Microbial Biodiversity Of Mineral Soils Planted With Young Oil Palms In Belaga, Sarawak.

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INTRODUCTION

Soil microbes are sensitive to the changes in the soil and play an important role in conserving soil productivity (Agnieszka *et al.*, 2012). Oil palm plantation has been thought to have impacts on the environment and ecosystem like all the other plantations, such as rubber (Cotter *et al*., 2009). Microbes in the soil are known to be sensitive to such agricultural activities. The development of molecular techniques such as PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis, 16S rRNA, phylogenic tree, fatty acids analysis and protein sequencing have assisted in the identification and classification of bacteria through direct DNA extraction from soil samples then sequencing the isolated DNA (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 coupled with denaturing gradient gel electrophoresis (PCR-DGGE).

METHODOLOGY

Soil sampling. Sampling for microbial biodiversity study at Sungai Asap, Belaga, Sarawak was obtained from 4 sites namely Biodiversity Strip 1 (hilly secondary jungle), Strip 2 (disturbed secondary jungle), Strip 3 (riparian area) and oil palm (OP) planted area of 1.5 years to 2.5 years (young palms). Sampling was carried out at respective GPS points from each site at depth of 0-15 cm.

DNA Extraction - Microbial DNA were extracted directly from soil, using GeneMatrix Soil DNA Purification kit' protocol (EURx Ltd., Poland) and quantified with Nanophotometer (Implen GMBH, Germany) with absorbance ratio of A260/A280.

PCR amplification of 16S rDNA and Denaturing Gradient Gel Electrophoreis (DGGE). The microbial DNA was amplified using universal 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(r) 907r (5'-ccc-cgt-caa-ttc-att-tga-gtt-t-3') (Muyzer et al., 1993). PCR products were separated on 1.0 mM of 6% (w/v) polyacrylamide (37.5:1; acrylamide: bisacrylamide) with a denaturing gradient of 40% to 80 % using the DCode DGGE System from Bio-Rad (Bio-Rad, USA). Microbial DNA successfully excised and purified from DGGE was 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 outsource for sequencing. Sequence similarity

searches on the sequence data were conducted using (BLASTn) of the NCBI GenBank database to identify the nearest relatives of the partially sequenced *16S rRNA* genes of excised bands.

Statistics for Biodiversity Index. The **Shannon-Weaver biodiversity index** (*H'*), is used to characterize species diversity in a community. The Shannon-Weaver diversity index is a general 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). **Berger-Parker Dominance Index.** The Berger-Parker Dominance index expresses the proportional importance of the most abundant species (the dominant species). It is simple measure of the numerical importance of the most abundant species (Hill, 1973).

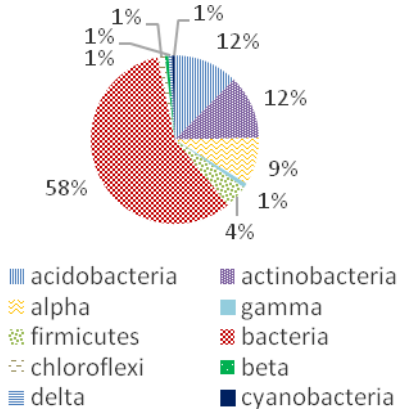
RESULTS AND DISCUSSION

Based on the data of total microbes analysed from Belaga, Sarawak, time-course of Shannon-Weaver prokaryotic biodiversity index showed an increase from early planting of oil palm to oil palm aged 2.5 years, from July 2009 to April 2010, from 7.627 to 7.773, respectively (Table 1). Index for biodiversity strip was high during early planting (July 2009), whilst, oil palm planted area increased gradually as the oil palm age increased to 2.5 years (April 2010). Though Berger-Parker Dominance index showed increase in dominancy of the oil palm planted area during palms aged 2.5 years, the dominancy was generally from the uncategorized phylum of Unclassified Bacteria. These Unclassified Bacteria may arise to be novel species in the occuring site.

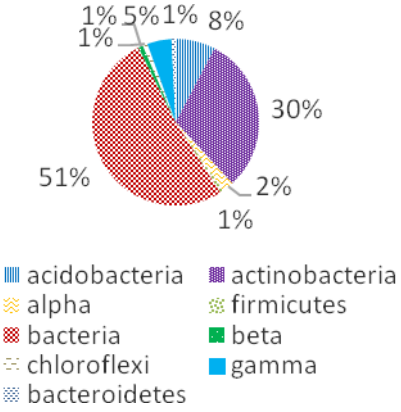
Table 1. Microbial Biodiversity Indices For Total Microbes On Mineral Soil Sample From Sg. Asap, Belaga, Sarawak.

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

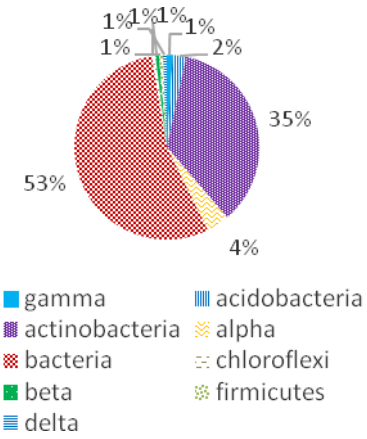
Note: *OPP – Oil Palm Cultivable Area, S1- Biodiversity Strip 1, S2-Biodiversity Strip 2, and S3-Biodiversity Strip 3.*



(A)



(B)



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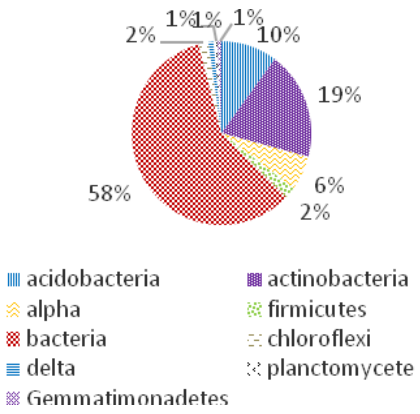

(D)

Figure 1. Prevalence of Prokaryote Phylum from Belaga 4th sampling (April 2010) for Biodiversity Strips versus Oil Palm sampled from Sg Asap, Belaga, Sarawak. Note; (A) Oil Palm at 2.5 years (B) Strip 1 (C) Strip 2 and (D) Strip 3.

Prevalence percentage of phylum indicates that, from early planting of 1.5 years old palm (July 2009) to palm aged 2.5 years (April 2010), Unclassified Bacteria was the dominant phylum amongst the prokaryotic population in all sites including the oil palm cultivated area (Figure 1). During early planting (July 2009), Actinobacteria group was the second most prevalent, followed by the Acidobacteria, Firmicutes, and α-Proteobacteria group which were found in all sites. Six months later, when the oil palm was 2.5 years (April 2010), Acidobacteria group (12%) was the second most prevalent, followed by the Actinobacteria (12%), α-Proteobacteria (9%) and Firmicutes (4%) (Figure 1). In all sites, species of Actinobacteria dominated 12-35% of the population in April 2010. There was a presence of minor phylum namely, Gemmatimonadetes, Planctomycetes, and Cyanobacteria which made up a more diverse microbial population in the OP

cultivated area. The total number of species population slightly decreased in all sites, where from early planting to oil palm aged 2.5 years, the value decreased from 132 to 116 species, as for the biodiversity strips, number of species also decreased from early planting of July 2009 to April 2010 due to heavy rainfall occurring during the month of April 2010 measuring at 344.43 mm^3.

CONCLUSIONS

The overall results showed that soil microbial biodiversity in the oil palm planting area increased slightly over time. In oil palm reaching the age of 2.5 years, the biodiversity index increased slightly from early planting whereas in biodiversity strips the index decreased slighty that maybe due to the heavy rainfall occurring during this period. Prevalence of minor phylum at this time showed that with the increase of oil palm age, there was an increase in the occurrence of new phylum groups.

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