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DETERMINATION OF LIPASE AND AMYLASE ACTIVITIES FROM WOOD DECAY FUNGI

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "Determination of Lipase and Amylase Activity from Wood Decay Fungi" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Lai Shu Ying (165019) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Fungal enzymes have long history of usage in various industries. Lipase and amylase are two of the most commercialized enzymes to date. Wood decay fungi are promising source of amylase and lipase. A number of fungi had been isolated from decayed tree bark. Isolates were screened with minimal media supplemented with specific carbon source, starch and tween 80 respectively.. Starch was used for screening of amylase producing fungi while tween 80 was used for screening of lipase producing fungi. Two amylase producing fungi identified to be *Aspergillus* sp. and *Fusarium* sp. were isolated. For lipase producing fungi, *Cladosporium* sp. and *Aspergilus* sp. were isolated. Fungal isolates were analyzed for its amylase and lipase activity. *Fusarium* sp. has highest amylase activity of 53.69U/ml while *Cladosporium* sp. reported highest lipase activity of 503.02 U/ml. Growth rate analysis was done to further verify the amylolytic and lypolytic capability of isolates.

ABSTRAK

Enzim kulat mempunyai sejarah penggunaan yang panjang dalam pelbagai industri. Lipase dan amilase adalah dua daripada enzim paling banyak dikomersilkan sehingga kini. Kulat dari kayu reput menjanjikan sumber amilase dan lipase. Beberapa kulat telah diasingkan daripada kulit kayu reput. Pengasingan kulat dari kayu reput telah dilakukan dengan media minimum yang ditambah dengan sumber karbon tertentu, kanji dan Tween 80. Kanji digunakan untuk pengasingan kulat yang menghasikan amilase manakala Tween 80 telah digunakan untuk mengasingkan kulat yang menghasilkan lipase. Dua kulat yang menghasilkan amylase telah dikenal pasti sebagai *Aspergillus* sp. dan *Fusarium* sp. Untuk kulat yang menghasilkan lipase, *Cladosporium* sp. dan *Aspergilus* sp. telah dikenal pasti. Kulat telah dianalisa untuk aktiviti amilase dan lipase. *Fusarium* sp. mempunyai aktiviti amilase tertinggi pada 53.69U / ml manakala *Cladosporium* sp. melaporkan aktiviti lipase tertinggi iaitu 503.02 U / ml. Analisis kadar pertumbuhan diperkenalkan bagi mengesahkan lagi kepupayaan amylolytic dan lypolytic kulat-kulat ini.

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LIST OF ABBREVATIONS

DDT	Dichlorodiphenyltrichloroethane
GRAS	Generally Recognized as Safe
PAH	Polycyclic Aromatic Hydrocarbons
PCP	Pentachlorophenol
TNF	Tumor Necrosis Factor
$CaCl_2$	Calcium chloride
$CuSO_4$	Copper(II) sulfate
$FeSO_4$	Iron(II) sulfate
HCl	Hydrochloric acid
IL	Interleukin
INF	Interferon
KH_2PO_4	Monopotassium phosphate
$MgSO_4$	Magnesium sulfate
ml	milliliter
$MnCl_2$	Manganese(II) chloride
NH_4NO_3	Ammonium nitrate
NK	Natural Killer
PDA	Potato Dextrose Agar
ZnSo4	Zinc sulfate

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Lipase and amylase are highly valuable biological catalytic agents. Their vast industrial and biotechnological application drove interest of scientific research to discover hidden potential of these enzymes (Singh & Mukhopadhyay, 2011; Pandey et al, 2000). Both amylase and lipase are classified under hydrolase. They break down complex polymer into simple monomer by addition of water molecules. Lipase acts on substrate lipid, mostly, triglyceride, diglyceride and monoglyceride in a lesser extent, while amylase catalyzes the breakdown of polysaccharide. α-amylase is ubiquitous in nature, possess by majority of higher organisms, plants and microrganisms (Kandra, 2003). It acts randomly on α-1,4-glycosidic linkages on polysaccharide.

Fungal genera which commonly used in lipase production include *Aspergillus, Penicillium, Rhizopus* and *Candida* (Singh & Mukhopadhyay, 2011). Bulk production of lipase is done through several types of fermentation process techniques namely submerged and solid-state and fed-batch fermentation (Singh & Mukhopadhyay, 2011). One of the simplest way to isolate lipase producing fungi is done by screening on tributyrin agar plate (Toscanon et al., 2011). A study from Contesinia et al., (2010) reported genus *Aspergillus* poses remarkable temperature and pH stability with addition to better fermentation time and enantioselectivity. *Aspergillus niger* is the most studied fungi in the production of amylase due to its

ability to utilize wide range of nutrient amylase (Abe et al., 1988). In the industry, amylase is produced mainly by submerged fermentation (Saranraj & Stella, 2013).

Wood decay fungi are fungi which feed on forest litter, wood and fallen trees (Blanchette, 1991). Most species of wood decay fungi can be found in humid and temperate tropical rain forest. Mode of feeding in fungi can be parasitic or saprophytic (Cooke & Whipps, 1980). Carbon cycle, nitrogen cycle and other forms of nutrient recycling into available forms for plants are almost impossible without involvement of wood decay fungi (Meiera et al., 2010). Other than its significant contribution on environment, wood decay fungi deserve recognition on its industrial and biotechnological contributions. One of the most important applications is as a source of enzymes (Sin et al., 2002).

Some of fungal metabolites are economically significant as it possess pharmacokinetic properties (Zjawiony, 2004). Thus, the economic value of fungi has far stretched from the foods and beverage industry, pulping industry to pharmaceutical industry (Esser, 2010). Screening for potential amylase and lipase producing isolates will open up a new potential source from different species of fungi. The fermentation capacity of fungi will give an insight on efficiency of wood decay fungal isolates on utilizing carbon source hence does the enzyme activity of lipase and amylase respectively (Machuca & Ferraz, 2001).

Degradation of wood lignin forms the basis of wood decay fungi classification system (Blanchette et al., 2009). Wood mainly consists of three types of carbon sources, which are cellulose, hemicellulose and lignin (Kirk & Farrell, 1987). In

1833, Theodore Hartig classified fungi according to their ability to degrade, degradation of wood is visible by naked eye. In this system wood decay fungi are divided into brown rot fungi, white rot fungi and soft rot fungi.

1.2 PROBLEM STATEMENT

Enzyme of fungus undergoes post modification compared to bacterial enzyme. Identification of diverse local strain with high enzymatic activity will add to further discovery of fungal isolate with high enzymatic activity and biodiversity.

1.3 OBJECTIVES

The objectives of this project are:

- To study and compare enzyme activity of lipase and amylase extracted from wood decay fungal isolates.
- To identify lipase and amylase producing wood decay fungi up to genus level.
- To investigate diversity of fungi in tropical country.

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