Bacterial Diversity and Community Structure of Banana Rhizosphere in Orang Asli Fields and Commercial Plantations
(Kepelbagaian Bakteria dan Struktur Komuniti Rizosfera Pisang di Kebun Orang Asli dan Ladang Komersial)

Jason Yee Neng Lee* & Irene Kit Ping Tan

ABSTRACT

Bacteria play an important roles in the soil ecosystem and in the rhizosphere, they are intricately linked to nutrient content and its accessibility to plants, plant protection and sometimes pathogenicity. Banana grows well in the tropics and it is popularly grown in Orang Asli (OA) (indigenous people) settlements. Banana is also grown in commercial plantations. In traditional planting practices, the OA do not add pesticide nor fertilizer to their crops which are planted for self-sustenance mainly. On the other hand, fertilizer and pesticide are added to commercial banana plantations to maximise yield. Rhizosphere bacteria from the banana plant, Pisang Nipah, grown in OA fields and commercial plantations were identified by clone library construction of the 16S rRNA gene. This was to determine whether farming practices influenced the bacterial community in the banana plant rhizosphere. Acidobacteria, Proteobacteria and Actinobacteria were found in all the soil. Other common phyla found in some soil (but not all) were Nitrospirae, Firmicutes, Bacteroidetes, Chloroflexi, Verrumicrobia, Gemmatimonadetes and Cyanobacteria. The bacterial diversity was a little more diverse in the OA fields than the commercial plantations. The latter had higher contents of nitrogen, phosphorus and potassium. These could have exerted selective pressure to reduce the bacterial diversity in the commercial plantations.

Keywords: Bacterial community; banana rhizosphere; commercial plantations; Orang Asli settlements; 16S cloning

ABSTRAK

Bakteria memainkan peranan penting di dalam ekosistem tanah dan rizosfera, sifat bakteria yang dinamik dan kompleks boleh memberi kesan positif atau negatif kepada tumbuh-tumbuhan. Pokok pisang boleh bertumbuh dengan baik di kawasan tropika dan juga popular ditanam di penempatan Orang Asli (OA) dan ladang komersial. OA mengamalkan penanaman tradisi dan tidak menambahkan racun perosak atau baja kepada tanaman mereka, terutamanya tanaman yang menghasilkan rezeki kepada mereka. Sebaliknya, ladang pisang komersial mengamalkan penambahan baja dan racun perosak untuk memaksimumkan hasil pertanian. Rizosfera bakteria yang dijumpai pada Pisang Nipah yang ditanam di kebun OA dan ladang komersial telah dianalisis dengan menggunakan klon gen 16S rRNA. Ini penting untuk menentukan sama ada amalan pertanian mempengaruhi komuniti bakteria di dalam rizosfera pokok pisang. Acidobacteria, Proteobacteria dan Actinobacteria dijumpai di dalam semua tanah yang dianalisis. Sementara itu, filum lain yang dijumpai di dalam analisis tanah adalah Nitrospirae, Firmicutes, Bacteroidetes, Chloroflexi, Verrumicrobia, Gemmatimonadetes dan Cyanobacteria. Terdapat lebih banyak jenis bakteria dijumpai di dalam OA berbanding dengan ladang komersial. Ladang komersial mengandungi lebih banyak kandungan nitrogen, fosforus dan kalium boleh menyebabkan kekurangan jenis bakteria di dalam ladang komersial.

Kata kunci: Klon 16S; komuniti bakteria; ladang-ladang komersial; penempatan Orang Asli; rizosfera pokok pisang

INTRODUCTION

Banana (genus *Musa* in the family Musaceae) grows well in tropical and subtropical regions, and constitute the principal food resources in the world, ranking just behind rice, corn and milk (Shyam et al. 2011). The genus *Musa* was classified into five sections (Ingentimusa, Australimusa, Callimusa, Musa and Rhodochlamys) but has been reclassified into three sections based on closer genetic variation which are Ingentimusa, Calimusa-Australimusa and Musa-Rhodochlamys (Wong et al. 2002). This report focuses on the rhizosphere of the Pisang Nipah variety (also known as Pisang Abu) which is commonly grown by the Orang Asli (OA) (indigenous people) as well as in commercial plantations.

The OA does not add fertilizer and pesticide to their banana fields but commercial plantations do to maximize yield. Broeckling et al. (2008) reported that fertilizer influenced soil microbial growth and activity and Beauregard et al. (2010) reported that repeated fertilizer applications to soil changed the soil microbial community as a result of changes to the soil physical, chemical and biological properties. Ge et al. (2008) reported that fertiliser application had significant impacts on the soil microbial

population and their activities by increasing the soil microbial biomass, decreasing nutrient content of the soil and lowering the soil quality. Lazcano et al. (2012) and Peacock (2001) reported that fertilised soil encouraged microbial growth as well as stimulated the changes of microbial community by increasing the Gram-negative bacteria. However, other studies (Okano 2004; Treseder 2008) stated that fertilizers had little or no effect on the soil microbial diversity and activities.

Scientists have acknowledged the importance of rhizosphere microorganisms to plant growth and health (Hirsch et al. 2013; Rovira 1991). Root exudates, mucilage and sloughed-off root cells provide a nutritional source for microbial cell multiplication and colonization of root surfaces and adjacent soil (Hirsch et al. 2013). The rhizosphere is a dynamic niche containing complex microbial communities and microbial members participate in a variety of beneficial and detrimental interactions with plants (Rovira 1991). Beneficial interactions include the roles microorganisms play in enhancing nutrient uptake by plants, stimulating plant growth and offering biological control of diseases. In contrast, microbial pathogens in the rhizosphere can impair plant health and decrease productivity in agricultural and forested environments.

In the past, before the advent of molecular biology, bacterial communities in environmental samples (soil and water) were assessed by culture-dependent methods i.e. the environmental samples were inoculated into various types of media and resultant cultures were streaked onto agar plates to obtain individual colonies. The colonies (termed isolates) would be purified and put through a series of biochemical tests to determine their physiological and metabolic properties. Based on these properties, the isolates would be identified. With developments in molecular biology techniques, pure cultures of isolates could also be identified based on DNA sequences. Nevertheless, analysis of bacterial community based solely on culturable bacteria has strong limitations. One major limitation is that more than 90% of bacteria in nature are not culturable in any media (Moyer et al. 1993). This may be due to the fastidious growth requirement of bacteria (Whitford et al. 1998). The culture-based technique is also limited by insufficient knowledge on how to reproduce natural conditions in the laboratory and how to create viable synthetic conditions for all organisms (Alain & Querellou 2009). Another limitation of culture-based techniques is that some environmental microbes may grow very slowly in the laboratory media as they may require longer time to adapt to the new media. Such microbes may not generate turbidity or single-cell colonies in a 'normal' period and would therefore not be isolated and studied (Leadbetter 2003).

In order to get a better analysis of the bacterial community, molecular techniques are often used now. These include clone library building (Head et al. 1998), Denaturing Gradient Gel Electrophoresis (DGGE) (Head et al. 1998), Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Liu et al. 1997), DNA microarrays (Fakruddin & Khanjada 2013) and Single Strand Conformation Polymorphisms (SSCP) (Fakruddin & Khanjada 2013). In contrast to culture-dependent techniques, molecular techniques are able to detect the total bacterial community in a sample because they analyse the nucleic acids which are extracted directly from the environmental samples. For our studies, we used the 16S rRNA gene which is a section of DNA found in all prokaryotes (bacteria and archaea). The 16S rRNA gene fragment is used as a common marker for bacterial identification because it is present in all bacteria, its function has not changed over time and its size of 1500 bp is large enough for informatics purposes (Janda & Abbott 2007; Ward et al. 1992). Direct amplification and analysis of 16S rRNA gene sequences have replaced cultivation as a way to compare the composition, richness and structure of bacterial communities. By itself, 16S rRNA gene sequencing has low phylogenetic power at the species level and poor discriminatory power for some genera, thus DNA relatedness studies are necessary to provide better resolution to these taxonomic problems (Janda & Abbott 2007).

Currently, there is limited documentation on the microbial diversity in the banana rhizosphere in Malaysia (Tripathi et al. 2012). This study aimed to assess the diversity of bacteria in the rhizosphere of banana plants (Pisang Nipah) grown in OA settlements and in commercial plantations by generating clone libraries of the 16S rRNA gene fragment. The bacterial diversity will be correlated with some environmental variables such as pH, nitrogen (N), phosophorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and sulphur (S).

MATERIALS AND METHODS

SOIL SAMPLING

Rhizosphere from banana plants (Pisang Nipah) was collected from two OA fields (Paya Mendoi, Pahang and Kampung Pian, Pahang) and two commercial plantations (Parit Serong, Selangor and Mersing, Johor). For each location, rhizosphere from four plants was collected. Each soil sample was collected in individual sterile Falcon tubes from around the roots, at a depth of 20 cm. The soil samples collected from each locations were observed to be brown loam. The tubes were placed in a cool box containing ice packs and brought back to the laboratory where they were stored at –20°C until used.

DNA EXTRACTION

The total DNA from the soil samples was extracted by using the MoBio Ultraclean Soil DNA Extraction Kit (MoBio Inc., Solana, CA) and quantified by using a biophotometer (Eppendorf). The extracted DNA were stored at –20°C until further use.

PCR AMPLIFICATION

Amplification of the 16S rRNA gene was performed by using the universal primers 27F (5'-AGA GTT TGA TCC

TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') to generate amplicons of ~1,500 bp. A total volume of 50 µL PCR mixture (Invitrogen) was prepared by adding 18.1 µL of master mix and 31.9 µL of diluted DNA template. The PCR program was set as follows – the template DNA was denatured at 95°C for 5 min, followed by 30 cycles of initial, annealing and extension processes. The initial process involved denaturing the DNA template at 94°C for 45 s. Annealing of the oligonucleotide primers was performed at 52.5°C for 30 s and extension of new DNA at 72°C for 90 s. After 30 cycles of these three processes, final extension was made at 72°C for 10 min after which the PCR product was stored at 4°C. The amplification products (~1500 bp) were checked on agarose gels.

CLONING AND SEQUENCING

The 16S DNA amplicons (PCR products) obtained from the rhizosphere of the four Pisang Nipah soils at the same site were pooled and purified by using MEGAquick-spin™ PCR and Agarose Gel DNA Extraction Kit (iNtRON Biotechnology, Korea). The purity was checked by agarose gel electrophoresis and measurement of optical density (OD) by biophotometer. The purified amplicons were ligated into pGEM®T Easy Vectors System (Promega, U.S.A.) and transformed into the TOP10 *Escherichia coli* (Invitrogen, U.S.A.). To compare bacterial diversity between sites, the number of clones picked was standardized (more than 100 clones for each clone library). The clones were then screened by restriction fragment length polymorphism (RFLP) with restriction enzyme *Msp*I (Thermo Scientific, U.S.A.). Representative clones with unique RFLP patterns were purified by using DNA-spin™ plasmid DNA purification kit (iNtRON Biotechnology, Korea) and sent to FirstBase Laboratories (Selangor, Malaysia) for single pass sequencing using the primers T7 promoter (5'-AAT ACG ACT CAC TAT AG-3'). The sequences were analyzed and compared to GenBank database at the National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST) program. The 16S rRNA gene sequences of our clones having 97% or higher similarity to GenBank sequences were considered to be of matching phylotypes. Sequences having 70% or less similarity to GenBank sequences were listed as unknown genus and species. The 16rRNA gene sequences of our clones were also deposited in the GenBank database with the accession numbers MF449372-MF449406.

PAST ANALYSIS

Surakasi et al. (2010) and Suyal et al. (2015) reported that the phylogenetic analysis of the clone sequences was carried out by assigning all the sequences to an operational taxonomic unit using distance-based operational taxonomic units (OTU). The diversity of OTU was further examined by using paleontological statistics (PAST) analysis (Ceteciogly et al. 2009). Shannon-Wiener diversity index was used to calculate Shannon index (H), evenness and the Simpson's index (D).

SOIL CHEMICAL ANALYSIS

The soil pH was measured in a 1:2 (w/v) mixture of soil in deionized water by using a pH meter (Eutech Instrument pH510). Total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and sulphur (S) in the soil were analysed at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang. Organochlorine and organophosphorus in the soil were also analysed in MARDI as indications of pesticides. Analysis of total nitrogen was analysed by using Kjedahl method, meanwhile, segmented flow analyser (SKALAR) was used to analyse the present of N, P, K Ca, Mg, Na and S. It was also reported that CHNS/O analyser was also used by MARDI to test the present of stated elements.

RESULTS AND DISCUSSION

SOIL BACTERIAL DIVERSITY BASED ON 16S CLONES

Figure 1 shows the bacterial phyla detected in the banana rhizosphere soil in the OA fields and commercial plantations. The major phyla were Acidobacteria, Proteobacteria, Actinobacteria, Nitrospirae, Firmicutes, Bacteriodetes, Chloroflexi, Verrucomicrobia, Gemmatimonadetes, Cyanobacteria and uncultured bacteria (phyla unknown). Acidobacteria constituted the largest phylum in the commercial plantations (Mersing and Parit Serong) (constituting 50 to 55.3%) but was less dominant in the OA fields (Kg. Pian and Paya Mendoi) (constituting 17.2 to 42.7%). Proteobacteria was more dominant in the OA fields (constituting 31.8 to 54.31%) compared to the commercial plantations (constituting 23.8 to 31.33%). Faoro et al. (2010) reported that Acidobacteria and Proteobacteria were the most common and abundantly distributed bacterial groups in soil. Some members of Acidobacteria were involved in nitrate and nitrite reduction, while some members of Proteobacteria were involved in denitrification (Aislabie & Deslippe 2013). Several Proteobacteria members could degrade organic acids, amino acids and sugars (Eilers et al. 2012).

The OA fields had higher proportions of Bacteroidetes (4.6 to 13.7%) compared to the commercial plantations (0 to 2%). Bacteroidetes were reported to be able to degrade complex organic molecules such as protein, starch, cellulose and chitin (Aislabie & Deslippe 2013). OA fields are farmed less intensively than commercial plantations. The OA also does not apply fertilizer and pesticide to their crops, so the soil would likely contain more complex degraded plant material. This could be a reason for the higher proportion of Bacteroidetes in the OA soil.

The proportion of Nitrospirae was found higher in a commercial soil in Parit Serong compared to the OA soils in both Kg. Pian and Paya Mendoi. Nitrospirae plays an important role in the nitrogen cycle. Oxidation of ammonia to nitrite and then to nitrate were facilitated by bacteria under the phylum Nitrospirae (Philippot et al. 2007). There is also a higher proportion of Verrucomicrobia in the commercial soil compared to the OA soil. The roles of Verrucomicrobia are poorly understood. van Passel et
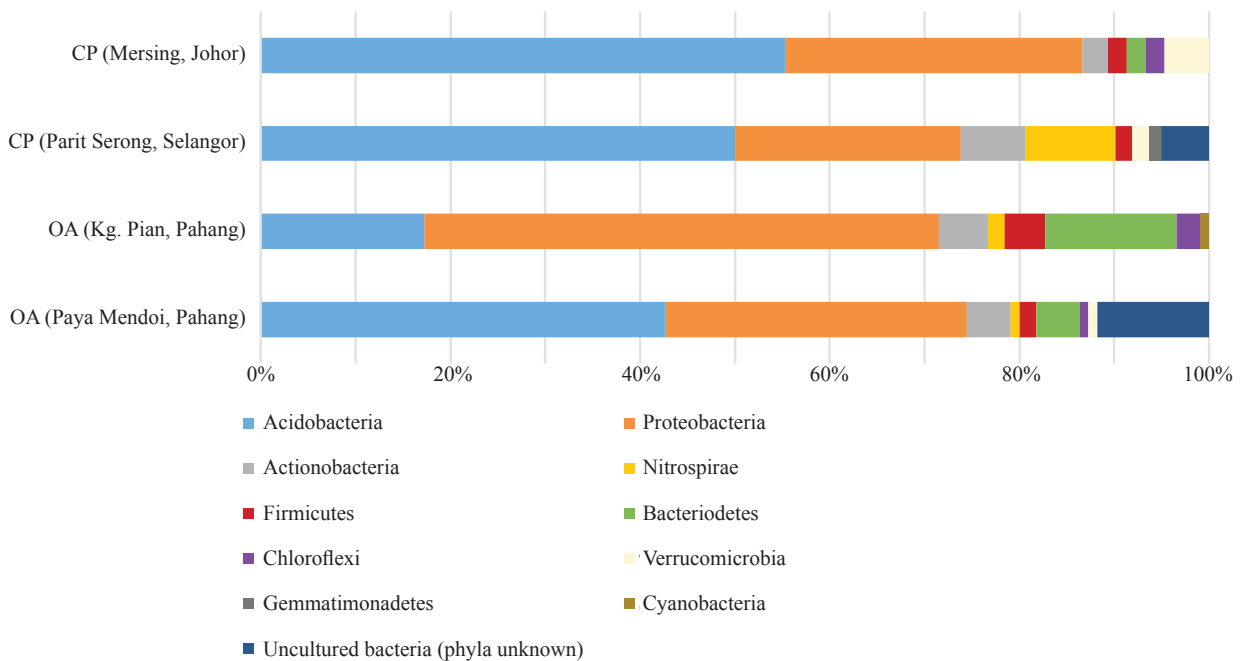
FIGURE 1. Banana rhizosphere bacterial community in OA fields (Kg. Pian and Paya Mendoi) and commercial plantations (CP) (Mersing and Parit Serong)

al. (2011) reported that some anaerobic members of this phylum might be involved in the fermentation of plant polysaccharides.

There is relatively little information on soil bacteria belonging to the phyla Firmicutes, Gemmatimonadetes and Chloroflexi because only few representatives of these phyla had been cultivated (Aislabie & Deslippe 2013). Firmicutes were found in the OA fields and the commercial plantations. Most Firmicutes are endospore-forming and some are lactic acid bacteria. Some of these bacteria such as *Bacillus* were capable of degrading different carbon sources including plant polysaccharides (Aislabie & Deslippe 2013). Some genera of Firmicutes are fermentative and have nitrogen-fixing characteristics (Aislabie & Deslippe 2013). Gemmatimonadetes bacteria are aerobic heterotrophs and are adapted to low soil moisture conditions (de Bruyn et al. 2011; Stark & Firestone 1995). Members of the genus Gemmatimonas were reported to be abundant in soil (Aislabie & Deslippe 2013). In our study, Gemmatimonadetes were found only in the commercial plantation in Parit Serong. On the other hand, Chloroflexi were found in both OA fields (Paya Mendoi and Kg. Pian) and in the commercial plantation in Mersing. Chloroflexi was reported to be abundant in soils but not easily obtained from culture-dependent experiments. They are aerobic heterotrophs and had been isolated on oligotrophic media in the form of slow-growing mini colonies (Davis et al. 2011).

The proportion of uncultured bacteria was higher in the OA field in Paya Mendoi (11.8%) compared to the commercial field in Parit Serong (5%). However, uncultured bacteria were not detected in the other OA field in Kg. Pian and the other commercial field in Mersing. Uncultured bacteria are those clones with 16S sequences which did not match any phylogroup sequences in public databanks. It is interesting to note that the OA field registered a higher proportion of uncultured bacteria which could be attributed to unknown or unclassified bacteria. This might reflect a more natural bacterial community in the OA rhizosphere which had not been disturbed by commercial farming practices (e.g. addition of fertilizers and pesticides).

Table 1 shows the phyla and genera that were detected by comparing the 16S sequences in the GenBank to the DNA sequences obtained from the rhizosphere of the OA fields and commercial plantations. The phyla Acidobacteria, Proteobacteria, Actinobacteria and Firmicutes occurred in all the soils. However, the types of genera within each of these phylum varied between the four soils. The phyla Nitrospirae, Bacteroides and Chloroflexi occurred in the OA field soils but not in all the commercial plantation soils. The phyla Verrucomicrobia, Gemmatimonadetes and Cyanobacteria appeared to be randomly present.

The results from Figure 1 and Table 1 indicate that the rhizosphere from the OA fields contained higher diversity in terms of bacterial phyla and genera compared to the rhizosphere from the commercial plantations. Furthermore, a higher percentage of uncultured bacteria occurred in one OA field compared to one commercial plantation, probably indicating that the commercial plantation soil might have exerted stronger selective pressure on the bacterial diversity.

The genera *Bradyrhizobium, Xanthomonas* and *Bacillus* were detected in both the OA field soils but not in the commercial plantation soils. *Bradyrhizobium* is a nitrogen-fixing bacteria and its presence in the OA field soils might reflect a soil capable of generating nutrients biologically (Hani et al. 1998; Rossum et

TABLE 1. Banana rhizosphere bacterial community in the OA fields and the commercial plantations based on the 16S DNA sequences. The bacterial phyla are denoted in bold; the indented non-bold letters denote bacterial genus

	GenBank Accession Number	OA Fields		Commercial Plantations	
		Paya Mendoi, Pahang	Kg. Pian, Pahang	Parit Serong, Selangor	Mersing, Johor
Acidobacteria	MF449372	+	+	+	+
Candidatus Solibacter	MF449373	+		+	
Proteobacteria	MF449374	+	+	+	+
Burkholderia	MF449375	+		+	
Bradyrhizobium	MF449376	+	+		
Dyella	MF449377	+			
Desulfoglaeba	MF449378			+	
Xanthomonas	MF449379	+	+		
Ralstonia	MF449380	+			
Steroidobacter	MF449381		+	+	+
Castellaniella	MF449382		+		
Dokdonella	MF449383		+		
Bordetella	MF449384		+		
Frateuria	MF449385		+		
Nevskia	MF449386		+		
Pseudoxanthomonas	MF449387		+		
Psuedolabrys	MF449388				+
Phenylobacterium	MF449389				+
Actinobacteria	MF449390	+	+	+	+
Micromonosporaceae	MF449391	+			
Microbacterium	MF449392		+		
Nitrospirae	MF449393	+	+	+	
Nitrospira	MF449394	+	+	+	
Firmicutes	MF449395	+	+	+	+
Bacillus	MF449396	+	+		
Sporosarcina	MF449397		+		
Oceanobacillus	MF449398				+
Bacteroidetes	MF449399	+	+		+
Chryseobacterium	MF449400	+			
Flavisolibacter	MF449401		+		
Chloroflexi	MF449402	+	+		+
Verrucomicrobia	MF449403	+		+	+
Gemmatimonadetes	MF449404			+	
Cyanobacteria	MF449405		+		
Uncultured bacteria (phyla unknown)	MF449406	+		+	

al. 1994). The commercial plantations which apply fertilizers on a regular basis might have discouraged the propagation of nitrogen-fixing bacteria in the soil. On the other hand, some genera detected in OA field soils but not in the commercial plantation soils are known to be plant pathogens. These include *Xanthomonas* and *Pseudoxanthomonas* which are associated with vascular wilt, cankers, leaf spot and fruit spot (Nadia et al. 2011), *Chryseobacterium*, an antimicrobial and pathogenic soil bacteria (Kirby et al. 2004) and *Ralstonia*, a plant pathogenic bacteria which cause wilt disease (Meng 2013). The absence of these plant pathogenic bacteria in the commercial plantation soils might be associated with the use of pesticides. The presumed selective pressure on the bacterial diversity in the commercial plantation soil (due to the use of fertilizer and pesticide) might also be a cause for the lower proportion of uncultured bacteria in those soil compared to the OA field soil. Little is known about such uncultured bacteria because their 16S DNA sequence does not match those of documented bacteria. Therefore they could be either beneficial or detrimental for crop growth, yield and health.

Determination of operational taxonomic units (OTU) is a method for comparing diversity from different clone libraries (Suyal et al. 2015). Based on 16S rRNA gene clone library analysis, the four rhizosphere (two from OA

TABLE 2. Comparative diversity analysis on 16S rDNA clone libraries using paleontological statistics

Diversity indices	OA fields		Commercial plantations	
	Paya Mendoi, Pahang	Kg. Pian, Pahang	Parit Serong, Selangor	Mersing, Johor
Individuals	117	165	167	135
Shannon_H	3.876	3.312	3.285	2.985
Menhinick	5.455	3.192	3.560	2.668
Margalef	12.180	7.834	8.793	6.116
Berger-Parker	0.103	0.206	0.102	0.096
Simpson_1-D	0.975	0.951	0.929	0.925

fields and two from commercial plantations) appeared to have similar bacterial richness and diversity with only slight differences (Table 2). The Shannon_H values were higher in the Paya Mendoi and Kg. Pian OA libraries (3.876 and 3.312, respectively) compared to the Parit Serong and Mersing commercial plantations libraries (3.285 and 2.985, respectively). A higher Shannon index value (H) correlates to higher number of unique species or greater species evenness (Shannon 1948). The Margalef and Menhinick indices for the Paya Mendoi OA field were higher than the other three locations. Margalef and Menhinick indices estimate the species richness independent of the sample size (Magurran 2004). The Berger-Parker index values were higher in the OA field soils compared to the commercial plantation soils. This index expresses the proportional importance of the most abundant species. Increase in the value of this index accompanies an increase in diversity and a reduction in dominance (Magurran 2004). With reference to the Simpson's index, values which are near zero indicate that the ecosystem is highly diverse or heterogeneous, and values which are near one indicate a more homogeneous ecosystem (Simpson 1949). In our study, the four soils show similar Simpson's index which was close to one.

SOIL CHEMICAL PROPERTIES

The pH values of the OA field soils ranged between 6.3 and 6.6 which were slightly higher than the pH of the commercial plantation soils ranging between 5.9 and 6.0 (Figure 2). This pH range is the norm in humid tropical soil (Brady & Weil 1999; Elisa et al. 2014). The slightly lower pH in the commercial plantation soils may explain the higher proportion of Acidobacteria in those soils compared to the OA field soils (Figure 1). Such a correlation was also observed by Lauber et al. (2009) who proposed that lower pH in the soil would contribute to higher Acidobacteria population. Soil pH is generally believed to be the best predictor of bacterial community composition and diversity (Rousk et al. 2010).

The rhizosphere in Parit Serong commercial plantation had higher contents of total N and K compared to the other three soils (Figure 2). This might reflect the addition of fertilizers to the commercial plantation, as acknowledged by the farmers. Like most cash crops, banana plants require large amounts of N, K, P, Ca and Mg to maintain high yield (Abdullah et al. 1999; Memon et al. 2010; Mia et al. 2010). A limitation in N uptake could lead to deficiency symptoms in the banana plants, therefore extra N must be frequently applied even to fertile soil (Robinson 1995). However, the other commercial plantation soil from Mersing showed lower levels of total N and K compared to Parit Serong commercial plantation and it was similar to the levels in the two OA field soils (Figure 2). A plausible reason would be that whatever fertilizer was added to the Mersing soil could have been used up by the plants or decomposed or washed away. The fertilisers appeared to have not accumulated in the soil.

No organochlorine and organophosphorus were detected in all four rhizosphere even though farmers of the two commercial plantations acknowledged adding pesticides to the banana crops. It could be concluded that the pesticides used did not accumulate in the rhizosphere. While slight differences in bacterial community (Figure 1 and Table 1) and diversity indices (Table 2) were observed among the four soils, the differences were not distinct between the OA field soils and the commercial plantation soils. This is a good news as the addition of fertiliser and pesticide did not affect the soil bacterial composition and diversity in the commercial plantations, using the OA field soils for comparison. Similar findings were reported by Dong et al. (2014).

CONCLUSION

In this study we addressed two main questions: What is the bacterial community profile in banana plant rhizosphere in OA fields and commercial plantations? and do the profiles differ? The 16S DNA clone library results showed that the main phyla found in all the banana rhizosphere were Acidobacteria, Proteobacteria and Actinobacteria. We observed that the bacterial diversity in the OA banana fields (Paya Mendoi and Kg. Pian) was different from that of the commercial plantations (Parit Serong and Mersing), the PAST analysis provided evidence of higher bacterial diversity in the OA banana fields compared to the commercial plantations.

As the rhizosphere samples were collected at different locations and on different dates, the bacterial composition could be influenced by geological and climatic conditions such as terrain and soil structure, rain and drought. The absence of pesticide indicators (organochlorine and
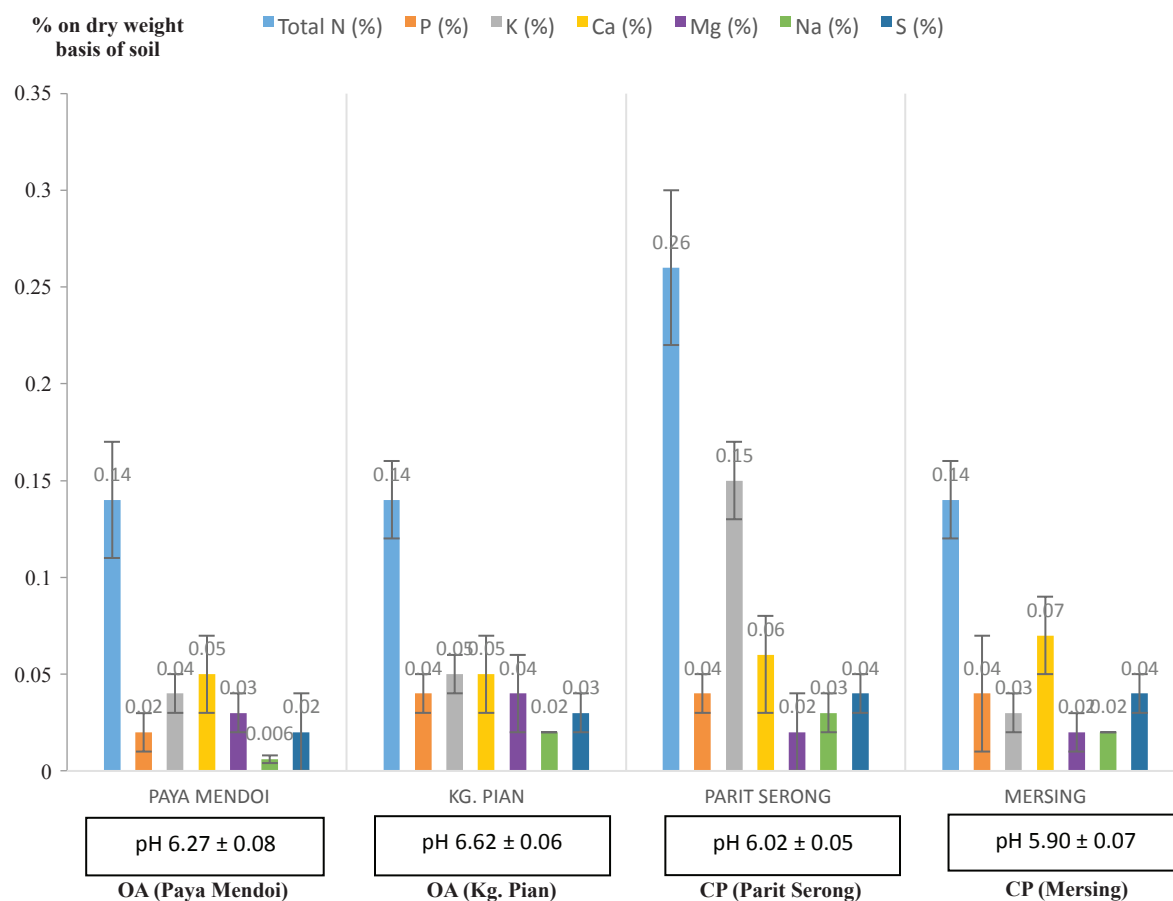
FIGURE 2. pH and some chemical composition of the OA field soils and commercial plantation soils

organophosphorus) in the commercial plantation soils might be due to degradation or washed away by rain. The addition of pesticides and fertilizers may increase plant production and hence organic matter levels in soil may also increase. This is generally beneficial as the soil nutrient sources are increased. However, it was also reported that addition of pesticides and fertilizers may contribute to toxic effects on soil microorganisms. Such effects may be direct or indirect, and are dependent upon several interacting factors such as the soil environment and the nature of the microbial populations. The dynamic nature of soil biology and the effects of environment will influence the fate of pesticides and fertilizers which, in turn, will influence the soil microbial community and function. Thus, it is difficult to draw conclusion about the impacts of various inputs in our agricultural systems. Long term application of fertilizer could affect the soil microbial diversity. It is known that members of the Actinobacteria and Proteobacteria groups decreased in population in long term fertilized soil. Our results show less bacterial varieties under the Proteobacteria group in the commercial plantation rhizosphere compared to the OA field rhizosphere.

This study established a library of 16S rRNA gene fragments from the banana plant rhizosphere of OA fields and commercial plantations as a means to indicate bacterial

composition. In a review of several studies where long-term effects of pesticides and fertilizers on soil microbial composition and function are still variable and unknown, future studies on rhizosphere bacterial communities could be strengthened by adopting newer molecular methods e.g next generation DNA sequencing (NGS) where it can perform better result in quantity and quality in a large amount of data generated.

REFERENCES

Abdullah, M.Y., Hassan, N.M., Mahmood, Z. & Talib, Z. 1999. Trend in foliar nutrient concentrations and contents and its implication on leaf area index development and yield in banana cultivar 'berangan'. In *Proceedings of the First National Banana Seminar*, edited by Zakaria, W., Mahmud, T.M.M., Siti, D., Khalijah, M.F., Nor Aini & Marziah, M. Awana, Genting, Pahang, Malaysia. pp. 95-105.

Aislabie, J. & Deslippe, J.R. 2013. Soil microbes and their contribution to soil services. In *Ecosystem Services in New Zealand - Conditions and Trends*, edited by Dymond, J. Lincoln, New Zealand: Manaaki Whenua Press. pp. 112-161.

Alain, K. & Querellou, J. 2009. Cultivating the uncultured: Limits, advances and future challenges. *Extremophiles* 13: 583-594.

Beauregard, M., Hamel, C. & St-Arnaud, M. 2010. Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. *Microbial Ecology* 59: 379-389.

Brady, N.C. & Weil, R.R. 1999. *The Nature and Properties of Soils*. 12th ed. Upper Saddle River, NJ: Prentice-Hall. p. 881.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K. & Vivanco, J.M. 2008. Root exudates regulate soil fungal community composition and diversity. *Applied and Environmental Microbiology* 74: 738-744.

Ceteciogly, Z., Ince, B.K., Kolukirik, M. & Ince, O. 2009. Biogeographical distribution and diversity of bacterial and archaeal communities within highly polluted anoxic marine sediments from the Marmara Sea. *Marine Pollution Bulletin* 3: 384-395.

Davis, K.E., Sangwan, P. & Janssen, P.H. 2011. Acidobacteria, Rubrobacteridae and Chloroflexi are abundant among very slow-growing and mini-colonyforming soil bacteria. *Environmental Microbiology* 13: 798-805.

de Bruyn, J.M., Nixon, L.T., Fawaz, M.N., Johnson, A.M. & Radosevich, M. 2011. Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. *Applied and Environmental Microbiology* 77: 6295-6300.

Dong, W.Y., Zhang, X.Y., Dai, X.Q., Fu, X.L., Yang, F.T., Liu, X.Y., Sun, X.M., Wen, X.F. & Schaeffer, S. 2014. Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. *Applied Soil Ecology* 84: 140-147.

Eilers, K.G., Debenport, S., Anderson, S. & Fierer, N. 2012. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology and Biochemistry* 50: 58-65.

Elisa, C.P.C., Fabyano, A.C.L., Janaina, F.A., Alinne, P.C., Cristine, C.B., Mercedes, M.C.B., Bertania, F.Q. & Ricardo, H.K. 2014. Soil Acidobacteria 16S rRNA gene sequences reveal subgroup level differences between Savanna like Cerrado and Atlantic Forest Brazilian biomes. *International Journal of Microbiology* 1: 1-12.

Fakruddin, Md. & Khanjada, S.M. 2013. Methods for analyzing diversity of microbial communities in natural environments. *Ceylon Journal of Science* 42(1): 19-33.

Faoro, H., Alves, A.C., Souza, E.M., Rigo, L.U., Cruz, L.M., Al-Janabi, S.M., Monteiro, R.A., Baura, V.A. & Pedrosa, F.O. 2010. Influence of soil characteristics on the diversity of bacteria in the Southern Brazilian Atlantic Forest. *Applied and Environmental Microbiology* 76(14): 4744-4749.

Ge, Y., Zhang, J., Yang, M. & He, J. 2008. Long-term fertilization regimes affect bacterial community structure and diversity of an agricultural soil in northern China. *Journal of Soil and Sediments* 8: 43-50.

Hani, A., Chantal, J.B., Nadia, G., Rock, C. & Roger, L. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). *Plant and Soil* 204: 57-67.

Head, M., Saunders, J.R. & Pickup, R.W. 1998. Microbial evolution, diversity and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. *Microbial Ecology* 35: 1-21.

Hirsch, P.R., Miller, A.J. & Dennis, P.G. 2013. Do exudates exert more influence on rhizosphere bacteria community structure than other rhizodeposits? *Molecular Microbial Ecology of the Rhizosphere* 22: 229-242.

Janda, J.M. & Abbott, S.L. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology* 45: 2761-2764.

Kirby, J.T., Sader, H.S., Walsh, T.R. & Jones, R.N. 2004. Antimicrobial susceptibility and epidemiology of a worldwide collection of *Chryseobacterium* spp.: Report from the SENTRY antimicrobial surveillance program (1997-2001). *Journal of Clinical Microbiology* 42(1): 445-448.

Lauber, C.L., Hamady, M., Knight, R. & Fierer, N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75: 5111-5120.

Lazcano, C., Brandon, M.G., Revilla, P. & Dominguez. J. 2012. Short-term effects of organic and inorganic fertilizer on soil microbial community structure and function. *Biology and Fertility of Soils* 12: 761-767.

Leadbetter, J.R. 2003. Cultivation of recalcitrant microbes: Cells are alive, well and revealing their secrets in the 21st century laboratory. *Current Opinion in Microbiology* 6: 274-281.

Liu, W.T., Marsh, T.L., Cheng, H. & Forney, L.J. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Applied and Environmental Microbiology* 63(11): 4516-4521.

Magurran, A.E. 2004. *Measuring Biological Diversity.* Oxford: Blackwell Publishing. p. 264.

Memon, N.N., Memon, K.S., Rachel, A.S.A. & Nafees, M. 2010. Status and response to improved NPK fertilization practices in banana. *Pakistan Journal of Botany* 42: 2369-2381.

Meng, F. 2013. The virulence factors of the bacterial wilt pathogen *Ralstonia solanacearum*. *Plant Pathology and Microbiology* 4(3): 168.

Mia, M.A.B., Shamsuddin, Z.H. & Mahmood, M. 2010. Use of plant growth promoting bacteria in banana: A new insight for sustainable banana production. *International Journal Agricultural and Biology* 12: 459-467.

Moyer, C.L., Dobbs, F.C. & Karl, D.M. 1993. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Applied and Environmental Microbiology* 60(3): 871-879.

Nadia, M.H., Jacques, M.A. & Koebnik, R. 2011. Adhesion mechanisms of plant-pathogenic Xanthomonadaceae. *Advances in Experimental Medicine and Biology* 5: 71-89.

Okano, Y. 2004. Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. *Applied and Environmental Microbiology* 70: 1008-1016.

Peacock, A. 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biology and Biochemistry* 33: 1011-1019.

Philippot, L., Hallin, S. & Schloter, M. 2007. Ecology of denitrifying bacteria in agricultural soil. *Advances in Agronomy* 96: 249-305.

Robinson, J.C. 1995. Systems of cultivation and management. In *Banana and Plantains,* edited by Gowen, S. London: Chapman and Hall. pp. 15-65.

Rossum, D., Muyotcha, A., Hoop, B., Verseveld, H.W., Stouthamer, A.H. & Boogerd, F.C. 1994. Soil acidity

in relation to groundnut-*Bradyrhizobium* symbiotic performance. *Plant and Soil* 163(2): 165-175.

Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R. & Fierer, N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *International Society for Microbial Ecology* 4: 1340-1351.

Rovira, A.D. 1991. Rhizosphere research - 85 years of progress and frustration. In *Beltsville Symposium in Agricultural Research: The Rhizosphere and Plant Growth,* edited by Keister, D.L. & Cregan, P.B. Dordrecht: Kluwer Academic Press. 14: 3-13.

Shannon, C. 1948. A mathematical theory of communication. *The Bell System Technical Journal* 27: 379-423.

Shyam, K.R., Ganesh, M.I., Rajeswari, R. & Harikrishnan, H. 2011. Utilization of waste ripe banana, and peels for bioethanol production using *Saccharomyces cerevisiae*. *Journal of Biosciences* 2(2): 67-71.

Simpson, E.H. 1949. Measurement of diversity. *Nature* 163: 688.

Stark, J.M. & Firestone, M.K. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology* 61(1): 218-221.

Surakasi, V.P., Anthony, C.P., Sharma, S., Patole, M.S. & Shouche, Y.S. 2010. Temporal bacterial diversity and detection of putative methonotrops in surface mats of Lonar crater lake. *Journal of Basic Microbiology* 50: 465-474.

Suyal, D.C., Yadav, A., Shouche, Y. & Goel, R. 2015. Bacterial diversity and community structure of Western Indian Himalayan red kidney bean *(Phaseolus vulgaris)* rhizosphere as revealed by 16S rRNA gene sequences. *Biologia* 70(3): 305-313.

Treseder, K.K., 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology Letters* 11: 1111-1120.

Tripathi, B.M., Kim, M., Dharmesh, S., Lee, L.C., Ang, L.H., Ainuddin, A.N., Go, R., Rahim, R.A., Husni, M.H.A., Chun, J. & Adams, J.M. 2012. Tropical soil bacterial communities in Malaysia: pH dominates in the equatorial tropics too. *Microbial Ecology* 64: 474-484.

Ward, D.M., Bateson, M.M., Weller, R. & Ruff-Roberts, A.L. 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. *Advances in Microbial Ecology* 12: 219-286.

Whitford, M.F., Forster, R.J., Beard, C.E., Gong, J. & Teather, R.M. 1998. Phylogenetic analysis of rumen bacteria by comparative sequence analysis of cloned 16S rRNA genes. *Anaerobe* 4: 153-163.

Wong, C., Kiew, R., Argent, G., Set, O., Lee, S.K. & Gan, Y.Y. 2002. Assessment of the validity of the sections in *Musa (Musaceae)* using AFLP. *Annals of Botany* 90: 231-238.

van Passel, M.W., Kant, R., Palva, A., Copeland, A., Lucas, S., Copeland, A., Lucas, S., Lapidus, A., Glavina del Rio, T., Pitluck, S., Goltsman, E., Clum, A., Sun, H., Schmutz, J., Larimer, F.W., Land, M.L., Hauser, L., Kyrpides, N., Mikhailova, N., Richardson, P.P., Janssen, P.H., de Vos, W.M. & Smidt, H. 2011. Genome sequence of the *Verrucomicrobium opitutus terrae PB90-1,* an abundant inhabitant of rice paddy soil ecosystems. *Journal of Bacteriology* 193: 2367-2368.

Institute of Biological Sciences
Faculty of Science
University of Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

Centre for Research in Biotechnology
for Agriculture (CEBAR)
University of Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

*Corresponding author; email: jasonleeyeeneng@hotmail.com