR 147737.1| Acidobacteria                 |
| 6   | *Aliidongia dinghuensis* 7M-Z19           | 167/168 (99%)    | NR 156087.1| Alphaproteobacteria           |
| 7   | *Microtetraspora niveoalba* Mt-3           | 164/169 (97%)    | NR 025998.1| Actinobacteria                |
| 8   | *Corynebacterium guangdongense* S01       | 171/175 (98%)    | NR 151871.1| Actinobacteria                |
| 9   | *Rhodovibrio sodomensis* DSI              | 166/168 (99%)    | NR 104763.1| Alphaproteobacteria           |
| 10  | *Microvirga subterranea* Fail4            | 167/168 (99%)    | NR 114297.1| Alphaproteobacteria           |
| 11  | *Microvirga ossetica* V5/3M               | 166/168 (99%)    | NR 156049.1| Alphaproteobacteria           |
| 12  | Uncultured *Rhodoplanes* sp. U000106899   | 168/168 (100%)   | FJ 037028.1| Alphaproteobacteria           |
| 13  | *Amorphus coralli* RS.Sph.026             | 167/168 (99%)    | NR 043544.1| Alphaproteobacteria           |
| 14  | *Desertibacter xinjiangensis* M71        | 165/168 (98%)    | NR 134017.1| Alphaproteobacteria           |
| 15  | Uncultured *Magnetospirillum* sp. L429    | 168/169 (99%)    | JX 295208.1| Alphaproteobacteria           |
Figure 5. Phylogenetic Trees Using the Neighbor-joining Method with Bootstrap 1,000 Repetitions.

16S rRNA gene-based dendrogram showing the phylogenetic relationship of 15 peat clones (boldface). Bootstrap values (1,000 data resamplings) of >50% are shown. The root was determined by using the 16S rRNA gene sequence of *Bacillus cereus* ATCC14579 NR 074540.1 as an outgroup. The scale bar represents 0.2 substitutions per nucleotide position.

Most of OTUs belonging to the Proteobacteria and can be separated into two sub-phyla. 9 OTUs belonging to the Alpha-proteobacteria group and 1 OTU belonging to the Epsilon-proteobacteria group. Alpha-proteobacteria were the most dominant group in all samples (60.7-67.6%), followed by Acidobacteria (13.4-19.5%), Actinobacteria (7.3-11.4%), and Epsilon-proteobacteria (8.4-11.7%)
(figure 4b). Meanwhile, in the genus level analysis, no dominant genus was found in all samples (figure 4a).

Base on the abundance, the Acidobacteria is associated with acidic environment [15]. Acidobacteria is one of the globally distributed and highly diverse phyla of the bacteria domain. These microorganisms live in pH 3.5 to 5.0, inhabit a wide variety of terrestrial and aquatic habitats and are particularly abundant in acidic soils, peatlands and mineral iron-rich environments Acidobacteria played an important role in plant polysaccharides degradation in deep drained oil palm plantation [15]. While Actinobacteria involved in activities related to plant physiology such as the degradation of macromolecules including cellulose, lignin, chitin, and starch by producing extracellular enzymes [16].

Furthermore, 3 OTUs are grouped into the Acidobacteria and two OTUs are Actinobacteria. Four of the 15 bands of DGGE related to the uncultured bacterium (Rhodoplane, Sulfurospirillum, Magnetospirillum, and Acidobacteria) with high (> 96%) similarity. There are several reasons why bacteria cannot be cultured using standard methods. Some bacteria are low in abundance and grow slowly, so they may be missed during standard microbiological cultivation [17]. Others are fastidious and have specific growth requirements that must be strictly followed. Bacterial growth may also be inhibited by microbial competition and compounds produced by other bacteria [17].

Magnetospirillum was a Gram-negative, magnetotactic, facultative anaerobic bacterium [18]. Respiratory nitrate reduction allows the oxidation of a substrate under anaerobic conditions. The bacterium is usually found near the oxic-anoxic transition zone, which is usually located at the sediment-water interface in freshwater environments or displaced upward into the water column in marine semi-anoxic environments [18].

Rhodoplane is a phototrophic purple nonsulfur bacteria that was taken from the main purification stage of a sewage treatment plant [19]. While, Sulfurospirillum spp. is anaerobic, free-living, metabolically versatile Epsilon-proteobacteria, many of which are known for their ability to respire toxic or environmentally harmful compounds. Several Sulfurospirillum spp. were found in contaminated sediments, wastewater plants, marine environments, or on biocathodes. The role of Sulfurospirillum in such environments is unclear [20].

Site A is a conventionally drained (50-70 cm) oil palm plantation, while site B is a shallow drained oil palm plantation (30-50 cm), and site C is an over-lodged forest affected by drainage, but frequently flooded during the wet season. According to Könönen et al. [21] the intensive land management (including deforestation and draining) leads to the surface peat becoming a more deficient biological environment. But our research showed the opposite results. Calculation of the Shannon diversity index (H’) observed that the highest bacterial diversity was at site A, followed by site B and site C.

The research about the microbial diversity in the peatland forest in Central Kalimantan also showed that the disturbed peat soil had tremendous bacterial diversity [21]. A previous study conducted in the same location also observed the highest population of culturable bacteria in drained oil palm plantation [9].

Enhanced draining and removal of the original vegetation cover and draining also upset the nutrient cycle between litter and live vegetation and may exacerbate the loss of nutrients. This impoverishment of nutrient-poor peat increases the need for fertilization for productive cultivation [23]. Management of oil palm plantations in site A and site B was carried out relatively intensively for 15 to 16 years by applying fertilizer continuously.

Based on the DGGE profile in this study, it could be observed that the conversion of peat forest to oil palm plantations affects the soil bacteria community. The pattern of bacterial communities in oil palm plantations at site A (conventional drained oil palm) shows a similarity to site B (shallow drained oil palm) due to similar management systems. However, the peat soil in site A has a higher bacterial diversity, and perhaps this is due to the difference in the drainage depth. The drainage for agriculture led to an aerobic condition, or the peat to become oxidized. It increased aerobic or heterotrophic microbes, including microbes that decompose organic matter. According to the opinion of Talukder and Sun [22], which explains that the number and soil microbe diversity was affected by the existence
of a soil management system. We estimated that drainage and fertilization in drained peatlands under oil palm plantations in Jambi encouraged bacterial activities and, hence, biodiversity, implying a high peat decomposition rate under this land use. It seems that drainage and fertilization, and organic matter inputs under oil palm plantations encouraged bacterial biodiversity. The high biodiversity also implies a high rate of peat decomposition under this land use.

4. Conclusions

Sequence analysis based on the 16S rRNA gene showed that 15 OTUs could be grouped into 3 phyla, i.e. Proteobacteria, Acidobacteria, and Actinobacteria. The alignment result of nucleotide shows that 4 of 15 bands of DGGE were identified as the uncultured bacterium (Sulfurospirillum, Acidobacteria, Rhodoplanes, and Magnetospirillum). Overall, the Shannon-Weaver biodiversity index showed that the conversion of peatlands to oil palm plantation increased bacterial biodiversity. It seems that drainage, fertilization, and organic matter inputs under oil palm plantations encouraged bacterial biodiversity. The high biodiversity also implies a high rate of peat decomposition under this land use.

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