IDENTIFICATION, CHARACTERIZATION AND APPLICATION OF THE POTENTIAL *TRICHODERMA* SPECIES LOCALLY ISOLATED FROM LAHAD DATU, SABAH

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ABSTRACT

Specific strains of *Trichoderma* species have been commercially applied as biocontrol agents against several plant pathogenic fungi due to their production of antifungal metabolites, competition for nutrients and space, and mycoparasitism. The main aim of this study was to identify the specific potential of *Trichoderma* strains from an oil palm plantation in Lahad Datu for the application in the biocomposting of oil palm empty fruit bunches. This compost able to enhanced plant growth performance and also produced high yield production. Generally, compost is not only a good biofertilizer but also a good biocontrol agent against soil-borne pathogens. Isolation of *Trichoderma* species from soil was done using dilution technique. A total of 138 colonies of fungus found, and only 97 isolates were physically identified as *Trichoderma* species. Apart from that, only 52 *Trichoderma* isolates were further identified based on the morphological characteristics and molecular data analysis. From the results, all 52 isolates were belong to eight *Trichoderma* species namely *Trichoderma asperellum, T. harzianum, T. koningiopsis, T. hamatum, T. theobromicola, T. erinaceum, T. viride* and *Hypocrea melanomagna.* Next, the lignocellulolytic activities of all isolates were studied based on their ability to degrade cellulose, lignin and starch and the best potential strains were selected as the decomposers. Strains 2H, 5D and 10L2 were found to give the largest halo zone in the order of 4.40 ± 0.46 cm for cellulose, 4.93 ± 0.06 cm for lignin and 7.47 ± 0.15 cm for starch, respectively. Based on these results, two strains (11B and SICCI) were selected for the composting of oil palm fibre. Over the four weeks of the composting period, analysis of pH of the compost was found to be slightly alkaline at the earlier stage and slowly become acidic. The temperature recorded was almost the same being the 24°C during the laboratory assay. Field experiments were done to test the effectiveness of the compost for 15 weeks by using chilli plants and two types of compost (Compost A and Compost B) together with a control group. Based on the results, *Trichoderma* propagules were found dominant with 72% abundancy in soil when compared to the other fungi. Compost A showed better results in length (90 cm), number of leaves (95.5), stem diameter (0.8 cm), branching root development, flower formation (21 during week 13), and yield of Chilli (58) compared to compost B and control. However, chili tree mixed with compost B had an average higher branch formation (7.8), higher percentage of moisture in plant (58.93 %) and longer root development (32.6 cm). Analysis of soil electrical conductivity showed it to be 50.40 µS/cm for compost A, 42.10 µS/cm for compost B and 40.11 µS/cm for the control, respectively. Higher CN ratio was found in compost A and B (3.21:0.26, 3.18: 0.26) as compared to control (2.71: 0.26). Analysis on week eight showed the ratio of NPK to be in the order, compost A (4.30) > compost B (2.79) > control (1.55). To conclude, either by using compost A or compost B, composting of oil palm fibres shows a great potential for application in agricultural fields to improve soil fertility, soil texture, enhance plant growth and high yield of production.

ABSTRAK

IDENTIFIKASI, PENCIRIAN, DAN APLIKASI SPESIS TRICHODERMA BERPOTENSI YANG DIISOLASI DARI LAHAD DATU, SABAH

Strain tertentu bagi spesis Trichoderma digunakan secara komersial sebagai agen kawalan biologi terhadap beberapa tumbuhan kulat patogen disebabkan kebolehannya menghasilkan metabolit antikulat, persaingan untuk nutrien dan ruang, dan mycoparasitisma. Tujuan utama kajian ini adalah untuk meningkatkan kesuburan tanah dan meningkatkan hasil tanaman dengan menggunakan biokompos baja dari bahan mentah tandan kosong kelapa sawit. Ia boleh meningkatkan prestasi pertumbuhan tanaman dan pengeluaran hasil tanaman pertanian yang tinggi. Pengeluaran kompos bukan sahaja biobaja yang baik tetapi juga agen kawalan biologi yang baik terhadap patogen bawaan-tanah. Terdapat 52 kultur telah dikenal pasti sebagai spesies Trichoderma berdasarkan karateristik fisiologi dan penelitian mikroskopik berdasarkan sampel tanah yang diambil dari ladang kelapa sawit di Lahad Datu, Sabah. Identifikasi di peringkat spesis Trichoderma yang ditemui telah dilakukan berdasarkan gabungan kaedah penilitian data morfologi dan ciri-ciri molekular telah menemui 11 spesis Trichoderma yang dinamakan sebagai Trichoderma asperellum, T. harzianum, T. koningiopsis, T. hamatum, T. theobromicola, T. erinaceum, T. viride dan Hypocrea melanomagna. Seterusnya, saringan lignosellulolitik berdasarkan keupayaan fungus tersebut untuk merendahkan kadar selulosa, lignin dan kanji telah dilakukan untuk memilih fungus yang terbaik supaya dapat digunakan sebagai pengurai kepada kompos. Sampel kultur 2H, 5D dan 10L2 menunjukkan zon halo terbesar; 4.40 ± 0.46 cm, 4.93 ± 0.06 cm dan 7.47 ± 0.15 cm terhadap penguraian enzim selulosa, lignin dan kanji. Berdasarkan keputusan ini, dua strain Trichoderma terbaik (11B dan SICCI) telah dipilih untuk menjadi pengurai untuk kompos. Empat minggu diperuntukkan untuk kompos mengurai dan analisis pH awal kompos mendapati pH sedikit alkali pada permulaan proses dan selepas itu menjadi sedikit asid. Suhu kompos yang direkodkan sepanjang empat minggu proses pengkomposan adalah 24 °C iaitu suhu makmal. Pokok cili telah digunakan untuk eksperimen di lapangan dan telah dilakukan selama 15 minggu dengan menggunakan dua jenis kompos bersama strain fungus yang telah dipilih dan kumpulan kawalan. Berdasarkan keputusan eksperimen, sejumlah 72% propagul Trichoderma telah ditemui dan setelah dibandingkan dengan pertumbuhan kulat dalam tanah yang lain propagul Trichoderma mendominasi jumlah populasi fungus di dalam tanah. Pokok cili yang dicampur dengan kompos A menunjukkan keputusan yang baik dari segi panjang pokok dengan purata 90 cm, jumlah daun (95.5), diameter stem pokok (0.8 cm), perkembangan akar yang lebih tersebar, pembentukan bunga (21 bunga terbentuk pada minggu 13), dan hasil buah cili (58) dihasilkan berbanding dengan tumbuhan dirawat dengan kompos B dan kawalan. Walau bagaimanapun, pokok cili yang ditambah dengan kompos B membentuk dahan yang lebih banyak (7.8), peratusan kelembapan dalam tumbuhan yang lebih tinggi (58.93%) dan perkembangan akar yang lebih panjang (32.6 cm). Analisis tanah terhadap kekonduksian elektrik mendapati 50.40µS/cm untuk kompos A, 42.10µS/cm untuk kompos B dan 40.11µS/cm untuk kawalan. Analisis pada minggu 8, nisbah NPK yang lebih tinggi

terdapat dalam kompos A (4.30)> kompos B (2.79)> kawalan (1.55). Oleh itu, kompos daripada kelapa sawit menunjukkan potensi yang besar untuk digunakan di dalam bidang pertanian untuk meningkatkan kesuburan tanah dan tekstur tanah, meningkatkan pertumbuhan tumbuhan dan hasil pengeluaran yang tinggi.

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LIST OF SYMBOLS

%	-	Percentage
±	-	Plus minus
°C	-	Degree celcius
µg	-	Microgram
µg / mL	-	Microgram per mililiter
µL	-	Microliter
µm	-	Micrometer
cm	-	Centimeter
g	-	Gram
h	-	Hour
mg	-	Miligram
min	-	Minute
mL	-	Mililiter
mM	-	Milimolar
mm	-	Milimeter
nm	-	Nanometer
rpm	-	Rotation per minute

LIST OF ABBREVIATIONS

A_{260}/A_{280}	-	A260 and A280 are the optical spectrometer measurement of absorbance at the wavelength of 260nm and 280nm respectively
AFLP	-	Amplified fragment length polymorphism
BCA	-	Biological control agent
BLAST	-	Basic Local Alignment Search Tool
bp	-	Base pair
C:N	-	Carbon to Nitrogen ratio
CFU	-	Colony forming unit
CTAB	-	Cetylmethylammonium bromide
ddH_2O	-	Double distilled water
DNA	-	Deoxyribonucleic acid
dNTP	-	Deoxynucleotides Triphosphates
EDTA	-	Ethylenediaminetetraacetic acid
GCPSR	-	Genealogical Concordance Phylogenetic Species Recognition
H_2O	-	Water
HCL	-	Hydrochloric acid
IGS	-	Intergenic Spacer Region
ISSR	-	Inter Simple Sequence Repeat
ISTH	-	International Subcommission on *Trichoderma* and *Hypocrea*
ITS 1	-	Internal Transcribed Spacer 1
ITS 2	-	Internal Transcribed Spacer 2
ITS 4	-	Internal Transcribed Spacer 4
K_2HPO_4	-	Dipotassium Phosphate
Kb	-	Kilo base
LSU	-	Large Subunit
MEGA	-	Molecular Evolutionary Genetics Analysis
$MgCl_2$	-	Magnesium chloride
$MgSO_4.7H_2O$	-	Magnesium sulphate
NaCl	-	Natrium chloride

NCBI	-	National Center for Biotechnology Information
NH_4NO_3	-	Ammonium nitrate
PCR	-	Polymerase Chain Reaction
PDA	-	Potato Dextrose Agar
pH	-	Negative decimal logarithm of the hydrogen ion activity in a solution
RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
RNA	-	Ribonucleic acid
rpm	-	Revolutions per minutes
rRNA	-	Ribosomal ribonucleic acid
sp.	-	Species
TBE	-	Tris borate ethylene diamine tetraacetic acid
TSM	-	*Trichoderma* Selective Media
UPGMA	-	Unweighted Pair Group Method with Arithmetic Mean
www		World Wide Web

CHAPTER 1

INTRODUCTION

1.1 Research Background

In general, fungi are well known for their special ability to degrade lignin in woody material (Beata, 2007). Specifically, the fungi genus *Trichoderma,* which can be found among all types of soil in the world, possess strong criteria as a biological control agent. This species is also able to compete strongly for space and nutrients amongst other organisms, produce toxins against phytopathogenic species (Mohd Zainudin *et al.,* 2008), and utilize antagonist properties against *Ganoderma boninense* (Siddiquee *et al.,* 2009). Study by Dayana Amirah *et al.* (2012) has proven that *T.virens* is one of the most effective fungi that could degrade organic matter into minerals.

Unfortunately, there have been some difficulties in the identification of *Trichoderma* at species level - so far, only morphological and cultural characters have been identified (Siddiquee *et al.,* 2007). Gams and Bissett (2002) have reported that the *Trichoderma* species have been characterized by their morphological characteristics with the advancement of molecular tools. Similar procedures have been adopted by several researchers (Rifai, 1969; Bissett, 1991a; Samuels, 2002) to characterize and differentiate among various members of the *Trichoderma* species. However, it is still very difficult to differentiate among members of the *Trichoderma* species, especially when it comes to their microscopic characteristics; for example, the phialide and conidia of this species are very similar to its teleomorph, the *Hypocrea* (Rifai and Webster, 1966). Therefore, some researchers (Chaverri and Samuels, 2003; Overton *et al.,* 2006) have agreed that to determine *Trichoderma* at species level, morphological characteristics alone are not enough for identification; given putative names are needed.

Identification in this species, which began in 1995, have been re-assessed following current advancements in molecular tools, the DNA sequencing and morphology-based taxonomy of *Trichoderma* species (Druzhinina and Kubicek, 2005; Samuels, 2005). Several molecular techniques such as analysis on the molecular markers (ITS sequencing analysis, RAPD), physiological (isoenzymes analysis), and phenetic characters have been employed to solve the confusion of *Trichoderma* taxonomy (Kuhls *et al.*, 1996; Samuels *et al.*, 1998; Lieckfeldt *et al.*, 1998; Kindermann *et al.,* 1998). The combination of phylogenetic and phenotypic data is crucial for the identification of *Trichoderma* species (Chaverri and Samuels, 2003; Jaklitsch, 2009; Poldma, 2011; Kim *et al.,* 2012).

In addition to that, fungi growth rates have always assisted in determining species. These are accessed by observing and recording specific features in fungi, such as their ability to sporulate abundantly, the presence of pustules, the formation of concentric rings, the presence of a distinctive coconut odour and the production of various types of secondary metabolites (Blaszczyk *et al.,* 2011).

One unique criteria present in the *Trichoderma* species involves biological control characteristics. According to Roath (2014), biological control was first described by Harry Smith in relation to the biological control of insects, where the suppression of insect populations by native or introduced enemies was observed. Biological control is thus defined generically as a population-levelling process in which an increase in the population of one species lowers the number of another within the same food chain. Biological control agents are non-toxic to humans, do not contaminate water and are very host-specific once they are colonized.

It is believed that *Elaeis guineensis jacq*, better known as the oil palm tree, grew wildly before it was cultivated as an agricultural crop. It was first introduced to Malaysia (then Malaya) by British colonizers around the year 1870. The Tennamaran Estate in Selangor became the first estate where oil palm trees were planted commercially in Malaysia. Through the Malaysian government's agricultural diversification programme in the early sixties, the oil palm industry grew rapidly to cut down the country's economic dependence on rubber and tin. The scheme also