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DETERMINATION OF CELLULASE AND PROTEASE ACTIVITY OF FUNGI ISOLATED FROM WOOD DECAY

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "Determination of Cellulase and Protease Activity of Fungi Isolated From Wood Decay" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Nasha Allysha Binti Ahmad Fazilah (162085) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

In nature, organic substances are found either as simple carbon or nitrogen molecules are found as complex polymers of these simple molecules. These substances may be of plant, animal or microbial origin. Simple organic molecules are utilized by the living organisms and get converted into cellular materials and finally enter into the mineral cycling. Substances such as starch, celluloses, hemicelluloses, proteins, fats and oils, pectin, lignin are the major complex organic substances and many of these are quite resistant to enzyme degradation. However, certain microorganisms produce enzymes that can hydrolyze such molecules into simple substances. Certain fungi for example, wood decay fungi are able to hydrolyze cellulose and protein. This study was aimed to observe the potential of particular wood decay fungi as a new source for cellulase and protease enzyme. There are a lot of fungal species that have not recorded yet. Comparison of the cellulolytic activity and the proteolytic activity between four isolates C1 and C2 (cellulolytic fungi) and P1 and P2 (proteolytic fungi) was done by studying the cellulolytic and proteolytic activity of the isolates. The cellulase activity for isolates C1 and C2 were 0.0017 and 0.00094 respectively, while the protease activity for isolates P1 and P2 were 0.1462 and 0.251 respectively. Isolate C1 was found to have the highest activity in cellulase assay and isolate P2 was found to have highest protease activity compare to isolate P2 in protease assay. From the microscopic identification, isolate C1 was assumed to be *Aspergillus niger* while other isolates cannot be identified. Thus, further research, such as molecular identification, for example, amplification using PCR and sequencing of 18S rDNA of the isolates are suggested.

ABSTRAK

Secara semula jadi, bahan-bahan organik yang terdapat sama ada sebagai karbon mudah atau molekul nitrogen dikenal pasti sebagai polimer kompleks molekul mudah. Bahan-bahan ini boleh menjadi tumbuhan, haiwan atau asal mikrob. Molekul organik yang mudah digunakan oleh organisma hidup dan dapat ditukar menjadi bahan selular dan akhirnya masuk ke kitaran mineral. Bahan-bahan seperti kanji, selulosa, hemiselulosa, protein, lemak dan minyak, pektin, lignin adalah bahan-bahan organik kompleks yang utama dan kebanyakan bahan-bahan ini adalah agak tahan kepada enzim degradasi. Walau bagaimanapun, terdapat mikroorganisma tertentu menghasilkan enzim yang mampu menghydrolisis molekul itu ke dalam molekul yang lebih mudah. Kulat tertentu sebagai contoh, kulat kayu reput dapat menhidrolisis selulosa dan protein. Kajian ini bertujuan untuk meninjau potensi kulat kayu reput tertentu sebagai sumber baru untuk enzim selulase dan protease. Ini kerana terdapat banyak spesies kulat yang belum direkodkan lagi. Perbandingan aktiviti cellulolytic dan aktiviti proteolitik antara empat kulat C1 dan C2 (kulat cellulolytic) dan P1 dan P2 (kulat proteolitik) dilakukan dengan mengkaji selulase dan aktiviti protease daripada pencilan. Aktiviti selulase untuk kulat C1 dan C2 adalah 0,0017 dan 0,00094 masing-masing, manakala aktiviti protease untuk kulat P1 dan P2 masing-masing adalah 0,1462 dan 0,251. Kulat C1 didapati mempunyai aktiviti yang tertinggi di dalam aktiviti selulase dan kulat P2 didapati mempunyai aktiviti protease tertinggi berbanding dengan kulat P2 dalam protease assay. Dari pengenalpastian mikroskopik, kulat C1 telah merupakan *Aspergillus niger* manakala kulat lain tidak dapat dikenal pasti. Oleh itu, kajian lanjut, seperti pengenalan molekul, sebagai contoh, amplifikasi menggunakan PCR dan penjujukan 18S rDNA daripada sample yang disyorkan.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Fungi are among the most important organisms in the world, not only because of their vital roles in ecosystem functions, but also because of their influence on humans and human-related activities (Mueller and Foster, 2004). Fungi form an essential role in the composition of organic matter and have fundamental roles in most terrestrial and some aquatic ecosystems and therefore they play an important role in biogeochemical cycles and in many food webs.

Dead wood is an essential component of stream ecosystems. Fallen tree trunks, branches and twigs regulate stream dynamics by increasing the retention of organic matter, and provide habitats for a large number of organisms, including fungi, insects and even fish (Tsui et al., 2000).

Wood decay fungi are often divided into three groups, namely white rots, brown rots, and soft rots (Hickman and Perry, 2010). Most of the lignocellulolytic fungi secrete extracellular enzymes released in the presence or absence of inducers in the media (Mtui and Nakamura, 2007)

Cellulose is a type of organic compound which is an important component in the production of non-fossil carbon source on earth such as food and biofuels. Cellulose can be degraded by cellular enzymes to form, reducing sugar glucose. Cellulolytic wood decay fungi are species of microorganism that can produce the cellulose enzyme and degrade wood (Lynd et al., 2002).

Proteolytic enzyme, also known as protease is a group of enzymes that break the long chainlike molecules of proteins into shorter fragments (peptides) and

eventually into their components, amino acids. Proteases catalyze hydrolytic reactions, in which protein molecules are degraded into peptides and amino acids. Their properties are very diverse because the group is large and complex (Kudryavtseva et al., 2008).

Microbial proteases represent one of the three largest groups of industrial enzymes and account for approximately 60% of the total enzyme sale in the world and they are the leaders of the industrial enzyme market worldwide (Rai and Mukherjee, 2010).

Malaysia is rich with diversity and fungi are included in the diversity. There still a lot of fungi species that have not recorded properly. Since fungi are eukaryotic organism, the enzyme produced by fungi might be having different enzyme activity depend on their species. This study was aimed to observe the potential of particular wood decay fungi as a new source for cellulase and protease enzyme. The specific objectives of this study were:

1. To isolate fungi from wood decay.
2. To screen for cellulase and protease producing fungi.
3. To determine the cellulolytic and proteolytic activity of the fungi isolated from wood decay.

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