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DIVERSITY OF SOIL GREEN ALGAE IN AN OIL PALM PLANTATION AT SUNGAI ASAP, SARAWAK, MALAYSIA

LIM CHIN TSONG

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By

LIM CHIN TSONG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DIVERSITY OF SOIL GREEN ALGAE IN AN OIL PALM PLANTATION AT SUNGAI ASAP, SARAWAK, MALAYSIA

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Faculty : Agriculture and Food Science (Bintulu)

Palm oil industry is the main contributor toward current Malaysia's economy. Sarawak, being the largest state in Malaysia, contribute about 26% of total oil palm planted in Malaysia. Sustainable agriculture is closely related to the efficient use of natural resources, which is heavily dependent on soil microorganism activities. Hence, soil microorganisms often act as early indicator of soil quality as they respond rapidly to changes related to soil management. Soil green algae are mostly photosynthetically active microorganisms having changeable community structure, regardless to the soil type and agricultural practices. Hence, in this study, the diversity of soil green algae was investigated in an oil palm plantation and compared with the nearby secondary forest. Total soil microorganism DNA was extracted and soil green algae 18S rDNA was amplified using ChloroF and ChloroR primers set. Denaturing gradient gel electrophoresis (DGGE) was used to study the diversity of soil green algae communities of the oil palm plantation and the nearby secondary forest at Sungai Asap, Sarawak. Shannon-weaver index revealed that the diversity in oil palm plantation (OP) and disturbed secondary forest (S2) were higher than undisturbed secondary forest. The diversity index (*H*') was increase over time for OP (3.1 to 3.3) and S2 (1.4 to 1.9), except undisturbed forest (S1: 1.8 to 1.6). Furthermore, species richness (1-D) was increased over time in S2 (2.3 to 3.0) and OP (3.1 to 3.3). Algae richness in S1 was nearly constant over time, which might be due to the soil in this area being undisturbed. In contrast, both OP and S2 were disturbed by planting and human activity, respectively. Cluster analysis based on operational taxonomic unit (OTUs) divided the banding patterns mainly into three main clusters, which were the three sampling sites. DGGE profile of OP was preferentially related to S2 during wet season (December) while it was closer to S1 during dry season (June). However, clusters similarity decreased

over time, suggesting green algae community of the three sampling sites became more distinct over the sampling period. Diversity indices were significantly correlated to soil exch. K, exch. Ca, exch. Mg and total N at OP which might due to the fertilization activity. A total of 126 prominent DGGE bands were excised and sequenced for phylogenetic analysis. The diversity of soil green algae in the OP was higher than the S2, followed by S1. Chlamydomonadales, Sphaeropleales, Chaetophorales, Trebouxiales and Chlorococcales were among the dominant order of green algae found in this sampling area. Therefore, this study delivered an overview of the composition, diversity, and community structure of soil green algae in oil palm plantation and the nearby secondary forest at Sungai Asap, Sarawak. Generally, the soil chemical properties of secondary forest, especially undisturbed soil, was better than OP. However, it can be concluded that OP soil is still healthy as the soil green diversity and richness was increased over time. The knowledge of soil green algae diversity was obtained and hence lead to the better understanding of soil health, especially in oil palm plantation. Further studies should be carried out to study sustainability of soil green algae as soil quality indicator.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

KEPELBAGAIAN ALGA HIJAU TANAH DALAM LADANG KELAPA SAWIT DI SUNGAI ASAP, SARAWAK, MALAYSIA

Oleh

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Industri minyak kelapa sawit telah memberi sumbangan yang besar kepada ekonomi Negara Malaysia. Sarawak merupakan negeri terbesar di Malaysia telah menyumbang lebih kurang 26% daripada jumlah kelapa sawit yang ditanam di seluruh negara. Pertanian lestari berkait rapat dengan penggunaan sumber semula jadi secara cekap, terutamanya aktiviti-aktiviti mikroorganisma dalam tanah. Mereka sering bertindak sebagai penunjuk awal kepada kualiti tanah kerena bertindak balas dengan cepat kepada aktiviti-aktiviti yang berlaku pada tanah. Pelbagai jenis mikroorganisma boleh dijumpai pada persekitaran tanah, terutamanya alga hijau. Ini disebabkan alga hijau lebih tertumpu di atas permukaan tanah kerena sifatnya yang memerlukan cahaya matahari untuk proses fotosintesis. Maka, dalam penyelidikan ini, kepelbagaian alga hijau di tanah telah dikaji dan dibandingkan dengan hutan sekunder yang berdekatan. Teknik elektroforesis berdasarkan kecerunan nyahasli gel (DGGE) telah digunakan bagi mengkaji kepelbagaian alga hijau tanah di ladang kelapa sawit di Sungai Asap, Sarawak dan hutan sekunder disekelilingnya. Indeks *Shannon-weaver* menunjukkan kepelbagaian species alga hijau pada ladang kelapa sawit (OP) dan hutan sekunder terganggu (S2) lebih tinggi daripada hutan sekunder tidak terganggu (S1). Index kepelbagaian (*H*') meningkat dari masa ke semasa di OP (3.1 kepada 3.3) dan S2 (1.4 to 1.9), kecuali S1 (1.8 kepada 1.6). Tambahan pula, kekayaan spesis (1-D) meningkat di S2 (2.3 kepada 3.0) dan OP (3.1 kepada 3.3). Kekayaan alga hijau di S1 hampir malar sepanjang kajian ini dijalankan, dan ini mungkin disebabkan oleh tanah di sini tidak diganggu. Ini berbeza dengan tanah di S2 and OP yang telah diganggu oleh aktiviti penanaman dan manusia. Analisis kelompok berdasarkan unit taksonomi operasi (OTUs) membahagikan corak jaluran kepada tiga kumpulan yang utama, iaitu tiga kawasan persampelan. Profil DGGE pada kawasan OP adalah berkait rapat dengan S2 pada musim hujan (Disember) tetapi lebih rapat kepada S1 semasa musim kering (Jun). Walau bagaimana pun, kelompok persamaan antara 3 kawasan persampelan ini menurun dari masa ke semasa, menujukkan komuniti alga hijau daripada tiga kawasan ini menjadi lebih jelas dan tersendiri.

Indeks kepelbagaian berkait rapat dengan bes tukarganti K, Ca dan Mg, dan jumlah N di ladang kelapa sawit, yang mungkin disebabkan oleh aktiviti pembajaan. Sejumlah 126 jalur dominan DGGE telah dipotong dari gel dan diproses dengan menggunakan teknik penjujukan DNA. Kepelbagaian alga hijau pada ladang kelapa sawit lebih tinggi daripada hutan sekunder terganggu (S2) diikuti dengan hutan sekunder tidak terganggu (S1). Hasil penyelidikan ini selaras dengan tingkah laku alga hijau kerana mereka lebih cenderung kepada kawasan terbuka. *Chlamydomonadales, Sphaeropleales, Chaetophorales, Trebouxiales* dan *Chlorococcales* adalah antara order alga hijau yang dominan di kawasan kajian ini. Penyelidikan ini telah memberi satu gambaran secara keseluruhan bagi komposisi, kepelbagaian dan strukur komuniti alga hijau tanah di ladang kelapa sawit di Sungai Asap, Sarawak dan hutan sekunder disekelilingnya. Secara umumnya, sifat kimia tanah di hutan sekunder lebih baik, terutamanya hutan tidak terganggu, adalah lebih baik daripada OP. Secara kesimpulannya, tanah OP masih sihat ini disebabkan kepelbagaian dan kekayaan alga hijau di sini masih mengingkat dari masa ke semasa. Maka, pengetahuan mengenai kepelbagaian alga hijau tanah ditingkatkan dan dijangka akan menerajui ke arah pengetahuan kualiti tanah terutamanya di ladang kelapa sawit. Kajian selanjutnya patut dijalankan bagi mengetahui kemampanan alga hijau sebagai penunjuk kualiti tanah.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ºC	degree Celsius
× g	gravity force
µg	microgramme
µL	microliter
µM	microMolar
1-D	Simpson's index
Avail.	available
BLAST	basic local alignment search tool
bp	base pair
C	carbon
Ca	calcium
CA	California
CEC	cation exchange capacity
CIRP	Christmas Island Rock Phosphate
cm	centimetre
Cu	copper
DGGE	denaturing gradient gel electrophoresis
dH_2O	distilled water
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
Exch.	exchangeable
Fe	iron
g	gramme
GC-clamp	Guanine and Cytosine rich sequence
H'	Shannon's index
ha	hectare
K	potassium
km	kilometre
L	litre
M	molar
MDS	multidimensional scaling
Mg	magnesium
MgCl2	magnesium chloride
mL	millilitre
mm	millimetre
mM	milliMolar
Mn	manganese
MPOB	Malaysian Palm Oil Board
N	nitrogen
Na	sodium
NGO	non-Government Organisation
NJ	neighbour-joining
No.	number
NPK	nitrogen (N), phosphorus (P), and potassium (K)
OTU	operational taxonomic unit
P	phosphorus
PCA	principle component analysis
PCR	polymerase chain reaction

PEG	polyethylene glycol
Rf	retention factor
SD	standard deviation
Sdn. Bhd	*Sendirian Berhad* (meaning "private limited")
SDS	sodium dodecyl sulphate
TAE	Tris-acetate-EDTA
TEMED	N,N,N',N'-Tetramethylethylenediamine
TN	total N
TP	total phosphorus
U	unit
U. V.	ultraviolet
UPGMA	unweighted pair group method using arithmetic averages
USA	United States of America
v/v	volume per volume
Ver.	version
w/v	weight per volume
Zn	zinc

CHAPTER 1

INTRODUCTION

Oil palm is known as one of the world's most fast expanding equatorial crops and palm oil contributes more than half of the total worldwide oils and fats. Palm oil is one of the major fat diets especially in South East Asia. Currently, over 80% of the global market is dominated by the two major palm oil exporting countries, Malaysia and Indonesia (Colchester and Chao, 2011). Malaysia accounts for about 39% of global palm oil production and about 44% of global exports (MPOC, 2015). However, this also attracts the attention from the oil palm detractors such as Greenpeace which bring up a lot of well researched evidences and try to hold the further expansion of oil palm industries. Thought, there is no negating that change of forest to commercial farm will give an impact to the environment in one way or another. Perhaps the great anxiety is the loss of biodiversity which is the species composition of flora and fauna, and not to exclude the microorganisms in the soil.

Soil microorganisms play a vital role in conserving soil quality and ecosystem health. Though, relatively little is known about the diversity and ecology of soil microbial communities (Giri *et al*., 2005). Therefore, it is important to develop effective methods to study the composition, distribution, and diversity of microorganisms in soil habitat for a wider understanding of soil quality. Soil community analysis has been limited in the past which due to only a small proportion of microbes from the natural habitat can be cultured in laboratories. The unculturable microbes will reveal the species of close related cultured microbes as well as those from virtually uncultured species. Therefore, molecular method which involved direct extraction of whole-community DNA from soil followed by polymerase chain reaction (PCR) amplicons on the targeted gene may give a clearer picture of the microbial communities in the soil. The molecular methods include denaturing gradient gel electrophoresis, amplified ribosomal DNA restriction analysis (ARDRA), ribosomal intergenic spacer analysis (RISA), *et cetera*, may give a clearer picture of the microbial communities in the soil. Hence, the use of molecular biology to survey soil microbes has great potential to give the overall pictures of microbes in the soil.

Algae are among the top five microorganisms found in soil and yet they are not been extensively studies especially in tropical region. Algae are common to all kinds of soil ecosystem (Soare and Dobrescu, 2010) and are visible in all tropical soil during favourable season (Ray and Thomas, 2012). Information about their biodiversity and natural succession affection can be obtained by monitoring their activity with regard to either biotic or abiotic. Hence, studying the ecology of the algae may provide a good way in accessing health of an ecosystem. Beside, algae are directly exposed to any physical as well as chemical changes to the soil as they usually concentrated on top of the soil due to their photosynthetic behaviour (Bérard *et al*., 2004). Green algae are the dominant group of algae of tropical soils (Messyasz, 2006) and any land usage will directly affect them

(Patova, and Dorokhova, 2008). Thus, green algae serve as one of the potential soil microbes to determine the soil health.

The island of Borneo is covered with vast tropical rainforests which believed to be more than 130 million years old. This island also known to be one of the utmost diverse tropical habitat found on the Earth. Sarawak, which is positioned just above the equator at the northwest of Borneo is the largest state in Malaysia. Which it makes up 37.5% of the total land in Malaysia. With it's extensive of land, more than 26% of oil palm is located here as the available lands in west Malaysia as well as Sabah are nearly exhausted. Hence, Sarawak plays an important role in food security and also the global demand for oils.

In this study, soil green algae community in Sungai Asap, Belaga, Sarawak was surveyed twice a year. One of the popular molecular fingerprinting methods, denaturing gradient gel electrophoresis (DGGE) was used to analyse the soil green algae community. Then, the 18S rDNA fragments were sequenced for phylogenetic analysis. Sampling was carried out in December 2010, June and December 2011 from three sites which consist of an oil palm plantation area (OP; aged 3 years old on December 2010) and two nearby secondary forests (S1: undisturbed and S2: disturbed). Hence, the aim of this study was to use culture-independent method to study the soil green algae composition of oil palm plantation and compare with the nearby secondary forests. The soil in the oil palm plantation is expected to have higher soil green algae diversity when compared to secondary forest especially the undisturbed soil, as this might due to fertiliser nutrient availability and sufficient of sunlight as a results of opened space. The findings would improve the understanding of soil green algae in the selected sampling sites. Thus, the objectives of this study were set as follows:

1. To determine the diversity of soil green algae based on the partial 18S rDNA sequences by using PCR-DGGE method;
2. To compare the diversity of soil green algae between oil palm plantation and the secondary forests. There may be a preamble at the beginning of a chapter. The purpose may be to introduce the themes of the main headings.

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LIST OF PUBLICATIONS

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