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)

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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

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 Ingemtumusa, 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 growth and activity.
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), phosphorus (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 Mensing, 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'-GTT TAC TTT GTG 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 MspI (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 purification kit (iNtRON Biotechnology, Korea). The purity was checked by agarose gels. 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 MspI (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.

SOIL BACTERIAL DIVERSITY BASED ON 16S CLONES

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 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.

PAST ANALYSIS

Surakasi et al. (2010) and Suyal et al. (2015) reported that the phylogenetic analysis of the clone sequences was further examined by using paleontological statistics (PAST) analysis (Cetecigoly et al. 2009). Shannon-Wiener diversity index was used to calculate Shannon index (H), evenness and the Simpson’s index (D).
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 phylgroup 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.

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

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
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.

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) and diversity in 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, while 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.

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

<table>
<thead>
<tr>
<th>Diversity indices</th>
<th>OA fields</th>
<th>Commercial plantations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paya Mendoi, Kg. Pian, Parit Serong, Mersing, Pahang, Pahang, Selangor, Johor</td>
<td></td>
</tr>
<tr>
<td>Individuals</td>
<td>117</td>
<td>165</td>
</tr>
<tr>
<td>Shannon_H</td>
<td>3.876</td>
<td>3.312</td>
</tr>
<tr>
<td>Menhinick</td>
<td>5.455</td>
<td>3.192</td>
</tr>
<tr>
<td>Margalef</td>
<td>12.180</td>
<td>7.834</td>
</tr>
<tr>
<td>Berger-Parker</td>
<td>0.103</td>
<td>0.206</td>
</tr>
<tr>
<td>Simpson_1-D</td>
<td>0.975</td>
<td>0.951</td>
</tr>
</tbody>
</table>

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 organophosphorus) 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).
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.

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