UNIVERSITI PUTRA MALAYSIA

CELLULASE ACTIVITY ON THERMOPHILIC FUNGI

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THESIS

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LIST OF ABBREVIATIONS

SSF	solid state fermentation	ρNPG	ρ-nitrophenol-β-glucoside
M	molar	mm	millilitre
GH	glycosidase hydrolase	mg	miligram
℃	degree centrigrade	μl	microliter
PDA	potato dextrose agar	IU/ml	international unit / mililitre
CMC	carboxymethyl cellulose	FPU	filter paper unit
nm	nanometre		
$NH_4H_2PO_4$	ammonium hydrogen phosphate		
KCl	potassium chloride		
$MgSO_4.7H_20$	magnesium sulphate heptahydrate		
NaCl	sodium chloride		
CI	cellulolytic index		
ml	millilitre		
w/v	weight per volume		
$(NH_4)_2PO_4$	diammonium phosphate		
g	gram		
KH_2PO_4	potassium dihydrogen phosphate		
$MgS0_4$	magnesium sulphate		
psi	per square inch		
rpm	revolutions per minute		
xg	centrifugal force		
Min	minute		
mM	mili molar		
DNS	3,5-dinitrosalicylic acid		
μmol	micro mole		

ABSTRACT

Thermophilic fungi produce thermostable enzymes which are very stable at extreme and elevated temperature. One of the enzyme produced by thermophilic fungi is cellulase. Interaction of three components of cellulase enzyme in degrading cellulose into its small subunits make cellulase became popular in industrial processes. The sample of thermophilic fungi isolated from hotspring area diluted using serial dilution method grow on potato dextrose agar after incubated at 50℃. The function grow of fungi on potato dextrose agar is to get the single colony of thermophilic fungi. The single colony of thermophilic fungi subcultured into carboxymethyl cellulose agar (CMC) to determine its cellulase production. The fungi identified through macroscopic and microscopic method in order to know the species of fungi. Cellulase comprise of three components which are endoglucanase, exoglucanase and β-glucosidase. The three components of cellulase tested in each specific assay such as filter paper assay, endoglucanase assay and β-glucosidase assay. Filter paper assay determine the total cellulase activity, endoglucanase assay determine endoglucanase activity and β-glucosidase assay determine β-glucosidase activity.

ABSTRAK

Kulat thermophilic menghasilkan enzim tahan panas yang sangat stabil pada suhu yang melampau dan tinggi. Salah satu enzim yang dihasilkan oleh kulat thermophilic adalah selulase. Interaksi tiga komponen enzim selulase dalam meleraikan selulosa ke subunit kecil membuat selulase menjadi popular dalam proses perindustrian. Sampel kulat thermophilic diasingkan dari kawasan kolam air panas dicairkan menggunakan kaedah pencairan bersiri tumbuh di kentang dekstrosa agar selepas dieram pada 50 ℃. Fungsi tumbuh kulat diatas kentang dekstrosa agar adalah untuk mendapatkan satu koloni kulat thermophilic. Satu koloni tunggal kulat thermophilic disubkulturkan ke carboxymethyl selulosa agar (CMC) untuk menentukan pengeluaran selulasenya. Kulat yang dikenal pasti melalui kaedah makroskopik dan mikroskopik untuk mengetahui spesies kulat. Selulase terdiri daripada tiga komponen iaitu endoglucanase, exoglucanase dan β-glucosidase. Ketiga-tiga komponen selulase diuji dalam setiap assay tertentu seperti penapis assay kertas, assay endoglucanase dan β-glucosidase assay. Penapis kertas assay menentukan keseluruhan aktiviti selulase, endoglucanase assay menentukan aktiviti endoglucanase dan β-glucosidase assay menentukan aktiviti β-glucosidase.

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1.0 INTRODUCTION

Fungi is an organisms called the decomposers grow in the soil or on dead plant matter where they play an important role in the cycling of carbon and other elements. Fungi also one of important organisms in biodiversity mainly in degrading plant materials such as cellulose, hemicellulose and lignin. Fungi degrade plant materials by secreting crucial enzymes that are stable in extreme environment. Thus fungi become popular in industrial use due to their useful enzymes. For instance, cellulase enzyme secreted by fungi used for exchange of biomass to fermentable sugars in biorefineries and in textiles, paper, and detergent industries (Karmakar and Ray, 2011; Kuhad *et al*., 2011).

The only one organism in eukaryotic group that have capability to survive at the elevated temperature was called thermophilic fungi. Thermophilic fungi are species that grow at a lowest temperature of 20°C or above, and a highest temperature of 50°C or above (Maheshwari *et al.*, 2000). Therefore they are the only representatives of mycoflora that can grow at high temperature above 45°C. Despite thermophilic fungi are able to grow at elevated temperature, but they offer faster growth rates as compared with mesophilic fungi, an organism that grows best in moderate temperature (Ashraf *et al*., 2007). Nevertheless, thermophilic fungi are not as extreme as in eubacteria and archaea, which are characterized by their ability to tolerate extreme temperature at 100°C and live in hydrothermal vents (Brock, 1995). The known orders of thermophilic fungi are Sordariales, Eurotiales, and Onygenales from phylum ascomycetes and order Mucorales from phylum zygomycetes (Berka *et al*., 2011; Morgenstern *et al.*, 2012).

A lot of information about the location of thermophilic fungi from various types of soils and in habitats where decomposition of plant materials takes place whether in tropical as well as temperate regions. In nature, thermophilic fungi are usually found in composts, piles of hays, wood chip piles, stored grains, animal dung, nesting material of birds and animals, snuff, municipal refuse, other environments that are self-heating due to degradation of plant materials, and other accumulations of organic matter where in the warm, humid, and aerobic environment that provides the basic physiological conditions to their development (Johri *et al*., 1999). In these habitats, thermophilic fungi may occur either as resting propagules or as active mycelia rely on the presence of nutrients and favourable environmental conditions. Thermophilic fungi can be measured based on their cellulolytic potential whether they are good cellulose-degraders or poor cellulose-degraders. Since such material often contains high concentrations of cellulose some good cellulose-degraders will degrade cellulose easily, but some poor cellulose-degraders seem to utilize sugars released by cellulolytic species in the biotope (Maheshwari *et al*., 2000). Therefore, thermophilic fungi can differ greatly in their cellulolytic potential.

Thermophilic fungi have a great capability to break down plant materials and polysaccharides constituents of biomass like cellulose and become the potential source of cellulolytic enzyme with scientific and commercial importance. Mesophilic fungi produce enzymes that are usually effective at temperature below than 50℃ whereas thermophilic fungi produce more thermostable enzymes that able to function at temperatures up to 70℃ (Murray *et al*., 2004; Parry *et al*., 2002; Venturi *et al*., 2002; Voutilainen *et al*., 2008). Several factors of thermophilic fungi that make the industrial process more economical such as their thermostable enzyme, ability to saccharify under non-aseptic conditions, and high rate of cellulolysis (Haggerdal *et al*., 1980; Merchant *et al*., 1988; Maheshwari *et al*., 2000; Sohail *et al*., 2009). Other than that, thermostable enzymes which have been isolated from these fungi, have found a number of commercial applications because of their overall inherent stability (Demirijan *et al*., 2001). Examples of thermophilic fungi that produce thermostable cellulases are Talaromyces emersonii (Murray *et al*., 2004; Voutilainen *et al*., 2010), Myceliophthora thermophila (Roy *et al*., 1990), *Chaetomium thermophilum* and *Acremonium thermophilum* (Voutilainen *et al*., 2008).

There are several problem statements arise in this study. Firstly, to solve problem of inadequate sources of industrially relevant thermostable enzymes. Secondly, stimulated isolation of a number of microbes from thermal environments in order to access enzymes that could significantly increase the window for enzymatic bioprocess operations. Another problem has to solve in technical products and processes, often in a very large scale using industrial enzymes from thermophilic fungi. There are also some hypothesis about this study. Firstly, thermophile cellulase are key enzymes for efficient biomass degradation. Their importance stem from the fact that cellulose swells at higher temperatures, thereby becoming easier to break down. Secondly, thermophilic fungi and their proteins are able to function at elevated temperatures (high temperatures, above 55°C). Thirdly, thermophilic enzyme rarely require toxic metal ions for functionality, hence creating the possibility to use more environmentally friendly processing. Lastly, thermostable enzymes offer robust catalyst alternatives, able to withstand the often relatively harsh conditions of industrial processing.

Therefore, the specific objectives of this study were :

1. To Isolate and identify thermophilic fungi from hotspring,
2. To screen the cellulolytic enzymes from the fungal isolates.

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