BACTERIAL DIVERSITY AND LIPASE PRODUCING BACTERIA IN FOREST AND OIL PALM PLANTATION AT SAROLANGUN JAMBI INDONESIA

MARINI WIJAYANTI

GRADUATE SCHOOL
BOGOR AGRICULTURAL UNIVERSITY
BOGOR
2015

DECLARATION OF THE SOURCE OF THIS DISSERTATION

I declare that this dissertation, entitled *Bacterial Diversity and Lipase Producing Bacteria in Forest and Oil Palm Plantation at Sarolangun Jambi Indonesia* is entirely my own work, assisted by a supervisory committee and has not been submitted in any form for another degree or diploma to any university or other tertiary institution of education. Where this dissertation draws on existing publications, those sources are cited in the text and listed in the references section.

Bogor, August 2015

Marini Wijayanti
G361110011

RINGKASAN

MARINI WIJAYANTI. Keragaman Bakteri dan Bakteri Penghasil Lipase dalam Hutan dan Perkebunan Sawit di Sarolangun Jambi Indonesia. Dibimbing oleh ANJA MERYANDINI, ARIS TRI WAHYUDI, and MUNTI YUHANA.

Deforestasi untuk pembukaan lahan perkebunan sawit paling masif terjadi di Asia Tenggara. Hal ini berdampak negatif pada keragaman hayati dan lingkungan. Tanah dan sedimen pada penelitian ini diperoleh dari lapisan atas tanah hutan dataran rendah dan perkebunan sawit, serta perairan yang disekitar situs tersebut. Hutan tersebut berlokasi di Taman Nasional Bukit Duabelas, dan perkebunan sawit sekitar hutan di Sarolangun Jambi Indonesia. Keragaman komunitas bakteri dari tanah dan sedimen perairan area hutan dan kebun sawit Sumatra dipelajari menggunakan pyrosequencing gen 16S rRNA dan indeks keragaman umumnya. Pendekatan filogenetik digunakan untuk mengungkap perubahan komunitas filotipe bakteri dan genusnya di kedua area. Pendekatan ekologis menggunakan nilai pH, kandungan Karbon (C) total, Nitrogen (N) total, Fosfor (P) tersedia dan keragaman bakteri menggunakan indeks Shannon dan Simpson, dan kelimpahan bakteri dengan indeks Chao1-ACE dan OTUs. Keragaman dan kelimpahan bakteri tanah di hutan dan kebun sawit tidak berbeda sebagaimana pH, C total, dan N total, sebagai faktor substrat yang tidak berbeda nyata. Tetapi, indeks kelimpahan bakteri di sedimen perairan area hutan justru lebih tinggi dari area kebun sawit.

Mayoritas sekuen berturut-turut Acidobacteria (56.33%), Proteobacteria (27.43%), Actinobacteria (7.11%), dan Cyanobacteria (5.55%) berasal dari tanah hutan, sementara Acidobacteria (50.11%), Proteobacteria (31.63%), Actinobacteria (7.58%), Chloroflexi (2.60%), dan Gemmatimonadetes (2.71%) dari tanah kebun sawit. Acidobacteria menjadi filotipe paling dominan di kedua habitat, karena pH tanah kedua areal tersebut sangat asam (3.77 – 4.80). Genera dari Alpha proteobacteria mendominasi di tanah hutan dan kebun sawit. Genus terbanyak di pohon filogenetik adalah Burkholderia dari Betaproteobacteria. Berbeda dari tanah, dalam sedimen perairan, mayoritas sekuen berturut-turut Proteobacteria (34.85%), Acidobacteria (32.67%), Nitrospirae (6.86%), Chloroflexi (4.31%), dan Actinobacteria (4.02%) dari area hutan, sementara dari area kebun sawit berturut-turut Acidobacteria (46.10%), Proteobacteria (25.86%), Nitrospirae (9.20%), Chloroflexi (4.99%), dan Actinobacteria (2.34%). Acidobacteria masih menjadi filotipe dominan di habitat sedimen perairan areal kebun sawit. Genera Alphaproteobacteria dan Betaproteobacteria mendominasi pada kedua pohon filogenetik gen16S rRNA baik di areal hutan maupun kebun sawit. Genus yang terdeteksi dominan pada pohon filogenetik bakteri asal tanah maupun sedimen area hutan dan kebun sawit adalah *Burkholderia*. Perubahan komunitas bakteri terjadi dalam transformasi hutan, meskipun lebih banyak ditemukan genus di pohon filogenetik bakteri tanah asal kebun sawit. Sebaliknya, komunitas di sedimen perairan transformasi hutan, sebagaimana sedimen perairan area hutan menunjukkan indeks keragaman, kelimpahan, filotipe dan genera yang lebih tinggi daripada yang ditunjukkan parameter tersebut dari area sawit.

Bakteri spesial di hutan dan lahan sawit adalah bakteri lipolitik. Enzim bakteri tersebut telah diaplikasikan di industri agroakuakultur, pangan, deterjen, farmasi, susu, maupun biodiesel-biokerosin. Informasi keragaman bakteri lipolitik asal tanah dan sedimen perairan di areal hutan dan perkebunan kelapa sawit, dan komposisi asam lemaknya diperlukan. Sebanyak 22 isolat terpilih dari tiga puluh dua isolat bakteri lipolitik yang tumbuh pada media selektif lipolitik, terdiri atas 11 isolat dari tanah lapisan atas dan 11 isolat dari sedimen air di hutan dan daerah perkebunan. Isolat-isolat bakteri diidentifikasi berdasarkan analisis gen 16S rRNA. Hasil identifikasi menunjukkan isolat-isolat tersebut terdiri atas lima genera yaitu *Burkholderia, Cupriavidus, Serratia, Acinetobacter,* dan *Kurthia*. Pada pohon filogenetik yang dibangun menggunakan metode *maximum likelihood,* isolat-isolat tersebut terdistribusi ke dalam tiga grup, yaitu grup *Burkholderia-Cupriavidus*, grup *Serratia-Acinetobacter*, dan grup *Kurthia*. Hasil analisis kromatografi gas (GC-FID) menunjukkan bahwa enzim lipolitik yang dihasilkan bakteri-bakteri tersebut memproduksi berbagai asam lemak. Beberapa isolat bakteri menghasilkan asam lemak esensial, seperti asam lemak: linoleat, linolenat, arakidonat, eikosapentanoat (EPA), dan dokosaheksanoat (DHA). Untuk masa depan, bakteri lipolitik dari hutan dan perkebunan sawit dapat dilanjutkan penelitian untuk kesejahteraan lingkungan dan manusia.

Kata kunci: *komunitas bakteri, bakteri lipolitik, hutan hujan dataran rendah, perkebunan sawit, Sumatra-Indonesia*

SUMMARY

MARINI WIJAYANTI. Bacterial Diversity and Lipase Producing Bacteria in Forest and Oil Palm Plantation at Sarolangun Jambi Indonesia. Supervised by ANJA MERYANDINI, ARIS TRI WAHYUDI, and MUNTI YUHANA.

Deforestation for oil palm plantation are the most invasive land use changes in Southeast Asia. It has impacted its rich and unique biodiversity. On the other hand, conversion of land to agricultural use such as oil palm plantations has even more negative impacts for environment. Soil dan sediment samples used in this study were obtained from topsoil in a lowland forest and on an oil palm plantation and from sediments in fresh water near these sites. The forest is located in Bukit Duabelas National Park, and the oil palm plantation is near the forest in Sarolangun District, Jambi Province, Indonesia. The diversity of bacterial communities from top soil and aquatic sediment lowland rainforest and oil palm plantation in Sumatra was studied using pyrosequencing of 16S rRNA gene and common biodiversity indices. Phylogenetic approach was used for revealing the community shift of bacterial phyla and genera in both areas. Ecological approach used soil pH, TC (Total Carbon), TN (Total Nitrogen), AP (Available Phosphorous) values measurement and bacterial diversity with Shanon and Simpson indices, and bacterial richness with Chao1-ACE index and OTUs. Bacterial diversity and richness in top soil lowland forest and oil palm plantation soil were not different, as soil pH, TC, and N as substrate factors were not different significantly. But, bacterial richness indices in aquatic sediment of forest area were higher than in plantation area.

The majority of sequences related to Acidobacteria (56.33%), Proteobacteria (27.43%), Actinobacteria (7.11%), and Cyanobacteria (5.55%) were from soil forest; whereas those related to Acidobacteria (50.11%), Proteobacteria (31.63%), Actinobacteria (7.58%), Chloroflexi (2.60%), and Gemmatimonadetes (2.71%) invented from soil of oil palm plantation. Acidobacteria was the most dominant phyla in both habitats, because soil pH in both areas was acidic (3.77 – 4.80 pH_{H_2O}). The genera of Alphaproteobacteria dominated in genera phylotype of bacterial 16S rRNA gene phylogenetic revealed both topsoil forest and oil palm plantation. The most genera in phylogenetic tree was *Burkholderia* from Betaproteobacteria. Differently from soil, in aquatic sediment, the majority of sequences related to Proteobacteria (34.85%), Acidobacteria (32.67%), Nitrospirae (6.86%), Chloroflexi (4.31%), and Actinobacteria (4.02%) were from forest; whereas those related to Acidobacteria (46.10%), Proteobacteria (25.86%), Nitrospirae (9.20%), Chloroflexi (4.99%), and Actinobacteria (2.34%) invented from oil palm plantation. Acidobacteria still was the most dominant phyla in oil palm sediment habitats. The genera of Alphaproteobacteria and Betaproteobacteria dominated in genera phylotype of bacterial 16S rRNA gene phylogenetic revealed both aquatic sediment of forest and oil palm plantation.

The most genera in phylogenetic tree of 16S rRNA gene from soil and aquatic sediment of both areas was *Burkholderia*. The bacterial community shift

occured in forest transformation, eventough the oil palm plantation showed more bacterial phyla and genera than the lowland forest. Contrary, the bacterial community shift occured in aquatic sediment of forest transformation, as like as the aquatic sediment from forest area showed more bacterial diversity index, richness index, phyla, and genera than these ones from oil palm plantation.

The special bacteria in forests and on oil palm plantations are lipolytic bacteria. Their enzymes have been applied in the agro-aquaculture, food, detergent, pharmaceutical, dairy, and biodiesel-biokerosene industries. The diversity of cultivable lipolytic bacteria from soil and aquatic sediment in a forest and on an oil palm plantation and their fatty acid products. Twenty-two isolates of lypolitic bacteria were selected from 32 isolates grown in lipolytic selective medium. The 22 consisted of 11 isolates from topsoil and 11 from aquatic sediment from the forest and plantation area. These isolates were identified by 16S rRNA gene-sequence data analysis. Taxonomically, they belonged to five genera: *Burkholderia, Cupriavidus, Serratia, Acinetobacter,* and *Kurthia*. The maximum likelihood tree showed that they are phylogenetically distributed in three clusters. They were clustered into three groups: the *Burkholderia-Cupriavidus* group, the *Serratia-Acinetobacter* group, and the *Kurthia* group. Their lipolytic enzymes formed various fatty acids after analysis by gas chromatography-flame ionization detector (GC-FID). Some isolates formed essential fatty acids, such as linoleic, linolenic, arachidonic, eicosapentanoic acid (EPA), and docosahexanoic acid (DHA). In future, lipolytic bacteria from forest and oil palm plantation can be continued research for human and environment welfare.

Key words: *bacterial communities, lipolytic bacteria, lowland rainforest, oil palm plantation, Sumatra-Indonesia*

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Marini Wijayanti

Dissertation
as one of the requirements of the PhD program
in the Major Microbiology

GRADUATE SCHOOL
BOGOR AGRICULTURAL UNIVERSITY
BOGOR
2015

Examiners in the Closed Examination:

1. Dr I Made Artika, MAppSc
2. Dr Iman Rusmana, MSi

Examiners in the Doctoral Promotion:

1. Dr Achmad Dinoto
2. Dr Iman Rusmana, MSi

Title of Dissertation : Bacterial Diversity and Lipase Producing Bacteria in Forest and Oil Palm Plantation at Sarolangun Jambi Indonesia

Name : Marini Wijayanti
Student ID : G 361110011
Major : Microbiology

Approved by the Supervisor Committee

Prof Dr rer nat Anja Meryandini, MS
Head of Committee

Prof Dr Aris Tri Wahyudi, MSi
Member of Committee

Dr Munti Yuhana, MSi
Member of Committee

Authorized by,

Head of Major Microbiology

Prof Dr rer nat Anja Meryandini, MS

Dean of Graduate School

Dr Ir Dahrul Syah, MSc Agr

Date of Closed Examination :
11 August 2015

Date of Doctoral Promotion :
28 August 2015

Date of Graduation : 0 SEP 2015

ACKNOWLEDGEMENTS

Alhamdulillahirabbil'alamin. It would not have been possible to complete this dissertation without the support, patience and guidance of the following people. It is to them that I owe my deepest gratitude.

- My promotor, Prof Dr rer nat Anja Meryandini, MS for her endless support, enthusiasm, knowledge and patience
- My co-promotor, Prof Dr Aris Tri Wahyudi, MSi for his support, guidance and knowledge during my graduate study at Bogor Agricultural University
- My co-promotor, Dr Munti Yuhana for his support, guidance, and input throughout finalizing my dissertation.
- Aquaculture Program, Faculty of Agriculture, Sriwijaya University and Indonesian Minister of Education for the doctorate scholarship and research funding (BPPS-DIKTI 2011 and Hibah Disertasi Doktor 2014)
- Dr Ir Gayuh Rahayu,MS and Prof Dr rer nat Anja Meryandini, MS as former Head and Head of Graduate Programe Microbiology in Bogor Agricultural University for their support during my study, respectively.
- Dr Iman Rusmana, MSi for his support and guidance to think as microbiologist
- Prof. Rolf Daniel and Martin Engelhaupt (R.I.P) for facilitating the molecular work at the Genomic Laboratory, the Institute of Microbiology and Genetics at George-August University, Göttingen, Germany, via CRC990 EFForTS project B02.
- Dr I Made Artika, MappSc and Dr Achmad Dinoto as examiners at the closed examination and the doctoral promotion.
- My fellow postgraduate students and friends both at Bogor Agricultural University Susan Soka, Tri Handayani Kurniati, Ifah Munifah, Roni Ridwan, Sipriyadi, Wahyu Ekasari, Violita, Ernin Hidayati, Dade Jubaedah and her husband Julisman, Ani Suryanti, Andi Dharmawan, Zamroni, Bu Catur, Bu Yayin, Bu Nina, and graduate students of BOT, AKU, and SDP, Nur Rohmah Kumalasari, Desniar, Siti Nuryati, Pak Jaka, Bu Dewi, Bu Heni, Bu Hely and the others for their support and friendship.

Finally, and most importantly, I take this opportunity to express my gratitude to my family for their love, unfailing encouragement and support. I would like to thank my husband, Mohamad Amin, for his patience during the ups and downs of my study. I would like to thank my daughter and son, Nurfahmia Marisa Amin and Muslih Munadi Amin, for their loves and supports. I thank my parents and sister, Budi Wijaya Adi, Sumarsi, and Marheni Wijayanti, for their love and faith in me. And also, I thank Amin's parents, for providing me with unending encouragement and support.

Bogor, August 2015

Marini Wijayanti

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Cont.

LIST OF APPENDIXES

1 INTRODUCTION

Background

Southeast Asia is one of the most important hot spots of biodiversity. It has been reported that in this region the above-ground diversity has been strictly affected by land use changes. Deforestation and agricultural intensification are the most invasive land use changes in Southeast Asia. In contrast to other tropical regions, Southeast Asia has the highest deforestation rate. It has impacted its rich and unique biodiversity. On the other hand, conversion of forest to agricultural use such as oil palm plantations has even more negative impacts for environment (Tripathi *et al.* 2012). Between 1999–2006, 3.5 million hectares of Indonesian forest were damaged per year. Between 2006–2010, 2 million hectares per year were damaged, and between 2010–2012, 300,000 hectares per year. In Jambi Province, Sumatra, Indonesia, land has been transformed from forest to temporary cropland and agro-forest (Partohardjono *et al.* 2003). Loss of environmental services provided by forests is a non-linear process in this province. A gradual simplification of complex agro-ecosystems to agro-forests with increased profitability might be threatened by the oil palm plantation industry (Mudiyarso *et al.* 2002).

The tropical low land forests are ecosystems that are rich in endemic species of flora, fauna and microbes like Bacteria. Lee-Cruz *et al.* (2013) reported that bacteria were grouped into operational taxonomic units (OTUs) at the 97% similarity level, and OTU richness and local-scale diversity (alpha-diversity) showed no difference between the various forest types and oil palm plantations. But, focusing on the turnover of bacteria across space, true diversity (beta-diversity) was higher in oil palm than in forest, whereas community dissimilarity-based metrics of β-diversity were only marginally different between habitats, suggesting that at large scales oil palm could have higher overall diversity (gamma-diversity) than forest, driven by a slightly more heterogeneous community across space. Clearance of primary and logged forest for oil palm did, however, significantly impact the composition of soil bacterial communities, reflecting in part the loss of some forest bacteria, whereas primary and logged forests did not differ in composition. The soil bacteria of tropical forest are to some extent flexible or resistant to logging, but that impacts of forest conversion to oil palm are more severe (Lee-Cruz *et al.* 2013). Fluctuations of diversity of bacterial community in lowland forest and oil palm plantation in Jambi, Indonesia, have not been scrutinized yet. The impact of lowland rainforest transformation to oil palm plantation on phylogenetic of soil and freshwater sediment bacterial communities in Sumatra, Indonesia has not been studied.

Land use has a low effect on the bacterial community composition. However, bacterial community composition and diversity was strongly correlated with soil properties, especially soil pH, total carbon, and C/N ratio. Soil pH was the best predictor of bacterial community composition and diversity across the various land use types, with the highest diversity close to neutral pH values. In

addition, variation in phylogenetic structure of dominant lineages (*Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Acidobacteria, and Actinobacteria*) is also significantly correlated with soil pH. Together, these results confirm the importance of soil pH in structuring soil bacterial communities in Southeast Asia. Soil bacteria in oil palm plantation is actually richer than primary forest (Tripathi *et al.*, 2012). Among the most abundant operational taxonomic units (OTUs), there was clear evidence of niche partitioning by pH. It showed that pH plays a major role in structuring tropical soil bacterial communities. Soil pH was the best predictor of diversity and community composition of bacteria, being a stronger predictor than land use (Tripathi *et al.*, 2013). The distribution of some bacterial groups and subgroups in different sites correlated with soil properties and vegetation. Soil organic matter (SOM), total nitrogen (TN), and specific plant group were important factors shaping the composition of bacterial communities (Li *et al.* 2014). Tsiknia *et al.* predicted that 31–79% of the spatial variation in microbial taxa abundance could be explained by the parameters measured, with total organic carbon and pH being identified as the most important (Tsiknia *et al.* 2014).

High fertilizer input is necessary to sustain high yields in oil palm agroecosystems, but it may endanger neighboring aquatic ecosystems when excess nutrients are transported to waterways. The low nutrient concentrations recorded in streams throughout the landscape indicated that the mature oil palm plantations in this study did not contribute to eutrophication of aquatic ecosystems. Soil type controlled dissolved inorganic N and total P fluxes, with greater losses of N and P from loamy-sand uplands than loamy lowlands (Comte *et al*. 2015). One reason was the conversion of the huge forest zone to oil palm plantations over the Johor River Basin which caused modifications in the surface soil layer and vegetation canopy. Land-use variation plays a vital role in local water cycle changes, especially for the water movement within the soil layer (Tan *et al*. 2014). The change of water nutrient cause change of microbial community in the water which is dominated in water sediment habitat. There are increasing oily substrates in soil and water as impact of forest transformation to oil palm plantation area which receive fallen palm oil fruit and oily water from Palm Oil Mill Effluent (POME).

Bacteria are important for most nutrient transformations in soil and are major drivers of bio-geochemical cycles in oil palms plantation. One of special bacteria in oil palm plantation is lipolytic bacteria. Fallen palm oil fruit and debris from the cell membranes of dead animals have provided lipolytic bacteria substrates for degrading and synthesizing many kinds of fatty acids in the soil of oil palm plantations and forests. Lipolytic bacteria have been found in forest grass and soil (Nacke *et al.* 2011), marine sediment (Hu *et al.* 2010; Peng *et al.* 2011), river surfaces (Wu *et al.* 2009), hot spring water (Tirawongsaroj *et al.* 2008), agricultural industry waste (Salihu *et al.* 2012), soil contaminated by wastewater treatment (Glogauer *et al.* 2012), and endophytic lipolytic bacteria of palm oil fruit (Djafar *et al.* 2010). Lipolytic bacteria secrete lipase, which is used by many industries. The majority of lipase currently used industrially has been isolated from cultivated bacteria.

Bacteria produce and secrete lipases, which can catalyze both the hydrolysis and the synthesis of long-chain acylglycerols. Lipolytic bacteria produce

extracellular lipases in their environments. These enzymes are capable of hydrolyzing and synthesizing carboxyl esters of long-chain acylglycerol (≥10 carbon atoms). They have applications in various industries, such as food, detergent, pharmaceutical, dairy, biodiesel-bio-kerosene, and bioremediation (Jaeger *et al.* 1999; Casas-Godoy *et al.* 2012). They can be developed in sustainable agro-aquaculture if the applications are cheaper than those used in industry. Lipases are produced by various organisms, including animals, plants, fungi, and bacteria. Bacterial enzymes are easier to manipulate in culture. Commercially, lipases from bacteria have been produced by *Burkholderia cepacia, Pseudomonas mendocina, Pseudomonas alcaligenes,* and *Chromobacterium viscosum* (Jaeger *et al.* 1999). The various lipase producing bacteria could be found in the forest and oil palm plantation areas. They will be an added value of the bacterial diversity in forest transformation to oil palm plantation.

Hypothesis

Transformation of low land forest (become oil palm plantation) make change of biota diversity. It makes shifting of their bacterial community in top soil and freshwater sediment around them. Bacterial community shift in both habitats might play a role as balancing nutrient in soil and water for natural environment sustainability. Utility of shifting bacterial community is founding unique bacteria for the community welfare.

The dominant bacteria in forest and oil palm plantation soil and sediment are lipase producing bacteria. Diversity of lipolytic bacteria in forest and oil palm plantation should be important to search superior candidate of lipase producing bacteria.

Objectives

The objectives of this study was to describe the diversity of bacteria from low land forest and oil palm plantation (transformation area) in soil and sediment habitats. In this study, phylogenetic approach for analyses the community shift of bacteria between both areas was used. Special bacteria in both areas is lipolytic bacteria. This study also described the diversity of cultivable lipolytic bacteria from soil and aquatic sediment in a forest and in the area of an oil palm plantation, their capability for producing lipases, and their fatty acid composition of product.

Novelty

The impact of lowland rainforest transformation to oil palm plantation on phylogenetic of soil and freshwater sediment bacterial communities in Sarolangun, Jambi, Sumatra, Indonesia has not been studied. This research studied how to use ecological and pyrosequencing approach for estimating bacterial community shift and deciding unique bacteria for developing in livelihood welfare such as lipolytic bacteria. Lipase producing bacteria which have been dominant shifted bacteria for forest transformation to oil palm plantation area, revealed shifting diversity of lipolytic product as various fatty acid for essentiall fatty acid production.

Outcomes

This bacterial diversity study gave several basic information for shifting bacterial communities in the transformation forest to oil palm plantation. The information could be used by the stake holder for recomendation of land use management, so they will give several policy for preserving the forest area and developing sustainable oil palm plantation for environment welfare.

This lipase producing bacteria study have given several information of lipase diversity invented from forest and oil palm plantation area for developing agro-aqua-industries that use or produce the bacterial lipase.

2 LITERATURE REVIEW

Bacterial Diversity in Shifting Environment, Transformation Forest to Oil Palm Plantation

The bacterial diversity is one of microbial diversity which play important role in the world. The microbial diversity is described as the number of different species and their relative abundance in a given community in a given habitat. The soil is considered as one of the major reservoirs of microbial diversity on our planet (Swift *et al.* 1998) with an estimated bacterial diversity of 10^4 to 10^6 taxa per 1 g of soil (Torsvik *et al.* 1990, Gans *et al.* 2005). Torsvik and Overeas (2002) found that 1 g of soil has 4000 different species of microbes and the bacterial population in top layers of soil can be more than 10^9 individual cells per gram soil. Microorganisms in soil are critical to the maintenance of soil function because of their involvement in key processes like soil formation, decomposition of organic matter, toxin removal and the cycling of carbon, nitrogen, phosphorus, and sulphur (van Elsas and Trevors 1997). Besides soil functions, microbes especially bacteria, are involved in every sphere of human life ranging from industry, medical, food, bioremediation, production of energy, and mining (Sharma *et al.* 2014). The bacterial diversity was affected by soil ecological processes which resulted by modifications of the soil physicochemical properties and vegetation cover (Rampeloto *et al*. 2013).

Land use systems generally results in modifications of the soil physicochemical properties and vegetation cover, which, in turn, affects soil ecological processes such as nutrient cycling and gas emissions but it is still unclear how these changes affect the soil microbial diversity. Moreover, microorganisms are one of the main active components of soil, regulating the nutrient cycling and affecting the plant productivity and stability of ecosystems. Since microorganisms are the main actors regulating nutrient cycling in soil, they directly affect plant productivity and the stability of ecosystems. The knowledge of how microbial diversity are influenced by soil management systems may help us to understand changes in carbon balance, energy flow, and greenhouse gas fluxes in these areas that have shifted. Such knowledge is fundamental for the sustainable management of forest and agricultural ecosystems in this threatened hotspot of biodiversity (Rampelotto *et al.* 2013).

The bacterial diversity of the forest soil was phylum rich compared to the agricultural soils, which are species rich but phylum poor. It is the most comprehensive examination to date of bacterial diversity in soil and suggests that agricultural management of soil may significantly influence the diversity of bacteria and archaea (Roesch *et al.* 2007). Bacteria not only make up a large proportion of the biological diversity of the rainforest environment but also are a fundamental component of nutrient cycling and productivity. Logging and forest conversion for agriculture drive changes to the soil chemistry; for instance, they can alter the pH, lead to the loss of soil carbon, and modify the C/N ratio and the content of phosphorus and calcium. Bacterial soil communities are in many ways a product of their chemical and physical environment and therefore can be affected by such physicochemical changes to the soil (Lee-Cruz *et al*. 2013)

The Shannon entropies of multiple communities can be averaged to give what is known in information theory as the ‘‘conditional entropy’’, Hα, of the set of communities. Because Shannon entropy is a concave function, Hα is always less than or equal to the gamma entropy Hγ, the entropy of the pooled communities. Though Hα is often called the ‘‘alpha diversity’’ in biology, it is of course really an entropy. It can be converted to the true alpha diversity by Equation:

$$^1D = \exp\left(-\sum_{i=1}^{S} p_i \ln p_i\right) = \exp {}^1D_\alpha = \exp(H_\alpha)$$

Likewise the amount of information provided by knowledge of the sample location is often called the beta diversity in biology but is actually an entropy. Like the alpha entropy, it can be converted to the true beta diversity by the Equation. The same tranformation also converts gamma entropy to true gamma diversity.

The relation between the Shannon alpha, beta, and gamma entropy follows directly from information theory:

$$H_\alpha + H_\beta = H_\gamma$$

By converting both sides of this equation to true diversities via the Equation, the relation between alpha, beta, and gamma diversity is obtained:

$$\exp(H_\alpha + H_\beta) = \exp(H_\gamma)$$

So,

$$(\exp(H_\alpha))(\exp(H_\beta)) = \exp(H_\gamma)$$

Or

(Alpha diversity)(Beta diversity) = (Gamma diversity)

Shannon or order 1 diversity thus necessarily follows Whittaker’s (1972) multiplicative law. The minimum possible beta diversity is unity, which occurs when all communities are identical. The maximum possible beta diversity is N, the number of communities; this occurs when all N communities are completely distinct and equally weighted. Alpha and beta diversity are independent of each other regardless of the community weights.

Forest logging cycles had no effect on alpha-diversity and a small positive effect on true beta-diversity. True beta-diversity, which compares the total OTU richness (alpha-diversity) in relation to the average OTU richness (beta-diversity) for each land use, was higher for twice-logged forests a singletons were removed. Combined, these results suggest that oil palm plantation soil has a higher turnover of bacterial diversity at large spatial scales and is more heterogeneous, whereas bacterial composition in forest soils tends to be more homogeneous. However, the results also suggest that the marginal increase in community dissimilarity beta-diversity in oil palm plantation soil is due to the presence of rare OTUs, because differences disappeared when singleton OTUs were removed (Lee-Cruz *et al.* 2013).

Soils from oil palm plantations had significantly lower abundances of *Acidobacteria* than primary forest, whereas *Actinobacteria* was more abundant in oil palm plantations than in all forests. Similar results have been found for these phyla in studies of soils in the Amazon and forested land in the United States (Bossio *et al*. 2005, Shange *et al*. 2012). *Acidobacteria* are oligotrophic organisms abundant in carbon-poor soils (Nemergut *et al.* 2010). Carbon and nitrogen content often decreases in cultivated soils, such as lower concentrations of total carbon and total nitrogen and a lower C/N ratio in soil from oil palm plantations than from forests. However, *Acidobacteria* are also strongly influenced by pH, favoring soils with pH values lower than 4. Primary forest soils had lower pH than oil palm plantation soils, probably promoting *Acidobacterial* abundance (Lee-Cruz *et al*. 2013). Oligotrophs exhibit slower growth rates and are likely to outcompete copiotrophs in conditions of low nutrient availability due to their higher substrate affinities. Copiotrophs preferentially consume labile soil organic C pools, have high nutritional requirements, and can exhibit high growth rates when resource conditions are abundant. It follows then that soils with large amounts of available organic C should favor copiotrophs while oligotrophs should predominate in soils where organic C quality and/or quantity is low. Bacteria belonging to the Acidobacteria phylum were most abundant in soils with very low resource availability (low C mineralization rates) and their relative abundances were lowered in an individual soil amended with high concentrations of organic C. In contrast to the oligotrophic Acidobacteria, the β-Proteobacteria, and Bacteroidetes exhibited copiotrophic attributes; their relative abundances were highest in soils with high C availability either as an intrinsic property of the soil or as a result of sucrose amendments. The metaanalysis provided further support for this ecological categorization, with the Acidobacteria less abundant, and the β-Proteobacteria (as well as the α- and γ-Proteobacteria) more abundant, in rhizosphere soils than in the comparatively C-poor bulk soils. These results do not suggest that every member of the Acidobacteria, β-Proteobacteria, and Bacteroidetes phyla are distinctly copiotrophic or oligotrophic. The dominant autotrophic ammonia oxidizers are β-Proteobacteria, they are likely to exhibit oligotrophic characteristics (Fierer *et al.* 2007)

In general, copiotrophic bacteria should have higher growth rates, a greater degree of variability in population size, and lower substrate affinities than oligotrophic bacteria. In environments where microorganisms are exposed to sustained environmental stress, particularly where the stress stems from low resource concentrations, oligotrophs are likely to outcompete copiotrophs. The

microbial succession on substrates rich in organic matter, such as leaf litter or other types of fresh organic detritus, should be dominated by copiotrophs in the earlier stages, with oligotrophs increasing in relative abundance as substrate quality and/or quantity declines over time (Jackson 2003). In contrast, the oligotrophs to be the first to colonize nutrient-poor substrates, such as mineral surfaces, with copiotrophs increasing in abundance as communities mature and develop in size (Fierer *et al.* 2007).

However, bacterial community composition and diversity was strongly correlated with soil properties, especially soil pH, total carbon, and C/N ratio. Soil pH was the best predictor of bacterial communitycomposition and diversity across the various land use types, with the highest diversity close to neutral pH values. In addition, variation in phylogenetic structure of dominant lineages (Alphaproteobacteria, Beta/Gammaproteobacteria, Acidobacteria, and Actinobacteria) is also significantly correlated with soil pH (Tripathi *et al*. 2012). Soil bacterial community composition and diversity of the six analyzed management types showed significant differences between the land use types grassland and forest. Furthermore, bacterial community structure was largely driven by tree species and soil pH. Land use in itself has a weak but significant effect on the bacterial community composition (Nacke *et al*. 2011).

Pyrosequencing Reveal Bacterial Community Shift

The biogeography and ecophysiology of bacteria can be drawn from the mere presence or absence of their 16S rRNA gene sequences in an ecosystem. The assessment of the diversity and biogeography of bacteria increase the perception of natural diversity beyond the level of 16S rRNA gene sequences (Jaspers and Overmann 2004). Bacterial diversity can be measured by cultivable and uncultivable approach. Most of the soil microorganisms cannot be cultured, and only a small fraction of soil microbial diversity is assessed. Problem solving of this limitation is using molecular biology and protein engineering strategies, such as meta-genomic (Glogauer *et al.* 2011). Metagenomic is a culture-independent genomic analysis of whole microbial communities inhabiting an exacting niche (Armougom and Raoult 2009). Cultivable approach can present quantitative yields and detailed information on diversity of bacterial communities, less than 1% of total microbial community is cultivable. Cultivation and molecular-based approaches can be used to study microbial diversity in two different environments such as sediment or soil with compared condition (Besaury *et al.* 2012). Culture-dependent methods have been complemented or replaced by culture-independent meta-genomic approaches. The use both molecular and cultivable based methods can give the opportunity to collect information on the composition of bacterial communities. Methods of meta-genomic such DGGE and pyrosequencing can be compared with cultivation method. Qualitative analysis of cost-benefits for three methods in study of bacterial community is described in Table 1. Culture-dependent and independent should be used as complementary, mainly if the objective of the study is related with risk assessment (Vaz-Moreirra *et al*. 2009).

If 454 pyrosequencing was compared with microarray, Roh *et al.* (2010) described in Table 2. Pyrosequencing is the first alternative to the conventional

Sanger method for *de novo* DNA sequencing. Pyrosequencing is a DNA sequencing technology based on the sequencing-by-synthesis principle. It employs a series of four enzymes to accurately detect nucleic acid sequences during the synthesis. Pyrosequencing has the potential advantages of accuracy, flexibility, parallel processing, and can be easily automated. Furthermore, the technique dispenses with the need for labeled primers, labeled nucleotides, and gel-electrophoresis. Pyrosequencing has opened up new possibilities for performing sequence-based DNA analysis.

Table 1 Qualitative analysis of cost-benefits for the three methods (Vaz-Moreira *et al*. 2009)

	Cultivable bacteria	DGGE	454 Pyrosequencing
Cost			
Time consumed	Medium	High	Medium
Equipment	Low	Medium	High
Reagent	Low	High	High
Benefits			
Taxonomical accuracy	High	Low	Low
Ease of performance and interpretation	High	Medium	Low
Bacterial diversity coverage	Low	Medium	High

Table 2 Pyrosequencing and DNA microarray in study comparative

	454 pyrosequencing	DNA microarray
•System type	Open architecture system	Closed architecture system
•Depth of sample coverage	Higher	Lower
•Appropriate throughput	Lower	Higher
•Ease of sample preparation	Simple to prepare PCR product or extracted DNAs	Complex preparation of target and microarray slides
•Cost efficiency with multiple sample	More expensive per sample	Less expensive per sample (for short oligo arrays)
•Ease of data handling analysis	Complex annotation and sorting of massive sequence reads	Simple analysis of signal intensities
•Applicability to study of species genomic relatedness	Potencially the best method with complete re-sequencing of microbial genomes	Currently the best for the DNA-DNA hybridization method
•Recommended application	In depth studies of unknown microbial communitydiversity	Routine studies of functional gene diversity across many samples (spatio temporal mapping of functional)

The method has been proven highly suitable for single nucleotide polymorphism analysis and sequencing of short stretches of DNA. Pyrosequencing has been successful for both confirmatory sequencing and *de novo* sequencing**.** By increasing the read length to higher scores and by shortening the sequence reaction time per base calling, pyrosequencing may take over many broad areas of DNA sequencing applications as the trend is directed to analysis of fewer amounts of specimens and large-scale settings, with higher throughput and lower cost (Fakruddin and Mannan 2013).

In most previous studies the effects of land use and soil properties on soil bacterial communities have been assessed by employing traditional molecular

methods such as Sanger sequencing-based analysis of 16S rRNA gene libraries or fingerprinting methods. These approaches are often limited to the analysis of a relatively small number of clones and a few different soil samples. Taking into account the large bacterial community size and the heterogeneity of soils, only a tiny fraction of the bacterial diversity was unraveled by these methods. Recently, high-throughput pyrosequencing of 16S rRNA gene fragments has been applied for in-depth analysis of soil bacterial communities (Nacke *et al*. 2011). PCR-amplified soil DNA for the bacterial 16SrRNA gene targeting the V1–V3 region was pyrosequenced using the 454 Roche machine.

Table 3 Pros and contras of pyrosequencing methods (Fakruddin *et al.* 2012)

Pros	Contras
Expedient (1 day)	Expensive
High throughput (>200000)	Short read for phylogenetic inference (next generation)
Sequence length can be as high as > 200bp	Bioinformatics cumbersome
Unlimited sample number	Relative high error rate (0.0098)
Simple frequency data	Long single dNTP strings unreliable (8bp linearity)
It is a fast method with real time read out that is highly suitable for sequencing short stretches of DNA	Long fusion primers may bring bias
It can generate sequence signals immediately downstream of the primer	.
Sample preparation and single-strand DNA process is also relatively rapid	.
The reagent costs are consideraly lower for sequencing short stretches of DNA compared to current available methods	.

Lipase Producing Bacteria

Lipases are a class of hydrolases which catalyze hydrolyses of triglycerides to glycerol and fatty acid on oil water interface, they are coded EC 3.1.1.3. This enzymes are capable of hydrolyzing carboxyl esters of long-chain acylglycerol (≥ 10 carbon atoms) and synthesizes long chain acylglycerols. They have applications in food, detergent, pharmaceutical, dairy industries, and biodiesel-bio-kerosene (Jaeger *et al.*1999; Casas-Godoy *et al.* 2012). Lipases are produced by various organisms, including animals, plants, and microorganisms. Lipases have been isolated from many bacteria including *Bacillus prodigiosus*, *Bacillus pyocyaneus*, *Bacillus fluorescens*, S*erratia marcescens, Pseudomonas aeruginosa*, and *Pseudomonas fluorescens.* Commercially, lipases from bacteria have been produced by *Burkholderia cepacia, Pseudomonas mendocina, Pseudomonas alcaligenes,* and *Chromobacterium viscosum* (Casas-Godoy *et al.* 2012). Lipase producing bacteria is bacteria which produce and secrete lipolytic enzymes, lipase.

Lipase have a catalytic triad consisting of a serine nucleophile, aspartate / glutamate as acid residue, and histidine. Subclass lipase consensus sequence G-X1-S-X2-G contains the catalytic serine is replaced by a sequence GDSL placed near the terminal N (Godoy *et al.* 2012). Lipolytic enzymes usually are extracellular enzymes that have a specific gene with the chaperonin. Lipase gene

(*lip*A) and specific foldase gene *lif* (*lip*B) forming an operon in *Acinetobacter* sp. XMZ-26 (Zheng *et al.* 2012). At *Acinetobacter* sp DYL129 are found three lipase genes (lipA1, lipA2, and lipA3) which located at down stream of the chaperon gene (lipBA1A2A3) to have a common consensus motif GHSHG, the lipase active site. This lipase is a potential for biodiesel production (Kim *et al*. 2008). Genes encoding bacterial lipase usually consists of *lip*A and *lip*B or *lip*BA, while for the type of Actinomycetes usually have *lip*R than *lip*A. LipR is a transcriptional activator protein of LipA. Position of *lip*R is in the region upstream of the promoter -35 *lip*A which serves as the main activator of type II (Evangelita-Martinez 2006). Based on amino acid sequence comparisons and biological character, prokaryotic lipolytic enzymes can be grouped into eight families, relatives first familie called as true lipase, an enzyme with the motif Gly-Asp-Ser-(Leu) [GDS (L)] which contains the active site Ser (GDSL) as a family second, family third, hormone sensitive lipase (HSL) family IV, and V until family VIII esterase (Arpigny and Jaeger 1999). Type of lipase gene have unique character for each other. Superior lipase can be gotten from selected of diversity of lipase producing bacteria.

Jaeger and Eggert (2002) have explained that bacterial lipases are extracellular enzymes and must therefore be translocated through the bacterial membrane to reach theirfinal destination. Figure 1 summarises the major secretion pathways for bacterial lipases. In Gram-positive bacteria, secreted enzymes have to cross just a single cytoplasmic membrane. Usually, these proteins contain a signal sequence, which directs their translocation via the Sec machinery. More recently, a second translocation pathway has been described to operate in both Gram-negative and Gram-positive bacteria, named the Tat pathway because proteins using this pathway contain a unique Twin arginine translocation motif in their signal sequence. In the *B. subtilis* genome, 188 proteins have been identified as being potentially secreted. These include two lipases of which LipA contains a Tat signal sequence, whereas the highly homologous enzyme LipB contains a Sec signal sequence.

Several Gram-negative bacteria are known to efficiently secrete extracellular lipases, among them *Pseudomonas* and *Burkholderia* species. In *Pseudomonas aeruginosa*, at least four main secretion pathways have been identified of which extracellular lipases use the type II pathway: after being secreted through the inner membrane via the Sec machinery they fold in the periplasm into an enzymatically active conformation. Periplasmic folding catalysts are needed to ensure the correct folding and proper secretion of lipases, these include specific intermolecular chaperones called Lif proteins (lipase-specific foldases). A lipase variant from *Pseudomonas* species KFCC 10818 carrying just the single amino acid exchange Pro112Gln folded into its active conformation and displayed 63% of the wild-type enzymatic activity even in the absence of its cognate Lif protein. If confirmed with other Lif-dependent lipases these findings may have important consequences for the construction of novel high-yield production host strains (Jaeger and Eggert 2002).

Lipases from *Pseudomonas fluorescens* and *Serratia marcescens* lack a typical N-terminal signal peptide. They are secreted by the type I secretion pathway (also named the ABC exporter) consisting of three different proteins. The lipase from *S. marcescens* is a biotechnologically important enzyme because it

catalyses with high enantioselectivity (E = 135) the asymmetric hydrolysis of (*rac*) *trans*-3-(4-methoxyphenyl) glycidic acid methyl ester yielding a key intermediate in the synthesis of diltiazem, a major pharmaceutical used as a coronary vasodilator. A thorough analysis of the lipase secretion process in *S. marcescens* revealed that a C-terminal Val-Ala-Leu motif and its location relative to the C terminus of the lipase greatly affect the secretion efficiency. The motif identified here is different from a previously described secretion motif, a glycine-rich repeat consisting of the nine-residue sequence Gly-Gly-X-Gly-X-Asp-X-U-X (where X is any amino acid and U is a large hydrophobic amino acid). Studies with the lipase from *Pseudomonas* species MIS38, which is similar to the *S. marcescens* and *P. fluorescens* lipases, clearly showed that this latter motif is needed for the binding of 12 Ca^{2+} ions, thereby inducing the folding of this lipase. Overexpression of additional copies of the ABC exporter provides a considerable increase in secretion of the lipase and therefore an increased yield of extracellular lipase protein, as demonstrated for *S. marcescens* and *P. fluorescens* lipases (Jaeger and Egert 2002).

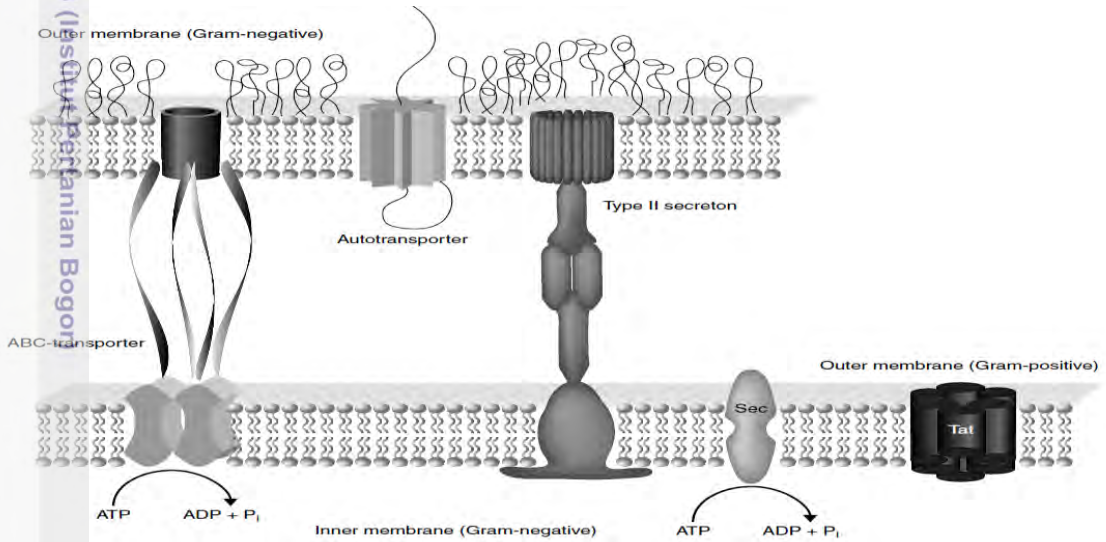


Figure 1 Pathways used by bacteria to secrete lipolytic enzymes. Gram-positive bacteria contain an inner or cytoplasmic membrane, Gram-negative bacteria additionally possess a second so-called outer membrane. The Sec and Tat secretion pathways mediate translocation of proteins through the inner membrane and are found in both Gram-positive and Gram-negative bacteria; the type I (ABC transporter-) and type II(secreton-) mediated pathways and the 'self-secreting' autotransporter enzymes are found in Gram-negative bacteria (Jaeger and Eggert 2002).

Lipase Diversity

Lipases are the most widely used class of enzymes in biotechnology. Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Lipases occur widely in nature, but only microbial lipases are commercially significant. The many applications of lipases include speciality organic syntheses, hydrolysis of fats and oils, modification of fats, flavor enhancement in food processing, resolution of racemic mixtures, and chemical analyses (Sharma *et al.* 2001).

This includes their applications in organic synthesis and kinetic resolution of racemic compounds. There are three main reasons (Kapoor and Gupta 2012):

a. Foremost reason, which is often overlooked, is that industrial preparations of many lipases were available in view of their applications in early industrial enzymology. The major early application was in fat splitting. Hence, when other areas like enzyme catalysis in low water media developed, these became a convenient and preferred choice.

b. Lipases are somewhat a unique class of enzymes in carrying out reactions often in heterogeneous media. Associated with this fact is that a very large number of lipases show the phenomenon of interfacial activation. The latter phenomenon distinguishes lipases from esterases. “To withstand the denaturing effect of the interface, lipases have evolved unusually stable structures that may survive even the effect of organic solvents”.

c. Lipases have broad substrate specificities. Esters of fatty acids as well as alcohols of various chain lengths are hydrolyzed. Similarly triglycerides formed from long chain fatty acids of varying chain lengths are also hydrolyzed. Apart from hydrolysis, lipases can also catalyze esterification, interesterification and transesterification in low water media. What has made lipases even more versatile is the fast developing area of catalytic promiscuity. Hence the above-mentioned third factor promises to be the main driver for further work in this area. This review looks at lipases in the context of their versatility as biocatalysts from the above perspective.

Lipases are serine hydrolases which act at the lipid–water interface. The catalytic triad is composed of Ser-Asp/Glu-His and usually also a consensus sequence (Glyx-Ser-x-Gly) is found around the active site serine. The three-dimensional (3-D) structures of lipases reveal the characteristic α/β-hydrolase fold (Nardini and Dijkstra 1999). Lipases naturally catalyze the hydrolysis of the ester bond of tri-, di-, and monoglycerides into fatty acids and glycerol (Figure 2). Nevertheless, as shown in Figure 2, they are also active on a broad range of substrates. In all cases, the reaction is carried out at the interface of a biphasic system reaction. This biphasic system results from the presence of an immiscible organic phase, containing the hydrophobic substrate, in water. Lipases, in thermodynamic favorable conditions (i.e., low water activity), also catalyze a large variety of synthesis reactions which can be classified into two main types of reactions, i.e., esterification and transesterification. As shown in Figure 2, esterification is the reaction where a fatty acid is linked, through the action of the enzyme, to an alcohol by a covalent bond, producing an ester and releasing a water molecule. Thio-esterification and amidation are similar reactions but with a thiol or an amine as substrates. Transesterification groups alcoholysis, acidolysis, aminolysis, and interesterifi cation reactions. Usually, these synthesis reactions occur in a medium with low thermodynamic water activity, the thermodynamic activity being a measure of the molecule availability in a solvent. The medium then consists in a free-solvent system (molten medium) or in an organic solvent. Finally, lipases are also capable of expressing other annex activities such as phospholipase, lysophospholipase, cholesterol esterase, cutinase, or amidase activities (Casas-Godoy *et al.* 2012).

Bacterial lipolytic enzymes were classified by Arpigny and Jaeger into eight families, I to VIII, according to their amino acid sequences and biological

properties (Arpigny and Jaeger 1999). This classification is still the reference most commonly used to assign a newly discovered enzyme to a family, though with certain modifications, because new families have been discovered through metagenomics. The current state of this classification of bacterial lipolytic enzymes is summarized in Table 1 (Lopez-Lopez *et al*. 2014). The Lipase Engineering Database (LED) has been designed to serve as a navigation tool for systematic analysis of the relationship of sequence, structure, and function of this rapidly growing, highly diverse protein class, and for the design of variants with optimized properties. The LED integrates information on sequence and structure (http://www.led.uni-stuttgart.de) (Fischer and Pleiss 2003).

Hydrolysis

Synthesis

a. Esterification

Esterification

Amidation

Thioesterification

b Transesterification

Acidolysis reaction

Aminolysis reaction

Alcoholysis reaction

Interesterification reaction

Figure 2 Reaction catalyzed by lipase (Casas-Godoy *et al.* 2012)

Table 4 Current bacterial lipolytic enzymes classification. Description of bacterial lipolytic families I-VIII in the Arpigny and Jaeger classification and new families and subfamily discovered by functional metagenomics (*) (Lopez-Lopez *et al*. 2014)

Family	Description
I	• Group of true lipases subdivided into six subfamilies: Pseudomonas lipases and relatives (subfamilies I.1, I.2 and I.3), Bacillus and Staphylococcus lipases and relatives (I.4 and I.5) and other lipases (I.6).
II	• Modified pentapeptide motif around the active serine: Gly- Asp-Ser-(Leu) [GDS(L)]. • Secreted and membrane-bound esterases
III	• Extracellular lipases and esterases.
EstA*	• Related to family III but different conserved motifs (pentapeptide GHSMG). • Discovered from surface seawater
IV	• Many members of this family show sequence similarity to mammalian hormone-sensitive lipase (HSL). • Typical motif HGG. • Lipolytic enzymes from psychrophilic, mesophilic and thermophilic origins. • EstB*: New subfamily in family IV with second active site glutamate (conserved sequence EXLLD) instead of the aspartate (DPLXD) of the representative members of family IV. It was discovered in surface sea water
V	• Conserved motif HGGG upstream of the pentapeptide motif GDSAG. • Sequence similarity with non-lipolytic enzymes: epoxide hydrolases, dehalogenases and haloperoxidases. • Esterases from psychrophilic, mesophilic and thermophilic origins.
EstF*	• Related to family V but with a modified pentapeptide, GTSXG, and different flanking regions around the HG motif and their own unique conserved sequence motifs. • Isolated from deep sea sediments
VI	• The smallest esterases known (23–26 kDa). • Sequence similarity to eukaryotic lysophospholipases.
VII	• Large bacterial esterases (55 kDa). • Sequence homology with eukaryotic acetylcholine esterases and intestine\ liver carboxylesterases.
VIII	• Similarity to several class C. -lactamases.
LipG*	• Presence of an Arg-Gly sequence in oxyanion hole instead of His-Gly, a signature sequence distinctive of filamentous fungal lipases. • Isolated from tidal flat sediments
LipEH166*	• Comprise newly discovered lipase LipEH166 of psychrophilic origin, and three putative open reading frames. • Isolated from intertidal flat sediments
EstY*	• Derived from pathogenic bacteria. First possible lipolytic virulence factors that do not belong to the GDSL family. • Isolated from surface river water

The diversity of lipolytic enzymes have caused the diversity of lipolytic bacterial product. It has been shown by diversity of fatty acid product.

3 MATERIALS AND METHODS

This study explored the bacterial diversity, their shifting from lowland forest to oil palm plantation using 454-pyrosequencing for unculturable top soil and aquatic sediment bacteria. The special oil palm plantation bacteria such as lipolytic bacteria were isolated for culturable bacteria. The steps of this study are described in Figure 3.

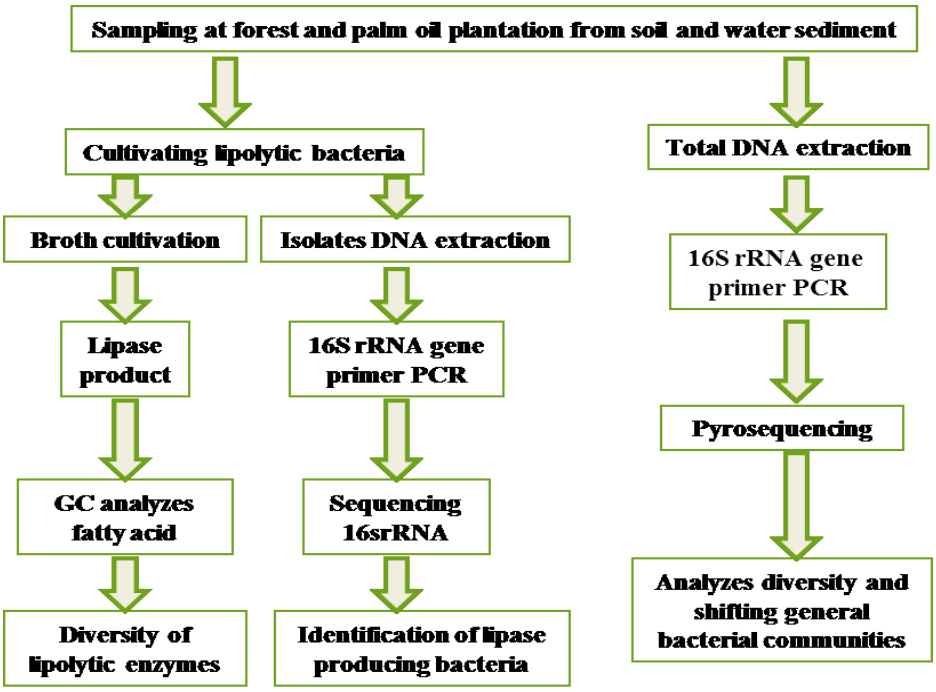


Figure 3 Diagram of general methods of this study

Soil and sediment sampling

Samples used for this research were derived from the topsoil of a lowland forest and an oil palm plantation and from sediment in fresh water near these sites. Soils in the Bukit Duabelas area are represented by clay Acrisols and in the oil palm plantation are represented by modified Acrisols (Guillaume *et al.* 2015).

The forest is located in Bukit Duabelas National Park, and the oil palm plantation is near the forest in Sarolangun District, Jambi Province, Indonesia. Notations used on the samples were BF1, BF3, and BF4 for the top soil of lowland forest area, and BO2, BO3, and BO4 for the top soil of oil palm plantation area. SBF1, SBF3 and SBF4 were notation used on the aquatic sediment sample from forest area, and SBO2, SBO3, and SBO4 for aquatic sediment of oil palm plantation area. Samples were collected in December 2012 and May 2014. In each of these six sub areas, small cores were collected in five replicates and triplicates of each core (approximately 10 grams) from topsoil and surface sediment (approximately 5 cm from the top), using a small shovel and tube (approximately 2 inches in diameter) (Figure 4).

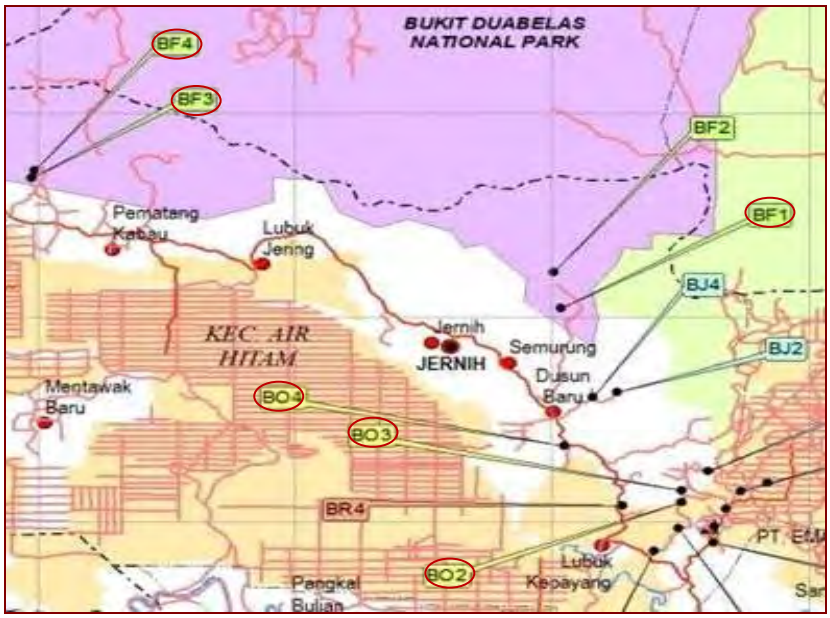


Figure 4 Map of Sampling Location from Lowland Forest (Bukit Duabelas National Park) and Oil Palm Plantation at Sarolangun, Jambi Province, Sumatra, Indonesia (between 102º32'00''–102º45'00'' East and 1º55'00''–2º50'00'' South)

The samples have been maintained at ± -30ºC. Samples from the same area were combined and mixed completely to produce one homogeneous sample. This study was done at Laboratory of Microbiology, Faculty of Mathematic and Natural Science, Bogor Agricultural University and Göttingen Genomics Laboratory, George August University of Göttingen, Germany.

Acidity of soil and sediment was in situ measured with a pH meter. All chemical parameters of soil (acidity, Carbon, Nitrogen, available Phosphorus) were determined according to ISRIC standards (van Reeuwijk 2002) at the Soil Research Institute, Bogor, Indonesia. All samples were kept on ice up to arrival at the laboratory and then were stored at 4ºC. Samples from the same area were combined and mixed completely to produce one homogeneous sample. These samples are BF (soil from lowland forest), BO (soil from oil palm plantation), SBF (aquatic sediment from lowland forest), and SBO (aquatic sediment from oil palm plantation).

DNA Extraction from soil/sediment

Total microbial community DNA was isolated from 10 g of soil or sediment per sample. For this purpose, the Mo Bio Power Soil DNA extraction kit (Mo Bio Laboratories, Carlsbad, CA) was used according to the instructions of the manufacturer. The Power Soil® DNA Isolation Kit from MO BIO Laboratories, Inc., was applied according to the manufacturer's instructions. The kit contained a special inhibitor removal solution for the removal of humic acids and other disturbing substances. A special solution was included which allows the removal of humic substances, respectively brown color from even the most difficult soil types (Ettenauer *et al.* 2012).

The concentration and quality of the DNA preparations were estimated by a NanoDrop spectrophotometer and by electrophoresis on 0.8% agarose gels.

Measurements were assessed using a NanoDrop® ND-1000 spectrophotometer (peqLab Biotechnologie GmbH, Linz, Austria). The analyses were performed according to the manufacturers' protocol and the extracted DNA of all triplicate sample amounts from the method was measured three times. Mean values were calculated for each sample amount. The purity of the extracted DNA was assessed by measuring the A260/A280 absorbance ratio which indicates the presence of impurities as protein, phenol, humic acids or other contaminants that strongly absorb at or near 280 nm. They were used for the electrophoresis and for the PCR amplifications.

454-Pyrosequencing

Pyrosequencing is a DNA sequencing method based on the sequencing-by-synthesis principle first described by Melamade and Wallace (1985). In principle, this method is based on the detection of the sequential incorporation of deoxyribonucleotides (dNTPs) during DNA synthesis (Ronaghi *et al.* 1996 and 1998). In general, the pyrosequencing method comprises four enzymatic steps: the DNA polymerase I bind at the primer of the single-stranded DNA template. During incorporation nucleotides inorganic pyrophosphate molecules (PPi) are released. Subsequently, this released PPi molecules are used to produce ATP molecules by the enzyme ATP sulfurylase. The next step is the conversion of the ATP molecules into a luminometric signal which is catalyzed by the luciferase enzyme and can be detected by a charge coupled device (CCD) camera resulting in a pyrogram. This enables detection of the light signal only in cases after successful base pair formation between the incorporated nucleotide and the template strand. Finally, unincorporated nucleotides and remaining ATP molecules are removed by the apyrase enzyme (Ronaghi *et al.*, 1998). A new reaction cycle can then be started by adding another dNTP. Eventually, the sequence of the template strand corresponds to signal peaks of the program (Figure 5). This sequencing method is applied in the 454 Life Science Pyrosequencing Platform providing a highly parallel sequencing platform. The 16S rRNA gene amplicons generated by PCR are immobilized by attaching them to DNA capture beads whereas each bead carries a unique single-stranded amplicon. Next, these beads are emulsified together with amplification reactions in a water-oil mixture to generate microreactors for amplification. Amplification leads to a manifold of copies of the respective sequence. Afterwards, the emulsion is broken whereas amplicons remain bound to the beads. The beads are loaded onto a picotiter plate containing ~ 1.6 million picoliter wells with a well diameter fitting only for one single bead.

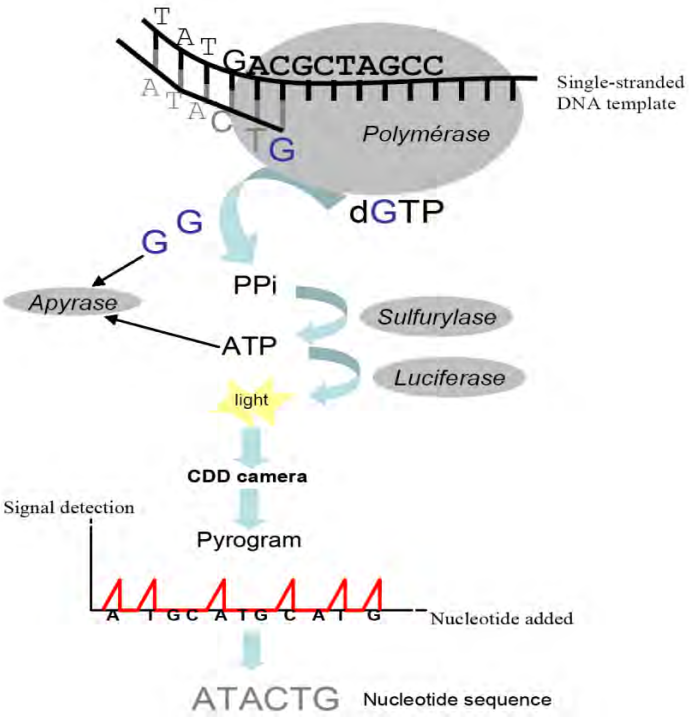


Figure 5 Schematic overview about the function principle of the pyrosequencing method (Armourgon, 2009).

In order to sequence multiple samples in parallel a barcoding strategy was applied (Figure 6). This approach is based on the association of a unique DNA sequence tag, called barcode, to the DNA template. During pyrosequencing the barcode is also sequenced allowing assignment of each sequence read to the respective sample origin. An entire list of barcode MIDs used in this study is shown in Table 5. The principle design of the sequencing primer is shown in Figure 7.

Table 5 List of barcode MIDs used for amplicon-based pyrosequencing of 16SrRNA gene amplicons.

Sample	Bacteria
BF	V3for-MID49
BO	V3for-MID50
SBF	V3for-MID51
SBO	V3for-MID52
All samples	V5rev

Primer name		Sequence	
V3for-B-MID49	5'	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCGATCGATACGGRAGGCAGCAG	3'
V3for-B-MID50	5'	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTAGCAGTATACGGRAGGCAGCAG	3'
V3for-B-MID51	5'	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTCACGTATACGGRAGGCAGCAG	3'
V3for-B-MID52	5'	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTATACATATACGGRAGGCAGCAG	3'
V5rev-B	5'	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCGTCAATTCMTTTGAGT	3'

Note: BF (soil from lowland forest), BO (soil from oil palm plantation), SBF (aquatic sediment from Lowland forest), and SBO (aquatic sediment from oil palm plantation)

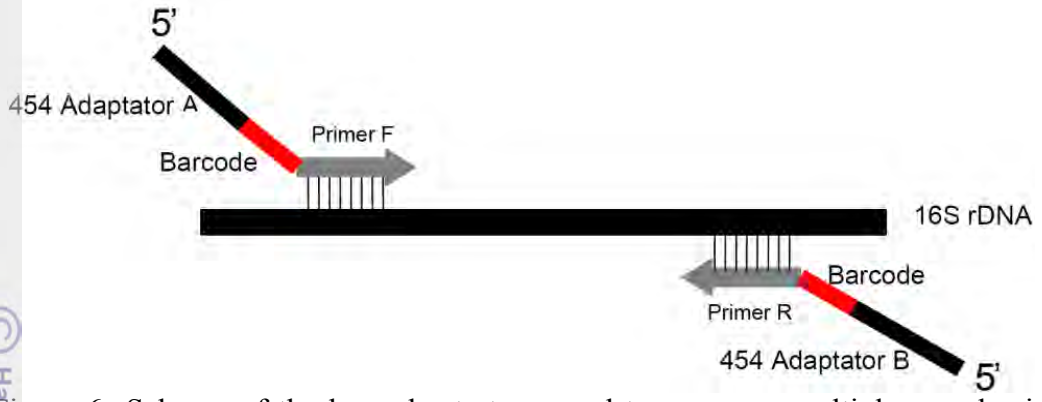


Figure 6 Scheme of the barcode strategy used to sequence multiple samples in parallel (Armourgon, 2009)

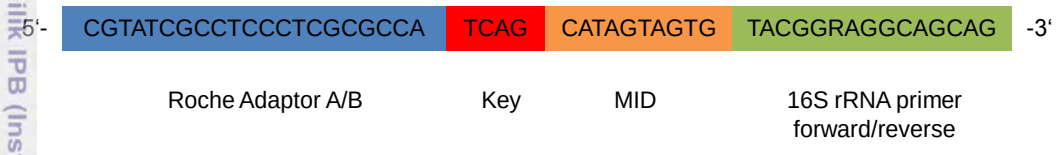


Figure 7 General design of the sequencing primer.

Note: The sequencing primer comprises four different oligonucleotides: the Roche Adaptor is located at the 5'-end whereas adaptor A is used in forward primer and adaptor B in the reverse primer. The adaptor is followed by a four bp key-sequence. The MID is a ten bp long oligonucleotide that is specific for each sample. Whereas, in case of the reverse primer always the same MID was used. At the 3'-end the oligonucleotide specific for 16S rRNA gene amplification of either bacterial or archaeal 16S rRNA genes is located. This schematic example shows the sequencing primer construct for the bacteria-specific forward primer of layer 1. For pyrosequencing a GS FLX System (Roche) was used together with the GS FLX Titanium Sequencing kit XLR and sequencing was carried out by the Göttingen Genomics Laboratory (G2L).

Bacterial 16S rRNA genes were amplified using the forward primer 343F (5'-TACG-GRAGGCAGCAG -3') and the reverse primer 909R (5'-CCGTCAATTCMTTTGAGT -3') (Liu *et al.* 2007; Wang 2009). Products were confirmed by subjecting 2 µl of each sample to electrophoresis on a 0.8% agarose gel. The PCRs were carried out in triplicate with 50 µl of the reaction mixture comprising 10 µl of 10-fold Phusion buffer, 1 µl of 10 mM dNTPs, 1 µl of each primer (1:10), 2-5 µl of diluted DNA sample, 0.5 µl of Phusion HS DNA Polymerase (2U/µl), and approximately 10 ng of DNA template by using the programmable thermo cycler (MJ Research PTC-200 Peltier Thermal Cycler, MJ Research, Inc., Malham, USA). The amplification program consisted of an initial denaturation step at 98°C for 5 min, followed by 25 cycles of denaturation at 98°C for 45 sec, annealing at 55-68°C for 45 sec, and elongation at 72°C for 30 sec, with a final extension step at 72°C for 5 min. The replicate PCR products of the same soil treating group were assembled within a PCR tube. The PCR products were visualized on agarose gels (0.8% in TAE buffer) containing ethidium bromide, and purified using a peqGOLD Gel Extraction Kit (PEQLAB Biotechnologie GmbH, Erlangen, Germany). In general, gel extraction was performed according to manufacturer's instruction. Quantification of the PCR products was performed by using the Quant-iT dsDNA BR assay kit and a Qubit

fluorometer (Invitrogen GmbH, Karlsruhe, Germany) as recommended by the manufacturer. The Goettingen Genomics Laboratory determined the sequences of the partial 16S rRNA genes by employing the Roche GS-FLX 454 pyrosequencer (Roche, Mannheim, Germany) and using picotiter sequencing plates subdivided into 8 parts (1 part per sample). Amplicons were sequenced as recommended in the instructions of the manufacturer for amplicon sequencing (Will *et al.* 2010).

In the quality filtering, several filtering options can be set, if sequences that were too short (less than 250 base pair) or too long (more than 1000 base pairs), or have more than 8 bases of the same base in a row had been filtered out of the total dataset. The Quality filtering had also given the number of raw sequence from the initial sequence dataset after sequencing (in the first line of the table) and the number of sequences after quality filtering (last line of the table) (Appendix 1). With this sequence number the next steps of the analysis had been done. Furthermore, the table explained the minimal, maximal and the average sequence length for each of the samples. The next step for processing the sequence data was to cluster the sequences into Operational Taxonomic Units (OTUs). That means that all sequences in all of the samples had been compared to each other and if there is similarity between the sequences, they had been grouped into one OTU. The grouping was based on a sequence similarity of 99 %. So, if some sequences were at least 99 % similar to each other than compared to other sequences in the dataset, they were grouped together. After grouping the sequences into OTUs, from each OTU one representative sequences had been picked. With this representative sequence from each OTU the taxonomic assignment had been made. This means that the representative sequences had been classified by their taxonomy. This taxonomic assignment made by using a database which include huge amount of already known 16S rRNA gene sequences. The database used for the taxonomic assignment is the SILVA SSURef NR 111 database (http://www.arb-silva.de/) (Quast *et al.* 2013). After taxonomic assignment, the number of sequences in this particular OTU for each of the samples, and the respective taxonomic classification, we calculated rarefaction curves as well as the Shannon index based on OTU picker data, by employing the RDP pyrosequencing pipeline (Magurran and McGill 2011, Cole *et al.* 2009). ACE and Chao1 indices (Chao and Bunge 2002) were calculated using the EstimateS program version 8.2.0 (http://purl.oclc.org/estimates).

Isolation of cultivable lipase-producing bacteria

Soil and sediment were serially diluted with NaCl 0.85% solution to 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Bacteria in this solution were spread on a selective medium with olive oil. The medium used for isolation and screening contains 2.5% olive oil (w/v); 0.2% glycerol (w/v); 0.002% rhodamine B, Merck (w/v); 1.5% peptone (w/v); 0.2% KH_2PO_4 (w/v); 0.01% $MgSO_4.7H_2O$ (w/v); 0.5% NaCl (w/v); 0.5% yeast extract (w/v); 3.0% agar (w/v); and 0.1% cycloheximide (w/v) (Djafar *et al.* 2010). The medium was adjusted to pH 5.5 with HCl 0.2 M. Plates are incubated at 28 °C for 3 d. Colonies on the plates were coded as BF, BO, SBF, and SBO. Colonies with orange fluorescent halos around them were purified by repeated streaking on the selective medium agar plates, and the pure culture of the single

colony was picked out for lipase production in liquid medium, without agar and rhodamine B.

Profile of fatty acid product using GC-FID

Cell culture samples (5 mL) were centrifuged at 4°C and 12,000 x g for 30 min, and the supernatants were assayed for lipase activity after NaCl saturation of each supernatant of solution. Two milligrams of recovered lipids are reconstituted in 1 mL of 0.5 M methanolic KOH and hydrolyzed at 80 °C for 1 h. Then, 1 mL of fresh 10% BF3 in methanol was added. Trans-esterification was performed at 100 °C for 20 min. After trans-esterification, 2 mL DH_2O and 1 mL hexane were added to the sample to quench the reaction. The recovered organic phase was pooled and spiked with methyl ester of 21:0 to a final concentration of 25.0 µg/mL. Products of reactions were determined using a standard fatty-acid methyl-ester mixture (FAME Mix) (10 mg FAME Mix per dichloromethane) by GC (Gas Chromatography) methods. An Agilent Technologies 6890 Gas Chromatograph with Auto Sampler, a 6890 Flame Ion Detector, and a Chemstation data system were used. The column was an HP Ultra 1 capillary column with length 50 (m) X 0.200 (mm) I.D. X 0.11 (µm) film thickness. Ovens used an initial temperature of 160 °C for 2 min, rose at 2 ºC/min to 240 ºC, rose at 10 ºC/min to 290 ºC and held for 10 min. Injection port, detector, and inlet temperatures were 250 ºC, 250 ºC, and 260 ºC, respectively. Carrier gas was Helium. The column mode was constant flow 1.5 µL/min., and the injection volume was 5 µL.

DNA Sequencing of 16S rRNA gene

Cells grown on broth selective medium (pH 6–7) for 24 h were harvested by centrifugation (10,000 x g, 3–5 min). The supernatant was discarded, leaving approximately 25 µL of liquid. The pellets were resuspended in mixer for 10 sec. Solution DNA was purified with Master PureTM DNA Purification Kit (EPICENTRE, Madison, WI) according to the manufacturer's protocol. The DNA was resuspended in 35 mL of TE buffer. A PCR amplifying the nucleotide of the *Escherichia coli* 16S rRNA gene was carried out with the forward primer, 341F (5'-CCTACGGRAGGCAGCAG-3'), and the reverse primer, 1114 R (5'-GGGTTGCGCTCGTTRC-3') (Liu *et al*. 2007). In a total volume of 50 µL, 30 µL DEPC-treated water and 2.5 µL DNA template were added (Altschul *et al.* 1997). PCR products were purified with a QIA quick spin kit (QIAGEN, Hilden, Germany) and were sequenced directly with a SequiTerm Excel II LC DNA sequencing kit (Epicentre Technologies, Madison, WI). An automated, infrared laser fluorescence sequencer (Model 4000 DNA Sequencer; Li Cor, Lincoln, NE) was used for sequencing. Sequences of each sample were analyzed by a BLAST homology search tool (Altschul *et al.* 1997).

Phylogenetic analysis

The Ribosomal Database Project and the BLASTN 2.0.6 algorithm of GenBank were employed to search for close evolutionary relatives of the 16S rRNA gene sequences of bacterial isolates. Alignments were generated with ClustalW. The partial 16S rRNA genes were sequenced at two different sites,

forward and reverse, with an overlap between the sequences. Therefore, it was possible to align both sequences from the same isolate to get a longer fragment of the 16S rRNA gene region. Aligning the two sequences led to a consensus sequence. Furthermore, false base pairs could be edited and exchanged, or added, if there were missing base pairs. The consensus sequence was copied and compared with an NCBI database sequences from isolates and reference sequences from known strains, and sequences from the results of the NCBI search were aligned with the Aligner program from http://www.arb-silva.de/ (http://www.arb-silva.de/aligner/). The phylogenetic tree was constructed with the MEGA5 program by using Maximum Likelihood methods. Bootstrapping (Felsenstein 1985) was used to estimate the reliability of phylogenetic reconstructions with 1,000 replicates.

4 RESULTS AND DISCUSSIONS

Result of this study was divided to three parts. Two part are diversity of soil bacteria and aquatic sediment bacteria from Bukit Duabelas low land forest and oil palm plantation area. The other is the potensial culturable bacteria, lipolytic producing bacteria from soil and aquatic sediment from the forest and the oil palm plantation.

Soil Bacteria in Forest and Oil Palm Plantation

Environmental variable and bacterial diversity indices

The compositions of soil bacterial communities present in two different soil systems derived from a forest site (site BF) and an oil palm plantation site (site BO) were assessed and compared using pyrosequencing-based analysis of 16S rRNA gene sequences. They could be influenced by soil chemical properties of samples from different land use types, the forest and oil palm plantation sites such as soil pH, Total Carbon (TC), Total Nitrogen (TN), Available Phosphorus (AP), and C/N ratio (Table 6). The t-test of two analyzed soil area showed no significant differences in some soil parameters (pH, TC, TN, and C/N ratio), but signifiant differences in available phosphorus (AP). The available P content of the soil of site BO (0.42%) was higher than that of site BF (0.02%). Available phosphorus varied significantly between forest and nonforest sites, while soil pH, total carbon, total nitrogen, and C/N ratio did not vary significantly across the landscape.

Table 6 Mean values (±standard deviation) of soil parameters for soil samples from the forest site (BF) and the oil palm plantation site (BO).

Sample sites	pH (H_2O)	pH (KCl)	TC (%)	TN (%)	AP(%)	C/N ratio
BF	3.77 ± 0.31	3.13 ± 0.47	3.93 ± 1.83	0.27 ± 0.06	0.02 ± 0.00	14.08 ± 3.50
BO	4.80 ± 0.79	4.43 ± 0.93	2.91 ± 0.86	0.22 ± 0.05	0.42 ± 0.13	13.15 ± 1.83

Forest conversion for agriculture drive changes to the soil chemistry. They can alter the pH, lead to the loss of soil carbon, and modify the C/N ratio and the content of phosphorus. Soil pH was found to be the best predictor of

bacterial community across forest and plantation area (Tripathi *et al.* 2012). Soil pH in both area were no significant differences, although soil pH of BO sample was higher than those of BF sample relatively. This condition could be considered by liming on the plantation. Worldwide acid soils cultivated with oil palm represent more than 50% of the total area. Under highly acidic soil conditions represented by Ultisols, which constitute the most important soils for oil palm growth in Tropical Asia and Tropical Latin America. Increasing rates of dolomite and $MgCO_3$ were found to reduce phytotoxic concentrations and activities of Al species and Mn in highly acidic soils (Cristancho *et al.* 2014).

Total carbon and C:N ratio were lower in oil palm soils than in forests, suggesting that in this case other factors that impact on acid specific bacteria, such as pH, had a stronger role than any effect the carbon concentration and C:N ratio might have had on the copiotropic bacteria, like as Proteobacteria (Lee Cruz *et al.* 2013). Oil palm plantation had been added with anorganic fertilizer (agricultural lime and phosporous fertilizer) for agricultural management. They changed increasing soil pH even though no significant differences between both areas. Based on Tropical Soil Quality Index (TSQI), Total Carbon (3.93 and 2.91 %) and Nitrogen (0.27 and 0.22 %) in both sites BF and BO were still in range of moderate-adequate levels, but Available P (214.33 and 4236.00 $mg.kg^{-1}$) in both sites were high – excellent range that reserve of available P in slightly acidic to alkaline soils, possible adverse effects to water quality from erosion of high P soils (Arifin *et al.* 2012).

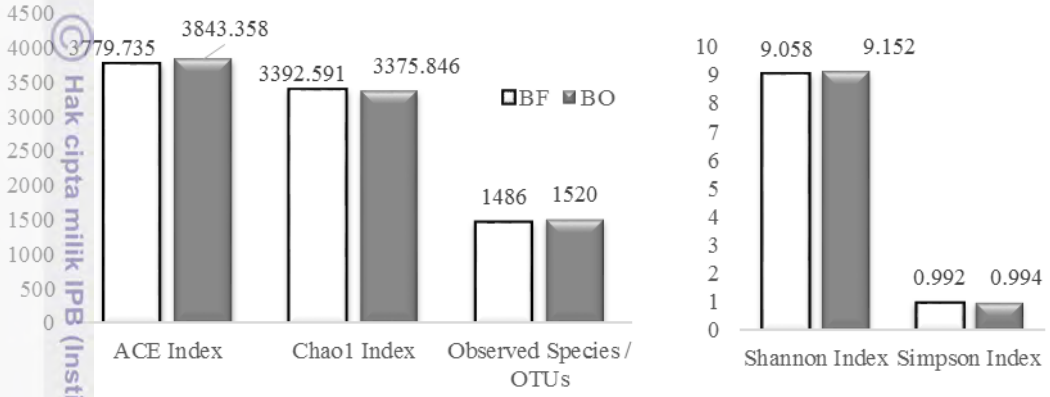
Phosphorus (P) is an essential element for all living beings as part of proteins, nucleic acids, membranes, and energy molecules, such as ATP, GTP, and NADPH. Depending on some environmental and biological factors, it can be the main growth-limiting nutrient (Azziz *et al.* 2012). Phosphorus is the second important key element after nitrogen as a mineral nutrient in terms of quantitative plant requirement. Although abundant in soils, its availability is restricted as it occurs mostly in insoluble forms. A substantial number of bacterial species exhibit P solubilization capacity. Available P in site BO increased nutrient quantity of soil bacteria at oil palm plantation. They impacted to increase on diversities and abundances of soil bacteria in site BO rather than that in site BF relatively.

Effect of Soil Properties on Bacterial Diversity

Pyrosequencing analysis of environmental samples can obtain much more sequences and OTUs than conventional cloning and sequencing methods. The higher OTUs and Shannon indices observed in this study demonstrated the usefulness of pyrosequencing analysis in revealing the bacterial diversity and richness from the tropical low land rainforest and the oil palm plantation as deforestation area. Libraries of sites BF and BO were composed of 1486 and 1520 OTUs, respectively (Figure 8). Rarefaction curves were generated at 1% cutoff to make a comparison of species richness between the two soil groups (Figure 9).

Based on Abundance-based Coverage Estimator (ACE) of both bacterial richness from site BF displayed relatively lower species richness than those from site BO, but based on Chao1 index, that from site BF displayed relatively higher

than from site BO (Figure 8). Figure 8 showed similar comparative values with regard to the diversity for the two sample groups, with higher level of diversities observed for samples from site BO. When the genetic distances were 0.01, the value of ACE, Chao1 and OTU for samples from site BF was 3779, 3392, and 1486, whereas that for samples from site BO was 3843, 3375, and 1520 respectively.



Richness-diversity estimates 1% dissimilarity

Figure 8 Bacterial richness indices of aggregates and whole soil at a genetic distance of 1%, expressed as the number of observed unique OTUs, ACE, Chao1, Shannon Index, and Simpson Index.

Both the Shannon and Simpson diversity indices revealed similar trends, with lower values observed for samples from site BF (9.058 and 0.992), when compared with those from site BO (9.152 and 0.994) relatively, but they were not differences considerably. It was confirming the similarity bacterial richness and diversity in both sites.

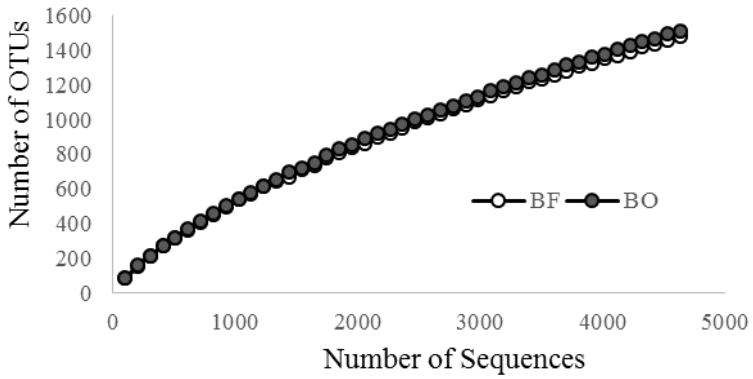


Figure 9 Rarefaction curves showing the observed number of operational taxonomic units (OTUs) at 1% dissimilarity for samples from sites BF and BO

A similar in the bacterial diversity was observed between the two sites, BF and BO, suggesting that these two soil systems are not different with diverse soil properties. The data obtained clearly demonstrated that the population of bacteria isolated from soils of site BF had slight lower diversity, when compared with that

from soils of site BO. The slight differences in the soil bacterial diversity might be explained by the slight differences in the concentration of TC and TN. Previous studies have shown that TC, TN, AP, and pH are important regulators of soil microbial community composition and activities in the tropical forest and vary land use type of agriculture area, included oil palm plantation area (Tripathi *et al.* 2012, Lee Cruz *et al.* 2013, Tripathi *et al.* 2013). In this study, similarity of bacterial OTUs and their richness and diversity showed that short term amendment of oil palm plantation, about eight until ten years, have not make considerably difference of implication in their soil nutrient quality.

Availability of C, N, and P make microbiological processes in soil of forest and oil palm plantation optimally. They acts as a water absorbent and binding agent for microbial biofilm and soil aggregate (Wei *et al.* 2011). The results of our study showed that oil palm plantation soil under fertilization management increased AP (Table 6). Change in soil P fertility with the application of fertilizer had effect on shifts in the composition of bacterial communities without affecting their richness in 8 years (Beauregard *et al.* 2010). The dissolving of inorganic P is usually ascribed to microorganisms which have the ability to acidify the local environment through the release of organic acid such as mobilization of insoluble soil inorganic P by *Pseudomonas* spp. is mediated through the extracellular production of gluconic acid (Uroz *et al.* 2009; Rice *et al.* 2012). The degradation of insoluble organic P by the production of specific enzymes, such as phosphomonoesterases (Nannipieri *et al.* 2011), phytases (Yao *et al.* 2012) and phosphonatases (Kamat *et al.* 2011), is also a significant aspect of P cycling in the soil. *Burkholderia,* and *Staphylococcus* had solubilization activity, which was associated with a decrease in the pH and the release of organic acids, such as gluconic, citric, succinic, and acetic acids (Acevedo *et al.* 2014).

The phosphorus solubilizing bacteria activity had caused decreasing soil pH in the forest and the oil palm plantation (3.77 and 4.80). Soil pH considered the bacterial community composition and diversity across the various land use types, with the highest diversity close to neutral pH values (Tripathi *et al.* 2012). Soil pH in this study for both areas was acidic, so that impacted to increase acidic bacteria composition.

Bacterial Community Composition in Forest and Oil Palm Plantation Soils

The 2457 classifiable sequences were affiliated with 23 phyla across the entire data set. The major phylum groups were those with a relative abundance of >2%. Figure 10 shows the phylum compositions of the two soil groups. Site BF was mainly composed of Acidobacteria (56.33%), Proteobacteria (27.43%), Actinobacteria (7.11%), and Cyanobacteria (5.55%). Site BO mostly comprised Acidobacteria (50.11%), Proteobacteria (31.63%), Actinobacteria (7.58%), Chloroflexi (2.60%), and Gemmatimonadetes (2.71%). Acidobacteria contributed to the majority of the community composition of both soil groups, indicating that sequences associated with Acidobacteria contributed to a highest percentage of community DNA.

The dissimilarities among the samples at the class level of taxonomic classifications were found in this study (Figure 11). Acidobacteria, Cyanobacteria

WD272, Alphaproteobacteria, and Gammaproteobacteria were the most dominant classes (> 5.00%) in site BF, accounting for 55.84%, 5.23%, 16.69%, and 8.08% of the total bacterial sequences, respectively.

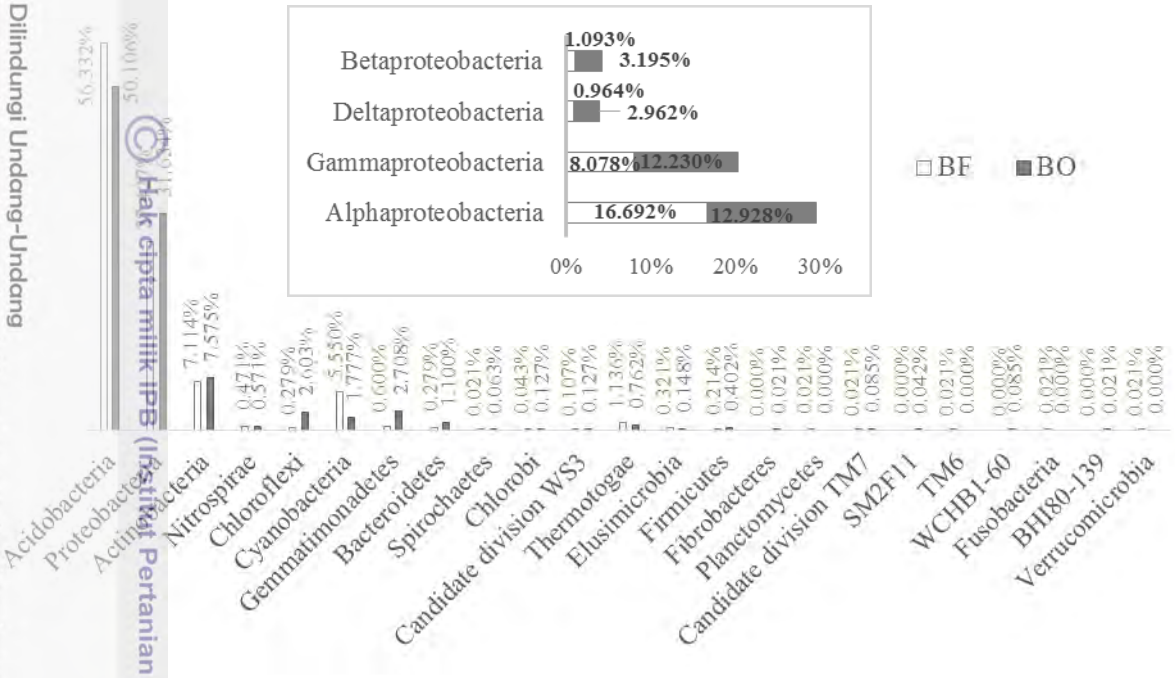


Figure 10 Average relative abundances of dominant bacterial taxa in samples collected at forest (BF) and oil palm plantation (BO) area.

In site BO, several classes were dominant (>5.00%) such as Acidobacteria, Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria, accounting for 49.30%, 5.12%, 12.93%, and 12.23% of the total bacterial sequences, respectively. Furthermore, the two areas revealed obvious similarity in the abundances of the class Acidobacteria of the most dominant phylum, Acidobacteria. Analyzing at the genus level showed an enrichment trend of beneficial bacterial groups in the soil. The relative abundance of any genus was <1% in each sample, implying high bacterial diversity in the two sample groups. Among the top 35 predominant genera in the soils evaluated (Figure 12), the seven greater bacterial genera were common in the BF sites: Acidobacteriales Candidatus *Koribacter*, Acidobacteriales uncultured bacterium, Candidatus *Solibacter* uncultured bacterium, Acidobacteria DA052 uncultured bacterium, Cyanobacteria WD272 uncultured bacterium, Rhodospirillales DA111 uncultured bacterium, and Xanthomonadales Sinobacteraceae uncultured bacterium. Five genera of them (except: *Solibacter* and Rhodospirillales DA111) were dominant in the BO sites too. However, the compositions of the bacterial community and the distributions of the dominant 34 genera were different between samples from sites BF and BO (Figure 12). The respective abundance of the 34 most represented genera in the samples from the two sites were examined (Figure 12), there were seven genera in BF and five genera in BO sites which more abundance than others genera in both sites.

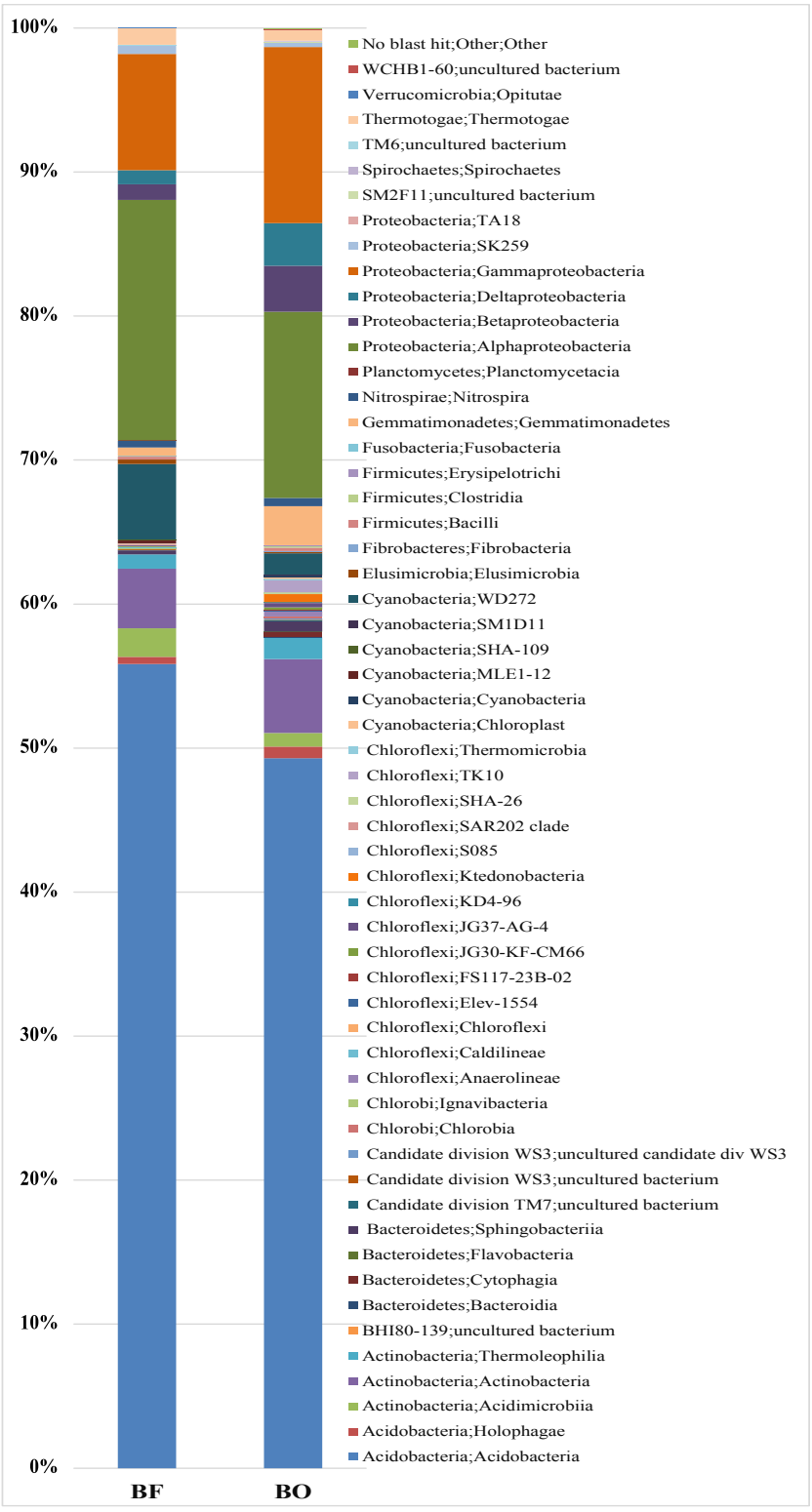


Figure 11 16S rRNA gene surveys revealed a clear distinction between the two bacterial class populations investigated. Comparison of the percentage of sequences (relative abundances) affiliated with different classes from sites BF and BO.

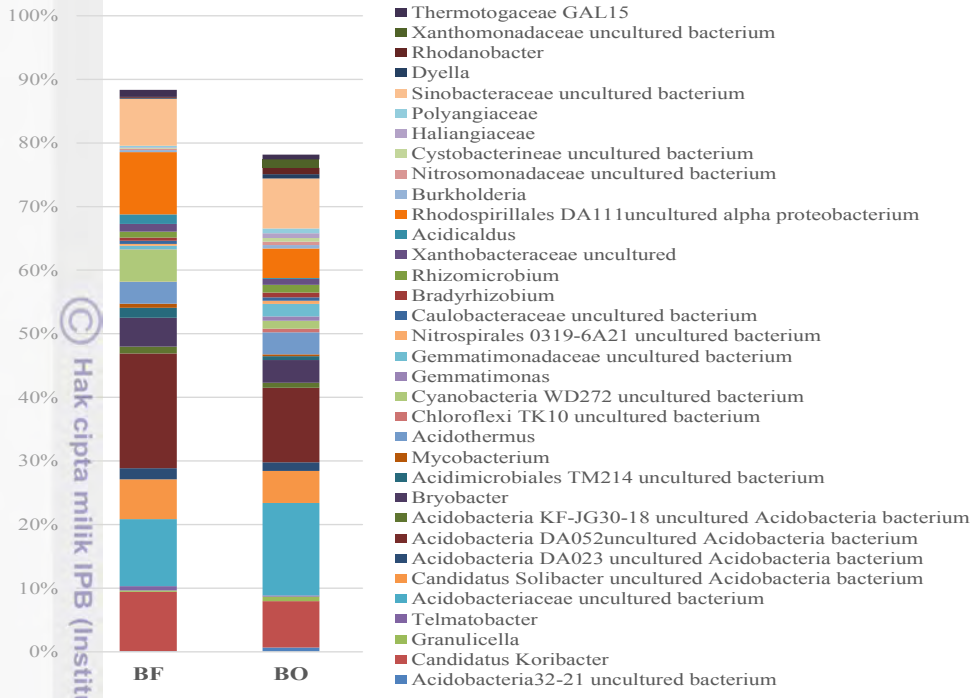


Figure 12 Comparison of the overall distribution of 34 predominant genera (more than 0.5% of total sequenes) within sites BF and/or BO.

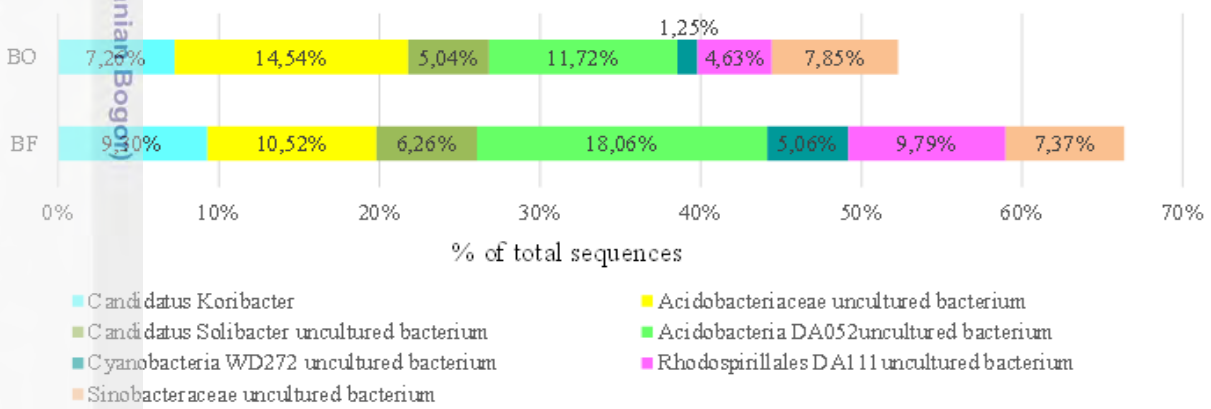


Figure 13 Comparison of the overall distribution of the selected 7 dominant genera (more than 5% of total sequences) from 34 predominant genera within sites BF and/or BO.

The seven majority of the sequences associated with Acidobacteria DA052 uncultured bacterium (18.06%), Acidobacteria uncultured bacterium (10.52%), Rhodospirillales DA111 uncultured bacterium (9.79%), Candidatus Koribacter (9.30%), Sinobacteraceae uncultured bacterium (7.37%), Candidatus Solibacter uncultured bacterium (6.26%), and Cyanobacteria WD272 uncultured bacterium (5.06 %) were found from forest site (BF). From oil palm plantation site, there were only five represented genera of the seven those from site BF which raised over 5% from total sequences.

They were Acidobacteriaceae uncultured bacterium (14.54%), Acidobacteria DA052 uncultured bacterium (11.72%), Sinobacteraceae uncultured bacterium (7.85%), Candidatus Koribacter (7.56%), and Candidatus

Solibacter uncultured bacterium (5.04%) which were obtained from site BO. Four genera dominated in both two sites were included to Acidobacteria phylum. The prokaryote dominant from both of two areas were *Acidobacteria.* These phyla are oligotrophic organisms abundant in carbon-poor soils. The *Proteobacteria*-to-*Acidobacteria* ratio was higher in oil palm plantation soils than in primary and once-logged forest soils. This ratio tends to be lower in oligotrophic soils and higher in soils with organic input, indicating the nutritional status of soil (Lee-Cruz *et al.* 2013). The relative abundances of Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria were positively correlated with soil N and C content, while acidobacterial relative abundance was correlated with pH factors. Carbon and nitrogen content often decreases, on the contrary, pH value often increases in cultivated soils (Waldrop *et al.* 2000, Nemergut *et al.* 2010).

This respect our study was no exception, with lower concentrations of total carbon and total nitrogen and a lower C/N ratio in soil from oil palm plantations than from forests, but the result showed that Acidobacteria phyla from forest sites were more abundance than those from oil palm plantation sites. Although, the carbon content in oil palm plantation soils was lower than in forests, pH value in forest soil was lower than in oil palm plantation. *Acidobacteria* are also strongly influenced by pH, favoring soils with pH of 4 (Jones *et al.* 2009, Lee Cruz *et al.* 2013). Secondary forest soils had lower pH (3.77 ± 0.31) than oil palm plantation soils (4.80 ± 0.79), probably promoting *Acidobacterial* abundance.

The dolomite supply modified only the pH and the calcium and magnesium contents, this allows us to claim that the modifications of the functional and/or taxonomical structure of the culturable bacterial communities highlighted in previous study resulted from this supply. Such effects on soil pH and Ca and Mg contents have already been observed after a dolomite supply in nutrient-poor soils. Moreover, the increase of soil pH and Ca and Mg contents improve the base saturation, increasing by this way nutrient availability for bacteria (Lepleux *et al.* 2013). Microbial communities represent a key component in the functioning of forested ecosystems, it appears important to better assess the effects of liming on microbial diversity. It has been previously suggested that the success of restoration could be estimated by measures of soil microbial communities. The results confirm previous observations showing that the ratio between Proteobacteria and Acidobacteria could be a microbial indicator of soil quality improvement. Both phyla are among the most diversified groups within the bacterial domain. Encompassing numerous culturable and well-described species, the Proteobacteria are characterized by a high metabolic diversity and are known to be involved in biogeochemical cycles in soils. The ratio between the relative abundance of Proteobacteria and Acidobacteria was higher in limed soils compared to the untreated soils. This large-scale field study documents the effects of a four year old liming on bacterial communities of forest soils by providing a common indicator based on analysis of metabolic potential activities of heterotroph communities and by identifying major taxonomic changes in bacterial community structure. (Clivot *et al*. 2012). In this study, the genera of oligotrophic bacteria that dominated in both two areas, BF and BO, were from Acidobacteria. The genera from Acidobacteria in BF were more abundance than those in BO relatively, because agricultural liming (such as dolomite) only occurred at BO area for preparing plantation soil.

Proteobacteria as copiotrophic bacteria phylum had different composition of classes between both areas BF and BO. Alphaproteobacteria were found higher in BF area than BO area, while Beta-, Gamma-, and Delta-proteobacteria were more abundance in BO than BF area. The Betaproteobacteria exhibited copiotrophic characteristic, their relative abundances were highest in soils with high C availability either as an essential element of the soil. The relative abundances of Acidobacteria decreased while the relative abundances of Betaproteobacteria and Bacteroidetes increased (Fierer *et al.* 2007). In this study, total Carbon in BO sites were lower than those in BF sites, but on the contrary, available Carbon might been higher in BO sites than those in BF sites. Fierer *et al.* showed that the abundances of Alpha-proteobacteria, Firmicutes, and Actinobacteria did not respond in any predictable modus to changes in C availability. Spain *et al*. (2009) found that uncharacterized proteobacteria distribution in soil-ecosystem were Gammaproteobacteria dominating uncontaminated soil/rhizospere, Alphaproteobacteria dominating wetland soil, Deltaproteobacteria dominating metal/radionucleotide mill tailing, and Betaproteobacteria dominating organic contaminated soil.

The Actinobacteria showed an increased abundance in oil palm plantations compared to forests (Lee Cruz *et al.* 2013). In this study, there was an exception, the Actinobacteria class Acidimicrobiia were higher in BF soil than those in BO soil. The others of Actinobacteria (Actinobacteria and Thermoleophilia) were relative lower in BF soil than those in BO soil. Soil pH in BF sites (more acidic than BO sites) might impacted on relative abundance highness of Acidimicrobiia than the others. Existence of Actinobacteria in forest and oil palm plantation had been influenced by anthropogenic through agricultural lime. Actinobacteria are one of the few groups of saprotrophic microorganisms that oxidatively depolymerize lignin, producing substantial soluble polyphenolics in the process. N deposition further elicited a significant membership change in forest floor and surface soil communities, as well as significant differences in the phylogenetic diversity of forest floor Actinobacteria. This shift in community composition occurred in concert with a slowing of plant litter decay, accumulation of soil organic matter, and a greater production of phenolic DOC. Eiselord and Zak (2010) concluded that changes in actinobacterial community composition may basis of biogeochemical responses to experimental N deposition in forest soil. Actinobacterial shift community in oil palm plantations areas had been characterized to the effects of anthropogenic intervention and forest conversion, as like as ectomycorrhizal fungal community (Kerfahi *et al.* 2014). The phylum *Actinobacteria* is very large and diverse in terms of its biology, ecology, and genetics, and it contains numerous organisms that are of great interest from medical, industrial, biotechnological, and environmental perspectives (Gao and Gupta, 2012). Combined with the lower relative proportion of Firmicutes and Actinobacteria in limed soils, such modifications could be involved in the shift previously observed toward more Gram-negative and fewer Gram-positive bacteria following soil liming (Clivot *et al*. 2012).

According to an indicator species analysis, the changes were largely explained by the decline in the relative abundances of Acidobacteria, Gemmatimonadetes and Verrucomicrobia with high level of nutrient (Cederlund *et al.* 2014). Gemmatimonadetes and Verrucomicrobia were not appeared as the

dominant at class and genera level, but only Gemmatimonadetes present as predominant genera at BO site. The Gematimonadetes genera from BO area was more abundance (>four folds) than those from BF area. It may showed impact of upraised soil pH and P content at soil of palm oil plantation area relatively. Their presence in environments with a wide range of nutrient concentrations and redox states suggests versatile metabolisms which have contributed to their cosmopolitan success. The highest relative abundances were observed near neutral pH, while low abundances were observed across all pHs, suggesting that after moisture, pH may act as a secondary constraint on *Gemmatimonadetes* in these soils (DeBruyn *et al*. 2011).

Phylogenetic of Bacterial Community for BF and BO sites

16S rRNA gene-based phylogeny showing representatives of all bacterial phyla from tropical low land secondary forest (Figure 14) and oil palm plantation (Figure 15) soil derived sequences have been obtained. The fifteen phyla of bacteria were found in BF site, while eighteen phyla of bacteria were found in BO site. More phyla in BO showed that bacterial diversity in BO was higher than those in BF. The BO phyla could be included in shifted bacterial community for adapting to forest transformation to oil palm plantation.

Although Acidobacteria were found as the highest abundance in forest and oil palm plantation area, Proteobacteria occupied the highest genera of bacterial community composition in both two areas (Figure 14 and Figure 15). Alpha proteobacteria has more genera than others proteobacteria. Alpha proteobacteria inhabited second highest phyla of bacterial community composition in surface and subsurface peat after the first highest phyla, Acidobacteria (Serkebaeva *et al.* 2013). Similarity of acidic soil in peat and both two study areas (low land forest and oil palm plantation areas) might impacted to similarity of highest phyla of bacterial community composition at representative sequences of genera in both two areas.

Alphaproteobacteria is the most abundant class, relative to total sequences, comprising 35–58% of Proteobacteria, but the most abundant orders contained no cultivated or characterized pure cultures (Spain *et al.* 2009). Low pH soils were dominated by Acidobacteria along with the Alphaproteobacteria. Alphaproteo-bacteria became more dominant at intermediate pH (Griffiths *et al.* 2011). This class had more genera than others because of their capability to survive in acidic soil and degrade herbicide. Liu *et al.* (2011) explained that Alphaproteobacteria like bacteria were isolated from pristine and non-contaminated soils and contain organisms related to *Bradyrhizobium* of Alphaproteobacteria. Oil palm plantation soil contained herbicide for treating unwanted vegetation surrounding areas.

The highest genera in phylogenetic tree (Figure 14 and 15) is *Burkholderia*. *Burkholderia* is one genus of genera of Betaproteobacteria. *Burkholderia* genera were found 9 phylotypes from 18 Burkholderiales in forest and 14 phylotypes from 37 Burkholderiales in oil palm plantation. These genera in BO were more variated than genera in BF. In this study, genera of *Burkholderia* were the most population of bacterial isolates from forest and oil palm plantation soil (Wijayanti *et al.* 2014).

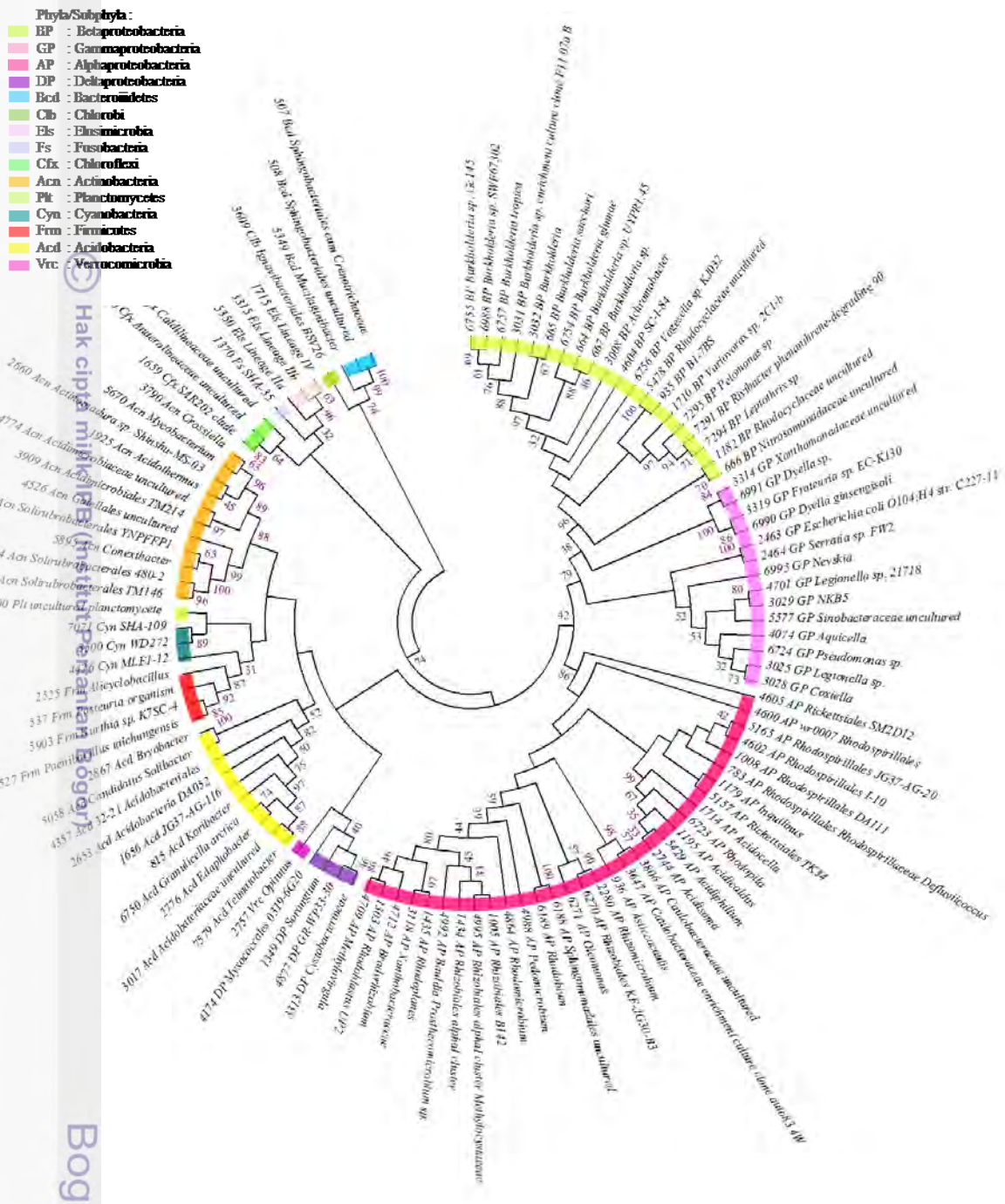


Figure 14 16S rRNA gene-based phylogeny showing representatives of all bacterial phyla from tropical low land forest soil derived sequences have been obtained.

Note : Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980). The tree with the highest log likelihood (-13181.3047) is shown. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6135)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 23.3440% sites). The analysis involved 111 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 394 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013). Bootstrap values (calculated from 1000 replicates) >30 are shown.

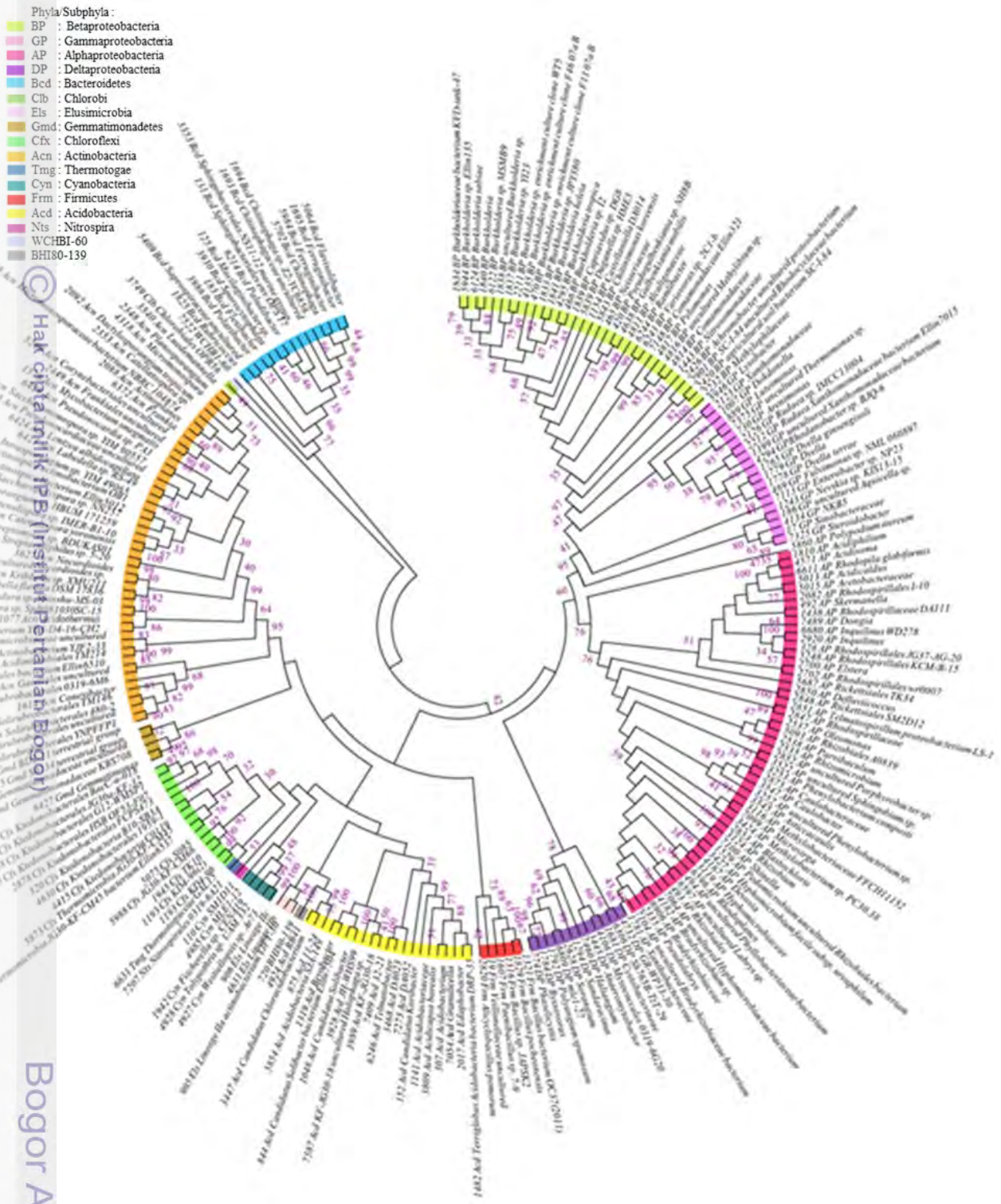


Figure 15 16S rRNA-based phylogeny showing representatives of all bacterial phyla from oil palm plantation soil derived sequences have been obtained.

Note : Molecular Phylogenetic analysis by Maximum Likelihood method The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980). The tree with the highest log likelihood (-26568.1997) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4944)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 27.0907% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 236 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 457 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013). Bootstrap values (calculated from 1000 replicates) >30 are shown.

In acidic forest soils, like in BF, bacteria should adapt to toxicity of aluminum (Al), which is solubilized by acidity. Kunito *et al.* isolated Al-resistant bacteria from acid forest soils using diluted tryptic soy broth agar plates with added Al and cycloheximide, and examined the relationship between their diversity and Al levels in soils. The diversity of Al-resistant bacteria was not related to Al concentrations in soils, and almost all bacteria found were assigned to the genus *Burkholderia*. Based on 16S rDNA sequences, 10 out of 11 isolated bacteria were assigned to the genus *Burkholderia*, and 1 to the genus *Acinetobacter* (Kunito *et al.* 2012). Stopnisek *et al.* tested the ability of 68 strains of *Burkholderia* belonging to 31 different species to grow at a pH range of 3.5-8. The abundance of the betaproteobacterial genus *Burkholderia* was consistent with the recent identification of this genus as an acid tolerant group that may outcompete other taxa in acidic. There was no significant correlation between pH and *Burkholderia* diversity. *Burkholderia* have developed pH tolerance mechanisms that enable them to survive and increase in environmental niches where many other taxa are inhibited, while they are outcompeted by faster growing microorganisms in less harsh conditions. Acid tolerance is a requirement for occurrence in low pH soils, but it is tempting to postulate that the preference of *Burkholderia* for such niches is not only the consequence of the ability to tolerate acidity, but the result of a strategy involving both tolerance to abiotic stress factors (such as higher toxicity of heavy metals) and to biological controls (e.g. the predominance of fungi) natural to such environments (Stopnisek *et al.* 2013).

In oil palm plantation area, genus *Burkolderia* reached the highest phylotype of genera in this study. In Malaysia, strains of the endophytic bacteria *Burkholderia cepacia, Pseudomonas aeruginosa and Serratia marcescens* also have potential to inhibit the spread of *Ganoderma orbiforme*. For eight months after inoculation, Basal Stem Root incidence was reduced by 42%, 76% and 54% in seedlings pre inoculated with either *B. cepacia* or *P. aeruginosa* or a mixture of both species, respectively (Mohammed *et al.* 2014). Some of the bacterial endophytes from the genera *Pseudomonas, Burkholderia,* and *Serratia* might have the potential to control *G. boninense*, as they were mostly found in healthy roots from symptomless palms (Bivi *et al.* 2010). These endophytic bacteria (*Pseudomonas aeruginosa* and *Burkholderia cepacia*) were tested for antagonistic effects on *Ganoderma boninense* that causes a serious disease in oil palm tree. The effect of these bacteria was expressed as severe morphological abnormalities in the hypha structures (Sundram *et al.* 2011). In this study, *Burkholderia* might have been phosphate solubilizing bacteria, growth promoting bacteria, and bioremediator bacteria. Strains of the genus *Burkholderia* occupy a wide range of ecological niches and have versatile properties of bioremediation, biocontrol, and plant growth promotion (Coenye and Vandamme 2003).

Xu *et al.* (2013) *Burkholderia phenoliruptrix* strain AC1100 (ATCC 53867) degrades a variety of recalcitrant xenobiotic, including 2,4,5-trichlorophenoxy-acetate. They presented a 7.8-Mb assembly of the genome sequence of this 2,4,5-trichlorophenoxyacetate degrading strain, which may provide useful information related to the degradation of chlorinated aromatic compounds (Xu *et al.* 2013). Burkholderia had been reported as xenobiotic and herbicide degrading bacteria. Several other common broad-spectrum herbicides for managing oil palm plantation are paraquat, glufosinate-ammonium and

glyphosate. All these herbicides are foliar applied, with paraquat activity being through contact, glufosinate-ammonium being partially systemic, and glyphosate being systemic (Mohamad *et al.* 2010). This genus could developed their capacity for adapting herbicide stress as mechanism of adaptation. The phenomenon of herbicide adapting bacteria could been associated with xenobiotic, such as 2,4-dinitrotoluene (DNT) for Burkholderia. *Burkholderia* sp. from environment had been reported that it could mineralized the xenobiotic compound DNT. They owed to the catabolic *dnt* genes borne by plasmid DNT (Perez-Pantoja *et al.* 2013). It is thus reasonable that the evolutionary roadmap for biodegradation of xenobiotic compounds like DNT was largely stimulated by mutagenic oxidative stress caused by damaged reactions of precursor enzymes with novel but structurally related substrates-to-be (Perez-Pantoja *et al.* 2013).

In this study, more *Burkholderia* phylotypes in oil palm plantation than in forest area showed more variant of genotype in this genus (Figure 16). Two species *Burkholderia* sp. enrichment culture clone F11 07a B and *Burkholderia tropica* were found in forest and oil palm plantation, but there were different phylotypes between each species in different areas. *Burkholderia tropica* in both area had closer phylotype than others. The species had capability to preserve their genotype. It could revealed the change of composition substrate made the shifting population in soil between both areas, but *B. tropica* could survived in several substrate alteration. Castro-Gonzales *et al.* found that *B. tropica* could as phophate solubilizing bacteria, produced sidephore, synthesized IAA, and recovered from cultures without enrichment of diazotrophs (Castro-Gonzales *et al.* 2011).

In Burkholderiales, there were three genera *Variovorax* sp. 2C1-b, *Pelomonas* sp., and *Achromobacter* which were found in both areas. *Variovorax* is desulfonator bacteria which plays a minor role in the mineralization of aromatic sulfonates in soil. Desulfonation is an important process in the sulfur cycle, through which organic sulfur compounds are mineralized, releasing S. Desulfonating bacteria are known to respond to inorganic S soil amendments (Inceoglu *et al.* 2013).

Pelomonas is a monad genus isolated from mud which able to fix nitrogen, oxidize hydrogen, and show autotrophic growth. *Pelomonas saccharophila* was reclassify from *Pseudomonas saccharophila* Doudoroff 1940 (Xie and Yokota 2005). Monard *et al.* found that active atrazine degraders were only detected in burrow linings after 2 days of ^{13}C-atrazine treatment, and corresponded to a unique phylotype highly similar to the strain *Pelomonas aquatica*. The existence of horizontal gene transfer in bacteria is important to consider when focusing on the relationship between diversity and function (Monard *et al.* 2011).

Achromobacter bacteria are gram negative, strictly aerobic, rod-shaped cells that are common inhabitants of the human intestinal tract and xenobiotic (polychlorinated biphenyl or arsenic) contaminated soil (Li *et al.* 2012). *A. xylosoxidans* was reported to have the ability to degrade catechol, biphenyl, and monoaromatic hydrocarbons (BTEX). *A. xylosoxidans* F3B as an endophyte of *Arabidopsis thaliana* in phytoremediation. They help the plant tolerate lethal concentrations of aromatic compounds and decrease catechol- and phenol-induced phytotoxicity. These types of functional endophytes could play important roles in phyto-protection and phytoremediation (Ho *et al.* 2012). *Achromobacter* sp.

strains MPS 12 capable of utilizing methylphosphonic acid (MP) or glyphosate (GP) as the sole sources of phosphorus were isolated from soils contaminated with these organophosphonates.

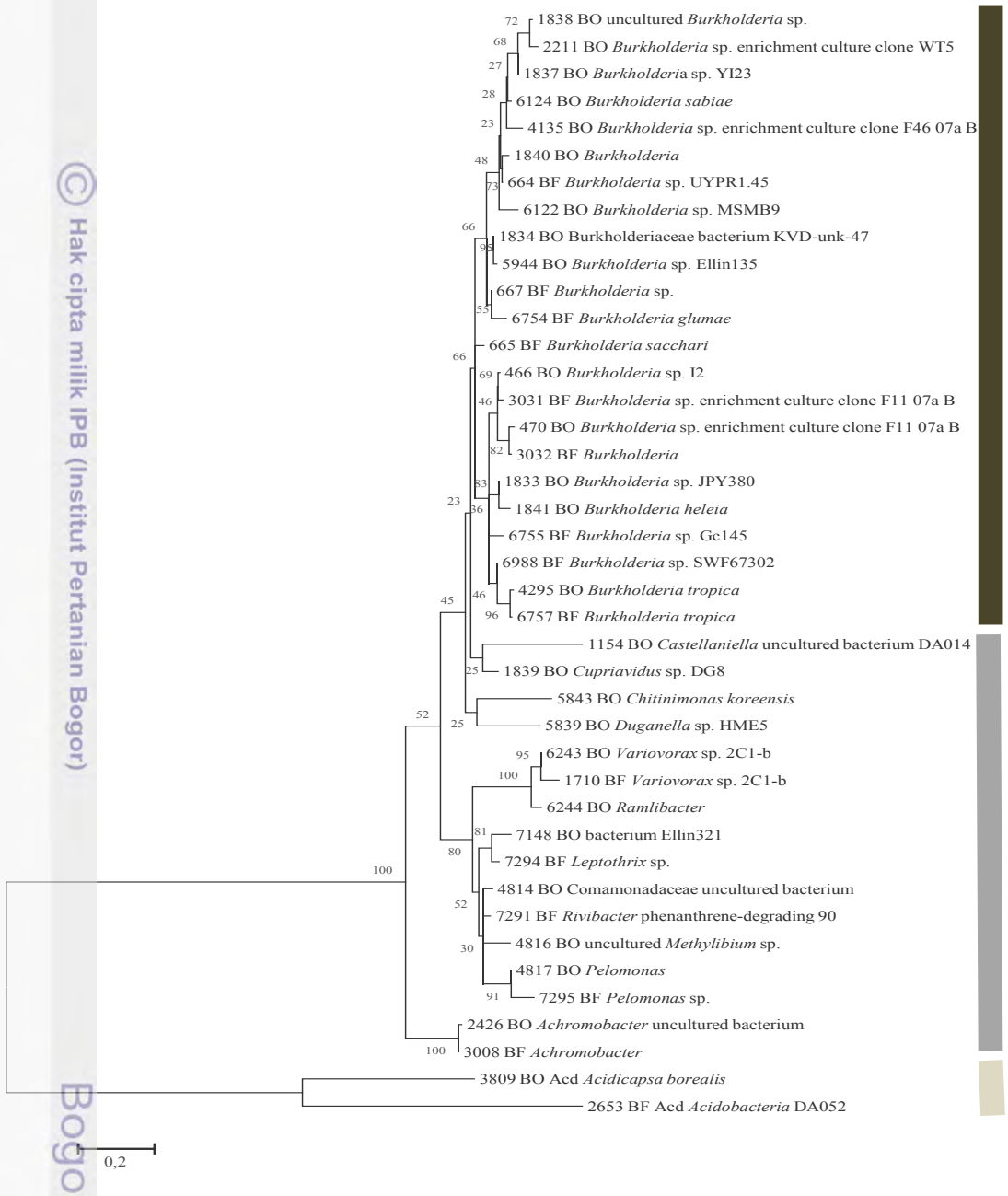


Figure 16 16S rRNA gene-based phylogeny showing representatives of Burkholderia (Dark grey), other Burkholderiales (Grey) and out group Acd (Acidobacteria) (Light grey) from soil derived sequences have been obtained from BF and BO areas.

Note : The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992). The tree with the highest log likelihood (-2885.0625) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.2478)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 32.5157% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 41 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 383 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013). Bootstrap values (calculated from 1000 replicates) >20 are shown.

At present, the most widespread synthetic Phosphonates is N-phosphono-methylglycine, or glyphosate (GP), which is used as an active component of a many herbicide formulations for oil palm plantation (Sviridov *et al.* 2012, Mohamad *et al.* 2010). The action of *Achromobacter* bacteria is on the high affinity transport system. Concerning the mechanisms underlying N acquisition by plants, an enhanced uptake of NO_3^- in roots of the plant inoculated with the soil-isolate *Achromobacter* bacteria with a consequent higher content of this anion in the plant tissues. So, this genus could be included in plant growth-promoting rhizobacteria (Pii *et al.* 2015). This capability caused this genus exist in forest and oil palm plantation area.

Sediment Bacteria in Forest and Oil Palm Plantation

Sediment microbial communities are responsible for a majority of the metabolic activity in river and stream ecosystems. Understanding the dynamics in community structure and function across freshwater environments will help us to predict how these ecosystems will change in response to human land-use practices (Gibbons *et al.* 2014). The forest transformation to oil palm plantation drive changes to the aquatic sediment around sites. It is likely that several different stages of the oil palm agriculture process will impact freshwater ecosystems. First, the clearance of land, and in particular forested areas, to create plantations will cause substantial disturbance of soil and loss of trees. This leads to sedimentation of streams and reduction in the amounts of essential leaves and woody inputs that stream organisms depend on. The loss of shading from riparian vegetation will likely cause increases in light and temperature beyond the tolerance limits of many organisms. Second, once oil palm has been established, pesticides and fertilisers routinely used on plantations may cause pollution of water as they run off the land. Third, processing oil palm fruit bunches produces palm oil mill effluent (POME), an acidic liquid containing solid plant material, oil and nutrients. If this is left untreated and released into waterways it could be very damaging to freshwater ecosystems (Luke 2014).

Environmental Variable and Bacterial Diversity Indices

The compositions of sediment bacterial communities present in two different soil systems derived from a forest site (site SBF) and an oil palm plantation site (site SBO) were assessed and compared using pyrosequencing-based analysis of 16S rRNA gene sequences. They could be influenced by sediment chemical properties of samples from different land use types, the forest and oil palm plantation sites such as soil pH, Total Carbon (TC), Total Nitrogen (TN), Available Phosphorus (AP), and C/N ratio (Table 7). The t-test of two analyzed soil area showed no significant differences in some sediment parameters (pH, TC, TN, and C/N ratio), but signifiant differences in available phosphorus (AP). The available P content of the sediment of site SBF (0.281%) was higher than that of site SBO (0.044%). Available phosphorus varied significantly between forest and nonforest sites, while soil pH, total carbon, total nitrogen, and C/N ratio did not vary significantly across the landscape.

Direct impacts on aquatic sediment ecosystems from oil palm cultivation include clearing of riparian vegetation for palm planting, sediment run-off from cleared land, and degraded water quality from pollutants (pesticides and fertilisers), mill tailings, and village activities around stream (Sheaves 2014). They can alter the pH, lead to modify the C/N ratio and the content of phosphorous in freshwater sediment. The sediment condition was influenced by aquatic column condition. Bacteria were related to Dissolved Organic Carbon (DOC) in the lower aquatic sites, but phosphorus in the upper aquatic. This is likely due to the input of bioavailable carbon in the upper aquatic leading bacteria to be P limited, which changes downstream to carbon limitation as DOC becomes more refractory (Hitchcock and Mitrofic 2014). Sediment pH in both area were no significant differences, although soil pH of SBO sample was higher than those of SBF sample relatively. This condition could be considered by liming on the plantation while receive the run off from oil plantation soil.

Table 7 Mean values (±standard deviation) of parameters for sediment samples from the forest site (SBF) and the oil palm plantation site (SBO).

Sample sites	pH(H_2O)	pH(KCl)	TC (%)	TN (%)	AP (%)	C/N ratio
SBF	4.500 ± 0.557	3.900 ± 0.361	3.540 ± 3.090	0.233 ± 0.150	0.281 ± 0.074	13.700 ± 3.480
SBO	4.800 ± 0.100	3.967 ± 0.208	1.725 ± 0.205	0.145 ± 0.005	0.044 ± 0.005	11.870 ± 1.000

Similarly in soil, total carbon and C:N ratio of freshwater sediment were lower in oil palm soils than in forest area, suggesting that in this case other factors that impact on acid specific bacteria, such as pH, had a strong role might have had on the copiotropic bacteria, like as Proteobacteria (Lee Cruz *et al.* 2013). The plantation run off had been run away with anorganic fertilizer (agricultural lime and phosphorus fertilizer for agricultural management) to downstream sediment. They changed increasing soil pH even though no significant differences between both areas. Available P in both sites were high–excellent range that reserve of available P in slightly acidic to alkaline soils, possible adverse effects to water quality from erosion of high P soils (Arifin *et al.* 2012).

Phosphorus is the second important key element after nitrogen as a mineral nutrient in terms of quantitative primer productivity requirement. Although abundant in water column, its availability is restricted as it occurs mostly in insoluble forms. A substantial number of bacterial species exhibit P solubilization capacity. Available P in site SBF increased nutrient quantity of sediment bacteria at forest area. They impacted to increase on diversities and abundances of soil bacteria in site SBF rather than that in site SBO relatively.

Effect of Sediment Properties on Bacterial Diversity

Pyrosequencing analysis of environmental samples can obtain much more sequences and OTUs than conventional cloning and sequencing methods. The higher OTUs and Shannon indices observed in this study demonstrated the usefulness of pyrosequencing analysis in revealing the bacterial diversity and richness from the tropical low land rainforest and the oil palm plantation as deforestation area. Libraries of sites SBF and SBO were composed of 2968 and

2186 OTUs, respectively. Rarefaction curves were generated at 1% cutoff to make a comparison of species richness between the two soil groups (Figure 18).

Based on ACE and Chao1 indices of both bacterial richness from site SBF displayed relatively higher species richness than those from site SBO (Figure 17). When the genetic distances were 0.01, the value of ACE, Chao1 and OTU for samples from site SBF was 11323, 10236, and 3057, whereas that for samples from site SBO was 7794, 7033, and 2428 respectively. Both the Shannon and Simpson diversity indices revealed similar trends, with higher values observed for samples from site SBF (11.175 and 0.999), when compared with those from site SBO (10.537 and 0.999) relatively, but they were not differences considerably. It was confirming the similarity bacterial richness and diversity in both sites.

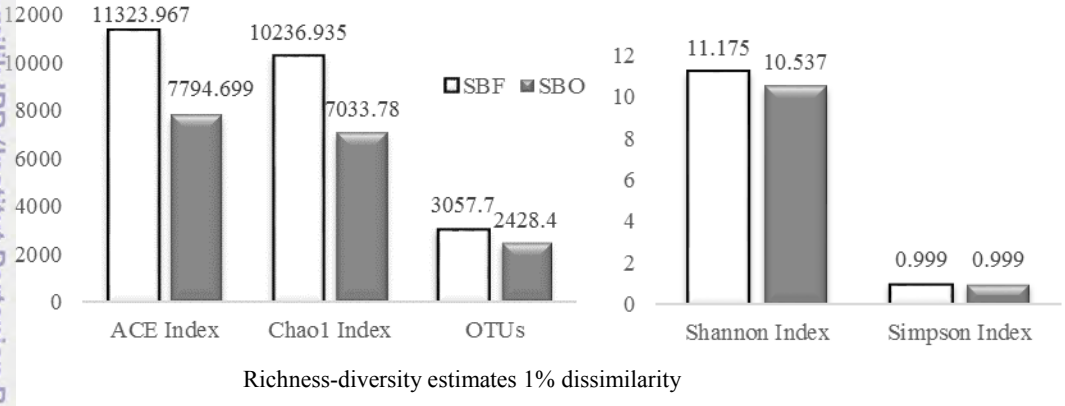


Figure 17 Bacterial richness indices of aggregates and whole sediment at a genetic distance of 1%, expressed as the number of observed unique OTUs, ACE, Chao1, Shannon Index, and Simpson Index.

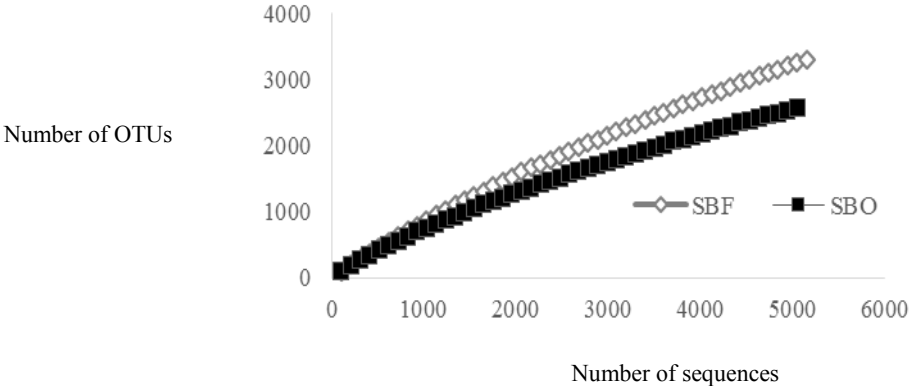


Figure 18 Rarefaction curves showing the observed number of operational taxonomic units (OTUs) at 1% dissimilarity for samples from sites SBF and SBO

A similar in the bacterial diversity was observed between the two sites, SBF and SBO, suggesting that these two systems are not different with diverse sediment properties. The data obtained clearly demonstrated that the population of bacteria isolated from sediment of site SBF had slight high diversity, when compared with that from soils of site SBO. The slight differences in the sediment bacterial diversity might be explained by the slight differences in the

concentration of TC, TN, and AP. Previous studies have shown that TC, TN, AP, pH, and oxic condition are important regulators of bacterial community composition and activities in the tropical forest and their transformation as oil palm plantation area (Tripathi *et al.* 2012, Lee Cruz *et al.* 2013, Tripathi *et al.* 2013, Mishra *et al.* 2014). In this study, freshwater sediment in both areas were included oxic and micro-oxic condition, because of stream water and shallow creek. Availability of C, N, and P make microbiological processes in sediment of forest and oil palm plantation optimally. They acts as binding agent for microbial biofilm and sediment aggregate (Wei *et al.* 2011). Sediment pH considered the highest diversity close to neutral pH values (Tripathi *et al.* 2012). Sediment pH in this study for both areas was acidic (pH 4.5-4.9), so that impacted to increase acidic bacteria composition.

The higher concentrations of inorganic nutrients in freshwater sediment at forest area (Table 7) was on the shifts in benthic microbial communities caused by natural decomposition of organic matter in aquatic sediment at the lowland forest. This study also showed that decreased concentrations of nitrogen and phosphorus associated with plantation run off decline benthic bacterial numbers (Figure 17). The toxic compounds may also be present in the effluent, and these may have inhibited bacterial populations. Toxic compounds of pesticides in the effluent could contribute to the reduction in bacterial diversity and species richness at the downstream locations of oil palm plantation area (Lu and Lu 2014). Moreover, rural effluent caused an increase in carbon of the sediment downstream of a rural creek in plantation area. However, decreases in the population size of sediment bacterial communities were also found in the plantation area. This indicated that toxic chemicals from effluents probably played major roles in biotic homogenization of sediment bacterial ecosystem (Lu and Lu 2014).

Bacterial Community in Forest and Oil Palm Plantation Sediments

The 5154 classifiable sequences were affiliated with 34 phyla across the entire data set. The major phylum groups were those with a relative abundance of >2%. Figure 19 shows the phylum compositions of the two soil groups. Site SBF was mainly composed of Proteobacteria (34.85%), Acidobacteria (32.67%), Nitrospirae (6.86%), Chloroflexi (4.31%), Actinobacteria (4.02%), Spirochaetes (3.38%), Chlorobi (3.36%), and Bacteroidetes (3.23%). Site SBO mostly comprised Acidobacteria (46.10%), Proteobacteria (25.86%), Nitrospirae (9.20%), Chloroflexi (4.99%), Spirochaetes (2.38%), and Actinobacteria (2.34%). Proteobacteria contributed to the majority of bacterial communities in forest sediment, eventhough Acidobacteria contributed to the majority of bacterial composition of oil palm plantation sediment.

The dissimilarities among the samples at the class level of taxonomic classifications were found in this study (Figure20). Acidobacteria, Alphaproteobacteria, Deltaproteobacteria, Betaproteobacteria, and Nitrospira were the most dominant classes (> 5.00%) in site SBF, accounting for 31.22%, 10.65%, 10.55%, 9.59%, and 6.86% of the total bacterial sequences, respectively. In site SBO, several classes were dominant (>5.00%) such as Acidobacteria, Nitrospira, Deltaproteobacteria, Alphaproteobacteria, and Betaproteobacteria, accounting for 42.94%, 9.20%, 8.65%, 7.35% and 6.11% of the total bacterial sequences,

respectively. The two areas sediment revealed obvious similarity in the abundances of the class Acidobacteria of the most dominant phylum, Acidobacteria, as like as in two areas soil.

Analyzing at the genus level showed an enrichment trend of beneficial bacterial groups in the soil. The relative abundance of any genus was <0.5% in each sample, implying high bacterial diversity in the two sample groups. Among the top 50 predominant genera in the soils evaluated (Figure 19), the ten greater bacterial genera were common in the SBF sites: Acidobacteria DA052 uncultured bacterium (7.56%), Acidobacteriales uncultured bacterium (7.42%), Bryobacter (3.73%), Spirochaeta (3.03%), Gammaproteobacteria Sinobacteraceae uncultured (3.02%), Acidobacteria DA023 uncultured bacterium (2.53%), Chlorobi Ignavibacteriales BSV26 uncultured bacterium (2.46%), Acidobacteria Candidatus Solibacter uncultured bacterium (2.42%), and Nitrospirales 4-29 uncultured bacterium (2.13%). Ten genera were dominant in the SBO sites : Acidobacteriales uncultured bacterium (11.67%), Acidobacteria DA052 uncultured bacterium (10.39%), Bryobacter (3.83%), Acidobacteria DA023 uncultured bacterium (3.77%), Gammaproteobacteria Sinobacteraceae uncultured (3.22%), Nitrospirales 0319-6A21 uncultured bacterium (3.05%), Acidobacteria Candidatus Solibacter uncultured bacterium (2.83%), Chloroflexi Caldilineaceae uncultured (2.63%), Nitrospirales 4-29 uncultured bacterium (2.40%), and Acidobacteria Candidatus Koribacter (2.24%). However, the compositions of the bacterial community and the distributions of the dominant 50 genera were different between samples from sites SBF and SBO. The respective abundance of the 50 most represented genera in the samples from the two sites were examined (Figure 21), there were seven genera in SBF and five genera in SBO sites which more abundance than others genera in both sites.

Five genera dominated in both two sites were included to Acidobacteria phylum. The dominant bacterial phyla from both of two areas were *Acidobacteria.* Jones *et al.* (2009) suggested that a strong influence of pH on microbial communities. Sediment or soil pH is also a strong predictor of acidobacterial abundance and community composition in the habitat (Jones *et al.* 2009). Similar with soil composition, in sediment, the relative abundances of Gammaproteobacteria were positively correlated with soil N and C content, while acidobacterial relative abundance was correlated with pH factors (Waldrop *et al.* 2000, Nemergut *et al.* 2010). *Acidobacteria* are also strongly influenced by pH, favoring soils with pH of 4 (Jones *et al.* 2009, Lee Cruz *et al.* 2013). Secondary forest sediment had lower pH (4.50 $\pm$ 0.56) than oil palm plantation soils (4.80 $\pm$ 0.10), probably promoting *Acidobacterial* abundance. But, the Acidobacteria in oil palm platation sediment were more abundance than in forest sediment, in contrast the proteobacteria in forest sediment were more abundance than in oil palm plantation. There were inffluent from rural and oil palm plantation to dowmstream. Bacterial community shift in the oil palm plantation sediment were influenced by the domestic and agrochemical pollutant. So, the acidobacterial abundance increased while bacterial diversity of plantation sediment decreased.

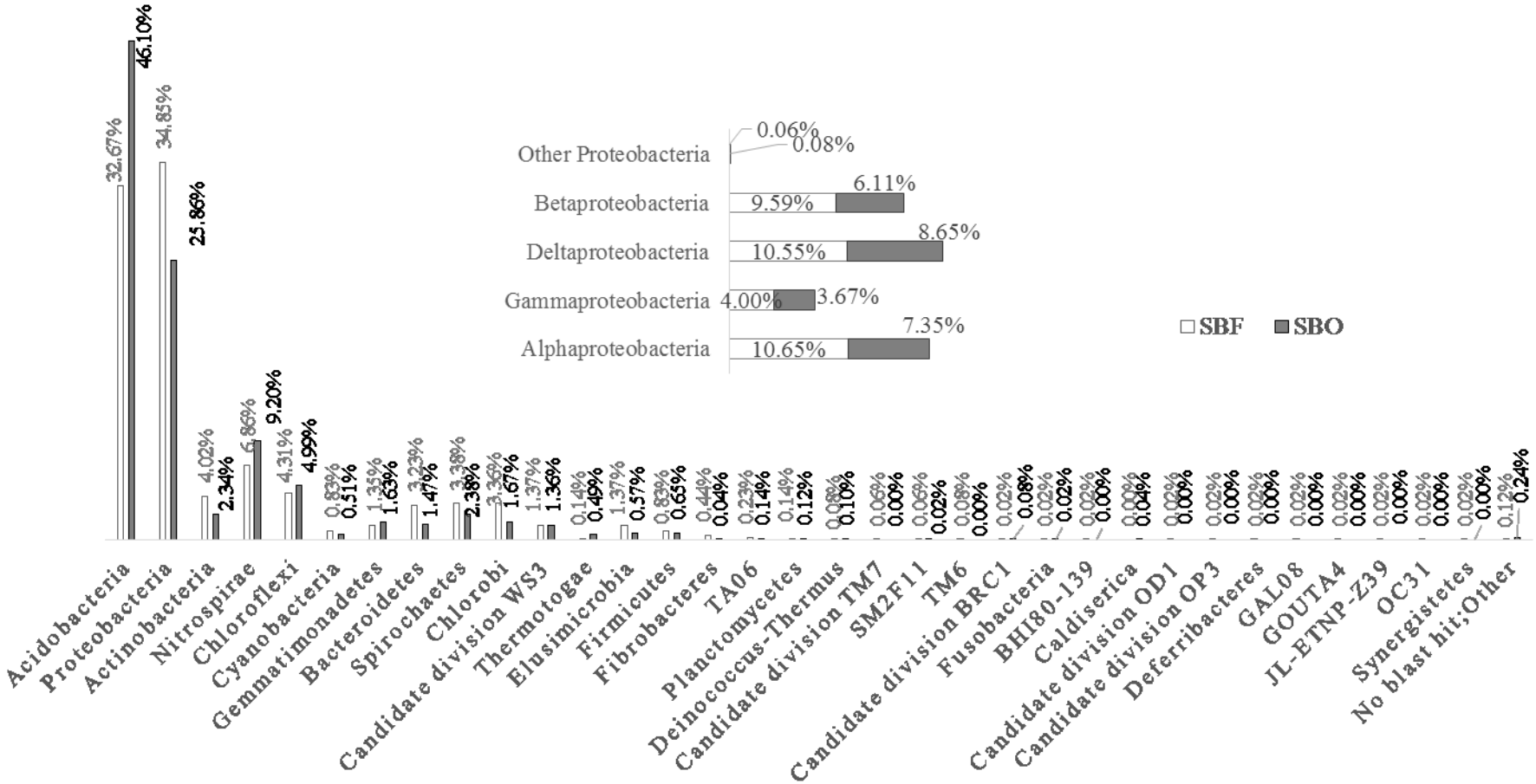


Figure 19 Average relative abundances of dominant bacterial taxa in sediment samples collected at forest (SBF) and oil palm plantation (SBO) area.

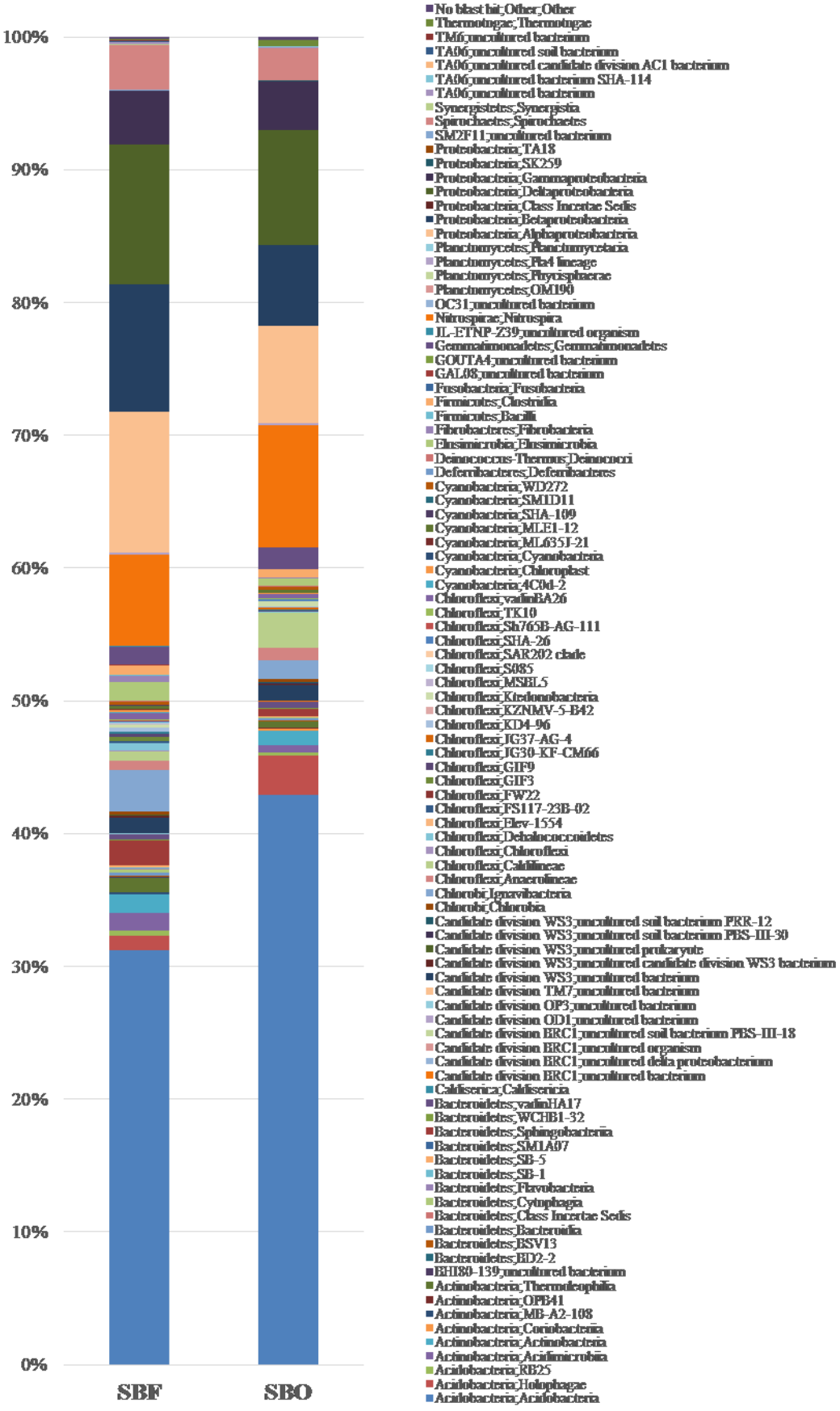


Figure 20 16S rRNA gene surveys revealed a clear distinction between the two bacterial class populations investigated. Comparison of the percentage of sequences (relative abundances) affiliated with different classes from sites SBF and SBO.

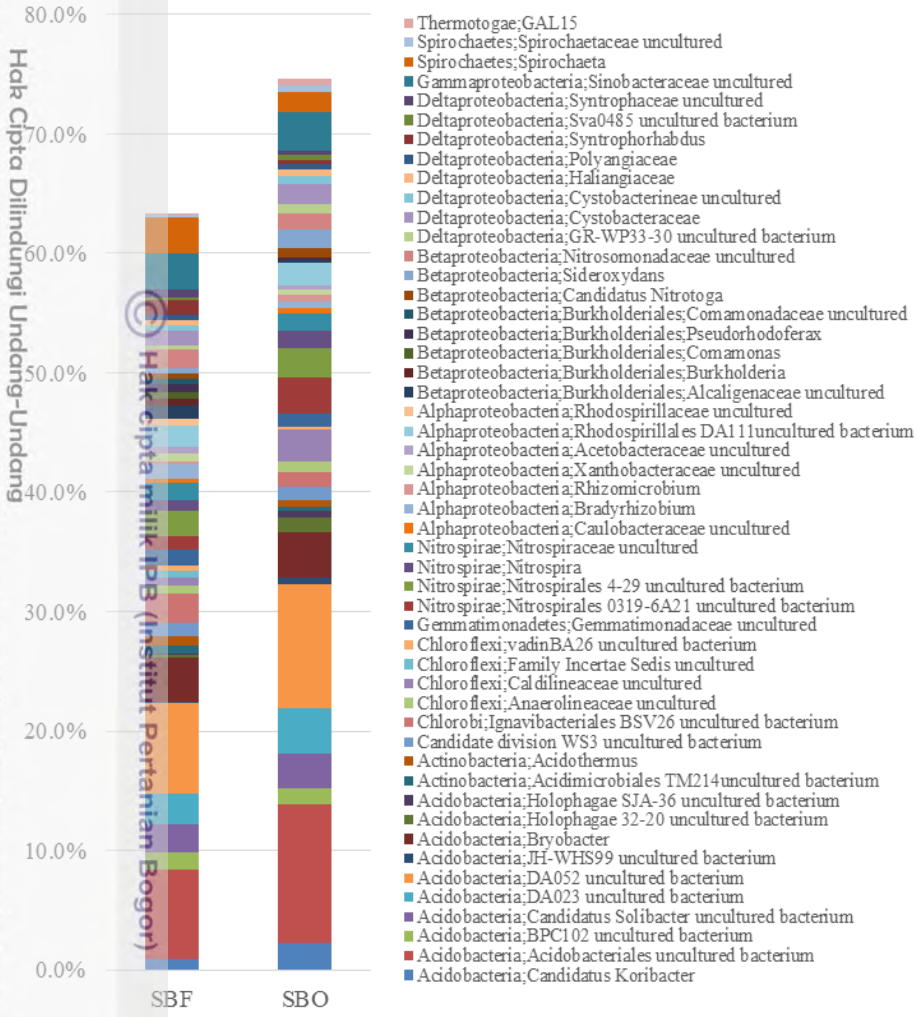


Figure 21 Comparison of the overall distribution of 50 predominant genera (more than 0.5% of total sequenes) within sites SBF and/or SBO.

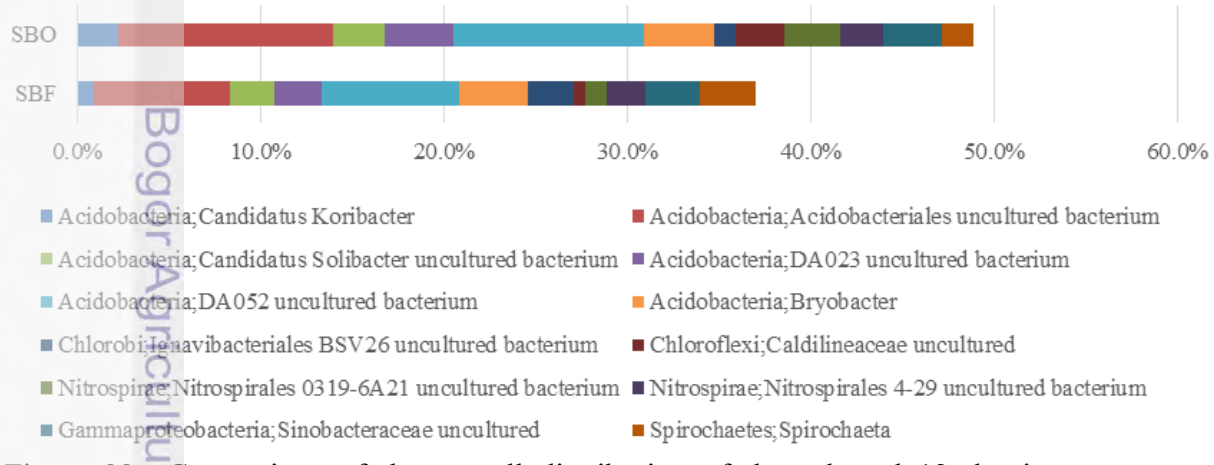


Figure 22 Comparison of the overall distribution of the selected 12 dominant genera (more than 2% of total sequences) from 50 predominant genera within sites SBF and/or SBO.

Mechanisms by which water table influences microbial communities are likely to be different in a depth profile, depending on oxygen availability. In the oxic zones, lowwater-table sites undergo more pronounced cycles of drying and wetting compared to high-water-table sites. Drying and wetting of peat leads to alternating aerobic and anaerobic physiological responses of the microbes. Thus, drying–wetting process selectively enriches those resilient members of microbial communities that can tolerate these changes both in physical environment and physiological functions. Examples of such successful resilient bacterial taxa are Actinobacteria and Firmicutes that have a very thick peptidoglycan layer to with stand changes in the physical environment and adapt to a broad range of oxygen availability (Mishra *et al.* 2014).

Phylogenetic of Bacterial Community for SBF and SBO sites

16S rRNA gene-based phylogeny showing representatives of all bacterial phyla from tropical low land secondary forest (Figure 23) and oil palm plantation (Figure 24) soil derived sequences have been obtained. The twenty phyla of bacteria were found in SBF site, while fourteen phyla of bacteria were found in SBO site. More phyla in SBF showed that bacterial diversity in SBF was higher than those in SBO. Proteobacteria was found as the highest phylum abundance in sediment of forest and oil palm plantation area. Proteobacteria might play an important role in water self-purification. Alpha and Beta-proteobacteria were more abundance than others class of Proteobacteria, Gamma and Delta-proteobacteria. The fewest abundance among classes of proteobacteria was Gammaproteobacteria in both the forest and plantation areas. The implication is that the *α*-, *β*-, and *γ* -*Proteobacteria* differ in their nutritional requirements, with the *γ –Proteobacteria* favored where substrate concentrations are high (Wargner *et al.* 1993, Zavarzin *et al.* 1991). Sites of this study had low carbon and nitrogen content (Table 3). Briee *et al.* (2007) showed that beta- and alpha-Proteobacteria were much more abundant in sediment than in plankton libraries, with the alpha-Proteobacteria nearly absent from water samples. Most delta-
Proteobacteria are sulfate reducers; they are abundant and play a cardinal role in anoxic settings, including meromictic and anoxic lakes (Briee *et al.* 2007). Sequencing the DNA of the recovered clones revealed a high prevalence of the Proteobacteria phylum (57% of analysed sequences) (Peixoto *et al.* 2011). In contrast with Peixoto statement, Gamma-proteobacteria being the fewest abundant group, followed by Deltaproteobacteria, Betaproteobacteria and Alphaproteobacteria, because limited inorganic and organic compound were in aquatic sediment at both sites. Alpha-, Betaproteo-bacteria and Cytophaga-Flavobacteria were influenced by pH, conductivity and temperature as well as by inorganic and organic carbon compounds, whereas phosphorous compounds and nitrate showed specific influence on single bacterial groups. These results can be used to predict future bacterial group shifts, and potential ecosystem functioning, in the landscapes under environmental transformation (Freimann *et al.* 2014). Beta- and partly Alpha-proteobacteria are often predominant in sediments in lower elevation streams (Kloep *et al.* 2006, Brablcova *et al.* 2013).

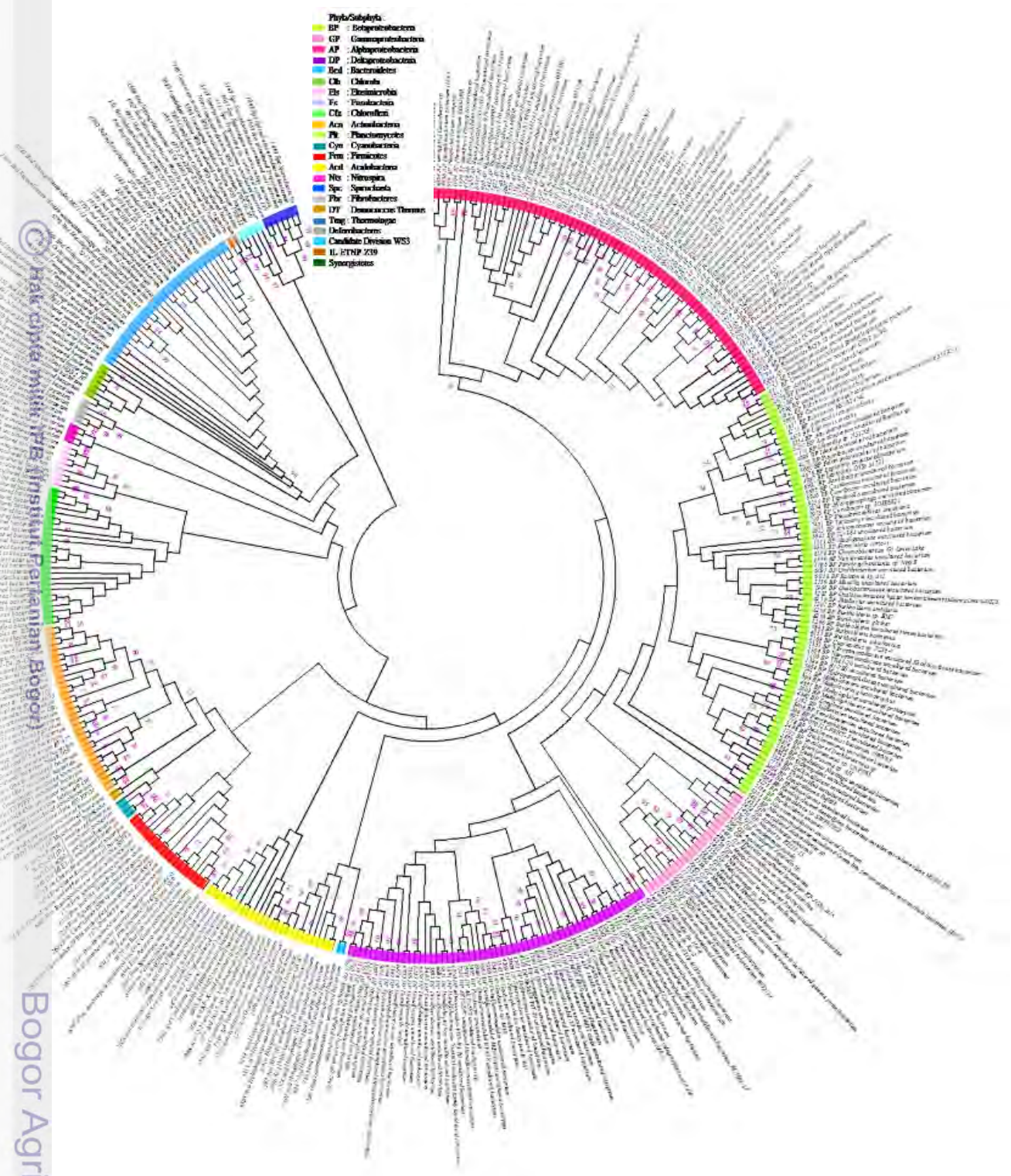


Figure 23 16S rRNA gene-based phylogeny showing representatives of all bacterial phyla in aquatic sediment from tropical low land secondary forest derived sequences have been obtained.

Note : Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980). The tree with the highest log likelihood (-40408, 3728) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0, 5578)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 12, 1163% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 412 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 380 positions in the final dataset. Evolutionary analyses were conducted in

Figure 24 16S rRNA gene-based phylogeny showing representatives of all bacterial phyla in aquatic sediment from oil palm plantation derived sequences have been obtained.

Note : Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar 2000). The tree with the highest log likelihood (-29292.3205) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4661)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.0000% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 266 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 427 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013).

Betaproteobacteria has been described as a diverse group dominating freshwater systems of different oligotrophic states and are highly competitive at the initial state of biofilm development. Betaproteobacteria also have been shown to be involved in the degradation of pollutants, and thus may provide beneficial ecosystem functions within the floodplains. Indeed, precipitation driven pollutant inputs may favor Betaproteo-bacteria. Biofilm bacterial assemblages in urban rivers have been shown to be dominated by Betaptoteobacteria and Cytophaga-Flavobacteria, which may be linked to their pollution load (Freimann *et al.* 2014). The alpha-proteobacteria composition taxa was more associated with phenanthrene. The phenanthrene, 16 PAH (polyaromatic hydrocarbon) and specific anthracene PAH, were more associated with the Actinobacteria group. These composition data are important for guiding the choice of potential groups for further bioremediation or for approaches to monitoring each type of pollutant in aquatic sediment ecosystems as similar to mangrove ecosystem (Peixoto *et al.* 2011). Alphaproteobacteria became more dominant at acidic to intermediate pH (Griffiths *et al.* 2011) in soil and aquatic sediment areas. This class had more genera than others because of their capability to survive in acidic soil and degrade herbicide. Similar with oil palm plantation soil, the freshwater sediment contained herbicide for treating unwanted vegetation surrounding areas.

The highest genera in phylogenetic tree (Figure 23 and 24) is *Burkholderia*. *Burkholderia* is one genus of genera of Betaproteobacteria, similar with in both soil areas. *Burkholderia* genera were found 8 phylotypes from 42 Burkolderiales in forest freshwater sediment and 4 phylotypes from 19 Burkholderiales in oil palm plantation freshwater sediment. In study of culturable bacteria, genera of *Burkholderia* were the most population of bacterial isolates from both areas, forest and oil palm plantation soil freshwater sediment (Wijayanti *et al.* 2014). Suárez-Moreno *et al.* (2012) found that the *Burkholderia* genus comprises 62 validly described species, and their taxonomy has been continuously revised. Two main clusters may be distinguished within the genus *Burkholderia*. One cluster comprises the *B. cepacia* complex (BCC), the "pseudomallei" group, and plant pathogens, as well as endosymbiotic species from phytopathogenic fungi. The second cluster contains non-pathogenic *Burkholderia* species associated with plants and/or the environment. The non-pathogenic plant-associated species could, therefore, constitute a single clade which contains closely related species. *Burkholderia* from this group are mostly associated beneficially with plants, although some species may also survive in sediments and bulk soil.

Remarkably, several species from this group can convert atmospheric nitrogen to ammonia via biological nitrogen fixation (BNF). In addition, most of them are catabolically versatile enabling them to degrade recalcitrant compounds, and thus to survive in environments with limited nutrient availability. They tolerate acidity and have a strategy involving both tolerance to abiotic stress factors (Stopnisek *et al.* 2013). Strains of the genus *Burkholderia* occupy a wide range of ecological niches and have versatile properties of bioremediation and biocontrol (Coenye and Vandamme, 2003). Bacterial sediment of streamline at oil palm plantation area must be more tolerance to ecological stress from pollutants (pesticides and fertilisers), mill tailings, and village activities around stream run off (Sheaves 2014).

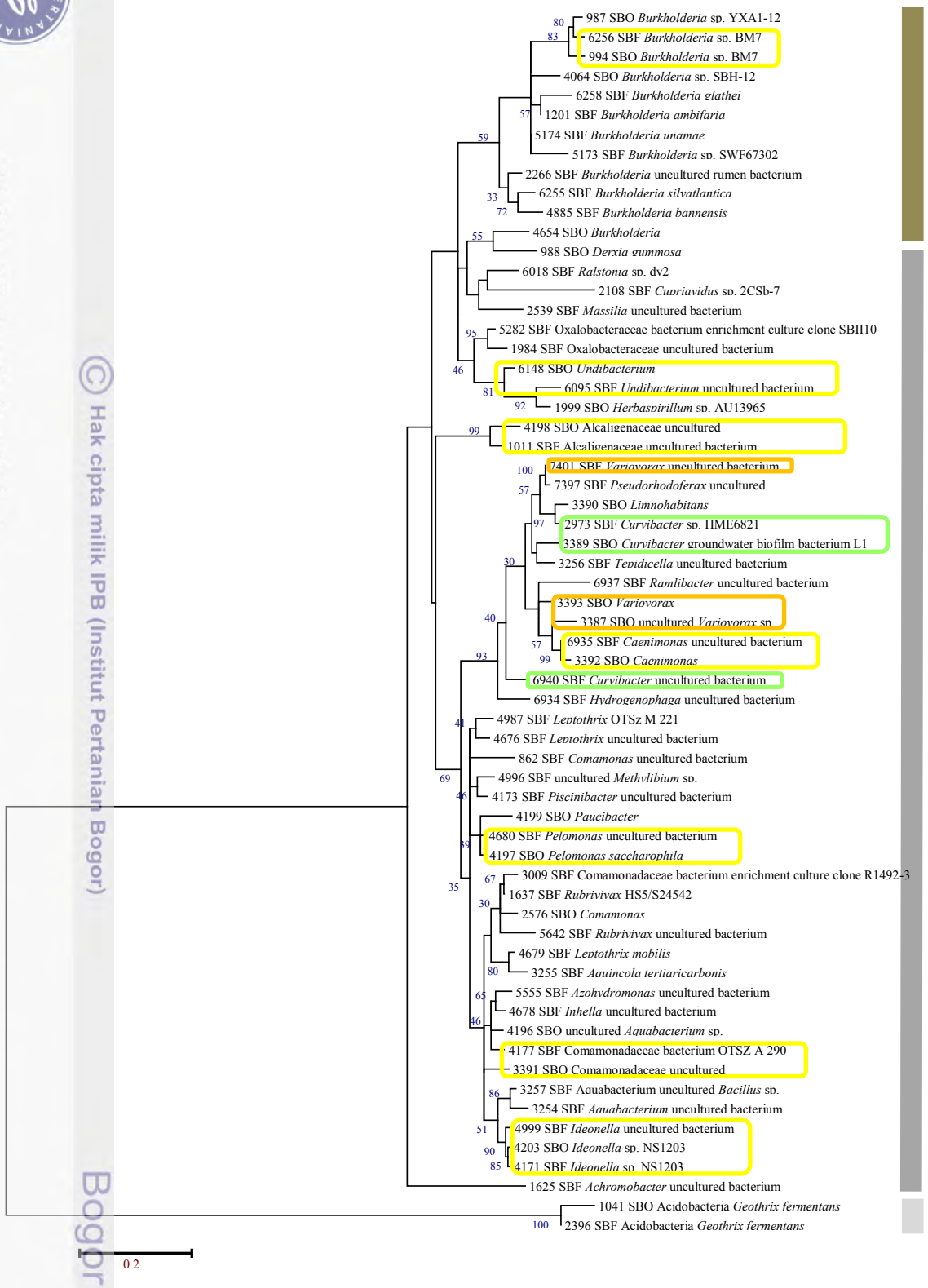


Figure 25 16S rRNA gene-based phylogeny showing representatives of Burkholderia (Dark grey), other Burkholderiales (Grey) and out group Acd (Acidobacteria) (Light grey) in soil derived sequences have been obtained from SBF and SBO areas.

Note : The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood (-3884.9881) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.2078)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 42.6432% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 63 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 372 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013).

The members of the family are heterotrophic and nonspore-forming gram-negative bacteria. They are mesophilic with the exception of some psychrophilic species. The cells are characteristically Gram-staining-negative, oxidase-positive, rod-shaped, motile by a single polar flagellum and chemo-heterotrophic (Sheu *et al.* 2014). The predominant habitat of the Undibacterium genus is the water and soil (Kim *et al.* 2014).

The genus *Curvibacter* was a Gram-negative, heterotrophic, aerobic, curved rod-formed bacteria. At present, genus *Curvibacter* comprises three recognized species, the type species *Curvibacter gracilis, Curvibacter delicatus* (formerly [*Aquaspirillum] delicatum*), and *Curvibacter lanceolatus* (formerly [*Pseudomonas*] *lanceolata*) (Ding and Yokota, 2004). The major characteristics of the genus Curvibacter are the cell shape is slightly curved rods, the flagella arrangement is polar or none, they are aerobic or microaerobic, the colony pigmentation is yellow-brown, and the ubiquinone is Q-8 (Ding and Yokota 2010). The genome of a *Curvibacter* bacterial species that is stably associated with *Hydra magnipapillata* and the *Chlorella* NC64 genome project, together with several large-scale expressed sequence tag (EST) projects have brought a molecular perspective on the *Hydra* holobiont (Bosch 2012).

Cells of *Variovorax* are motile using flagella and occur as single cells or in pairs. The dimensions of the straight to slightly curved rods amount to 0.3–0.6× 0.7–3.0 μm. The colonies are yellow because of carotenoid pigments. They tend to be slimy and shiny. *Variovorax* is Gram-negative and oxidase and catalase positive. The genus is aerobic or facultative anaerobic, and cells are generally able to store poly (3-hydroxybutyric acid) as an intracellular carbon source for periods in which extracellular carbon sources are scarcely available (Satola *et al.* 2013).

Caenimonas are Gram-negative, strictly aerobic, non-motile rods bacteria. Catalase- and oxidase-positive. Nitrate is reduced to nitrite and thiosulfate is oxidized to sulfate. No aerobic chemoautotrophic growth with hydrogen as substrate. Contain phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol and an unknown amino groupcontaining lipid as polar lipids. The major isoprenoid quinone is ubiquinone-8 (Q-8). Major polyamines are 2- hydroxyputrescine and putrescine. This genus was isolated from activated sludge performing enhanced biological phosphorus removal in a sequencing batch reactor (Ryu *et al.* 2008).

Pelomonas is betaproteobacteria for anaerobic benzene biodegradation similar with *Pseudomonas,* while aerobic bacteria for benzene biodegradation include *Pseudomonas*, *Comamonas*, *Alcaligenes*, *Acinetobacter* and *Burkholderia* spp. (Liou *et al.* 2008). Freshwater sediment in forests and their transformation, oil palm plantation, have micro aerophilic condition for bacterial growth. They contain several anthrophogenic pollutant. Microaerobic and anaerobic of benzene degrading bacteria play a crucial role for biodegradation process in water table and or water sediment. *Ideonella* is the chlorate-respiring bacterium and a facultative anaerobe bacteria that can use both oxygen and chlorate as terminal electron acceptors (Lindqvist *et al.* 2015). Chlorate (ClO^{3-}) and perchlorate (ClO^{4-}) are toxic compounds, mostly of anthropogenic origin, which have been rhappeneleased into soils and waters (Franca *et al.* 2015). This condition almost occured in stream of forest and oil palm plantation as domestic run off.

In soil and aquatic sediment of forest and oil palm plantation area, the special genera of bacteria were *Burkholderia.* The *Burkholderia* genus includes species

whose ecological properties can differ enormously from each other, and new species colonizing amazingly different habitats are continuously discovered (Paganin *et al.* 2011). The genus is one of cultivable bacteria and important lipolytic bacteria which multifunction for human and environment welfare.

Soil and Aquatic Sediment Bacteria Reveal Shifting Bacterial Comunities in Forest and Oil Palm Plantation

Based on both habitat, the data of environmental parameter showed that soil and sediment acidity determined bacterial communities. The most abundance of bacterial phylotype in top soil and aquatic sediment at forest and oil palm plantation was Acidobacteria. The pH value of both habitat and sites showed about 3.5 – 5.0. It was included in acid soil and acid sediment. Increasing one pH value means decreasing concentration of ion Hydrogen (H^+). It impact to transport system of bacteria in situ. The acidity has been determining factor for bacterial community composition. Tropical Soil Quality Index (TSQI) determined that pH value 3.01 to 4.0 is index 0, strongly acid. It is only the most acid tolerant plants can grow in this pH range and then only if organic matter levels are high enough to mitigate high levels of extractable Al and other metals. If the pH value 4.01 to 5.5, they are included index 1 TSQI. It is moderate acid, growth of acid intolerant plants is affected depending on levels of extractable Al and other metals (Amacher *et al*. 2007).

Total Nitrogen and Total Carbon were not different between soil and sediment in forest and oil palm plantation. TN and TC categorized as index 1 in TSQI. They were included in moderate adequate level. It means that both habitat could supported the bacterial metabolism. Soil macro and micro-organism utilized and decomposed, and released various types of enzymes involve in nutrients cycling which in turn is released into the soil. Arifin *et al.* (2012) explained that at monoculture plantation such as planted plot, it has lower quality index compared to mix-planted forest like enrichment planting due to only one type of tree planted. Singular species trigger only certain population of soil microbes because not all microorganisms have the same requirement of nutrients from the same forest litter from one type of tree species. Furthermore, same type of forest litter available on the forest floor will cause faster rate of microorganism growth spreading all over the plots. Problem always arise on the infestation of pest and disease on monoculture plantation because of the same or similar biochemical and physiological characteristic of the tree species planted which enhanced the insect's infestation or bacteria and fungi inoculation. At 0 to 15 cm depths, the forest soil was considered the most fertile with abundance of organic matter, humus with soil microbes. Abundance of soil microbes also found to be more concentrated at 0 to 15 cm depth due to many plant roots at that soil layer. Most of the soil quality evaluations focus on the topsoil rather than subsoil because most of the biology and biochemical reactions in the soil occurs in the topsoil (Arifin *et al*. 2012).

Decreasing Total Carbon and Nitrogen (TC and TN) content in oil palm plantation showed organic and/ or nitrogen fertilizer had not been used in maintenance of oil palm plantation. Composition of total Carbon and total Nitrogen in soil were different compared to aquatic sediment. In soil, Carbon and Nitrogen material are still in unsolved form, but in sediment, they are more solved form. The

C and N in sediment could be easier used by microbe in situ than those in soil, even though their concentration are less. So, it was reliable that bacterial richness and diversity in aquatic sediment were higher than in soil. On the other hand, Available Phosphorus (AP) had been increased exceed twenty fold in soil and decreased about seven fold in aquatic sediment from forest area to oil palm plantation area. The available P in both area and habitat, had been included high in TSQI. It is excellent reserve of available P in slightly acidic to alkaline soils, possible adverse effects to water quality from erosion of high P soils (Amacher *et al.* 2007). Available P in soil forest area had not affect to aquatic sediment because they followed the stream of water, they could not been precipited at aquatic sediment.

Phosphorus level could influenced bacterial richness and diversity at phylotype level. Rarefaction curve of bacterial OTUs among four habitats (Figure 26) showed that the high bacterial richness belonged to SBF (forest aquatic sediment) among other habitats. Bacterial richness in sediment was higher than those in soil. It could be influenced by condition of aquatic sediment (microoxic and pH value) and their available phosphorus for bacterial community. The available phosphorus in aquatic sediment was easier for absorbing in transport system of bacteria than in soil. The bacterial richness of sediments were higher than soils, eventough the available phosphorus of oil palm plantation area between sediment and soil habitats were not different significantly.

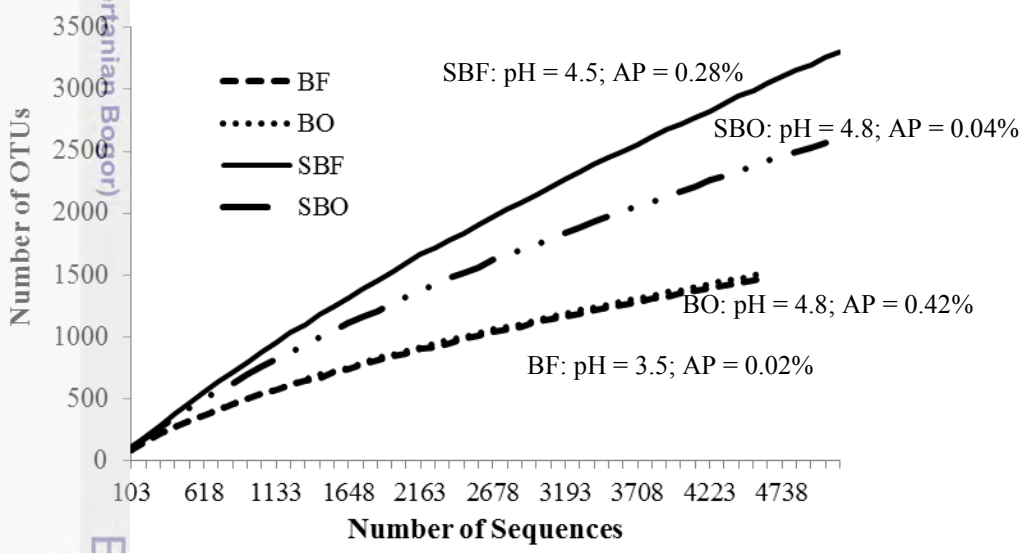


Figure 26. Rarefaction curves showing the observed number of operational taxonomic units (OTUs) at 1% dissimilarity for samples from sites forest (BF, SBF) and oil palm plantation (BO, SBO), with means of pH and available phosphorus (AP) values .

Figure 26 showed the highest number of bacterial OTUs among four habitats (BF, BO, SBF, and SBO) is bacterial number of sequences in aquatic sediment from forest area. It was reasonable, because the forest aquatic sediment had pH value exceed 4, and the available P raised high level (based on TSQI). In aquatic habitat, the available P has been used bacteria and other biotas easily, because of higher solubilisation, if it compared to terrestrial habitat. They showed that bacterial communities shift were more sensitive to respond the land use change to be oil palm

plantation in aquatic sediment than those in top soil area. It could be caused by the forest included secondary lowland rainforest. It has high level of rainfall, so that the run off water could wash the topsoil bacteria, especially in rainy season which occured almost all year.

Based on the 16S rRNA gene, bacterial richness and diversity in soil did not decrease from pristine vegetation to agricultural uses, but its composition shifted with land use change (Lammel *et al*. 2014, Lee-Cruz *et al*. 2013). Clearance of forest for oil palm plantations did, however, significantly impact the composition of soil bacterial communities, reflecting in part the loss of some forest bacteria. This might suggest that while some factors tend to make bacterial composition more distinct between oil palm plantations and forests, there are others that promote considerable variation in bacterial composition in oil palm plantation soils (Lee-Cruz *et al*. 2013). This is partially supported by the variation partitioning results, in which soil parameters (i.e., pH and available phosphorus) explained the largest proportion of variation in OTU abundance with this variation being neither spatially structured nor related to land use.

Lee-Cruz and colleague's results suggest that diversity of forest soil bacteria is partly either resilient or resistant to land use change. Past deforestration cycles appear to have no long-term effects, which is promising for the recovery of logged forests soils and for the continued provisioning of ecosystem functions. This reinforces the general picture obtained by studying larger organisms. Even clearance of forest for oil palm plantation does not depress the overall diversity of bacteria (Lee-Cruz *et al*. 2013). However, differences in certain taxonomic groups and changes in community composition between forests and oil palm plantations indicate that oil palm cultivation does have some negative impacts, direct in macrobiota, and undirect in bacteria. The negative impact of oil palm plantation must be limited. Our suggestions based on this study are:

1. To restrict extensivication of oil palm plantation with determining forest as buffer area for environment carrying capacity.
2. Using biofertilizer, bioherbicides, and agricultural lime during maintenancing the oil palm plantation.
3. To evaluate the assessment of environmental welfare and sustainable during developing and maintenancing oil palm plantation and buffer forest area.

If the bacterial community retains its function in spite of changes in its composition due to cultivation, then ecosystem processes might be little affected. This could represent good possibilities of long-term restoration of rainforest, both in cases where oil palm plantation lands are purchased to reconnect forest fragments or more generally should the economics of oil palm change such that these lands are abandoned. It is role which used in development of oil palm plantation at Malaysia (Lee-Cruz *et al*. 2013).

One of economic of oil palm change is development of oil palm plantation and mill. They give some impact to the environment with oily environment, so that lipase producing bacteria might survive, grow, and degrade the oily contaminated soil, water, or sediment. Lipase producing bacteria had been special bacteria in oil palm plantation. It will be desribed in the next.

Lipolytic Bacterial Isolates Obtained from Forest and Oil Palm Plantation

There were 32 culturable isolates of soil and aquatic sediment from the forest and the oil palm plantation, grown on lipolytic bacteria selective medium. Preliminary data showed that 22 isolates were growing on purely olive-oil lipolytic bacteria medium. Rhodamine B medium caused formation of a lytic zone around the colony of lipolytic bacteria in one qualitative assay. Isolates of lipolytic bacteria were separated according to the source of the sample, topsoil from the forest and the oil palm plantation accounted for 6 and 5 isolates, respectively, and aquatic sediment from the forest and the oil palm plantation accounted for 4 and 7 isolates, respectively. The lipolytic bacteria from sediment in water in the forest and at the oil palm plantation showed more genera compared that from top soil (Table 8).

Three genera of bacteria, *Burkholderia*, *Serratia*, and *Kurthia,* were found in forest samples. Three genera of bacteria, *Burkholderia*, *Cupriavidus*, and *Acinetobacter,* were found in oil palm plantation samples. *Burkholderia* was found in both forest and plantation. This genera also dominated in both topsoil and aquatic sediment habitats. Isolates of cultivable lipolytic bacteria from topsoil in the forest area (BF) and in the oil palm plantation area (BO) were dominated by *Burkholderia* (Table 8). Members of this genus are also found in a variety of ecological niches. They inhabit humid areas, industrial zones, and the rhizosphere, existing in symbiosis with various plants and mushrooms (Stoyanova *et al.* 2007). The gram-negative, rod-shaped bacteria were found in acidic forest soil (Otsuka *et al.* 2011) and burned forest soil (Nion and Toyota 2008). The soil in the forest is acidic soil, with a pH value of 3.1–5.0. Oil palm plantation soil also was acidic, with an average pH value of 5.0. The plantation area is suspected to be a burned forest area.

Initial purification of bacterial lipolytic isolates showed that lytic zones indicate the diversity of bacterial lipolytic capabilities. It could not be detected that some isolates even had lytic zones, as was the case with isolates BF02, BF06, SBF15, SBO03, and SBO12. Isolates SBF11, SBF12, SBO01, and SBO04 had formed lytic zones of more than 10 mm. These isolates came from aquatic sediment. The diameters of lytic zones in isolates from the forest were greater than those from the oil palm plantation.

Phylogenetic analysis of 22 identified isolates of lipolytic bacteria resulted in three clusters (Figure 27). The first cluster is dominated by all bacterial isolates closely related to *Burkholderiales* in the forest and oil palm plantation area, including in the topsoil and aquatic sediment. This cluster consisted of the genera *Burkholderia* and *Cupriavidus*. The second cluster consisted of the genera *Serratia* and *Acinetobacter*. Those isolates closely related to the genus *Serratia* were acquired from forest aquatic sediment (Table 8), whereas those closely related to *Acinetobacter* were acquired from oil palm plantation aquatic sediment. The third cluster consisted of only one Bacillales, the genus *Kurthia*. The isolate *Kurthia* BF02 was obtained from forest soil.

Table 8. Isolates of lipolytic bacteria from forest and oil palm plantation soil and aquatic sediment and their lytic zones

Code of isolates: Taxon assignment	Identity (%)	Diameter of lytic zone (mm)
BF01: *Burkholderia* sp.	99	7.75 ± 0.35
BF02: *Kurthia* sp.	100	Nd
BF03: *Burkholderia cepacia*	99	6.25 ± 0.35
BF04: *Burkholderia* sp.	99	7.50 ± 0.71
BF06: *Burkholderia oxyphila*	99	Nd
BF11: *Burkholderia* sp.	99	7.25 ± 0.35
BO01: *Burkholderia cepacia*	100	6.00 + 1.41
BO03: *Burkholderia* sp.	100	7.50 + 0.00
BO04: *Burkholderia* sp.	99	7.25 + 0.35
BO11: *Burkholderia vietnamiensis*	99	7.50 + 1.41
BO12: *Burkholderia pyrocinia*	100	6.00 ± 0.00
SBF02: *Burkholderia* sp.	99	3.75 ± 0.35
SBF11: *Serratia* sp.	99	17.00 ± 1.41
SBF12: *Serratia* sp.	99	15.75 ± 0.35
SBF15: *Burkholderia* sp.	99	Nd
SBO01: *Burkholderia* sp.	99	14.00 ± 0.71
SBO02: *Burkholderia* sp.	99	7.50 ± 0.71
SBO03: *Acinetobacter* sp.	98	Nd
SBO04: *Acinetobacter* sp.	99	10.25 ± 2.47
SBO06: *Cupriavidus* sp.	99	5.25 ± 1.06
SBO11: *Acinetobacter* sp.	98	7.50 ± 0.71
SBO12: *Acinetobacter* sp.	99	Nd

Note: Blast result from NCBI with query cover 100% and e-value 0.0
nd = none determined (isolates could grow on lipolytic medium, but their lytic zone was < 0.5 mm)

The first cluster of phylogenetic tree consisted of isolates closely related to the genus *Burkholderia,* and only isolate SBO06 was closely related to the genus *Cupriavidus* (Figure 27). *Burkholderia* is a phylogenetically coherent genus within a beta-proteobacterial subphylum first described by Yabuchi at 1992 (Paganin *et al.* 2011) by transferring seven species from the genus *Pseudomonas*. If they were isolated from their environment, they may have formed non-pathogenic associations with plants, such as *Burkholderia cepacia*. In recent years, new *Burkholderia* species have been reported as nitrogen fixing, non-pathogenic, plant growth promoting bacteria, and have been proposed as candidates for biotechnological applications (Paganin *et al.* 2011). *Burkholderia* species are producers of lipolytic enzymes. Lipolytic enzymes, which have been produced by *Burkholderia cepacia* and *Burkholderia glumae*, could be classified in the lipolytic family 1.2 (family 1 and subfamily 2) as true lipases (Jaeger and Eggert 2002).

In the first cluster, the isolate SBO06 is closely related to the genus *Cupriavidus*. The isolate formed lytic zones with diameters of less than 10 mm (Table 8). *Cupriavidus* cells are gram-negative, peritrichously flagellated rods, non-sporulating, non-fermenting bacterium originally inhabiting soil. They were obtained from aquatic sediment in the oil palm plantation area. Their metabolism was oxidative, thus, they were found only in shallow river sediment. Several amino

acids are their sole carbon and nitrogen sources. The genus which resistance to various metals is widespread.

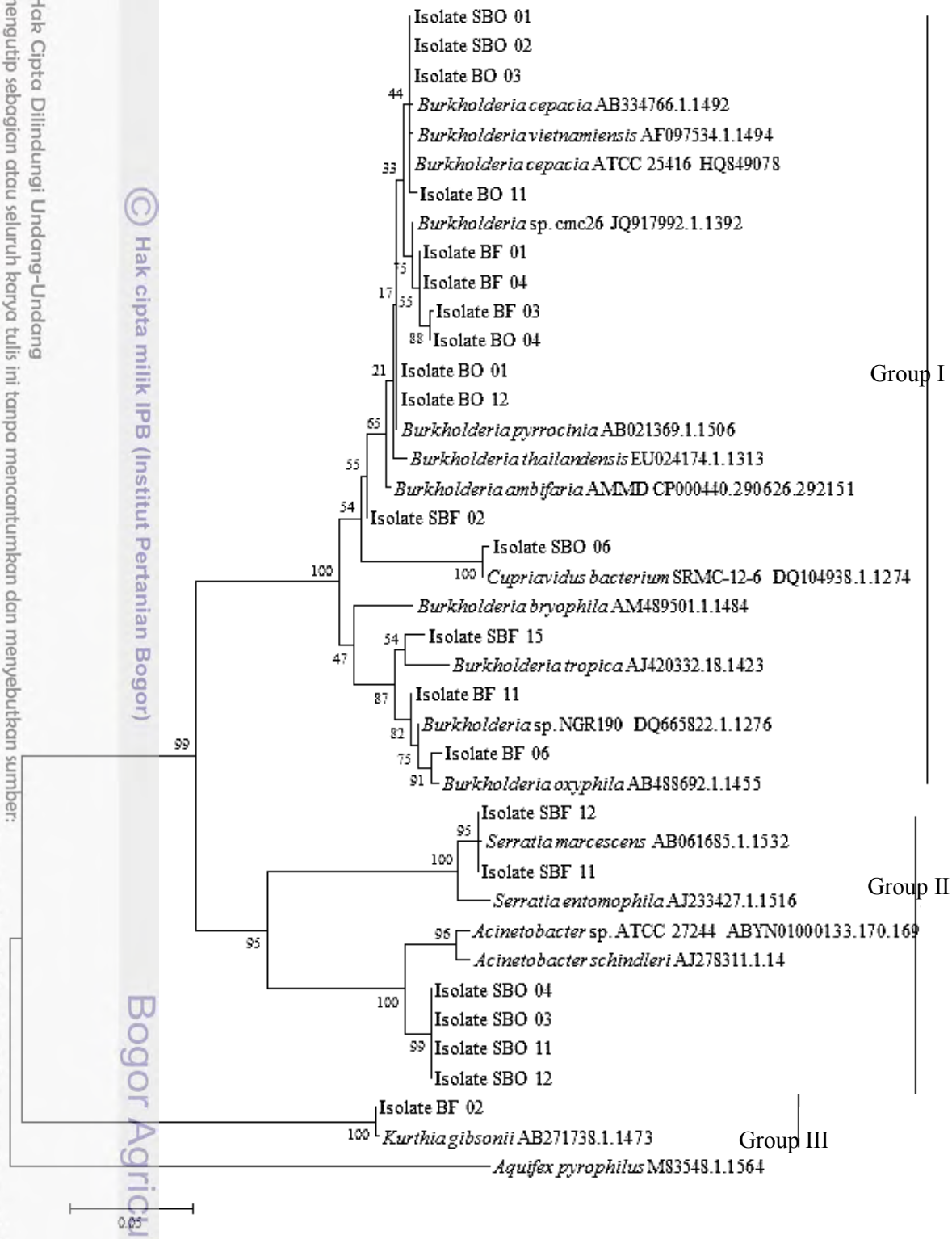


Figure 27 Phylogenetic tree constructed by Maximum Likelihood method based on 16s rRNA gene sequences data of 22 lipolytic bacterial isolates.

Note: The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). The tree with the highest log likelihood (-3986.6116) is shown. The analysis involved 40 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 745 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.* 2011).

The genus *Cupriavidus* is included in the biodiversity of various *Burkholderia cepacia*-like bacteria (Vandamme and Coenye 2004). They are beta-proteobacteria that have been identified as capable of establishing nitrogen-fixing nodules on legumes (Hirsch and Fujishige 2012). The isolate SBO06 could be a candidate for a biofertilizer for agriculture.

Family 1 included true lipase from bacteria, which is defined as a carboxylesterase that catalyzes the hydrolysis and synthesis of long-chain acylglycerols, with trioleoylglycerol being the standard substrate (Jaeger and Eggert 2002). Subfamily 2 showed that expression in an active form of lipases depends on a chaperone protein named lipase-specific foldase, usually characterized by a slightly larger size (33 kDa) owing to an insertion in the amino acid sequence forming an anti-parallel, double-beta strand at the surface of the molecule (Arpigny and Jaeger 1999). Large-size molecules could have more difficulty crossing agar medium polymer; thus, their isolates might not be able to make large lytic zones in rhodamine B agar medium (Table 8).

The second cluster of phylogenetic tree consisted of isolates close to the genera *Serratia* and *Acinetobacter*. These were found only in sediment. *Serratia* isolates, SBF11 and SBF12, were found in forest sediments, and *Acinetobacter* isolates, SBO03, SBO04, SBO11, and SBO12, were found in sediments in the oil palm plantation area. *Serratia* is a motile, gram-negative, rod-shaped, facultative anaerobe, commonly found in soil, water, air, plants, and animals. In this study, *Serratia* isolates were found only in aquatic sediment, because they are facultative anaerobes. One characteristic of aquatic sediments is that they are micro-aerophilic, having close to semi-anaerobic conditions. *Serratia marcescens* releases an extracellular lipase and phospholipase into the medium (McMahon *et al.* 2012). The purified lipase is a dimer with two homologous subunits, of which the molecular mass is between 52–65 kDa, and the pI is 4.2. The optimal pH was shown to be pH 8–9, and the optimal temperature was shown to be 45 ^{o}C (Gupta *et al.* 2004, Zhao *et al.* 2008). In this study, the isolates SBF11 and SBF12 could form lytic zones of the greatest diameter (Table 8). Both isolates could be the best lipolytic bacteria candidates among our isolates.

Isolates SBO03, SBO04, SBO11, and SBO12, from oil palm plantation aquatic sediment, were phylogenetically close to the genus *Acinetobacter*. Organisms belonging to the genus *Acinetobacter* often are considered ubiquitous in nature, given that they can be recovered from almost all soil and surface water samples and can be pathogenic to human skin (Howard *et al.* 2012). These isolates are gram-negative, catalase-positive, oxidase-negative, non-motile, non-fermenting coccobacilli. This genus has been known to be involved in biodegradation, leaching, and removal of several organic and inorganic, man-made hazardous wastes (Abdel-El-Haleem 2003). *Acinetobacter* has been reported that produces lipase and specific foldase with optimal acidity of pH 9–10, although they produce lipolytic enzymes of pH 4–8. Lipases of *Acinetobacter* have molecular masses of from 23 to more than 200 kDa (Arpigny and Jaeger 1999, Abdel-El-Haleem 2003, Gupta *et al.* 2004). Isolates SBO04 and SBO11 had lytic zones of from 7.5–10.25 mm (Table 8). The diversity of their molecular masses could account for their forming various diameters of lytic zones. The extracelullar enzyme of isolate SBO04 formed the essential fatty acids linoleic and arachidonic acid (Table 10). The isolate SBO11

formed eicosapentanoic acid (EPA). Every strain of *Acinetobacter* might have had different types of lipases; thus, their fatty-acid products could vary by type.

The third cluster of phylogenetic tree showed that isolate BF02, from forest soil, was closest to the genus *Kurthia* (Figure 27). This isolate is a gram-positive, aerobic, rod-class *Bacilli* family *Planococcaceae* (Roux *et al.* 2012). The genus *Kurthia* originally was found in forest soil, rhizospheric soil of tea bushes, contaminated soil, sediment, water, and animals (Lalitha and Surendran 2004, Stackerbrandt *et al.* 2006, Pawar *et al.* 2012, Sharma *et al.* 2012). It could produce lipolytic enzymes, esterase lipase (C8) and esterase (C4), which are produced by *K. gibsonii* and *K. massilliensis*, although *K. zopfii* and *K. sibirica* could produce these enzymes weakly (Roux *et al.* 2012). The isolate BF02 could not produce lipase for hydrolyzing long fatty acids to short fatty acids, but it formed longer fatty acids for an essential fatty-acid product. The genus *Kurthia* was a potential lipase-producing bacteria that produced a great quantity of lipases, like *Bacillus* (Salihu *et al.* 2012).

Diversity of fatty acid production

The results of the GC-FID tests of supernatant separated from the bacteria biomass were shown at Appendix 2. Their unwanted and essential fatty acid yield of product per substrate were shown at Table 9. It shows results for soil and aquatic sediment lipolytic bacteria (Appendix 2). Linolelaidic methyl ester (C18:2n6trans) dominated the composition of fatty acids in the olive-oil substrate without lipolytic bacteria. All bacterial isolates showed diversity of fatty-acid products. Arachidic acid methyl ester (C20:0) was a fatty acid that dominated production of lipolytic bacteria from both the forest and the oil palm plantation. Isolate BO12 derived from oil palm plantation soil was closest to *Burkholderia* sp. (Figure 26). Isolates from soil and aquatic sediments contained fatty acids to at least 8 carbon chains (C8:0), including isolates BF04, BF06, BO03, BO04, BO12, SBO03, SBO06, SBO11, and SBO12. Those from the soil were close to the genera *Burkholderia,* and those from aquatic sediment, to *Acinetobacter*. The genera *Burkholderia* and *Acinetobacter* have been reported to have lipid metabolism for degrading and synthesizing various fatty acids (Otsuka *et al.* 2011, Santala *et al.* 2011). Lipolytic bacteria produce various fatty acids for arranging their cell structures, surviving environmental stress, and forming biofilm.

Essential fatty acids, such as linoleic acid (C18:2n6c), gamma linolenic acid (C18:3n6), arachidonic acid (C20:4n5), eicosa pentaenoic acid (C20:5n3), and docosahexaenoic acid (C22:6n3) were formed by isolates from both soil and aquatic sediment in both the forest and plantation area. Linoleic acids concentrations higher than substrate with olive oil (no lipolytic bacteria isolates) were contained in products of isolates BF06. Linoleic acid was produced by isolates BF01, BO01, BO12, and SBO04. The most linoleic acid was produced by isolate BO04. Isolates BF01, BO01, BO04, SBO04, and SBO11 from sediment. Most isolates were able to form EPA, except isolates from sediments, such as isolate SBF11. The highest EPA value was found in isolate SBO11, which was close to the genus *Acinetobacter*. The highest docosahexaenoic acid (DHA) value was produced by isolate SBF15, which was close to the genus *Burkholderia*. Isolates BF04, BF06, SBO01, and SBO04 could not synthesize DHA. Isolate SBO06, which was closest to the genus *Cupriavidus*, could synthesize essential fatty acids, such as EPA and DHA.

Cupriavidus pauculus has been reported to be able to produce true lipase (C14), ester lipase (C8), and esterase (C4), but others could not produce all three kinds of lipolytic enzymes simultaneously (Paganin *et al.* 2011). They produced catalase and oxidase to protect EPA and DHA from oxidative attack.

Polyunsaturated fatty acids (PUFAs) play an increasingly important role as biomedical and nutraceutical agents. Many of them belong to the essential fatty acids, uptake of which is required for membrane-lipid and prostaglandin synthesis. Microbial lipases are used to enrich PUFAs from animal and plant lipids, such as "menhaden," tuna, or borage oil. Free PUFAs and their mono- and diglycerides are used to produce a variety of pharmaceuticals, including anti-cholesterol-emics, anti-inflammatories, and thrombolytics (Jaeger and Reetz 1998). Bacterial lipases can discriminate against Omega-3 PUFAs, such as EPA and DHA, and, hence, lipase-catalyzed hydrolysis has been used for production of Omega-3 PUFA concentrates (Kapoor and Gupta 2012).

Table 9. Yield of unwanted and essential fatty acid produced by isolates of lipolytic bacteria from soil and aquatic sediment bacteria per substrate (olive oil)

Isolates	Yield [p]/[s]						
	C18:1n9t☆	C18:2n6t☆	C18:2n6c	C18:3n6	C20:0	C20:5n3	C22:6n3
BF01: *Burkholderia* cmc 26-1	6.75%	-70.42%	59.95%	-2.04%	0.43%	0.02%	0.07%
BF02 : *Kurthia sp. HD4.16*	-3.15%	-15.13%	-0.17%	-0.72%	0.56%	0.01%	0.02%
BF03 : *Burkholderia cepacia str.GXN152*	12.80%	-70.42%	-1.17%	5.85%	0.14%	0.13%	0.24%
BF04 : *Burkholderia* cmc 26-2	16.31%	-70.42%	-1.17%	3.51%	0.09%	0.09%	-0.04%
BF06 : *Burkholderia oxyphila*	-11.31%	4.14%	0.46%	-0.06%	1.02%	0.01%	-0.02%
BF11 : *Burkholderia sp NGR190*	8.26%	-70.42%	-1.17%	6.75%	-0.13%	0.13%	0.26%
BO01 : *Burkholderia cepacia / ambiforia*	12.27%	-70.42%	79.75%	2.41%	0.09%	0.10%	0.12%
BO03 : *Burkholderia sp. STJ14*	-0.45%	-70.42%	-1.17%	-1.34%	0.07%	0.28%	0.21%
BO04 : *Burkholderia* cmc 26-3	16.68%	-70.42%	107.85%	3.45%	-0.08%	0.08%	0.15%
BO11: *Burkholderia vietnamiensis strAU4i*	18.16%	-70.42%	-1.17%	-1.91%	0.16%	0.08%	0.00%
BO12 : *Burkholderia sp. F-6*	6.07%	-70.42%	56.46%	1.54%	0.10%	0.03%	0.08%
SBF02 : *Burkholderia* cmc 25	-11.06%	-44.90%	-0.72%	-1.29%	0.57%	0.00%	0.03%
SBF11 : *Serratia sp. NE17* -1	5.29%	-70.42%	-1.17%	4.46%	0.29%	-0.02%	0.13%
SBF12 : Serratia sp. NE17 -2	13.97%	-70.42%	-1.17%	9.81%	1.04%	0.15%	0.28%
SBF15: *Burkholderia sp. Enrich. cul.clone F45_07a_B*	-10.93%	-20.43%	-0.27%	-0.80%	0.70%	0.00%	0.33%
SBO01 : *Burkholderia sp. CCUG 57241*- 1	12.70%	-70.42%	-1.17%	-2.00%	0.24%	0.06%	-0.04%
SBO02 ; *Burkholderia sp. CCUG 57241* - 2	19.65%	-70.42%	-1.17%	5.29%	0.21%	0.10%	0.16%
SBO03 : *Acinetobacter sp. LMG V90* - 1	9.40%	-70.42%	-1.17%	1.16%	-0.13%	0.58%	0.05%
SBO04 : *Acinetobacter sp. LMG V90* -2	4.43%	-70.42%	46.75%	0.15%	0.56%	0.01%	0.00%
SBO06 : *Cupriavidus sp. SaCRH16*	-11.05%	-33.00%	-0.28%	-1.05%	-0.11%	0.53%	0.22%
SBO11: *Acinetobacter sp. LMG V90* -3	-11.10%	-43.68%	-0.70%	-1.18%	0.22%	1.37%	0.00%
SBO12 : *Acinetobacter sp. LMG V90* -4	-11.30%	-69.88%	-1.16%	-2.13%	-0.12%	0.00%	-0.04%

Note: ☆ : unwanted fatty acid (trans fatty acid), (-) : decreasing product of fatty acid.

The diversity of lipases produced from bacterial isolates shows that lipolytic bacteria cannot hydrolyze long fatty acids to short fatty acids, but they can form essential fatty acids with longer acylglycerol and longer fatty acid methyl ester (Appendix 2). Bacterial lipases have been reported that could synthesize important organic compounds. The lipase from *B. cepacia* is a catalyst in organic synthesis for the kinetic resolution of racemic mixtures of secondary alcohols in hydrolysis,

esterification, and trans-esterification (Gupta *et al.* 2004). Their capabilities can be used in several industries for food, feed, and bioenergy.

5 CONCLUSION

Bacterial diversity and richness in lowland forest and oil palm plantation soil were not different if soil and freshwater sediment pH, C, and N as substrate factors were not different significantly. Acidobacteria was the most dominant phylum in both areas, because soil pH in both areas was acidic. The genera of Alphaproteobacteria dominated in genera phylotype of bacterial 16S rRNA genes phylogenetic revealed both soil forest and oil palm plantation. Acidobacteria and Proteobacteria were the most dominant phyla in oil palm plantation and forest aquatic sediment areas, respectively, because of anthropogenic run off. The genera of alphaproteobacteria dominated in genera phylotype of bacterial 16s rRNA genes phylogenetic revealed both soil and sediment of forest and oil palm plantation. The bacterial shift community was occured in forest transformation, even though the oil palm plantation had more phyla and genera of bacteria than the lowland forest in soil, on the contrary in sediment. It was caused by plantation management, such as agricultural lime, herbicide, and others handling oil palm plantation.

Lipolytic bacteria isolated from soil and aquatic sediment in a forest and on an oil palm plantation at Sarolangun District, Jambi Province, Indonesia taxonomically belong to five genera and phylogenetically, are distributed in three groups: the *Burkholderia-Cupriavidus* group, the *Serratia-Acinetobacter* group, and the *Kurthia* group. Their lipolytic enzymes formed various fatty acids. Some isolates formed essential fatty acids, such as linoleic, linolenic, arachidonic, EPA, and DHA. In future, lipolytic bacteria from forest and oil palm plantation can be continued research for human and environment welfare.

Suggestion of this study can be divided in two part, the first is for further studying in bacterial ecology and lipase producing bacteria, the second one is for land use management. For studying bacterial communities revealed environment shift by pyrosequencing, the data should be wide and long term studies with measuring the alpha, beta, and gamma diversity and vary analytical methods for reducing biases. For lipase producing bacteria should be explored their potential lipase activity for production of essential fatty acid or eliminating trans fat. For land use management of oil palm plantation should be used buffer forest system for stabilizing of environment carrying capacity, sustainability, and ecosystem welfare.

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Appendix 1.

Overview Quality Filtering of 454 Pyrosequencing Data

Overview Quality Filtering	MEN057MID49 - BF	MEN058MID50 - BO	MEN059MID51 - SBF	MEN60MID52 SBO
Number raw input seqs	7885	6674	7488	7122
Length outside bounds of 250 and 1000	1376	725	1121	1025
Num ambiguous bases exceeds limit of 6	0	0	0	0
Mean qual score below minimum of 25	28	17	27	25
Max homopolymer run exceeds limit of 8	144	25	199	96
Num mismatches in primer exceeds limit of 2	33	9	3	16
Number of sequences with identifiable barcode but without identifiable reverse primer:	4106	2402	4365	4204
Sequence length details for all sequences passing quality filters:				
Raw len min/max/avg	250.0/640.0/536.0	250.0/626.0/553.3	250.0/660.0/535.0	250.0/648.0/538.1
Wrote len min/max/avg	225.0/615.0/505.0	225.0/601.0/518.4	225.0/635.0/504.8	225.0/623.0/508.1
Total number seqs written	6304	5898	6138	5960

Appendix 2.

Composition of fatty acids (microgram methylester/mg supernatant with olive oil) that measured by GC-FID were produced by isolates of lipolytic bacteria from top soil in forest and oil palm plantation area ,cultured in broth medium with olive oil

	Fatty acid from lipase product (µg/mg)																																			
Code : Species	C8:0	C10:0	C11:0	C12:0	C13:0	C14:0	C14:1n5c	C15:0	C15:1n5c	C16:0	C16:1n7c	C17:0	C17:1n7c	C18:0	C18:1n9t	C18 :1n9c	C18:2n6t	C18:2n6c	C20:0	C18:3n6	C20:1n9	C18:3n6	C21:0	C20:2n6	C22:0	C20:3n3	C22:1n9	C20:3n3	C20:4n5	C23:0	C22:2n6	C24:0	C20:5n3	C24:1n9	C22:6n3
Substrat with olive oil	1.96	0.00	0.00	151.42	0.00	0.00	7.86	6.53	9.97	0.00	303.65	5.18	141.48	0.00	591.33	0.00	3682.70	61.20	0.00	112.61	10.60	0.00	15.78	13.72	0.00	11.15	16.56	14.43	6.67	14.90	44.59	2.05	1.21	0.00	2.27
BF01 : *Burkholderia* cmc 26-1	0.00	0.00	0.00	101.97	0.00	0.00	9.58	2.76	0.00	0.00	466.76	12.39	113.75	0.00	944.49	0.00	0.00	3196.52	307.22	6.11	27.73	0.00	7.85	30.18	0.00	10.90	6.23	14.62	29.06	2.21	6.36	2.75	2.00	0.00	5.82
BF02 : *Klebsiella* sp. HD4.16	0.00	0.00	0.00	0.24	0.00	0.00	1.89	6.01	0.00	0.00	202.43	0.00	138.74	0.00	426.49	0.00	2891.35	52.14	0.00	74.82	24.09	0.00	12.56	12.13	0.00	15.57	32.77	13.13	36.07	2.82	2.74	1.71	1.52	0.00	3.21
BF04 : *Burkholderia cepacia* str.GXN152	0.00	0.00	5.73	200.09	0.00	4.45	14.47	3.88	0.00	223.55	1107.06	26.96	141.26	41.75	1260.97	0.00	0.00	0.00	16915.63	418.55	62.65	0.00	11.35	83.14	0.00	32.48	107.64	25.34	13.96	1.86	7.56	28.60	7.95	0.00	14.96
BF05 : *Burkholderia* cmc 26-2	2.76	0.00	6.01	130.71	0.00	0.00	5.75	7.80	0.00	0.00	722.87	18.82	155.67	27.68	1444.07	0.00	0.00	0.00	10896.29	296.23	101.99	0.00	15.55	66.97	4.96	63.23	133.50	21.52	11.41	5.97	2.83	24.88	5.83	21.77	0.00
BF06 : *Burkholderia caryophila*	2.25	0.00	3.95	70.79	0.00	0.00	6.79	0.00	0.00	58.27	303.15	11.70	159.68	540.13	0.00	0.00	3899.10	85.00	0.00	109.72	8.14	0.00	0.00	17.36	0.00	3.61	4.01	16.80	60.25	3.32	10.35	5.56	1.49	0.00	1.39
BF07 : *Burkholderia sp.NGR190*	0.00	0.00	0.00	0.65	0.00	4.07	14.76	3.27	12.74	315.93	1227.99	29.84	120.12	23.31	1023.28	0.00	0.00	0.00	573.27	465.52	37.57	0.00	6.23	88.77	4.02	18.91	113.36	26.10	0.00	2.29	14.52	0.00	7.87	0.00	15.84
BF01 : *Burkholderia cepacia / ambiforia*	0.00	0.00	4.44	90.95	0.00	4.35	14.93	3.98	7.10	138.15	655.76	16.07	141.65	30.28	1232.96	0.00	0.00	4232.13	394.30	238.45	0.00	26.92	10.05	51.73	0.00	18.08	65.69	21.63	11.37	2.58	8.52	16.29	6.67	1.87	8.30
BF03 : *Burkholderia sp. STJ14*	2.61	3.13	7.04	7.86	3.35	0.00	24.81	8.60	0.00	171.87	1261.33	24.55	147.38	18.43	567.85	0.00	0.00	0.00	10960.34	42.76	71.88	21.01	29.83	64.54	7.73	28.55	52.44	6.17	10.18	44.90	1.63	6.46	15.84	0.00	13.36
BF04 : *Burkholderia* cmc 26-3	2.14	0.00	6.00	100.22	0.00	0.00	7.36	8.55	0.00	168.94	866.03	21.01	154.26	22.37	1463.49	0.00	0.00	5701.54	457.04	293.28	32.90	0.00	9.95	58.30	0.00	15.46	75.56	23.87	2.55	6.78	9.17	18.10	5.53	0.00	10.00
BF11: *Burkholderia vietnamiensis str.AU1*	0.00	0.00	4.30	0.52	0.00	0.00	33.72	10.75	0.00	177.11	952.16	23.21	150.81	97.44	1540.96	0.00	0.00	0.00	11671.41	12.80	49.18	0.00	12.21	60.10	23.19	18.92	82.28	24.02	14.79	6.66	15.02	20.48	5.29	16.37	2.52
BF12 : *Burkholderia sp. F-6*	25.33	3.63	1382.47	0.00	0.00	9.56	3.94	0.00	0.00	0.00	527.61	10.31	149.98	0.00	908.96	0.00	0.00	3013.80	283.83	193.21	16.41	0.00	10.60	31.27	0.00	12.06	2.42	18.38	12.01	5.20	10.33	1.75	2.96	4.31	6.32

Composition of fatty acids (microgram methylester/mg supernatant with olive oil) that measured by GC-FID were produced by isolates of lipolytic bacteria from aquatic sediment in forest and oil palm plantation area,cultured in broth medium with olive oil

	Fatty acid from lipase product (µg/mg)																																			
Code : Species	C8:0	C10:0	C11:0	C12:0	C13:0	C14:0	C14:1n5c	C15:0	C15:1n5c	C16:0	C16:1n7c	C17:0	C17:1n7c	C18:0	C18:1n9t	C18 :1n9c	C18:2n6t	C18:2n6c	C20:0	C18:3n6	C20:1n9	C18:3n6	C21:0	C20:2n6	C22:0	C20:3n3	C22:1n9	C20:3n3	C20:4n5	C23:0	C22:2n6	C24:0	C20:5n3	C24:1n9	C22:6n3
Substrat with olive oil	1.96	0.00	0.00	151.42	0.00	0.00	7.86	6.53	9.97	0.00	303.65	5.18	141.48	0.00	591.33	0.00	3682.70	61.20	0.00	112.61	10.60	0.00	15.78	13.72	0.00	11.15	16.56	14.43	6.67	14.90	44.59	2.05	1.21	0.00	2.27
SBF02 : *Burkholderia* cmc 25	0.00	0.00	0.00	1.42	0.00	0.00	3.02	2.00	7.70	16.30	103.94	0.00	161.20	194.88	12.99	0.00	1334.50	23.50	0.00	45.27	3.91	0.00	0.00	5.02	0.00	2.11	6.30	13.92	36.55	6.72	3.15	0.87	1.17	0.00	3.88
SBF11 : *Serratia sp. NE17 -1*	0.00	0.00	0.00	95.37	13.72	0.00	32.73	11.21	51.99	186.69	989.99	24.96	157.27	0.00	868.08	585.73	0.00	0.00	13871.27	345.79	45.95	0.00	11.08	0.00	69.31	15.97	77.17	27.45	22.05	2.14	6.12	17.57	0.00	14.56	8.97
SBF12 : *Serratia sp. NE17 -2*	0.00	0.00	5.20	120.99	0.00	0.00	49.95	13.83	0.00	0.00	22.91	41.45	160.79	0.00	1321.99	0.00	0.00	0.00	0.00	625.45	42.62	5.91	8.70	117.42	0.00	16.23	132.80	28.81	60.92	1.93	6.37	35.27	8.93	4.13	16.82
SBF15: *Burkholderia sp. enrichment cul.clone P-5-07a_B*	0.00	0.00	0.00	0.00	0.00	0.00	4.30	7.28	0.00	32.00	182.71	0.00	161.47	375.46	19.58	0.00	2614.09	46.96	0.00	70.65	0.00	0.00	0.00	10.66	0.00	0.00	12.94	13.96	43.22	5.50	5.80	3.78	1.34	0.00	19.55
SB001 : *Burkholderia sp. CCUG 57241 -1*	0.00	0.00	7.91	0.60	0.00	0.00	23.20	8.44	0.00	146.26	718.44	13.12	164.49	36.76	1255.75	0.00	0.00	0.00	8953.49	7.80	36.81	0.00	9.63	55.25	0.00	15.42	67.23	26.99	19.00	1.63	11.98	14.96	4.36	11.46	0.00
SB002 : *Burkholderia sp. CCUG 57241 -2*	0.00	0.00	5.44	0.49	0.00	0.00	26.44	2.93	10.07	192.77	2.01	21.68	0.00	29.73	1619.05	0.00	0.00	0.00	12898.41	389.03	46.35	0.00	4.65	69.81	0.00	17.76	8.16	28.72	17.51	1.48	0.00	2.28	6.48	0.00	10.81
SB003 : *Acinetobacter sp. LMG V90 -1*	5.30	0.00	9.72	100.34	0.00	0.00	8.75	5.08	0.00	87.22	2.16	0.00	0.00	0.00	1082.80	0.00	0.00	0.00	6109.93	173.11	9.78	0.00	0.00	25.22	0.00	0.00	5.66	44.93	0.00	3.62	10.16	9.08	31.36	0.00	4.95
SB004 : *Acinetobacter sp. LMG V90 -2*	0.00	0.00	0.00	66.68	0.00	0.00	7.97	0.00	0.00	88.06	387.37	10.31	148.12	0.00	823.21	0.00	0.00	2506.01	190.67	120.20	11.04	0.00	8.37	21.77	0.00	4.64	25.13	23.79	35.72	18.19	0.00	6.51	1.77	0.00	2.08
SB006 : *Gordonia sp. SoCRH16*	1.28	0.00	3.40	9.74	0.00	0.00	8.64	1.66	0.00	56.76	152.26	4.32	144.24	0.00	13.64	0.00	1957.11	46.34	0.00	57.49	3.65	0.00	0.00	7.67	0.00	0.00	0.00	19.00	0.84	21.11	0.00	1.43	28.89	2.16	13.85
SB011: *Acinetobacter sp. LMG V90 -3*	1.25	0.00	2.32	63.60	0.00	10.40	10.80	2.16	7.54	0.00	126.94	10.70	1363.08	194.20	10.72	0.00	1398.27	24.69	0.00	50.66	6.84	0.00	0.00	4.90	0.00	1.54	4.50	13.27	17.94	0.88	10.10	1.92	72.90	0.00	2.30
SB012 : *Acinetobacter sp. LMG V90 -4*	0.02	0.00	0.05	1.27	0.00	0.00	0.22	0.00	0.15	0.00	2.54	0.21	27.25	3.88	0.21	0.00	27.96	0.49	0.00	1.01	0.00	0.00	0.00	0.10	0.00	0.00	0.09	0.27	0.36	0.02	0.20	0.04	1.46	0.00	0.05

Note: C4:0 and C6:0 have 0.00 µg/mg fatty acid methyl ester from lipase product of all bacterial isolates in this study.

CURRICULUM VITAE

Marini Wijayanti was born in Nganjuk, September 10th, 1976 as the second daughter from Budi Wijaya Adi and Sumarsi. She obtained her Undergraduate of Fisheries, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University in 2000 and Master of Biotechnology in 2003 from Bogor Agricultural University. She is currently a faculty member of Faculty of Agriculture in Sriwijaya University, South Sumatra, Indonesia since 2001.

In 2011, she started obtaining her doctorate degree in Microbiology at Bogor Agriculture University. For upgrade the study, her promotor Prof Dr rer nat Anja Meryandini ask to her to join CRC990 EfforTS-IPB since 2012 and sent her to work a part of her study at Genomic and Applied Micobiology Laboratory at George-August University Goettingen Germany with Prof Rolf Daniel invitation in 2013. She got her funding for this research from BPPS Dikti (2011-2015) and Hibah Disertasi Doktor (2014) from Ministry of Research and Higher Education of Republic of Indonesia via Sriwijaya University.

The results of this study were oral presented in International Seminar I-KUSTARS at Kassesart University, Thailand (March, 2014) and ICOBIO 2015 at Bogor Agricultural University, Indonesia (August, 2015). Recent scientific publications include:

1. Wijayanti M, Meryandini A, Wahyudi AT, Yuhana M. 2014. Diversity and the Composition of Fatty Acids of Lipolytic Bacteria Isolated from Soil and Aquatic Sediment in a Forest and on an Oil Palm Plantation. *Makara J. Sci*. 18(3) : 71 – 78.
2. Wijayanti M, Wahyudi AT, Yuhana M, Engelhaupt M, Meryandini A. 2015. Impact of Bukit Duabelas rainforest transformation to oil palm plantation on phylogenetic of soil bacterial communities in Sarolangun Sumatra, Indonesia. (*In process*)
3. Wijayanti M, Wahyudi AT, Yuhana M, Engelhaupt M, Meryandini A. 2015. Bacterial communities in aquatic sediment from Bukit Duabelas rainforest and oil palm plantation at Sumatra, Indonesia. (*In process*)