CRC 990
Ecological and Socioeconomic Functions of Tropical Lowland Rainforest Transformation Systems
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ABS

Isolation and Amplification of 16S rRNA Gene for Metagenomic Analysis from Oil Palm Rhizosphere in Different Locations (Based on Soil Textures)

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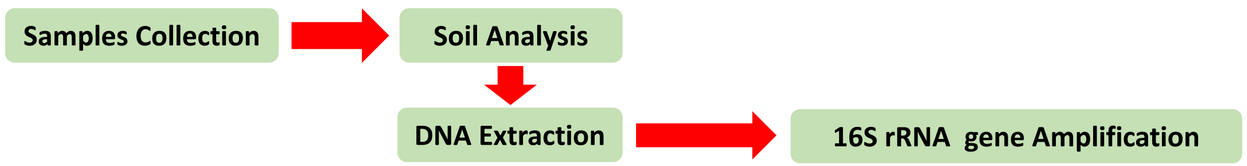
Introduction

Soil microbes, especially bacteria, play key roles in ecosystems and influence a large number of important ecosystem processes, such as nutrient acquisition cycling, driving plant productivity and soil formation, biocontrol of some diseases, and fertilizer. Rhizosphere (root zone) is an important habitat for bacteria to play their function as plant growth promoter and plant protector from pathogen infection. Large area of oil palm plantation in Jambi with a wide variety of soil textures will be a good habitat for soil bacteria. There is an assumption that differrent soil texture lead to different diversity of soil bacteria in oil palm root zone. This study aimed at investigating the diversity of soil bacteria in oil palm rhizosphere at different soil textures.

Materials and Methods

- This study was conducted in PTPN6 oil palm plantation in Batanghari Regency, Jambi Province; followed by a laboratory work at the Faculty of Science and Technology UNJA.
- Soil samples was collected from 26 different locations at 5 - 10 cm depth from top soil, then stored at -20 ^{o}C until further use.
- DNA extraction from soil samples using powersoil DNA isolation kit; the results were observed under Spectrophotometer followed by PCR procedure. Bacterial 16S rRNA genes were amplified using forward primer 343F and reverse primer 909 R.



Results and Discussion

Soil Textures

Eight class of soil textures were found in this study: sandy loam, sandy clay, loamy sandy, silty clay loam, silty loam, clay, sandy, and sandy clay loam (Figure 1), with loamy sandy and silty clay were dominant. Some of genomic DNA purity obtained in electrophoresis visualization were about 1,2 - 1,7 and more than 3000 bp in size. However, the 16S rRNA genes were not yet obtained.

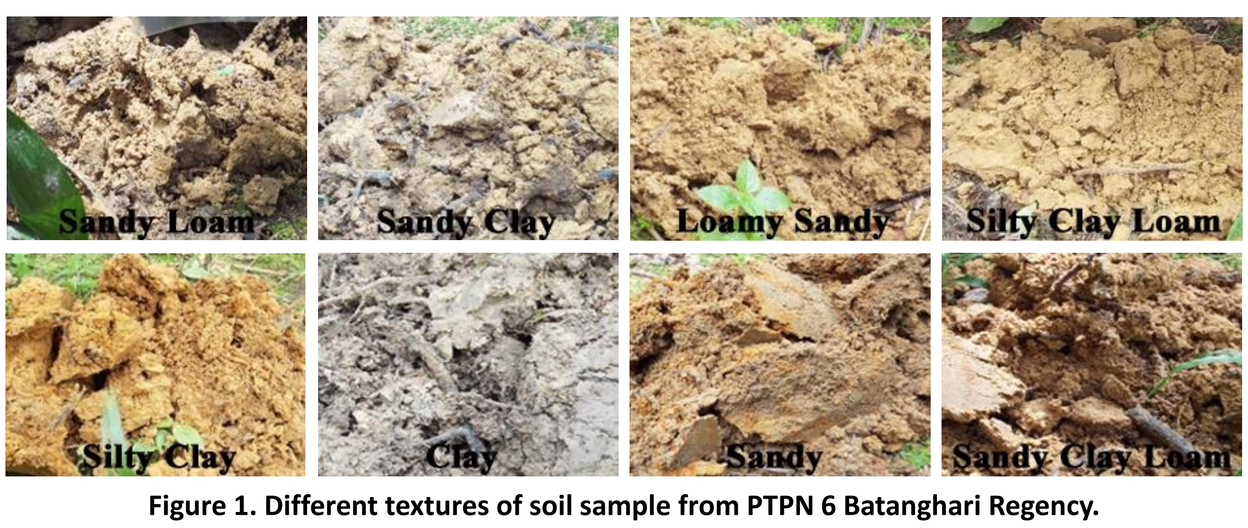


Figure 1. Different textures of soil sample from PTPN 6 Batanghari Regency.

Texture is as a factor keeping oxygen and humidity of soil, and therefore, creating a microenvironment for microbes and called micro-colony which have a specific characteristic. Micro-colony existence will create the cycles of substances such as carbon, nitrogen, phosphorus, etc. in nature. This condition gives benefits for plant such as providing vitamins, amino acids, auxins, and gibberellins in order to boost plant growth, and providing antibiotics to prevent pathogenic microbe attacks.

The sandy-dominant texture attracts more water and organic compounds and facilitates release of nutrition for plant, resulting in root growth and penetration in to soil easily. While clay-dominant texture will make sticky soil when wet and harden when dry, causing root penetration is difficult. The presence of microbes could make better condition. Our investigation revealed that silty clay loam, loamy sandy and sandy loam textures is high in bacterial content, and is expected to create good environment for root system as previously reported by Hamarashid *et al*. (2010).

Soil temperatures were 26^{o}C - 31^{o}C, pH were 6 - 7 and R_H were 5% - 75%. The soil temperature could affect soil volume, gases diffusion, water viscosity, surface tension, solubility of substances, and cellular reaction. Optimum temperature is needed for stability in bacterial respiration, enzymatic reaction, membrane permeability, and secondary metabolite production; and some rhizosphere bacteria will die at ≥ 40^{o}C (Lynch 1983). Different soil pH could determine diversity of bacteria (Zhalnina et al. 2014), but in our study the pH was neutral due to lime application, and show no effect on bacterial diversity in PTPN6 oil palm plantation. The loamy sandy soil had lowest R_H than others. Soil R_H was affected by rainfall and climate, and a wide range of soil R_H was presumably contributes to bacterial diversity in soil.

DNA genomes Qualities and Quantities

The purity of genomic DNA were 1,2 - 1,7 (Table 1) and denoted low DNA quality except of bacterial DNA from sandy clay loam. The purity below 1,8 indicated high protein contaminant or affected by pyrimidine and purine bases conjugation bond. The Best DNA purity should be between 1,8 - 2,0 indicating no protein, phenol or RNA contamination (Sambrook and Russell 2001). To confirm the spectrophotometer result, DNA genomes were measured by electrophoresis gel agarose, but it shown rather different pattern. It displays visualization of bacterial DNA from silty clay loam, loamy sandy and sandy loam, but did not from others. The molecular weight of genomes was larger than 3000 bp. While DNA genomes quantity based on measurement with spectrophotometer was 59,19 µg mL^{-1} in average.

Table 1. DNA (purity) ratio in λ260nm and λ280nm wavelength of bacterial genomic DNA

No.	DNA Sample from different soil textures	Ratio λ260nm/ λ280nm
1.	Sandy loam	1,17
2.	Sandy clay	1,18
3.	Loamy sandy	1,17
4.	Silty clay	1,17
5.	Silty clay loam	1,18
6.	Sandy clay loam	1,75
7.	Sandy	1,18
8.	Clay	1,18

Amplification 16S rRNA Genes

Amplicon of 16S rRNA as a PCR product is the main subject that will be used to metagenomic analysis. These soil bacteria genes should be about 650 bp (depend on the bacteria) in size (Farrelly *et al*.. 1995). But in this study, contaminated genomic DNA prevented them to appear in electrophoresis visualization, and cannot be processed to the next step (sequencing and metagenomic analysis). So, it need more optimation procedure in order to get good 16S rRNA genes amplicon from PCR amplification.

Conclusion

Eight class of soil textures were obtained in this study: sandy loam, sandy clay, loamy sandy, salty clay, salty clay loam, sandy clay loam, sandy and clay. Some of genomic DNA purity were 1.2 – 1.7, and their electrophoresis visualization was more than 3000 bp in size. The 16S rRNA genes, however, was not obtained yet. We suggest to carry out fusther optimation procedure to continue to sequencing and metagenomic analysis.

References

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