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Bacterial dynamic profile in rhizosphere of *Acacia mearnsii* during early time transplantation

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Abstract. Land degradation is one of the major environmental problems in the agricultural sector. On the other hand, the increasing human population needs more and more agricultural products. For that reason, land reclamation using a plant-based approach showing fast-growing capability should be considered as one of the strategies for solving that problematic issue. *Acacia mearnsii* is one of the fast-growing plants and has high adaptability to some environmental stresses such as drought and fire. Furthermore, the species has shown its ability to increase soil nutrition indicated by its symbiotic capability with some nitrogen-fixing bacteria. In order to exploit and maximize such character, understanding bacterial population dynamics in the rhizosphere zone should ease the use of the species for phytoremediation purposes. A bacterial dynamic profile in the rhizosphere area was identified using the amplicon metagenome sequencing platform. The sequencing result shows *Acacia mearnsii* has an impact on the dynamic profile of the bacterial population in the rhizosphere, especially nitrogen-fixing bacteria.

Keywords: metagenome, 16S rRNA, *Acacia mearnsii*, phytoremediation, land degradation

1. Introduction

Land degradation that occurs today can threaten human life. On the other hand, the population of humans in the world continues to increase every year. This increase in the human population will need a lot of agricultural products ranging from clothing to food. Agricultural land degradation causes a decrease in agricultural products so it must be handled to ensure the needs of agricultural products can be fulfilled. It is important for land reclamation efforts to be carried out so that critically degraded lands can be reused especially for the agricultural sector. The revegetation method is one of the ways to fix degraded lands, revegetation uses certain plants that have the ability to grow fast and have high adaptability in an unsupportive environment or infertile land. With its ability, the revegetation process will easily increase land fertility. One of the plant species that can be used for such purpose is *Acacia mearnsii* [1].



A. mearnsii is a woody tree plant belonging to the family *Fabaceae*. Fabacean mostly is known as a pioneer species that grows on marginal land and can increase soil nutrition [2]. The beneficial activity is caused by its ability to form symbiotic development with nitrogen-fixing bacteria at its rhizospheric area [3]. Such symbiotic relationship plays an important role in repairing nitrogen content in soil and could produce plant growth-promoting substances [4].

A. mearnsii's ability in a symbiotic relationship with bacteria can be studied by observing the diversity of rhizosphere bacteria. A conventional method such as the isolation of bacteria by culture can only identify the fraction of microorganisms from the environmental sample so there are many information and biological prospects which potentially remain unexplainable from the diversity of microorganisms that cannot be cultured [5]. This makes the study of microorganism diversity using nonculture methods important to be carried out. The metagenomic technique is a nonculture method that helps study microorganism diversity easily and provides high throughput data so that more comprehensive information about microorganism diversity and its population dynamic can be accessed [6]. The technique is based on the direct amplicon analysis of a specific DNA region target from the bacterial genome. The amplicon DNA sequence, for instance, the 16S rRNA region of various bacteria is provided by doing direct sequencing without any culturing of the bacterial cell prior to its subsequent sequencing step. This makes amplicon sequencing needs lower costs and resources compared to shotgun metagenomic sequencing [7] [8].

Metagenomic data of the 16S rRNA gene accessed from the rhizosphere of *A. mearnsii* is expected to give more insight into understanding its potential contribution to improving soil nutritional status. In the end, this species can be used for land reclamation so critically degraded lands can be remediated in the future.

2. Materials and Methods

2.1. Land preparation

The research was done at Alahan Panjang, Solok, West Sumatra. The land used for research is fallow land which had not been used for any crop cultivation. Two plots were prepared for each plot which has 4 x 4 m in size. The first plot was prepared for bulked soil sampling (control, no treatment) and the second plot was prepared for *A. mearnsii* treatment. The soil was not tilled and only mowed using a mowing machine.

2.2. Seedling preparation

A. mearnsii seedlings are collected from the growing tree around the area of research. Seedlings with 15 cm in height were collected and planted on polybags for 1 month and maintained until they reached 20 cm in height.

2.3. *A. mearnsii* planting

A. mearnsii seedlings that had 20 cm in height were chosen for planting at the research plot. The plants were grown in a hole with a dimension of 30 x 30 cm in length and width and 20 cm in depth. A total of 25 *A. mearnsii* seedlings were planted in the plot.

2.4. Soil sample collection

Soil rhizosphere sampling was done at 3 points of growth. The first sampling was collected before plantation, while the second and third sampling was performed at 45 and 90 days after seedling transplantation (DAST). The rhizosphere soil sample was taken from the region around 15 cm in distance and 30 cm in depth from the root zone. The sample was carefully detached from the root and collected in a plastic container. The rhizosphere soils from 3 samples were bulked and gently mixed using gloved hands. In order to avoid DNA contamination from non-bacterial organisms the debris was separated, and the 50-gram soil sample was then carefully stored in a sterile falcon tube stored at -80°C until metagenome analysis. Similar handling was done for the bulked soil.

2.5. Soil DNA extraction

The collected rhizosphere soil sample and the bulked soil were processed for metagenome analysis. The bacterial DNA was extracted using ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). Amplification of the V3-V4 DNA region of 16S rRNA was performed using primer combination 341F 5'CCTAYGGGRBGCASCAG3' and 806R 5'GGACTACNNGGGTATCTAAT3'. The amplicon product was sent for sequencing using Illumina NovaSeq 6000 at NovoGene, China.

2.6. Data Analysis

Assignment of the sequences based on their unique barcode, then the barcode and primer sequences were truncated. The paired-end sequences were then merged using FLASH (V1.2.7.4 <http://ccb.jhu.edu/software/FLASH>). In order to obtain high-quality clean tags. The chimeric sequences were filtered according to QIIME (V1.7.0, http://qiime.org/scripts/split_libraries_fastq.html) quality-controlled process [9]. UCHIME algorithm (UCHIME Algorithm http://www.drive5.com/usearch/manual/uchime_algo.html) was used to compare the tags with the GOLD database to detect and remove chimera sequences [10]. Then the effective tags were finally obtained.

UPARSE software (UPARSE v7.0.1001 <http://drive5.com/uparse>) [11] was used for sequence analysis using all the effective tags. The same OTUs were assigned from the sequences with $\geq 97\%$ similarity. Species annotation at different taxonomic levels was performed using MOTHUR software against the SSUrRNA database of SILVA Database (<http://www.arb-silva.de>) (Threshold:0.8~1) [12].

3. Results and Discussion

A total of 858,435 reads and 646,177 high-quality reads were obtained from 5 soil samples which resulted in 12,864 OTUs. The prokaryotic OTUs in the rhizosphere were also present in bulked soil but there is some difference in the relative abundance of the OTUs between bulked soil and rhizosphere soil.

3.1. Bacterial dynamic profile

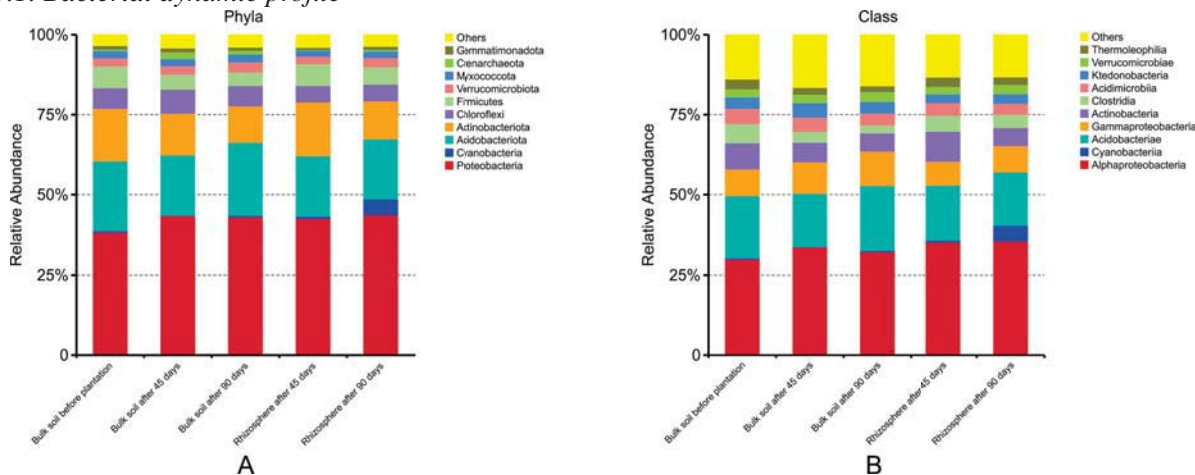


Figure 1. Dynamic of bacteria population in bulked and rhizosphere soil samples grouped on the different taxonomic levels. Phyla (A) and Class level (B).

The bacterial dynamic profile shows the top 10 different types of bacteria from different taxonomic levels, starting from phyla, class, order, family, and genus level. Figure 1A and 1B show a bacterial dynamic profile in bulked and rhizosphere soil samples. At the phyla level, the relative abundance of *Proteobacteria* is highest from each sample and increases after periods of time both at the bulked and rhizosphere soil. On average *Proteobacteria* tend to be higher in rhizosphere soil than in bulked soil.

Other bacterial phyla like *Actinobacteriota* and *Firmicutes* is also higher at the rhizosphere than bulked soil although not differ significantly. Other bacterial phyla like *Cyanobacteria* is significantly higher in rhizosphere soil than bulked soil and increases after periods of time increasing from 0.7% at 45 DAST to 5% at 90 DAST after *Acacia* transplantation. The *Acidobacteriota*, *Chloroflexi*, *Verrucomicrobiota*, *Myxococcota*, *Crenarcheota*, and *Gemmatimonadota* were also present in both the rhizosphere and bulk soil samples.

Based on class grouping, the class of *Alphaproteobacteria* has the highest abundance compared to all samples. The class *Alphaproteobacteria* has a higher relative abundance at the rhizosphere soil than bulked soil and the abundance is increasing after periods of time both at bulked and rhizosphere soil. The bacterial population of class *Cyanobacteria* has increased during 45 DAST interval ie. A significant increase in bacterial population occurred after 90 DAST of transplantation, while the other classes do not differ significantly.

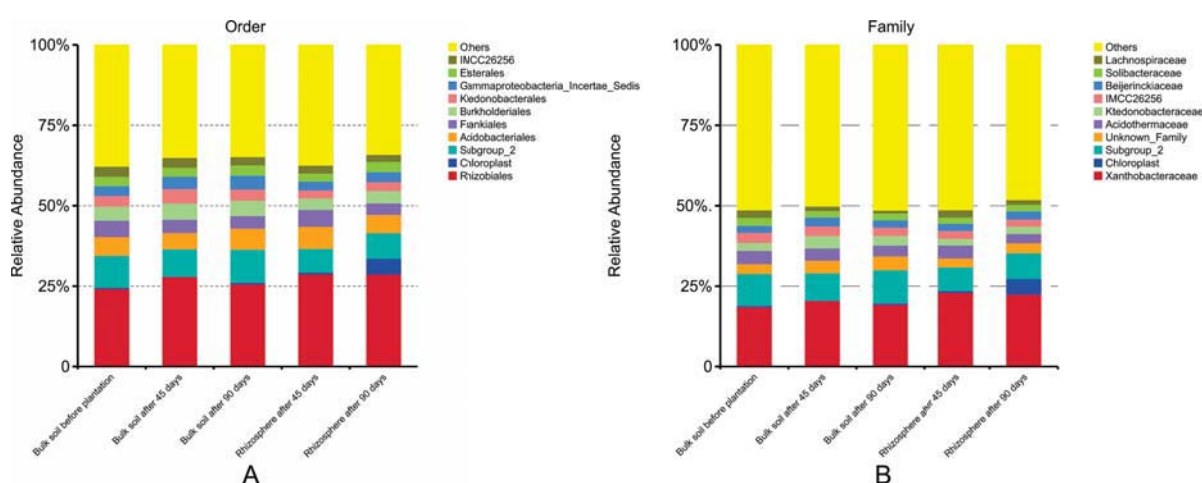


Figure 2. Dynamic of bacteria population in *bulked* and rhizosphere soil samples grouped on order (A) and family level (B).

Figure 2A and 2B show the bacterial relative abundance at the order level and family level, at the order level the relative abundance of *Rhizobiales* is the highest order compared to all orders in both samples (bulked and rhizosphere soil). The relative abundance of *Rhizobiales* is a bit higher in rhizosphere soil and increases after 90 days after seedling transplantation (DAST). Interestingly, in both taxonomic levels (order and family) we found the relative abundance data from the "chloroplast" in both ordo and family. It is assumed that plant debris is still existing in the sample, meaning that the cleaning procedure before the metagenome sequencing step is still not successful. The relative abundance of *Xanthobacteraceae* is higher in the rhizosphere soil than in bulked soil. The differentiation occurred since *Acacia* seedling transplantation (45 DAST) but was almost constant in 90 DAST. This data shows that *Acacia* transplantation probably contributes to the increase of *Xanthobacteraceae* in the rhizosphere. The family of *Xanthobacteraceae* is known as gram-negative, rod-shaped, sometimes twisted chemoorganotrophic or facultative chemolithoautotrophic bacteria. They can be motile but this family also involves non-motile bacteria. Some genera of this family are known to be able to fix N_2 under decreased O_2 pressure [8].

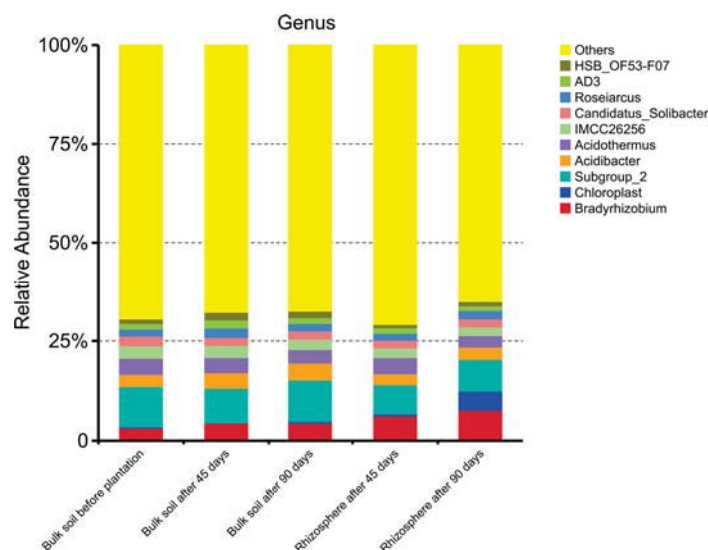


Figure 3. Dynamic of bacteria population in *bulk*ed and rhizosphere soil samples grouped on genus level

Figure 3 shows genus *Bradyrhizobium* shows an obvious population dynamic change over time. This happens especially in the rhizosphere zone where *Acacia mearnsii* roots are growing. A significant increase in the relative abundance of the genus *Bradyrhizobium* can be seen in 90 DAST compared with the situation in the initial state where *A. mearnsii* was still did not yet exist as well as in 45 DAST. In the control condition (bulk^{ed} soil) increasing in the relative abundance of the genus *Bradyrhizobium* happened only during the first phase (0-45 days) and was relatively constant after 45 DAST. Data from figure 3 shows interesting insight in terms of the power of the V3-V4 region of the 16S-rRNA gene as a discriminator in the bacterial metagenome analysis. The 16S rRNA gene has been complained to have low phylogenetic power at the species level and poor discriminatory power for some genera [9]. Data from this research shows that in fact only about 30% of the sequence population can be successfully grouped into a specific genus level, while the rest of the 70% are still grouped as others. However, this could also be caused by the limitation of V3-V4 sequence information available in the database. A complete big database supporting such needs should be able to overcome that drawback [13].

4. Conclusion

The *Acacia mearnsii* has an impact on the dynamic profile of the bacterial population in the rhizosphere at the different taxonomic levels. The bacterial community associated with early-time transplantation of *A. mearnsii* rhizosphere is nitrogen-fixing bacteria.

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