Bacterial Biodiversity in Oil Palm Plantation and Different Forest Ecosystems in Mineral Soil in Sarawak

Shamsilawani, A B*; Siti Ramlah, A A* and Mohd Shawal, T M*

ABSTRACT

Changes in the soil properties due to cultivation causes rapid changes in the microbial communities and activities in the soil. Most of this soil microbial population are sensitive to the changes in soil; therefore, some microbiological parameter such as microbial biodiversity can be used as an indicator for soil quality. By applying 16S rDNA and denaturing gradient gel electrophoresis (DGGE), we investigated the bacterial composition in an area planted with oil palm in mineral soil in Belaga, Sarawak. Overall Shannon-Weaver biodiversity index showed that soil bacterial biodiversity in the oil palm planted area increased slightly after clean clearing. When palms reached the age of 2.5 years, the biodiversity index increased from early planting compared to the biodiversity in the strip areas. Prevalence of minor phylum showed that with the increase of oil palm age, there was an increase in the variations of new phylum groups contributing to the diverse population of soil bacteria in the oil palm area.

ABSTRAK

Perubahan tanah akibat daripada aktiviti pertanian mengakibatkan berlakunya perubahan populasi dan aktiviti mikrob di dalam tanah. Kebanyakan mikrob tanah ini adalah sensitif kepada perubahan dalam tanah. Oleh itu, parameter seperti indek biodiversiti mikrob boleh digunakan sebagai satu penunjuk untuk mengukur kualiti tanah. Primer 16S rDNA dan kaedah 'denaturing gradient gel electrophoresis' (DGGE), telah digunakan bagi mengkaji komposisi bakteria di kawasan penanaman sawit dan kawasan strip biodiversiti pada tanah mineral di Belaga, Sarawak. Secara keseluruhan indek biodiversiti Shannon-Weaver menunjukkan bahawa biodiversiti bakteria di kawasan sawit meningkat sedikit selepas pembersihan kawasan tanpa kaedah pembakaran. Apabila sawit mencapai umur 2.5 tahun, indek biodiversiti meningkat berbanding penanaman awal sawit dan kawasan strip biodiversiti. Kehadiran filum minoriti menunjukkan dengan peningkatan umur sawit, variasi kumpulan filum baru juga meningkat dan seterusnya ia menyumbang kepada peningkatan kepelbagaian populasi bakteria tanah kawasan penanaman sawit berbanding strip biodiversiti.

Keywords: oil palm plantation, microbial biodiversity, 16S rDNA, denaturing gradient gel electrophoresis, forest ecosystem.

INTRODUCTION

Changes in the soil properties due to agricultural activities frequently lead to extensive damage to the top soil, soil compaction and erosion. These changes are often associated with reduction in the biodiversity and abundance of soil microbes, including bacterial communities. Soil microbes are sensitive to the changes in the soil and play an important role in preserving soil productivity (Agnieszka *et al.*, 2012). Oil palm plantation is believed to have impacts on the environment and ecosystem like all the other plantations, such as rubber (Cotter *et al*., 2009). Soil microbes are known to be sensitive to such agricultural activities. The development of molecular methods such as PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis of 16S rRNA gene, phylogenetic tree analysis, fatty acid analysis and protein sequencing have aided in the identification and classification of bacteria through direct DNA extraction from soil and DNA sequencing (Saman *et al*., 2010). Hence, this study was conducted to investigate whether microbial biodiversity was affected during early planting of oil palm on mineral soil by analysing 16S rDNA gene using polymerase chain reaction combined with denaturing gradient gel electrophoresis (PCR-DGGE).

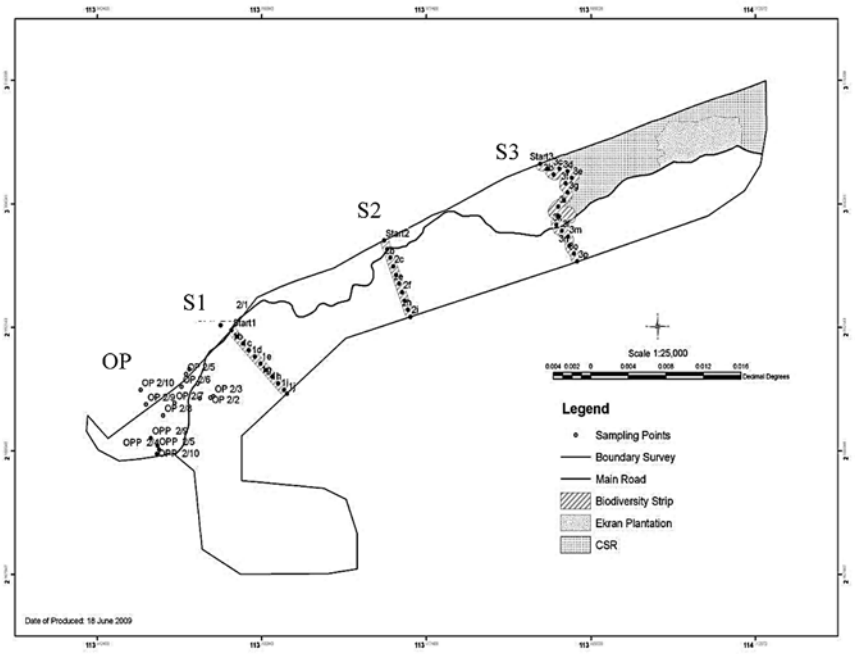
METHODOLOGY

Soil Sampling

Sampling of soil for microbial biodiversity study at Sungai Asap, Belaga, Sarawak was obtained from four sites consisting of, Biodiversity Strip 1 (hilly secondary jungle), Strip 2 (disturbed secondary

* Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.
E-mail: ramlah@mpob.gov.my

jungle), Strip 3 (riparian area) and oil palm (OP) planted area (*Figure 1*). Sampling was done at the respective GPS points of each site at depth of 0-15 cm. Soil sampling was conducted twice in July 2009 and in April 2010.



Note: Oil Palm Plantation (OP), Strip1 (S1), Strip 2 (S2) and Strip 3 (S3).

Figure 1. GPS points for soil sampling conducted in Belaga Sarawak.

DNA Extraction

Microbial DNA was directly extracted from soil, using GeneMatrix Soil DNA Purification kit' protocol (EURx Ltd., Poland). A total of 0.5 grams of soil was weighed and used in DNA extraction. The soil was added with 250 µl of soil extraction buffer, 50 µl soil lysis and 2 µl of Proteinase K and then vortexed on a flat-bed vortexer for 5 min. The solution was incubated at 65ºC for 10 min. Protein precipitation reagent was added and DNA solution was precipitated on ice for 10 min. After 13 000 rpm centrifugation, for 10 min., 100 µl of supernatant was transferred onto spin column and centrifuged for 5000 rpm to obtain filtrate. The filtrate was obtained and spin column was discarded. The DNA precipitation solution of 6 µl was added and mixed at room temperature for 5 min. The tubes were centrifuged for 5 min. at 13 000 rpm and supernatant was discarded to collect the pellet. Pellet was washed with 500 µl washing solution and inverted to mix and then spun at 13 000 rpm for 3 min. Washing step was done twice. The DNA pellet was air-dried and resuspended in 100 µl 1XTE buffer. The concentration of DNA was quantified using Nanophotometer (Implen GMBH, Germany) at absorbance ratio of A260/A280.

Amplification of 16S rDNA

The microbial DNA was amplified using universal bacterial 16S rDNA primers, 341f/907r, forward (f) primer with GC-clamps, 341f (5'-cgc-ccg-ccg-cgc-gcg-gcg-ggc-ggg-gcg-ggg-gca-cgg-ggg-gcc-tac-gg-agg-cag-cag-3') and reverse primer, 907r (5'-ccc-cgt-caa-ttc-att-tga-gtt-t-3') (Muyzer *et al*., 1993). Amplification mixture with this two set of primers had final volume of 25 µl and contained 10 pmol of each primer, 100 mM dNTPs, 1X PCR buffer, 50 mM Mg_2Cl, 0.3% BSA and 2.5 units of Taq polymerase. The amplification began with an initial 94ºC denaturation for 2 min, followed by 35 cycles of annealing at 94ºC for 30 s, 52.1ºC for 30 s, 72ºC for 30 s, final extension at 72ºC for 2 min., and finally held at 4ºC.

Denaturing Gradient Gel Electrophoreis (DGGE)

The PCR products were run on 1.0 mM of 6% (w/v) polyacrylamide (37.5:1; acrylamide: bisacrylamide) with a denaturing gradient of 40% to 80% (100% denaturant corresponds to 7 M urea and 40% [vol/vol] deionized formamide). An amount of 30 µl PCR products with 10 µl loading dye was pipetted into the individual lanes and DGGE was performed at 60ºC and 60V with 1X TAE buffer for 16 hr. Microbial DNA was excised and purified from DGGE and then re-amplified using 16S rDNA primers, 341f (without GC-clamp) (5'- cct-acg-gga-ggc-agc-ag-3') and reverse(r) 907r (5'-ccc-cgt-caa-ttc-att-tga-gtt-t-3').

Sequencing analysis. The PCR products were sequenced and the sequence similarity searches were performed using BLASTn of the NCBI GenBank database to identify the nearest relatives of the partially sequenced 16S rRNA genes.

***Statistics for biodiversity index*. Shannon-Weaver biodiversity index** (*H'*) was applied to characterise species diversity in a community. The Shannon-Weaver diversity index is a common diversity index which increases with the number of species and which is higher when the mass is distributed more evenly over the species (Hill, 1973). The **Berger-Parker Dominance index** expresses the proportional importance of the most dominant species. It is a measure of the numerical importance of the most abundant species (Hill, 1973).

RESULT AND DISCUSSION

Based on the data of total microbes analysed, the Shannon-Weaver prokaryotic biodiversity index showed an increase only in the oil palm planted area, from 7.627 at palm age 1.5 years to 7.773 when the palm reached 2.5 years (*Table 1*). The Berger-Parker Dominance index showed an increase in abundant species of microbes in Strip 1, followed by oil palm planted area, Strip 2 and Strip 3. The increase in dominant species was generally from the unspecified phylum of Unclassified Bacteria (*Figures 1* and *2*). These Unclassified Bacteria groups may arise to be novel species in the study sites.

Prevalence of prokaryotic phylum indicated that, unclassified bacteria was the dominant phylum amongst the prokaryotic population in all sites including the oil palm cultivated area (*Figures 1* and *2*). In early sampling of July 2009, Actinobacteria group was the second most prevalent, followed by the Acidobacteria, Firmicutes, and ***α*-**Proteobacteria groups which were found in all sites (*Figure 1*). However, in oil palm planted area, in which the palm age was at 1.5-year, Firmicutes were found abundant at 25% due to the abundance of organic materials left to rot naturally in the field after clean-clearing.

Nine months later, when the oil palm was at age 2.5 years, Acidobacteria and Actinobacteria groups (12%) were the second most prevalent, followed by ***α*-**Proteobacteria (9%) and Firmicutes (4%) (*Figure 2*). Data analysed in April 2010 showed that in all sites, the Actinobacteria was the most dominant phylum consisting between 12%-35% of the microbial population. There was a presence of the minor phylum, Cyanobacteria, which caused the palm cultivated area to be more diversed in microbial population than other sites. However, the total number of species population differed from 132 species in early planting of oil palm to 116 species, when the oil palm was getting older at 2.5 years. The same trend in the microbial species population was also analysed in all of the biodiversity strips area. The fluctuation was possibly due to heavy rainfall occurring during the month of April 2010 measuring at 344.43 mm which was also reported by Ram *et al.* (2013) who studied microbial populations at different depths of normal and sodic soils. The study suggests that, in the rainy season, increasing moisture leads to an anaerobic condition which were not favourable, especially for the growth of aerobic microbes. Another finding concluded that microbial biomass, C, N and P were low during the rainy season, which further limits the availability of nutrients to soil microbes, thereby reducing their immobilisation for microbial growth (Barbhuiya *et al.*, 2004).

TABLE 1. MICROBIAL BIODIVERSITY INDICES FOR TOTAL MICROBES IN MINERAL SOIL FROM SG ASAP, BELAGA, SARAWAK

Sites	Shannon-Weaver Biodiversity Index				Berger-Parker Dominance Index			
	OPP (1.5 yr)	Strip 1	Strip 2	Strip 3	OPP (2.5 yr)	Strip 1	Strip 2	Strip 3
July 2009	7.627	8.26	8.002	8.070	0.3958	0.2863	0.3173	0.5202
April 2010	7.773	7.322	7.337	8.003	0.4735	0.3545	0.4148	0.4894

Note: OPP – Oil Palm Plantation, S1- Biodiversity Strip 1, S2-Biodiversity Strip 2, and S3-Biodiversity Strip 3.

CONCLUSION

The findings showed that soil microbial biodiversity in the oil palm cultivated area slightly increased over time. When, oil palm reached the age of 2.5 years, the biodiversity indices increased compared

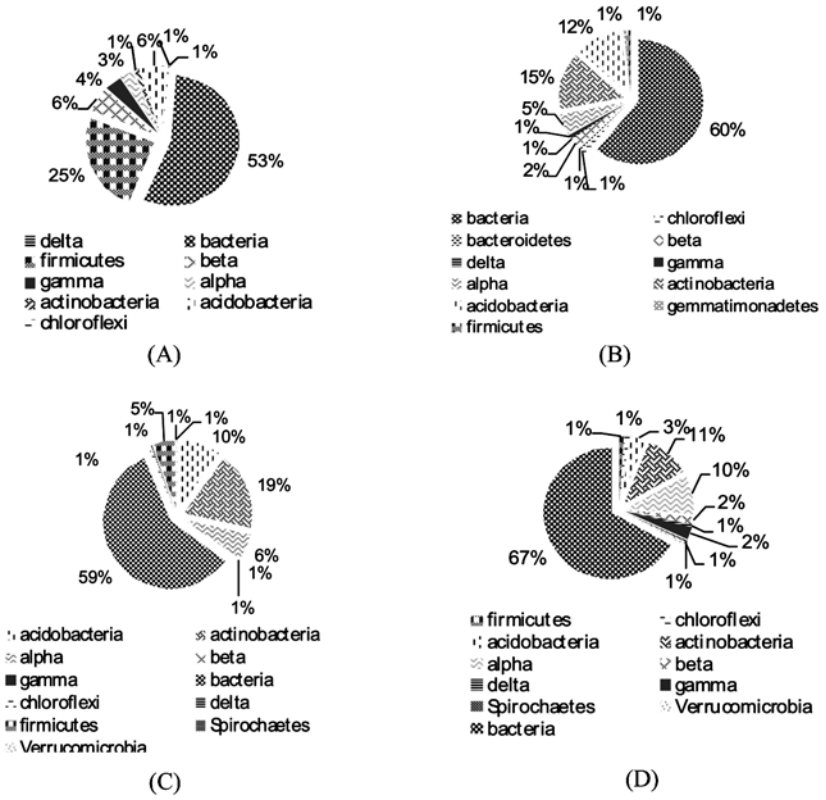


Figure 1. Prevalence of soil prokaryotic phyla sampled on July 2009 from (A) Oil Palm area with 1.5 years old palm (B) Strip 1 (C) Strip 2 and (D) Strip 3.

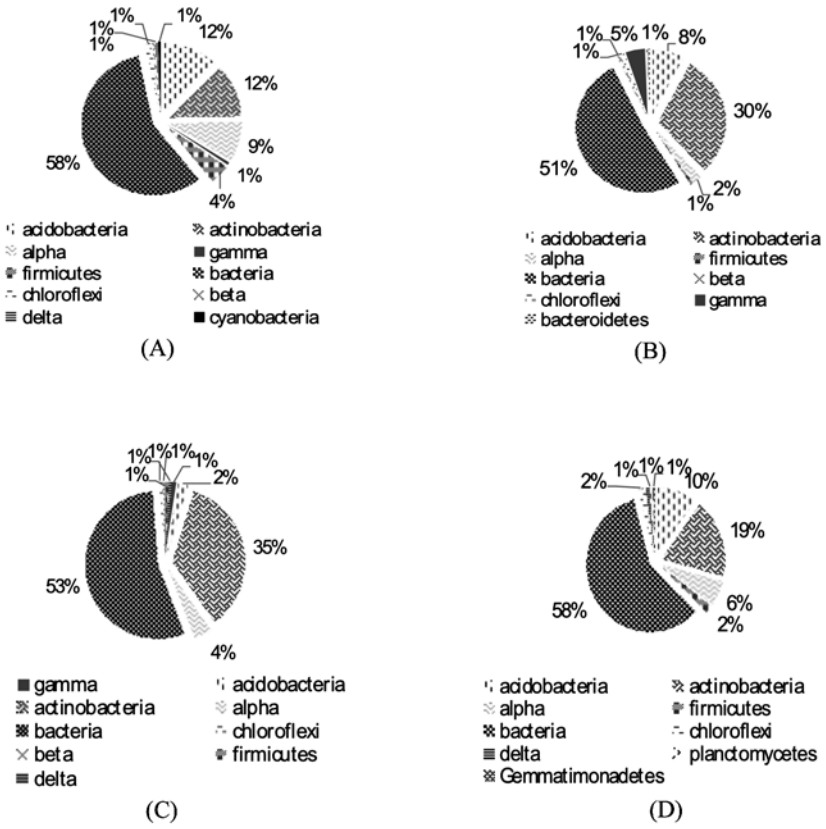


Figure 2. Prevalence of soil prokaryotic phyla sampled on April 2010 from (A) Oil palm area with 2.5 years old palm (B) Strip 1 (C) Strip 2 and (D) Strip 3.

to biodiversity strips areas. Prevalence of minor phylum at this time showed that with the increase of oil palm age, there was an increase in the incidence of new phylum groups.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB and the Director of Biological Research Division of MPOB for permission to publish this article.

REFERENCES

AGNIESZKA, W; ZOFIA, S; ALEKSANDRA, B and ARTUR, B (2012). Evaluation of factors influencing the biomass of soil microorganisms and DNA content. *Open Journal of Soil Science.* http://dx.doi.org/10.4236/ojss.2012.21010

BARBHUIYA, A R; ARUNACHALAM, A; PANDEY, H N; ARUNACHALAM, K; KHAN, M L and NATH, P C (2004). Dynamics of soil microbial biomass C, N and P in disturbed and undisturbed stands of a tropical wet-evergreen forest. *European Journal of Soil Biology, 40*: 113-121.

COTTER, M; MARTIN, K and SAUERBORN, J (2009). How do renewable products impact biodiversity and ecosystem services – the example of natural rubber in China. *Journal of Agriculture and Rural Development in the Tropics and Subtropics. Vol. 110 (1)*: 9-22.

HILL, M O (1973). Diversity and evenness: a unifying notation and its consequences. *Ecol. 54*: 427-431.

MUYZER, G; DE WAAL, E C and UITTERLINDEN, A G (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol. 59(3)*: 695-700.

RAM, R L; MAURYA, B R and SHARMA, P K (2013). Seasonal variation in microbial population at different depths of normal and sodic soils of Varanasi. *International Journal of Innovative Research and Development*, 2: 1870-1880.

SAMAN, S; SLATTERY, P and SAMAN, S (2010). Microbial biodiversity investigation techniques. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* (A Mendez-Vilas ed.). p. 1546-1550.