UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF LACCASE BY A LOCALLY ISOLATED FUNGUS FOR BIODEGRADATION OF SELECTED AGROWASTES

ANG KONG NIAN

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By

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

May 2007

Specially dedicated to

my beloved parents and my family members

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

PRODUCTION OF LACCASE BY A LOCALLY ISOLATED FUNGUS FOR BIODEGRADATION OF SELECTED AGROWASTES

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May 2007

Chairman: **Associate Professor Suraini Abd Aziz, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

The present study was conducted to screen the significant laccase (ligninolytic enzyme) producers from locally isolated white-rot fungi using agitated and non-agitated culture conditions. The highest laccase producer, identified as *Pycnoporus* sp. (strain S16) was selected for the optimisation of laccase production with particular attention to the effect of physiological factors on laccase production using submerged culture technique. Laccase was characterised to assist in formulating the best condition for the bioconversion of agro-wastes. Significant laccase production was obtained under carbon-depleted condition and dependent on culture morphology. The culture with high laccase activity was observed with the presence of small mycelial pellets with 1–2 mm diameter. The pellet formation and optimum laccase production influenced by various physiological factors are investigated. The best culture condition obtained consisted of 5 g/L of glucose, 12 mM of nitrogen, inoculum concentration of 8×10^6 spores/mL, initial pH 5, incubation temperature of

37°C and agitation speed of 100 rpm. Under the above culture conditions, the time of fermentation was shortened from 16 days to 7 days with maximum laccase activity of 225 U/L. The characterisation study of concentrated laccase revealed the importance of an acidic condition for the highest laccase activity. The biodegradation study showed that some locally available agro-waste materials are suitable for soluble sugar production and that rice bran gave the best results with 1.90 g/L of soluble sugar produced.

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

PENGHASILAN ENZIM LACCASE DARIPADA KULAT TEMPATAN UNTUK BIODEGRADASI SISA PERTANIAN TERPILIH

Oleh

ANG KONG NIAN

Mei 2007

Pengerusi: **Profesor Madya Suraini Abd Aziz, PhD**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Kajian dijalankan untuk menyaring kulat daripada kumpulan ‘white-rot’ yang berpotensi menghasilkan enzim laccase dalam kultur goncangan dan statik. Kulat daripada kumpulan ‘white-rot’ yang berpotensi menghasilkan enzim laccase yang tinggi telah dikenal pasti sebagai *Pycnoporus* sp. (strain S16) telah dipilih untuk proses pengoptimuman. Kesan pelbagai faktor fisiologi terhadap penghasilan laccase telah dijalankan untuk membangunkan fermentasi terbaik. Enzim yang diperoleh dikaji sifat enzimnya untuk merumus suatu keadaan yang sesuai bagi penguraian sisa pertanian. Penghasilan enzim laccase dikesan apabila kultur dalam keadaan kehabisan sumber karbon dan bergantung kepada morfologi kultur. Kultur yang mempunyai aktiviti laccase tinggi biasanya berkait rapat dengan kehadiran pelet mycelia dengan diameternya antara 1–2 mm. Keadaan fermentasi terbaik mengandungi glukosa sebanyak 5 g/L, nitrogen sebanyak 12 mM, kepekatan inokulum sebanyak 8×10^6 spora/mL, pH permulaan pada 5.0, suhu pengeraman

pada 37°C dan pengoncangan sebanyak 100 rpm. Dalam kultur sedemikian, masa untuk fermentasi dipendekkan daripada 16 hari kepada 7 hari untuk penghasilan maksimum enzim laccase sebanyak 225 U/L. Kajian sifat enzim menunjukkan aktiviti enzim laccase adalah tinggi apabila keadaan persekitarannya berasid. Dalam kajian penguraian, beberapa jenis sisa pertanian tempatan telah dikaji dan sesetengah sisa pertanian telah menunjukkan potensi untuk digunakan bagi penghasilan gula terlarut di mana dedak padi memberikan nilai yang tertinggi sebanyak 1.90 g/L gula terlarut.

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Finally, I express my gratitude to my parents, family members and housemates for their unfailing support and encouragement throughout my study.

I certify that an Examination Committee has met on 23th May 2007 to conduct the final examination of Ang Kong Nian on his Master of Science thesis entitled "Production of Laccase by A Locally Isolated Fungus for Biodegradation of Selected Agro-wastes" in accordance with Universiti Petanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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ANG KONG NIAN

Date: 1 August 2007

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis-(3-ethyl-benzthiazoline-6-sulphonate)
BSA	Bolvine serum albumin
C	Carbon
CO_2	Carbon dioxide
$CuSO_4$	Copper sulphate
cm	Centimetre
g	Gram
g	g force
H_2O_2	Hydrogen peroxide
kDa	kilo Dalton
L	Litre
Lip	Lignin Peroxidase
MFCB	Mycelium free cultured broth
min	Minute
mL	Millilitre
mm	Millimetre
M	Molar
MnP	Manganese Peroxidase
nm	nanometer
O_2	Oxygen
P. chrysosporium	*Phanerochaete chrysosporium*
PKC	Palm kernel cake
rpm	Rotation per minute

U	Unit
μL	Microlitre
w	weight
v	volume
β	Beta
p	Para
%	Percentage
°C	Celsius
ε	linear molar absorption coefficient

CHAPTER 1

INTRODUCTION

Bioconversion of lignocellulosic agro-wastes is an interesting topic which covers a lot of aspects, not only the economical value but also including the environmental issue. Various useful products which possess high commercial value like fermentable sugar, animal feed and biocompost have been produced through the bioconversion process.

Lignocellulosic material is a very complex structure and consists of lignin surrounding the cellulose polymer which protects it from microbial attack. In nature, white-rot fungi are the most well known microorganisms which able to degrade wood effectively by the secretion of ligninolytic enzymes (Kirk and Cullen, 1998). The ligninolytic enzyme or so-called lignin peroxidase was discovered in 1983 from white-rot fungus *Phanerochaete chrysosporium*. The discovery of this enzyme has led to the development of ligninolytic enzymes studies and improved the understanding of the role of ligninolytic enzymes in lignin biodegradation.

Laccase is a type of ligninolytic enzymes which is commonly found in white-rot (basidiomycetes) fungi. Laccase is a polyphenol oxidase which has a broad substrate specificity and able to degrade various phenolic compounds. For over last two decades, the applications of laccase have received much attention from industrial and biotechnological areas. The fields involved with the applications of laccase are

including food industry, pulp and paper industry, textile industry and nanobiotechnology (Couto and Herrera, 2006).

The ability of laccase in lignin biodegradation was not only limited in oxidising the phenolic compounds. Previous study has shown that laccase was able to play a role in oxidising the non-phenolic compounds with the presence of a suitable redox mediator such as ABTS (Bourbonnais and Paice, 1990). In the past, lignin peroxidase was the only ligninolytic enzyme that has the ability to degrade non-phenolic lignin structure.

Malaysia as an agricultural based country produces a lot of lignocellulosic agro-wastes per year. Some of the lignocellulosic agro-wastes produced are rich in nutritional value and have potential to be converted into various useful products through bioconversion process. In Malaysia, the applications of ligninolytic enzymes in the bioconversion of agro-wastes are still limited especially for laccase. Lack of the basic knowledge for the application of ligninolytic enzymes in the bioconversion process is one of the known factors. In order to improve this condition, research on the application of ligninolytic enzymes must be constantly carried on since there is still a lot of potentials of laccase in various applications of biotechnological areas.

Screening work for strains that are suitable for biotechnological application becomes important. Most of the previous ligninolytic studies carried out employed characterised fungal strains, often from established culture collection. There were only a small numbers of studies carried out on wild type strains.

In research and application, sometimes it could be hindered by the rather low yield of the enzyme formed by wild type strains. The expression of ligninolytic enzymes requires several nutritional and cultural conditions (Kirk, 1980; Kirk and Farrell, 1987). Various studies have been done to improve the understanding of the fungal physiological requirement in order to induce their enzyme expression.

In this study, the physiology of the laccase production from locally isolated white-rot fungi was investigated. The understanding of the physiological requirement for the laccase production is important because it provides basic knowledge which describes the pattern of the laccase expression by white-rot fungi in nature. Therefore, to obtain a high yield of enzyme expression, the environmental set-up for the fungal growth must be matched with their physiological requirement.

The objectives of this study are as follows:

1) To screen for laccase producer from locally isolated white-rot fungi.
2) To optimise the laccase production from locally isolated white-rot fungus using submerged culture technique.
3) To characterise the concentrated laccase.
4) To evaluate the performance of the concentrated laccase for bioconversion of lignocellulosic agro-wastes.

CHAPTER 2

LITERATURE REVIEW

2.1 Agro-wastes in Malaysia and Its Utilisation – An Overview

From the past, many studies which have been carried out were mainly focused on the efficiency of the utilisation of agro-wastes (Jalaludin *et al*., 1991). Biocomposting is one of the methods used to utilise the agro-wastes efficiently. The product of biocomposting is a biofertiliser which can be used for plantation and it is commercially available today. Microbial conversion of agro-wastes for the production of animal feeds is also one of the methods that has been given attention. Some of the lignocellulosic agro-wastes are high in nutritional value with great potential to be used as animal feed.

The conversion of the agro-wastes usually involved the degradation of lignocellulosic materials into smaller compounds. Generally, degradation of the lignocellulosic material is a slow process and it involves complex enzymatic reaction. Some part of the lignocellulosic materials, like lignin is resistant to degradation and limits the lignocellulosic degradation rate. There are only few oxidative enzymes known to have the ability to break this lignin barrier and are only produced by certain microorganisms (Pérez *et al*., 2002).

2.2 The Effect of Structure and Composition of Lignocellulosic Materials on Degradation

The term of lignocellulose is usually used to indicate the material that has not been processed or treated in any way that would disrupt the physical and chemical fine structure of matrix (Dunlap and Chiang, 1980). In nature, lignocellulose accounts for the major part of biomass. Thus, its degradation is essential for the operation of the global carbon cycle. Lignocellulosic materials are including agricultural residues, forestry residues, portions of municipal solid waste and various industrial wastes.

Lignocellulosic materials are formed from three main components: cellulose, hemicellulose and lignin. The composition of these three components is varies from one plant species to another. The lignocellulosic materials are able to be broken down by certain species of fungi and bacteria through the hydrolytic (cellulases and hemicellulases) and oxidative (ligninolytic) enzyme reactions (Eriksson *et al*., 1990).

2.2.1 Lignin

Lignin is a generic name given to complex aromatic polymers which forms major component of vascular plant tissues. In terms of weight it is second only to cellulose among renewable organic materials and in terms of energy content it might well be the single most abundant. In nature, lignin is mostly found as an integral part of the plant cell wall, embedded in carbohydrate polymer matrix of cellulose and hemicellulose.