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ISOLATION AND SCREENING OF PECTINASE PRODUCING THERMOTOLERANT FUNGI

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk **"Isolation and Screening of Pectinase Producing Thermotolerant Fungi"** telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Wajihah Bt. Ahmad Zamri (161840) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Pectin is a substance that has a very complex macromolecule and it can be found abundantly in the primary cell wall of the plants. Enzyme that helps to break down this polysaccharide substrate is known as pectinase enzyme and it catalyzes the pectin degradation through de-esterification and depolymerisation reactions. One of the current challenges in agricultural and fruit juice processing industries is the increasing of pollution problems due to the abundant amount of organic waste materials from agricultural and fruit juice processing industries. Besides that, there is also extensive demands of pectinase production for the extraction and clarification of fruit juices industry. Therefore, potential fungi with a high pectinolytic activity as well as its thermostability should be isolated and discovered so that they can be exploited for the degradation of organic waste materials in environment and their potential applications in the industry can be explored. This pectinase enzyme can be synthesized by thermotolerant fungi isolated from watery soil. This research study is designed to explore the potential of other strains of thermotolerant fungi that can produce pectinase and to study their pectinolytic activity. Comparison of the pectinolytic activity between thermotolerant fungi was done by studying the pectinolytic activity of each thermotolerant fungus. The polygalacturonase activity for isolates CP1 and CP2 were 1.3129 Uml^{-1} and 0.8856 Uml^{-1} respectively. The specific enzyme activity for CP1 is 23.5587 Umg^{-1} while for CP2 is 21.7635 Umg^{-1}. Isolate CP1 was found to have a higher enzyme activity in polygalacturonase enzyme activity. For the identification of thermotolerant fungi, both isolates were identified as *Aspergillus fumigatus* but from different strains.

ABSTRAK

Pektin adalah bahan makromolekul yang sangat kompleks dan banyak terdapat di dinding sel utama tumbuh-tumbuhan. Enzim yang membantu untuk memecahkan substrat polisakarida ini dikenali sebagai enzim pectinase dan ia mendegradasi pektin melalui proses penyahpolimeran. Cabaran yang dihadapi oleh industri pertanian dan pemprossesan jus buah-buahan adalah masalah peningkatan pencemaran yang disebabkan oleh pengumpulan bahan buangan sisa organik daripada industri tersebut. Selain itu, terdapat juga permintaan yang tinggi terhadap pengeluaran enzim pectinase khususnya untuk tujuan pengekstrakan daripada industri jus buah-buahan. Oleh itu, kulat yang berpotensi dalam menghasilkan aktiviti pectinolytic yang tinggi serta ciri "thermostability" perlu dikenalpasti supaya dapat dieksploitasi untuk tujuan mendegradasi bahan-bahan buangan organik dan juga mengenalpasti aplikasi yang berpotensi dalam industri. Enzim pectinase boleh disintesis oleh kulat 'thermotolerant' yang diambil daripada sampel tanah yang berair. Kajian penyelidikan ini dijalankan untuk meneroka potensi jenis kulat "thermotolerant" lain yang boleh menghasilkan pectinase dan mengkaji aktiviti pectinolytic mereka. Perbandingan aktiviti pectinolytic antara kulat "thermotolerant" dilakukan dengan mengkaji aktiviti pectinolytic setiap kulat "thermotolerant" tersebut. Aktiviti polygalacturonase bagi isolat CP1 dan CP2 ialah 1.3129 Uml^{-1} dan 0.8856 Uml^{-1}. Aktiviti enzim spesifik untuk CP1 ialah 23.5587 Umg^{-1} manakala bagi CP2 ialah 21.7635 Umg^{-1}. Isolat CP1 mempunyai aktiviti enzim yang lebih tinggi untuk aktiviti enzim polygalacturonase. Dalam proses mengenal pasti kulat "thermotolerant", kedua-dua isolat telah dikenal pasti sebagai *Aspergillus fumigatus* tetapi daripada "strain" yang berbeza.

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CHAPTER 1

INTRODUCTION

Currently, thermostable enzymes become the topic of interest due to their property to withstand the thermal inactivation which is suitable to be applied in industrial sectors (Singh et al., 2000). Such enzymes are stable in organic solvents because they are being produced by thermophilic or thermotolerant microorganisms in which these microorganisms have the intrinsic thermal stability (Palomo et al., 2004). Kumar (2004) stated that the characteristics of environment suitable for thermophilic microbes are high temperature, alkaline pH and poor nutrient source of the soil.

Pectinases that are being synthesized by microorganisms are commonly being used in the industrial sector and they are important nowadays because of their economic value (Darah et al., 2013). In industries, pectinases are being used in applications such as improving juice yields, degumming of plant fibers, scouring of cotton, extraction of vegetable oil and waste water treatment (Mehta et al., 2013). Reddy and Sreeramulu (2012) stated that the most important enzyme in the juice industry is the microbial pectinase. They also mentioned that only microorganisms that can produce a sufficient amount of pectinase are being used in industry and exploited commercially.

Pectinase is a type of enzyme that can break down the pectin structure (Pasha et al., 2013). Pectin is a heteropolysaccharide that consists of α-1, 4 linked galacturonate chains associated with a major percentage of methyl esterification, and it acts as an important cementing agent that hold the fiber to the stalk (Das et al.,

2010). Pectinase enzymes are grouped based on their mode of action or substrate specificity and those groups are pectinesterase (EC 3.1.1.11), polygalacturonase (EC 3.2.1.15), pectate lyase (EC 4.2.3.2) and pectin lyase (EC 1.4.2.10) (Cao, 2012).

According to Farahbakhsh (2013), there are two types of fermentation process for the production of enzymes which are solid-state fermentation and submerged fermentation. Renge (2012) defined submerged fermentation as the cultivation of microorganisms in liquid nutrient broth in order to release the desired enzymes. As for solid-state fermentation, it is a fermentation of solid substrates at low water activities and also moisture levels in order to provide growth and metabolism reaction of microbes (Ali and Zulkali, 2011).

1.1 Problem Statement

In recent years, there are demands for enzymes that are able to withstand the harsh and extreme conditions in industrial process. In industrial sectors, most of the industrial enzymes are synthesized by mesophiles. These enzymes have limited properties in industry such as minimal stability at the extreme conditions related to temperature, pH and ionic strength. In order to overcome this problem, one of the alternative ways is to isolate and study the thermostable enzymes produced by thermotolerants since they are stable at high temperature conditions. Besides that, pectinase enzymes provide a major contribution in fruit juice extraction and clarification processes. The wide application of pectinases in industry has increasing from time to time. This has led to extensive demands of pectinases in increasing the fruit juice yield and its clarification. However, there are very few pectin-degrading thermotolerant fungal species that have been isolated and identified. Abundant amount of organic waste materials generated from agricultural and fruit juice

processing industries has resulted in the sudden increase in pollution. This pollution problem has become a worldwide issue and caused some concern from all over the world. Therefore, this research study is conducted in order to explore other strains of thermotolerant fungi that can produce pectinase and to study their pectinolytic activity.

The highlight of this project is to determine whether thermotolerant fungi isolated from the selected site are able to produce pectinase enzyme by using the selected method and technique. The hypothesis of this research is that thermotolerant fungi are able to produce thermostable enzymes such as pectinases.

1.2 Objectives

The objectives of this study are:

a. To isolate the thermotolerant fungi from the environment.
b. To observe the thermotolerant fungi macroscopically and microscopically for tentative identification.
c. To screen for the pectinase enzyme producing strain.
d. To study the activity of pectinase enzyme from thermotolerant fungi.

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