UNIVERSITI PUTRA MALAYSIA

SOIL MICROBIAL POPULATIONS IN FELDA OIL PALM PLANTATION IN JENGKA 24, MALAYSIA

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FP 2015 37

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

September 2015

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the Degree of Doctor of Philosophy

**SOIL MICROBIAL POPULATIONS
IN FELDA OIL PALM PLANTATION IN JENGKA 24, MALAYSIA**

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September 2015

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The main objective for this research was to analyse soil microbial diversity in the oil palm plantation in order to understand the soil microbial community which could help to formulate new and innovative management of the basal stem rot (BSR) disease. Soil sampling was conducted at FELDA Jengka 24. Sampling areas include sites with empty fruit bunches (EFB) application, high BSR incidence and no BSR incidence. For comparisons, soils from the adjacent forest were sampled. Plate count technique was adopted for evaluation of overall soil microbial populations. Functional microbes were evaluated using specific media. Identification and characterisation of functional microbes were carried out by molecular means. BIOLOG EcoPlate was used to analyse microbial community. Plate count data (Colony forming unit = CFU) were subjected to Student's *t*-Test for significance test. Optical density (OD) values from the BIOLOG EcoPlate were subjected to Principal Component Analysis (PCA). Results showed that significant difference was not detected for fungal populations in EFB applied versus non-EFB applied sites. Similar observations were recorded for bacterial and actinomycetes. This has indicated that EFB application didn't seem to affect fungal, bacterial and actinomycetes populations. Although non-significant differences were reported for fungal, bacterial and actinomycetes in high BSR versus BSR-free sites, observations that prompt further investigations were discovered. Fungal population in high BSR sites fluctuates, ranging between $3.2x10^2$ to $2.3x10^5$ CFU/gram, as opposed to stable bacterial and actinomycetes populations. Sites with low fungal but high bacterial count could be potential indicator for BSR infestation. Phosphates solubilisers assessments revealed that they were not influenced by BSR incidence, supported by non-significant difference with average population of $1x10^5$ CFU/gram. Similar observations were recorded for nitrogen fixers. Although not significantly different, lignin degraders in BSR-free sites (average $1.3x10^3$ CFU/gram) were higher than BSR infested sites (average 63 CFU/gram). This has suggested that soils with low lignin degraders are conducive for BSR. EFB applied sites recorded with significantly higher lignin degraders population, indicating EFB contributing to lignin degraders. *Pseudomonas* and *Bacillus* bacteria were not affected by BSR

incidence indicated by significance test. EFB applications seemed to contribute to both with high and consistent population with average count of $2.13x10^6$ CFU/gram and $1.2x10^4$ CFU/gram for *Pseudomonas* and *Bacillus* respectively. *Trichoderma* sp. fungi with average $2.8x10^4$ CFU/gram population was not affected by BSR incidence and EFB application. A total of 27 microbes from various functional groups were identified including bacteria (*Burkholderia* sp., *Klebsiella* sp., *Bacillus* sp., *Chryseobacterium* sp., *Streptomyces* sp., *Chromobacterium* sp., *Pseudomonas* sp.) and fungi (mainly from orders Hypocreales and Eurotiales). PCA for microbial community assessment have revealed the closely related microbial communities in oil palm cultivated sites. In contrast, sparsely tabulated data points recorded for forests soils indicated low relative similarity of microbial communities. These results were further supported by analysis of carbon sources utilisation. The PCA analyses for EFB and non-EFB applied sites yielded non-distinct difference, indicating high relative similarity of microbial communities in both sites. It has also indicated that the application of EFB might not cause drastic changes to the soil microbial community. Similarly, PCA analyses for microbial communities in high BSR and BSR free sites yielded high relative similarity. The closely related microbial communities in BSR and BSR free sites also indicated that BSR disease infestation did not seem to affect soil microbial communities. Both comparisons above demonstrated presence of stable resilient microbial communities in oil palm soils. This research will provide valuable information on soil microbial community in oil palm soil and draw leads for future research.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

POPULASI MIKROB TANAH DI LADANG KELAPA SAWIT FELDA JENGKA 24, MALAYSIA

Oleh

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Objektif utama kajian ini adalah untuk menganalisa kepelbagaian mikrob tanah dalam tanah kelapa sawit bagi memahami komuniti mikrob dalam tanah supaya boleh membantu merumus cara menangani penyakit repur pangkal kelapa sawit (BSR) yang baru dan inovatif. Pengambilan sampel tanah telah dijalankan di FELDA Jengka 24. Tempat pengambilan tanah termasuk kawasan penggunaan tandan sawit kosong (EFB), kawasan dengan kejadian penyakit BSR tinggi dan kawasn yang bebas dari BSR. Untuk perbandingan, sampel tanah juga telah diambil dari hutan yang terletak di bersebelahan. Kaedah pengiraan plat telah digunakan untuk penilaian populasi mikrob tanah secara menyeluruh. Mikrob berfungsi juga dinilai menggunakan media tertentu. Pengenalpastian dan pencirian mikrob berfungsi telah dijalankan dengan menggunakan cara molekul. BIOLOG EcoPlate telah digunakan untuk menganalisa komuniti mikrob. Data yang didapati daripada kaedah pengiraan plat dalam bentuk "Colony Forming Unit" (CFU) telah dianalisa dengan menggunakan Student's *t*-Test. Keputusan yang didapati daripada BIOLOG EcoPlate adalah dalam bentuk "optical density" (OD) pula dianalisa menggunakan kaedah statistik yang dipanggil "Principal Component Analysis" (PCA). Keputusan analisa menunjukkan bahawa perbandingan jumlah populasi kulat di antara kawasan pengunaan EFB dan kawasan yang tidak menggunakan EFB tidak menunjukkan perbezaan yang signifikan. Pemerhatian yang sama juga direkod bagi populasi bakteria dan actinomycetes. Ini telah menunjukkan bahawa pengunaan EFB tidak menjejaskan populasi kulat, bakteria dan actinomycetes. Walaupun perbezaan yang tidak signifikan telah dilaporkan untuk kulat, bakteria dan actinomycetes di kawasan perbandingan antara kawasan kejadian BSR tinggi dengan kawasan bebas BSR, pemerhatian untuk siasatan lanjut juga telah ditemui. Populasi kulat di kawasan kejadian BSR tinggi adalah turun naik di sekitar $3.2x10^2$ hingga $2.3x10^5$ CFU/gram. Populasi bakteria dan actinomycetes pula dilaporkan stabil. Kawasan dengan populasi kulat yang rendah tetapi populasi bakteria tinggi berkemungkinan jadi tanda untuk serangan BSR. Penilaian populasi mikrob berfungsi terutamanya mikrob pelarut fosfat menunjukkan bahawa ia tidak dipengaruhi oleh kejadian BSR, disokong dengan keputusan perbandingan populasi secara purata 1x105 CFU/gram yang tidak signifikan.

Pemerhatian yang serupajuga didapati untuk mikrob pengikat nitrogen. Walaupun dengan perbezaan yang tidak signifikan, populasi pereput lignin di kawasan bebas BSR (purata 1.3x103 CFU/gram) adalah lebih tinggi daripada kawasan kejadian BSR tinggi (purata 63 CFU/gram). Ini telah mencadangkan bahawa kawasan dengan populasi pereput lignin yang rendah adalah kondusif untuk penyakit BSR. Kawasan penggunaan EFB telah mencatatkan populasi pereput lignin yang tinggi dan signifikan, menunjukkan penyumbangan dari EFB kepada pereput lignin. Bakteria *Pseudomonas* dan *Bacillus* tidak dipengaruhi oleh kejadian BSR seperti yang disokong oleh ujian signifikasi. Penggunaan EFB didapati menyumbang kepada kedua-dua bakteria tersebut dengan purata populasi yang tinggi dan konsisten sekitar $2.13x10^6$ CFU/gram dan $1.2x10^4$ CFU/gram bagi *Pseudomonas* dan *Bacillus* masing-masing. Kulat *Trichoderma* sp. juga tidak dipengaruhi oleh kejadian BSR dan aplikasi EFB dengan purata populasi $2.8x10^4$ CFU/gram. Sejumlah 27 mikrob dari pelbagai kumpulan mikrob berfungsi telah dikenalpasti. Ianya termasuk bakteria (*Burkholderia* sp., *Klebsiella* sp., *Bacillus* sp., *Chryseobacterium* sp., *Streptomyces* sp., *Chromobacterium* sp., *Pseudomonas* sp.) dan kulat (kebanyakan dari orders Hypocreales and Eurotiales). Analisis PCA untuk penilaian komuniti mikrob mendapati bahawa komuniti mikrob di kawasan tanaman kelapa sawit adalah berkait rapat. Sebaliknya, taburan titik data bagi kawasan hutan yang jarang menyatakan komuniti mikrob yang tidak berkaitan (rendah persamaan). Keputusan ini juga disokong oleh analisis penggunaan sumber karbon. Analisis PCA untuk kawasan penggunaan EFB dan kawasan tanpa penggunaan EFB juga menunjukkan perbezaan yang tidak ketara, menunjukkan komuniti mikrob di kedua-dua kawasan yang lebih berkaitan (tinggi persamaan). Ini juga bermaksud aplikasi EFB berkemungkinan besar tidak menyebabkan perubahan ketara pada komuniti mikrob tanah. Begitu juga dengan PCA analisis untuk memperbandingkan komuniti mikrob di kawasan kejadian BSR tinggi dan kawasan bebas BSR. Komuniti mikrob di kedua-dua kawasan dengan persamaan yang tinggi menunjukkan bahawa serangan penyakit BSR tidak akan mempengaruhi komuniti mikrob dalam tanah. Kedua-dua perbandingan di atas menunjukkan bahawa adanya komuniti mikrob yang stabil dan tahan dalam tanah kelapa sawit. Penyelidikan ini akan memberikan informasi tentang komuniti mikrob di tanah kelapa sawit yang bernilai dan akan memberi petunjuk untuk kajian masa akan datang.

ACKNOWLEDGEMENTS

My heartiest thanks to all, particularly to my supervisor Associate Professor Dr. Zainal Abidin Mior and Associate Professor Dr. Radziah Othman Ahmad for his valuable time spent and endless efforts in guiding me to the success and completion of this research. Endless support and constructive comments from my supervisory committee members Professor Dr. Sariah Meon and Dr. Lum Keng Yeang are much appreciated.

I am in debts to CABI South East Asia for funding and supporting the whole of my study programme. I am grateful and honoured to be part of CABI SEA. Not to forget FELDA Global Ventures (previously known as FELDA Agricultural Services Sdn. Bhd.) for providing the sampling sites and Science Fund (Project number 05-01-04-SF0208) for providing the research funding.

Last but not least, much appreciation to my wife and parents for their patience and love in supporting my study.

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

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LIST OF ABBREVIATIONS

ARDRA	Amplified Ribosomal DNA Restriction Analysis
ARISA	Automated Ribosomal Intergenic Spacer Analysis
BIOLOG-PM	BIOLOG Phenotypic Micro-arrays
BSA	Bovine Serum Albumin
BSR	Basal Stem Rot
BUG	BIOLOG Universal Growth Media
CCA	Canonical Correspondence Analysis
CFU	Colony Forming Unit
CIRP	Christmas Island Rock Phosphate
CLPP	Community Level Physiological Profile
DCA	Detrended Correspondence Analysis
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide TriPhosphate
EFB	Empty Fruit Bunch
FAME	Fatty Acid Methyl Ester
FASSB	FELDA Agriculture Services Sdn. Bhd. (Private Limited)

FELDA	Federal Land Development Authority
G-	No BSR Incidence
G+	High BSR Incidence
GC content	Guanine-Cytosine content
GN	Gram Negative
GP	Gram Positive
IPC	International Planter's Conference
ITS	Internal Transcribed Spacer
KOH	Potassium Hydroxide
LCC	Leguminous Cover Crop
MPOB	Malaysian Palm Oil Board
NA	Nutrient Agar
OPBC	Oil Palm Biomass Center
P&C	Principles and Criteria
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PIPOC	International Palm Oil Conference

PLFA	Phospholipid Fatty Acid
PM	Phenotypic Micro-array
POME	Palm Oil Mill Effluent
RAPD	Random Amplified Polymorphic DNA
rDNA	Ribosomal Deoxyribonucleic Acid
RFLP	Restriction Fragment Length Polymorphism
RISA	Ribosomal Intergenic Spacer Analysis
rRNA	Ribosomal Ribonucleic Acid
RSPO	Roundtable on Sustainable Palm Oil
SEM	Scanning Electron Microscope
SSCP	Single Strain Conformation Polymorphism
TBE	Tris-Borate-EDTA
TEM	Transmission Electron Microscope
TGGE	Temperature Gradient Gel Electrophoresis
T-RFLP	Terminal Restriction Fragment Length Polymorphism
TSA	Tryptic Soy Agar
UPM	Universiti Putra Malaysia
USA	United States of America

VF	Virgin Forest/Jungle

CHAPTER 1

INTRODUCTION

All organisms in the biosphere depend on microbial activity (Pace, 1997) and soil microbial biodiversity constitutes an important component of the world's biodiversity. Plant-soil microorganisms interactions ranging from mutualism to parasitism are known to have important consequences for both plant growth and health. Soil microbes play important roles in these interactions and have been utilized as bio-indicators for assessing soil health and sustainable productivity in cropping systems (Moscatelli *et. al.*, 2005). However, there is a need for more research to improve and enhance knowledge on soil microbial diversity and the major functions played by soil microbial communities. Varieties of essential functions in the soils such as nitrogen fixation and phosphate solubilisation are championed by different group of microorganisms or possibly even one. The simple yet efficient cell structure of the microorganisms and their sensitivity to the surrounding environment has been acknowledged. Soil microbes are the first organisms to react to changes in the environment due to their short life cycle and quick turnover. Therefore, they are considered the best candidates to be indicators for soil health and sustainability of cropping systems. An understanding of the relationships between functional microbial diversity and current agricultural practices may contribute to scientific information to elucidate the sustainability of oil palm plantations.

In this research, basal stem rot (BSR) disease of oil palm was chosen as one of the key components. It is caused by the fungal pathogen, *Ganoderma boninense* that had created a major setback in oil palm industries particularly in Malaysia. This disease causes serious economic losses due to palm stand loss and reduction in productivity as well as economic lifespan of oil palm. Over the years, the BSR disease has thrived from the coastal soils and making advance into the inland and peat soils (Rao *et.al.*, 2003). The concerns were worsened when the fungus caused serious damage to young oil palm trees as well. To date, there has been no effective method to manage BSR. Areas like the biology of the fungus, epidemiology (spreading of the disease, either via root contact or basidiospores, monokaryotic and dikaryotic mycelia) have been well studied over the years. Understanding the relationships between the BSR fungus and the surrounding soil microbial communities could assist future innovative options for improving the management of the BSR disease.

Hypothetically, the disturbed ecosystems (oil palm plantations) would have distinctly different microbial population profiles as compared to undisturbed ecosystem (forest). Applying this hypothesis, if the oil palm plantation is affected by the BSR disease, this shall be reflected by the microbial populations in the soil. Interventions to the environment, including fertilisers and EFB applications should also be reflected. Forest soils taken from the forest in adjacent to the plantation were included as reference points where

efforts to restore the diversity of soil microbes e.g. EFB applications should steer the similarity of soil microbial profiles of oil palm soils closer forest soils.

This research was therefore undertaken with these points in view and supported by the following specific objectives:

i) To evaluate soil microbial populations in oil palm soils in relation to BSR disease incidence and standard agronomical practices (chemical fertiliser and EFB application) with inclusion of isolation, identification and characterisation of functional microbes

ii) To assess microbial community of oil palm soils in relation to BSR disease incidence and standard agronomical practices (chemical fertiliser and EFB application)

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BIODATA OF STUDENT

Low, Ying Chiang was born in 1981 at Malacca (Melaka), a town where the history of Malaysia started centuries ago. A small piece of land with merely two meters wide and three meters length has triggered his interest in agriculture since he was a small boy. Upon completing his primary and secondary education, he has chosen to pursue his interest in agriculture by taking up the opportunity offered by Universiti Putra Malaysia to further his study in Diploma in Agriculture. This happened in the year 2000. This has given him a chance to further his interest in agriculture and has strengthened his knowledge in the similar field, particularly in plant protection. He has continued his study in Bachelor degree in Bio-Industry in same university without hesitation. During this period, he has decided to be a "Plant Doctor" and chosen his major in plant protection in the final year of his first degree. Graduated with first class honours in year 2005, he has joined CAB International (Southeast Asia Regional Centre) as research assistant in the same year. Here, his knowledge acquired from the Universiti Putra Malaysia have been fully utilised and has been groomed further as Plant Pathologist. During this period, CAB International has granted him an opportunity to pursue his doctoral degree while still being a full time staff. Over the years working with in CAB International, experiences gained include skills and knowledge in microbial characterization and identification using BIOLOG systems and also microbial diversity studies and data analysis. Involvements in different projects have also helped to gain more knowledge in plant pathology involving both mycology and bacteriology. He has been involved in various national and international projects which are mainly related to plant pathology (isolation, pathogenicity test and fulfilment of Koch's Postulates), environmental microbial studies involving diversity assessment and characterisation (using BIOLOG Phenotypic Micro-array) and biological resource collection projects. He is also actively involved in global project led by CAB International called "PlantWise" where he is tasked to train Plant Doctors and establish Plant Clinics in Cambodia, Myanmar, Thailand and Vietnam.

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