DEVELOPMENT OF *Mycorrhizae* LIKE spp. AND *Trichoderma* spp. COCKTAIL FOR DEGRADATION OF OIL PALM EMPTY FRUIT BUNCH

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A dissertation submitted in fulfilment of the requirements for the award of the degree of Master of Science (Biotechnology)

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13TH JANUARY 2020

DEDICATION

This thesis is dedicated to my parents, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my family, who taught me that even the largest task can be accomplished if it is done one step at a time.

ACKNOWLEDGEMENT

In the name of Allah, the most gracious, the most merciful.

Alhamdulillah, Thanks to Almighty Allah SWT, whom with His willing giving me the opportunity to complete this study.

In preparing this thesis, I was in contact with many people, researchers, academicians, and practitioners. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my thesis supervisor, Prof Madya Dr. Madihah Md Salleh, for encouragement, motivation, guidance, critics and friendship throughout my research.

I would like to express my appreciation to the management of Jigawa State College of Education Gumel, Jigawa State for the giving to me opportunity to undertake this study. Special appreciation goes to Tetfund (Nigeria).

I am also grateful to my beloved parents, family, colleagues and lab mates for their tolerance, guidance and patient during my life time at UTM and postgraduate student should also be recognised for their support. Unfortunately, it is not possible to list all of them in this limited space. I am grateful to all my family member.

ABSTRACT

The accumulation of Oil Palm Empty Fruit Bunch (OPEFB) in large quantity contributes to environmental pollution. The usual means of disposing this waste such as burning in incinerators leads to secondary pollution. The present study aims to find an efficient and practical approach to revalorize OPEFB as renewable and cost effective substrate for production of lignocellulolytic enzymes. A total of nine Mycorrhizae like fungi and six Trichoderma isolates stock were collected from Biorefinery Technology Research Laboratory. The isolates were studied for biocompatibility on Potato Dextrose Agar (PDA) and Mandel medium. The solid state fermentation was conducted using mono and mixed-culture of Mycorrhizae CS2 like fungi and Trichoderma A4 sp. and untreated OPEFB along with four different media. The activity of enzymes and reducing sugar during fermentation process was analyzed. The biocompatibility test showed that Mycorrhizae spp. and Trichoderma spp. could grow together on both PDA and Mendel medium. The highest enzymes activities were produced at 76% final moisture content, 500µm substrate, initial pH 7.0, inoculum size 1×10^7 , incubation at 30°C and consortium growth on OPEFB supplemented with Mandel medium. The highest CMCase and xylanase activities of mixed culture was 38.67 U/g and 111.88 U/g and single culture was CS2 37.66 U/g and 100.59 U/g both in Mandel medium. Interestingly, highest lignin peroxidase activity (0.000323 U/g and 0.000215U/g) were recorded from mono cultures of CS2 on medium B and C respectively. The highest reducing sugar production of 0.0383 U/g and 0.0785 U/g were recorded from single CS2 and mixed cultures both from mandel medium. The finding of the study suggest that OPEFB is a cheap renewable substrate that can be used for lignocellulolytic enzyme production and also indicates that Mycorhizae and *Trichoderma* consortium could be a good alternative for enzymes production.

ABSTRAK

Pengumpulan sisa tandan buah kelapa sawit (OPEFB) yang banyak telah menyumbang kepada pencemaran alam sekitar. Kaedah yang biasa digunakan adalah dengan pembakaran dalam incinerator yang boleh menyumbang kepada pencemaran sekunder. Kajian ini bertujuan mencari pendekatan yang effective dan praktikal untuk mengguna semula tandan buah kelapa sawit sebagai substrat yang diperbaharui dan kos efektif untuk pengeluaran enzim lignoselulolitik. Sebanyak sembilan Mycorrhizae spp. dan enam isolat *Trichoderma* dikumpulkan dari Makmal Penyelidikan Teknologi Biorefineri. Kajian biokopatibility telah dijalan dengan menggunakan 4 Mikorizae dan 4 Trichorderma di atas media PDA dan Mandel. Penapaian dalam keadaan pepejal dilakukan dengan menggunakan kultur tunggal dan kultur campuran campuran fungi seperti Mycorrhizae CS2 dan Trichoderma sp. A4 serta OPEFB yang tidak dirawat bersama dengan empat media yang berbeza. Ujian biokompatibiliti menunjukkan bahawa Mycorrhizae spp. dan Trichoderma spp. boleh tumbuh bersama di atas PDA dan media Mandel. Aktiviti enzim lignocsellulolytic tertinggi dihasilkan pada 76% lembapan, saiz substrat 500 μm, pH awal 7.0, saiz inokulum 1×10⁷ spore /ml serta inkubasi pada 30°C. Pertumbuhan konsortium *Mycorrhizae* spp. dan *Trichoderma* spp. pada OPEFB dalam medium Mandel. Aktiviti CMCase dan xylanase adalah tertinggi dalam kultur campuran iaitu 38.67 U/g dan 111.88 U/g. Kultur tunggal Mikorizae CS2 menghasilkan cellulose and xylanase sebanyak 37.66 U/g dan 100.59 U/g dalam medium Mandel. Walaubagaimanapun altiviti lignin peroksidase adalah sangat rendah (0.000323 U/g dan 0.000215 U/g) samada pada kultur tunggal atau campuran pada medium B dan C. Aktiviti gula peruduksi tertinggi 0.0383 U/g dan 0.0785 U/g dicatatkan dari Mikorhizae CS2 tunggal dan kultur campuran dari medium Mandel. Hasil kajian menunjukkan bahawa tandan buah kelapa sawit dan konsortium Mycorhizae dan Trichoderma berupaya untuk menghasilkan enzim lignocellulosik dengan menggunakan kaedah SSF.

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LIST OF ABBREVIATIONS

°C - Degree Celsius

AMF - Arbuscular Mycorrhizae Fungi

CMCase - Carboxymethyl-cellulase

DNS - Dinitrosalicyclic acid

g - Gram h - hour

H₂SO₄ - Sulphuric acid

HCl - Hydrochloric acid

H₂O₂ - Hydrogen Peroxide

L - Liter

LiP - Lignin peroxidase

min - Minute mL - Milliliter

MnP - Manganese peroxide

NA - Not available

NaOH - Sodium hydroxide

nm - Nanometer

PDA - Potato Dextrose Agar

rpm - Rotation per minute

SSF - Solid-State Fermentation

U/g - Unit of enzyme per gram

UTM - Universiti Teknologi Malaysia

v/v - Volume per volume

w/v - Weight per volume

μL - Micro liter

μm - Micro meter

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Interaction between *Mycorrhizae* and *Trichoderma* is environmentally friendly to reduce agrochemical damage of the product and has synergistic effect of plant disease as biological control (Martínez-Medina *et al.*, 2009). Microbial inoculants interaction of *Mycorrhizae* spp. and *Trichoderma* spp. has the capability to promote growth of plan height, shoot, root, phosphorous uptake for numerous biochemical, physiological function and resistance of plant pathogen (Tchameni *et al.*, 2011). Microorganism play vital role as intermediate for recycling of lignocellulosic materials, degradation, also use as means of waste management toward environmental pollution. The development of microbial advancement and technology has many role toward the efficiency and utilization of renewable materials and use with considerable success in many area such as biocontrol agent industrial fermentation, plant growth hormones, antibiotic production and others (Kaur *et al.*, 2013).

Mycorrhizal fungi could promote and sustain the oil palm growth either by its present in soil naturally or through pre-plantation management of Mycorrhizal. This technique will ensure the establishment of new crops and do not exhaust soil nutrients rapidly (Kaur et al., 2014). The use of biocontrol agents such as Trichoderma spp. requires particular attention because of the possibility that these antagonists fungi interact not only with plant pathogens fungal but also with Mycorrhizal fungi such as AMF (Sharma et al., 2016; Vosátka and Gryndler, 1999). The free-living microbial inoculants such as Trichorderma spp. could also stimulate the Mycorrhizal spp. colonization.

1.2 Objectives of the Study

The objectives of this study are:

- a. To perform the biocompatibility test of *Mycorrhizae* like spp. and *Trichorderma* spp. in solid Mandel medium and Potato Dextrose Agar (PDA).
- b. To identify the consortium growth of *Mycorrhizae* like spp. and *Trichorderma* spp. based on lignocellulolytic enzyme production performance in different medium using Oil Palm Empty Fruit Bunch (OPEFB) as a carbon source.
- c. To analyse the lignocellulolytic enzymes and reducing sugar production by consortium growth of *Mycoryzae* like spp. and *Trichorderma* spp. in different medium using Oil Palm Empty Fruit Bunch (OPEFB) as a substrate.

1.3 Problem Statement

As the lignocellulose biomass contain polysaccharide which hydrolyzed by enzymatic or acidic processes. Acidic hydrolysis has disadvantages toward the generation of hazardous acidic waste and it is difficult to recover fermentable sugar. Enzymatic hydrolysis is more efficient and preferred, the process is under rapid development and has high capability of improvement in cost and efficiency for utilization of biomass. Usually, the enzymes production is very expensive, as a result of 40-60% production cost is raw materials. The enzymes cost economics can be brought down by some strategies such as use of cheaper and abundant lignocellulose biomass, solid state fermentation is cost effective, and use of microbial for higher enzymes production (Dhillon et al., 2012). Lignocellulosic biomass uses in biorefinery as raw materials for production of enzymes, chemicals, and bioenergy. Difference sources would generate lignocellulosic feedstock which include empty palm oil fruit bunch, sugarcane bagasse, wheat brand straw, corn straw, wood, corn cobs and even newspaper, waste paper from office. The significance of biorefinery is bioconversion of lignocellulosic biomass to chemicals and bioenergy (Liguori and Faraco, 2016; Sperandio and Ferreira Filho, 2019). Cheap sources of renewable biomass into medium for the production of lignocellulose enzymes could help to decrease the production costs of enzymatic complexes that can hydrolyse lignocellulose residues for the formation of fermented syrups, thus contributing to the economic production of bioethanol (Camassola and Dillon, 2007).

1.4 Significance of the Study

Recent studies reveal that formulation of microbial bioinoculants for bio fertilizer and bio pesticides as a substitute agrochemicals in developing countries are at early stage of investigation (Mishra and Arora, 2016). Currently there is challenges of many features of co-culture need to understand and address the co-culture toward commercial enzymes production (Sperandio and Ferreira Filho, 2019). Development of lignocellulosic materials conversion to polymers and fine chemicals is another big challenges (Isikgor and Becer, 2015).

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