SCREENING OF CELLULOLYTIC AND XYLANOLYTIC FUNGI FOR ENZYMES COCKTAIL STUDIES OF *GANODERMA BONINENSE* INHIBITION

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Specially dedicated to my supportive families and friends

Thank you for all love and concern

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ABSTRACT

Ganoderma boninense is a basidiomycetes white rot fungus which causes basal stem rot (BSR), one major disease in oil palm plantation in Malaysia. The objective of the present study was to screen the most potential fungus that has a capability to produce crude xylanase and cellulase to be used as a biological control for *Ganoderma boninense*. Twenty four different strains of fungi were obtained from the Biorefinery Technology Research Laboratory of Faculty Biosciences and Medical Engineering, Universiti Teknologi Malaysia, Johor. They were screened for their potential to degrade cellulose and hemicelluloses. The screening of the fungi were based on the diameter of holozones on red color of congo red and the formation of yellow-opaque area on the carboxymethlycellulose(CMC) and xylan agar, respectively. Among these strains, five potential fungi which showed the maximum enzyme activities for endoglucanase (CMCase), filter paper degrading enzyme (FPase), beta-glucosidase, xylanase and reducing sugar were selected to produce enzyme cocktail on untreated oil palm trunk will be further study through its performance in solid state fermentation. The potential enzyme producers from fungi were CT2, *Pycnoporus cinnabarinus*, EFB1, EFB2 and TG6 that produced 304.04 U/g of CMCase, 13.25 U/g of FPase, 83.15 U/g for β-glucosidase,523.10 U/g of xylanase and 164.04 U/g of reducing sugar respectively. Cellulase and xylanase cocktail produced from these five fungi were mixed and applied on the *Ganoderma boninense* at ratio 1:1:1:1:1 were made up to 0.025mL in the culture plates. However, the inhibition of *Ganoderma boninense* when treated with cellulase and xylanase cocktail was undetected in this study.

ABSTRAK

Ganoderma adalah merupakan basidiomykota kulat reput putih yang menyebabkan penyakit yang serius kepada kelapa sawit di Malaysia iaitu reput pangkal batang. Objektif di dalam kajian ini adalah melakukan penyaringan ke atas kulat yang berpotensi untuk menghasilkan enzim mentah sellulase dan xylanase yang akan digunakan sebagai kawalan biologi ke atas *Ganoderma boninense*. Sebanyak dua puluh empat strain kulat yang diambil daripada Makmal Penyelidikan Biorefineri, Fakulti Biosains dan Kejuruteraan Perubatan, Universiti Teknologi Malaysia, Johor. Kesemua strain ini dilakukan proses penyaringan berdasarkan keupayaan untuk menghuraikan sellulosa dan hemisellulosa. Penyaringan kulat tersebut adalah berdasarkan diameter holozon berwarna merah yang terbentuk daripada congo merah dan legap kuning pada karboksimetil sellulosa (CMC) dan agar xylan masing-masing. Lima jenis kulat yang berpotensi menghasilkan aktiviti endoglukanase (CMCase), enzim pengurai kertas turas (FPase), beta glukosidase, xylanase dan gula penurun yang maksimum telah dipilih untuk menghasilkan koktel enzim ke atas batang kelapa sawit yang tidak dirawat melalui proses fermentasi keadaan pepejal. Pengeluar enzim daripada kulat yang dikenalpasti berpotensi adalah CT2, *Pycnoporus cinnabarinus*, EFB1, EFB2 dan TG6 yang masing-masing menghasilkan 304.04 U/g CMCase, 13.25 U/g FPase, 83.15 U/g β-glukosidase, 523.10 U/g xylanase dan 164.04 U/g gula penurun. Koktel sellulase dan xylanase ini dicampurkan dan digunakan untuk merencatkan pertumbuhan *Ganoderma boninense* pada nisbah 1:1:1:1:1 menjadikan jumlah di dalam piring petri sebanyak 0.25ml.. Walau bagaimanapun, perencatan *Ganoderma* b*oninense* apabila dirawat dengan menggunakan koktel sellulase dan xylanase tidak berjaya di dalam kajian ini.

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et. al	and friends
BSR	Basal stem rot
CMC	Carboxymethyl cellulose
CMCase	Carboxymethyl cellulose
FPase	Filter paper culture enzyme
SSF	Solid state fermentation
SmF	Submerged fermentation
H	Hour
µ	Micro
nm	Nanometer
°C	Degree celcius
PNPG	p-nitrophenyl β-D-glucoside
Rpm	Rotation per minute
U	Unit activity enzyme
DNS	Dinitrosalicyclic acid
MW	Molecular weight

m	Slope
L	Liter
mL	Mililiter
cm	Centimetre
g	Gram
NaOH	Sodium hydroxide
NaCl	Sodium chloride
PDA	Potato dextrose agar
OD	Optical density
BSA	Bovine serum albumin
v/v	Volume per volume
w/v	Weight per volume
µL	Micro liter

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Malaysia has been the second largest oil palm plantation areas after Indonesia (Chong *et al*., 2012) and one of the largest contribution for an economic crops in our industrial. The amount of biomass produced reached 225 million tonnes per annum with 4.5 million ha of oil palm (Mohamed and Alimon, 2012). The rate estimation of palm oil production in our country is more than twelve million tonnes (Najafpour et al., 2007). The important oil palm producing countries reported by (Paterson et al., 2013) are Indonesia, Malaysia, Nigeria, Democratic Republic of Congo, the Ivory Coast, Brazil, Colombia, Costa Rica and Ecuador.

In palm oil industry, the main residues are created from two major sources of processing which is from plantations or field residues and from palm oil milling. Oil palm trunks (OPT) and oil palm fronds (OPF) were categorized as field residues while empty fruit bunch (EFB), palm kernel cake (PKC), palm oil mill effluent (POME), palm press fibre (PPF) and shell are created from palm oil milling process (Zahari *et al*., 2012). Approximately 5 million tonnes of agricultural wastes were generated from the industry (Ibrahim, 2006). Therefore, all the co-products produced

from the palm oil industry need to be processed further to prevent the land pollution as well as to reduce a detrimental impact by convert it into value added product by using microorganism degradation.

There are many products had been produced from the agricultural waste. For example, fiber can be used to make pulp, paper and particle boards. Empty fruit bunch can be used to produce energy and the fertilizer while the oil palm trunk can be made as a furniture, particle board and also biofuels (Ng *et al.* 2011).

One of the most important diseases attacking the oil palm in Malaysia is a *Ganoderma* white rot. This disease caused by one of the pathogen which is called *Ganoderma boninense.* Azadeh *et al.* (2010) stated that cultural, chemical and clean clearing strategies only focused on the way to minimize the incidence of BSR in replanting, prolonging, the productive life of infected palms and delaying the duration of *Ganoderma* infection**.** The other method to reduce the chemical products application in agriculture is by using the biological control from the microorganism.

Ganoderma white rot disease may be controlled by the enzyme inhibitors. *Ganoderma* white rot can damage many living trees and wood which were used for manufacturing industry. *Ganoderma* can infected rubber trees and tea which developed red root rot disease in both of these plants (Zakaria *et al*., 2009) . This fungus also may attack the part of oil palm and seriously infects undamaged trees. It can infects palms as young as 12-24 months after planting and serious infects on palms of 4-5 years age, usually in replanted areas (Bivi *et al*., 2010). Research by Abdullah *et al*. (2012) mentioned that our industry would lose about RM80 million each year if 2.5 percent of the total acreage of oil palm plantation in Malaysia is suffered by this disease.

Ganoderma species can cause a major disease in palm oil industry. One of the major parts in the disease process is a lignin biodegradation which produce carbon dioxide and water molecule. Once lignin was degraded, fungus obtained energy from the cellulose (Paterson, 2007). This fungus species are grown in wood and classified as wood decaying fungi. It degrades cellulose, hemicellulose and lignin. *Ganoderma* is known as polypores because they possessed tiny pores underside their cap where the reproductive spores were hiding. The caps of the spores will always change when the age of that species were changed where it spongy hardening to a shiny when fresh while smooth woody structure when it was matured (Figure 1.1). The colour of the caps also always changed which were ranges from brown to yellowish (Wei *et al.*, 2003). The surface of the pore was cream in colour while the spores were brown colour.

Figure 1.1 The *Ganoderma boninense* basidiomycete (Abdullah *et al*., 2012)

However, *Ganoderma* species were not listed among the group of edible mushrooms because of its physical appearance. Its fruiting bodies were always thick, corky and tough and do not have the fleshy texture characteristics of true edible fungi.

Dry rot occurs when *Ganoderma* species attack the palm roots and spread to the stem of the palm. If the branch of palm is shedding, the wound surface will be attacked by airborne spore and this is how *Ganoderma* starts their lives. As the consequences, the transportation and absorption of nutrients by roots were disturbed. When the fungus degrade the lignin, which is one of the component of wood, it will form a white cellulose and this is one of the sources for white rot fungi to get their energy.

Applications of enzymes and microorganism can be used for degradation of lignobiomass of palm oil to produce value added products such as polyoses, organic acid, biomaterials, biofuel, bioenergy and biolignin. The cost effective process developed for inhibition of *Ganoderma* species growth can be patented for the future. One of the effective ways to overcome this problem is through the application of green technology using microbial or enzymatic reaction. Enzymes production from the microbial reaction with the agrowaste was sustainable due to the renewable and ubiquitous nature of biomass and to be an excellent carbon source in microbial enzyme production (Bivi *et al*., 2010).

1.2 Objectives of study

a) To screen the potential defense related enzymes producing fungi capable to inhibit *Ganoderma boninense*.

b) To apply crude cellulase and xylanase cocktail produce by fungi CT2, *Pycnoporus cinnabarinus*, EFB1, EFB2 and TG6 for *Ganoderma boninense* inhibition.

1.3 Problem of statement

Ganoderma spesies is one of the fungi which capable to attack palm oil. One of the major diseases in oil palm is Basal Stem Rot (BSR). *Ganoderma boninense* attacks the root system of oil palm from second and subsequent planting cycles. Oil palm has 25 to 30 years of economic life span but this disease can kill more than 80% of stands by the time they are half-way through a normal economic life. The infection of this disease resulted in crop loss up to 45% of the yield of oil palm (Bivi *et al.*, 2010). Basal stem root in oil palm can be controlled effectively by management practices in the early stage of disease development.

1.4 Significance of study

The normal conventional ways to solve *Ganoderma boninense* problems in oil palm industry are by using pesticide and burning which would cause disaster for the surrounding. These activities would cause massive air pollution or even release the toxic compound to soil, environment and give dangerous impact to the human's health. Green technology by using microbial or enzymatic reaction will become the replacement for the chemical pesticide in order to treat *Ganoderma boninense*. The cost-effective process developed for inhibition of *Ganoderma boninense* growth can be patented for the future. The treatment of *Ganoderma boninense* with cellulase and xylanase cocktail do not give bad influence the environment and therefore provides a glimmer of hope to environmentalist.

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