UNIVERSITI PUTRA MALAYSIA

DIVERSITY OF SOIL CILIATES AT OIL PALM PLANTATION IN SUNGAI ASAP, SARAWAK, MALAYSIA

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FSPM 2015 15

DIVERSITY OF SOIL CILIATES AT OIL PALM PLANTATION IN SUNGAI ASAP, SARAWAK, MALAYSIA

By

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

October 2015

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

DIVERSITY OF SOIL CILIATES AT OIL PALM PLANTATION IN SUNGAI ASAP, SARAWAK, MALAYSIA

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LEE TING TING

October 2015

Chair : Wong Sing King, PhD
Faculty : Agriculture and Food Sciences (Bintulu)

Palm oil industry is continuously making significant contribution to Malaysia's economy and oil palm cultivation area is expanding especially in Sarawak. Thus research on the condition of plantation soil is needed to ensure its sustainability. In this research, soil ciliates (Protozoa: Ciliophora) was studied because they are potential bioindicators soil health and quality. Soil ciliates diversity at oil palm plantation and two secondary forests (biodiversity strip 1 and biodiversity strip 2) were studied using the semi-nested polymerase chain reaction reaction-denaturing gradient gel electrophoresis (PCR-DGGE) approach targeting for the small subunit ribosomal DNA (18S rDNA). A total of 480 soil samples were collected from December 2010 to July 2012. Microorganisms total DNA was extracted from all soil samples and ciliate 18S rDNA was amplified in semi-nested PCR using primer pairs EukA/CilDGGE-r and GC-CilF/CilDGGE-r. Amplified sequences were separated on polyacrylamide gel using DGGE approach. A total of 290 predominant DGGE bands were excised and subjected to sequencing. These sequences matched with ciliates in NCBI GenBank database with similarity between 91% to 100%. Phylogenetic analysis revealed 73 species which affiliated to seven classes, 17 orders, 27 families and 34 genera. Classes Spirotrichea, Litostomatea and Colpodea were the top three dominant groups followed by Oligohymenophorea, Nassophorea, Armophorea and Heterotrichea. Four ciliate classes were found in biodiversity strip 1 while six classes were found in both biodiversity strip 2 and oil palm plantation. Statistical analyses revealed that community structure and diversity of soil ciliates in three study sites showed spatial and temporal variations. Community structure of soil ciliates in plantation was more similar to biodiversity strip 2 than biodiversity strip 1. Meanwhile, the diversity indices in the plantation were moderately diverse as compared to biodiversity strips 1 and 2. Moreover, the diversity indices among the sites were statistically indistinguishable after two years of study. There was no obvious correlation of the spatio-temporal changes of soil ciliate diversity indices with the environmental variables studied herein. Only Simpson index of soil ciliates diversity at plantation was significantly negatively correlated to rainfall. This research provides an overview of the composition, diversity and community structures of soil ciliates at oil palm plantation and forests in Sungai Asap, Sarawak. These have resulted in an increased knowledge of the diversity of soil ciliates and are then expected leading towards knowing the soil quality especially at oil palm plantation and provide

valuable knowledge for the development of sustainable oil palm plantation management.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

KEPELBAGAIAN CILIATA TANAH DI LADANG KELAPA SAWIT DI SUNGAI ASAP, SARAWAK, MALAYSIA

By

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Industri minyak sawit memberi sumbangan yang penting secara berterusan kepada ekonomi negara Malaysia dan kawasan penanaman kelapa sawit semakin meningkat terutamanya di negeri Sarawak. Maka, penyelidikan terhadap keadaan tanah ladang kelapa sawit perlu dijalankan untuk menjamin kelestariannya. Dalam penyelidikan ini, ciliata tanah (Protozoa: Ciliophora) telah dikaji kerana mereka merupakan penunjuk biologi yang berpotensi terhadap kesihatan dan kualiti tanah. Kepelbagaian ciliata tanah di ladang kelapa sawit dan hutan-hutan sekunder (jalur biodiversiti 1 dan jalur biodiversiti 2) telah dikaji dengan menggunakan kaedah tindak balas rantai polimerase-elektroforesis gel gradien nyahasli (PCR-DGGE) yang bersasar pada 18S rDNA. Sejumlah 480 sampel tanah telah dikumpul dari Disember 2010 hingga Julai 2012. DNA mikroorganisma telah diekstrak daripada semua sampel tanah dan 18S rDNA ciliate diamplifikasikan dalam 'semi-nested PCR' yang melibatkan dua pasangan primer EukA/CilDGGE-r dan GC-CilF/CilDGGE-r. Kemudian, jujukan DNA dipisahkan dalam gel poliakrilamid dengan menggunakan teknik DGGE. Sejumlah 290 jalur dominan DGGE telah dipotong dari gel dan diproses dengan menggunakan teknik penjujukan DNA. Jujukan-jujukan DNA ini berpadanan dengan ciliata dalam bank gen NCBI dengan peratusan keserupaan dalam lingkungan 91%-100%. Sebanyak 73 spesies yang tergolong kepada tujuh kelas, 17 order, 27 famili dan 34 genera telah ditemui menerusi analisis filogenetik. Spirotrichea, Litostomatea dan Colpodea merupakan tiga kelas dominan yang utama dan diikuti oleh Oligohymenophorea, Nassophorea, Armophorea dan Heterotrichea. Sebanyak empat kelas ciliata telah dijumpai di jalur biodiversiti 1 sementara enam kelas telah dijumpai di kedua-dua jalur biodiversiti 2 dan ladang kelapa sawit. Analisis statistik mendedahkan bahawa struktur komuniti dan kepelbagaian ciliata tanah di ketiga-tiga kawasan kajian menunjukkan variasi ruang dan masa. Struktur komuniti ciliata tanah di ladang kelapa sawit didapati lebih menyerupai struktur komuniti ciliata tanah di jalur biodiversiti 2 berbanding dengan jalur biodiversiti 1. Sementara itu, indeks kepelbagaian menunjukkan bahawa kepelbagaian ciliata tanah di ladang kelapa sawait adalah sederhana berbanding dengan jalur-jalur biodiversiti 1 dan 2. Tambahan pula, indeks kepelbagaian di antara ketiga-tiga kawasan kajian tidak dapat dibezakan secara statistik selepas dua tahun kajian. Selain itu, tiada korelasi yang jelas yang mengaitkan perubahan ruang dan masa bagi indeks kepelbagaian ciliata tanah dengan pembolehubah persekitaran. Hanya terdapat satu kekecualian dimana indeks Simpson di ladang kelapa sawit mempunyai korelasi

yang negatif secara signifikan dengan taburan hujan. Penyelidikan ini memberi satu gambaran keseluruhan untuk komposisi, kepelbagaian dan struktur komuniti ciliata tanah di ladang kelapa sawit dan hutan di Sungai Asap, Sarawak. Ini telah meningkatkan pengetahuan kepelbagaian ciliata tanah dan dijangka akan menerajui ke arah mengetahui kualiti tanah terutamanya di ladang kelapa sawit. Dengan adanya pengetahuan yang berharga ini, pembangunan pengurusan ladang kelapa sawit yang lestari dapat dilaksanakan.

ACKNOWLEDGEMENTS

Praise and thank to God for being the all-sufficient One who has led me all the way to the completion of my study. Various people have contributed to the success of this research and I would like to express my gratitude to them.

I would like to express my gratitude to my supervisors, Dr. Wong Sing King, Dr. Lau Wei Hong and Dr. Siti Ramlah Ahmad Ali for their guidance, suggestions, advice and help during the course of this research and in the completion of my thesis. I would also like to thank Malaysian Palm Oil Board (MPOB) for the financial support in the form of research grant. Special thanks to Dr. Ahmed Osumanu Haruna for his help and guidance in the statistical analyses in this research.

I would like to extend my thanks to all my friends especially Lim Chin Tsong, Sharron Wong, Lau Hsien Loong and Amelia Tang for their help and support. Finally I wish to express my deepest gratitude to my parents, sisters and brother for their love, support and encouragement throughout the period of my study at UPM Bintulu campus.

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

BLAST	Basic local alignment search tool
bp	Base pair
CEC	Cation exchange capacity
D	Simpson index
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
GC-clamp	Guanine and cytosine rich sequence
H	Shannon index
kb	Kilobase pair
MDS	Multidimensional scaling
Mg	Magnesium
min	Minute
MPOB	Malaysian Palm Oil Board
N	Nitrogen
NCBI	National Center for Biotechnology Information
NGO	Non-governmental organization
NJ	Neighbor Joining
OP	Oil palm plantation
P	Phosphorus
PAH	Polycyclic aromatic hydrocarbon
PCR	Polymerase chain reaction
rDNA	Ribosomal DNA
18S rDNA	Small subunit rDNA in eukaryotes
S	Species richness

S1	Biodiversity strip 1
S2	Biodiversity strip 2
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SSU rDNA	Small subunit rDNA
UPGMA	Unweighted pair group method using arithmetic averages

CHAPTER 1

INTRODUCTION

1.1 Background

Oil palm industry is an economic backbone of Malaysia. It had contributed RM52.7 billion of Malaysian Gross National Income (GNI) in 2009 and is seeking an increment of about 237% by 2020 (Jabatan Perdana Menteri, 2010). Currently, Sarawak is the second largest oil palm planted state and is expected to double the plantation area in coming years (Jasmine, 2011). As palm oil industry is progressing, it has received negative perception as environmentally damaging industry and is unwelcomed by the environmental organizations. Although the industry itself has identified the adverse impacts of the industry on the environment but there is always solutions for problems and room for improvement (Khor, 2013).

In palm oil industry, healthy soils are not only able to sustain oil palm cultivation but also to increase oil palm yields and reduce the production costs. However, intensive land use for oil palm cultivation and unsustainable soil management practices will result in soil degradation. Degraded soil causes decline in productivity, deterioration of water and air quality, migration and food insecurity (FAO, 2008). Therefore, soils need to be managed sustainably in order to perform their functions and to meet the growing world demand of oil palm products. At the same time, it is important to carry out research on oil palm plantation soils to know the condition of soils and to assess soil health and quality throughout time to protect soil resources and to ensure the sustainability of soils.

Soil Protozoa, known as ubiquitous single-celled protists, are one of the major microorganisms found in soils which play key roles in soil food webs and processes. Due to their importance in soils and unique physiological characteristics, protozoa have been proposed as ideal bioindicators of ecosystem changes. Furthermore, there was evidence of using protozoa as indicators of water and soil quality (Dopheide *et al*., 2009; Lara *et al*., 2007a). Thus, studies of protozoan diversity can be powerful indicators for assessing and monitoring environmental changes (Foissner, 1999).

Denaturing gradient gel electrophoresis (DGGE) is a powerful molecular method in assessing protozoan diversity and community structure. DGGE not only can overcome the limits of traditional morphology-based method but also able to provide new insights into protozoan diversity and community structure (Lara and Acosta-Mercado, 2012; Shimano *et al*., 2012). DGGE has been applied successfully in assessing protozoan diversity in various environments such as animal rumens, freshwater, marine and soils. For the application of molecular method, molecular marker 18S rDNA has been frequently used in the study of protozoa and was proven to be a suitable and excellent molecular marker in studying protozoan diversity.

Thus in the present study, diversity of soil protozoa (ciliates) was investigated by studying the molecular marker gene (18S rDNA) of ciliates using a semi-nested polymerase chain reaction – denaturing gradient gel electrophoresis (PCR-DGGE) method. Through this research, the diversity of soil ciliates in oil palm plantation was

compared to the secondary forests to reveal the effect of land use towards the diversity of soil ciliates. The diversity of ciliates also might serves as an indicator to reflect the condition of managed agriculture soil in oil palm plantation. Information gained from this study enable the planters and policy makers in understanding the current status of soil in oil palm plantation, implementing the policy frameworks for sustainable plantation soil and also contributing in future development of sustainable oil palm plantation management.

1.2 Objectives of the Study

1. To characterize the composition of soil ciliates based on the partial 18S rDNA sequences using a semi-nested PCR-DGGE method.
2. To compare the diversity of soil ciliates between oil palm plantation and secondary forests.

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