BIOCHEMICAL AND BIOTECHNOLOGICAL STUDIES FOR SOME MICROORGANISMS

By

FATMA ABDULAZIZ ALI

B.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2011

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SUPERVISION SHEET

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ABSTRACT

In order to obtain microorganisms for degradation of the agricultural wastes, six native Egyptian fungal strains were isolated, morphologically identified and screened for cellulose biodegradation potential which was determined as endoglucanase or as carboximethylcellulase (CMCase) producers. The most promising isolate (*Aspergillus terreus)* with cellulolytic activity 2.26 U/mL was selected for molecular characterizations based on sequencing of internal transcribed spacer (ITS). The result confirmed that the strain is 99.8% homology with *A. terreus.* Further optimization experiments revealed that 35ºC is the optimum temperature for cellulase production and raised the enzyme activity up to 3.19 U/mL. Out of two organic nitrogen sources; peptone and yeast extract, the peptone at concentration 6g/l was found to be the optimum nitrogen source for cellulase production with the highest activity 4 U/mL. Whereas, the different four carbon sources: microcellulose, corn stalks, wheat straw and rice straw showed significant differences in the enzyme activity with values 11.07, 9.68, 7.87 and 3.71 U/mL, respectively at pH 3. The maximum enzyme activity was recorded to be within 5-7 days of incubation, depending on the type of carbon sources. Eventually, the optimization of different incubation conditions raised cellulolytic activity from 2.26 U/mL up to 11.18 U/mL. Then the enzyme was submitted to three purification steps. Ammonium sulfate fractionation raised the specific activity from 394 U/mg of the crude enzyme up to 574 U/mg. The next step was dialysis, which elevated the specific activity to 895 U/mg. The last step was gel filtration using Sephadex G-25. The Sephadex increased the specific activity to 1262.7 U/mg. To confirm the enzyme purity, SDS-PAGE electrophoresis was carried out and the enzyme appeared as a single band on the gel with molecular weight ~66kDa.

key words: *Aspergillus terreus*, corn stalk, endoglucanase, purification, rice straw, SDS-PAGE, wheat straw.

DEDICATION

I dedicate this work to my parents, my brothers, sisters and to my greatest teacher ever; my cousin Dr. Omima Mohammed for their patience and help, for all the support they lovely offered before and during my post-graduate studies.

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INTRODUCTION

In Egypt, the agricultural wastes cause several problems; they can be incinerated in the field creating air and soil pollution problems. The most crops producing the highest quantities of wastes are rice (rice straw), sugarcane (sugarcane bagasse), corn (corn cobs), cotton (cotton straw), and wheat (wheat straw), banana and orange peels (Shaaban and Nasr, 2018).

The volume of the agricultural wastes is estimated by about 35 million tons per year, of which about 23 million tons of vegetarian wastes (4 MT of corn wastes, 6 MT of wheat wastes, 3 MT of rice straw and others) are produced annually (FAO, 2001and Ministry of State for Environmental Affairs, 2017).

A massive amount of these agricultural wastes is burning every year over Egypt's skies in October and November, which causes a black smog and health problems such as respiratory diseases and allergies (Abdulrahman and Huisingh, 2018). It is clearly this practice also, not environmentally sustainable, which lead to emission of greenhouse gases (GHGs) like carbon dioxide, methane and nitrous oxide. GHGs can trap the heat in the atmosphere and cause the greenhouse effect (Mathur and Srivastava, 2019).

Recently, these Agro-wastes can beneficially be used, not incinerated or disposed in a land fill. Agricultural wastes are mainly composed of lignocellulosic materials remained after collecting the valuable parts of crops. Lignocellulosic constitutes of certain carbohydrates in the form of cellulose (35- 45%) and hemicellulose

(25-40%). In spite of the availability of large amounts of the agricultural wastes, Egypt suffers from a lack in the materials of industrial values, green biofuel and in animal feed, and is imports to uses of them annually to fill these gaps. So, it has become necessary to activate the attention to recycle the agricultural wastes of the crops that represent a large proportion of wastes. It is necessary, also, to activate the most suitable means of converting those wastes into materials with an economic value (Shaaban and Nasr, 2018).

The strategies of biotechnology techniques are used to convert these agricultural wastes into valuable products by degradation of cell wall cellulosic materials, with cellulase enzymes system. Various microorganisms such as aerobic and anaerobic bacteria, white rot fungi, soft rot fungi, and anaerobic fungi can produce complete active cellulase enzyme systems. Microorganisms frequently produce multiple of cellulase types that vary in their molecular characteristics. Fungal native enzymes are in the top of common commercially available cellulases enzymes, as well most of industrial cellulases are produced by *Trichoderma* and *Aspergillus* species (Behera and Ray, 2016).

The cellulase enzyme system comprising; endoglucanases (EG, EC3.2.1.4), exoglucanase or cellobiohydrolases (CBHI and CBHII, EC3.2.1.91), and β-glucosidases (BGL, EC 3.2.1.21). These enzymes act together in cascade to degrade cellulose into mono sugar units (glucose) (Srivastava *et al.*, 2018a).

The production of cellulolytic and hemicellulolytic enzymes on economical scale is being more important for applications in the textile industry for cotton softening and denim finishing, in laundry detergents

for color care and cleaning, in the food industry for mashing, in the pulp and paper industries for drainage improvement and fiber modification, animal feeds production and they even used for biofuel and pharmaceutical applications (Shah *et al.*, 2015a).

To avoid agro-wastes problems in Egypt, this study aimed to isolate some native Egyptian fungi strains which have high ability to produce cellulase enzymes and identify the superior cellulase producer isolate by sequencing of the ribosomal ITS region. To achieve this aim, different cultivation media contained varies agricultural wastes were used. Also the physical growth conditions of the fungal strains were optimized and compared in terms of the cellulase enzyme activity. Additionally, the purification of the produced cellulase enzyme and its characteristics were investigated.

REVIEW OF LITERATURE

1. Agricultural wastes

Nowadays, in Egypt due to its over population density, which lead to intensive cultivation of food crops by using horizontal and vertical expansion in order to maximize the return of the agricultural production. Egyptian land yielding crops two or three times a year, the major crops include wheat, maize, rice, cotton, clover, sugar cane, beans, and soybeans (El-Mashad *et al.*, 2003 and Said *et al.*, 2013). Thus, it has produced a huge amount of celluloses agricultural and horticultural wastes (Agro-wastes). The accumulation of such wastes annually without any treatment represents a bad environmental impact and a waste of an important economic resource (Hassan *et al.*, 2014).

Agro-wastes are the amount of crop that leftovers after the harvest of the main product. They are leaves, stem and shelves which are considered as rough plant by-products with big size and relatively poor in protein, fat and other nutrient compounds (Shaban *et al.*, 2010 and Said *et al.*, 2013). Globally, annually produced agro-wastes are almost five billion metric tons of biomass made from agriculture and agro-based food industries. These wastes include rice bran, rice straw, sugarcane bagasse, fruits and vegetable wastes, wheat bran, cotton leaf scraps, etc. Also almost 1.3 billion tonnes, represent approximately one third of the globally produced food, are lost or wasted every year. Although these wastes contain high nutrients, it can become breeding grounds for disease-causing microbes. Thus, it is very important to deal