

PRODUCTION OF ENZYMES FROM WHEAT STRAW BY *Aspergillus niger* AND *ASPERGILLUS TERREUS*

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ABSTRACT

Aspergillus niger and *Aspergillus terreus* were tested for production of hydrolytic enzymes (cellulolytic enzymes, alpha-amylase and amyloglucosidase). Three different media of wheat straw were used. These occurred by cultivation of *A. niger* or *A. terreus* for 6 days with shaking (~ 120 rpm) at 30°C. *A. terreus* produced little higher quantities of alpha-amylase and amyloglucosidase than *A. niger*, but *A. niger* was superior for the production of cellulolytic enzymes. The maximum products of enzymes activities during cultivation of *A. niger* in medium III were 3.1 and 1.4 U/ml of alpha-amylase and amyloglucosidase ; 4.8 and 4.8 mg sugar / ml / 24 h of C₁ and Av enzymes ; 8.9 and 16.4 mg sugar/ml/h of C_x and X enzymes, respectively and 3.3 mg/ml of reducing sugar.

Keywords: *Aspergillus niger* ; *Aspergillus terreus* ; cellulolytic enzymes ; alpha-amylase ; amyloglucosidase ; wheat straw.

INTRODUCTION

Considerable amounts of lignocellulose are discarded in the form of straw as it has a very low nutritional value for ruminant livestock, Milstein *et al.* (1981). Straw from cereals are of great interest as raw materials for the microbiological industry, (Stakheev *et al.*, 1986) *Aspergillus niger* has been reported to convert rapidly a variety of substrates to useful products, (Hang *et al.*, 1975). *Aspergillus terreus* produces cellulases and is able to ferment hexoses, pentoses and disaccharides such as cellobiose and hemicellulose, depending on the type of agriculture residues and hydrolysis method used. *A. terreus* are known to ferment sugar to ethanol, (Pushalkar and Rao 1998). Microorganisms that produce both cellulase and ethanol are of particular importance in the direct microbial conversion of biomass, (Ahn and Lynd 1996). Production of the enzyme is a major cost factor. Cellulase productivity must be improved to make the process economically attractive, (Tanaka *et al.*, 1980). Cellulase enzymes have a variety of industrial application and are potentially effective for processing biomass feedstocks. The cost of cellulase enzymes is widely considered an importance factors in the commercialization of lignocellulose biomass, (Nieves *et al.*, 1998). Glucoamylase is one of the most important industrial enzymes, (Temesvari *et al.*, 1993). Amylase enzymes are important in view of their potential biotechnological application in starch processing industries, (Ali *et al.*, 1990a). The surfactant, Tween 80 which has been reported to be of value in the production and recovery of the enzyme cellulase, was shown to be detrimental to the degradation of cellulose in culture, (Romanflli *et al.*, 1975).

The aim of the present work is to compare *A. niger* and *A. terreus* for their ability to produce hydrolytic enzymes from wheat straw by cultivation in simple media for 6 days with shaking (~ 120 rpm) at 30°C.

MATERIALS AND METHODS

Microorganisms : *Aspergillus niger* NRR – 326 was obtained from the United States Dep. Agric., Culture Collection, Peoria, Illinois. *Aspergillus terreus* was donated from Plant Disease Dep. Minist. Agric. Egypt. Stock cultures of potato dextrose agar (PDA) slants were used for preservation of microorganisms at 4°C and subcultured at two months intervals.

Inocula: Agar plugs of profus *A. niger* or *A. terreus* growth were inoculated into 100 ml steril fermentation medium each contained in 250 ml flask.

Substrate: Milled wheat straw (~ 0.5 cm in length, in a laboratory mill) was used in this study.

Fermentation media: Production of enzymes (cellulases, alpha-amylase and amyloglucosidase) were carried out by using the following media:

- 1) Cultivation of *A. niger* or *A. terreus* on 4% wheat straw as the sole carbon source and in the presence of 1% Tween 80 (medium I).
- 2) Corn steep liquor (CSL) medium: CSL medium as recommended by Kadam and Newman (1997) which contained 0.3% CSL, 2.5 mM MgSO₄ – 7H₂O and supplemented with 4% wheat straw (medium II).
- 3) Roch – Chui and Hang medium (1990) which contained 10 g wheat straw, 10 g CaCO₃ and 100 ml. water added to 250 ml flask (medium III).

All the above mentioned media were sterilized in autoclave at 121°C for 20 min., inoculated with agar plugs of profus *A. niger* or *A. terreus* and incubated in shaker (~ 120 rpm) for 6 days at 30°C. Then the culture filtrated on nylon cloth and the filtrate was used for enzymes assays.

Assays of enzymes activities:

- 1) Amyloglucosidase activity was determined according to the method described by Nagasaka *et al.* (1998).
- 2) Alpha-amylase activity was evaluated according to Hayashida and Teramoto (1986) method.
One unit (U) of enzyme activity was defined as the amount of enzyme releasing one micromole of reducing sugar as glucose per ml of culture filtrate per min. under the assay conditions. Reducing sugars as glucose was determined by a sub-microdetermination method described by James and Marvin (1949).
- 3) Activities and composition of cellulolytic enzymes in cultures filtrates were evaluated as the method described by Galas *et al.* (1981).
C_x; (endo-glucanase, saccharified CMC); enzyme activity = mg sugar/ml enzyme/h
C₁; (exo-glucanase, degrades Solka Floc SW – 40) ; enzyme activity = mg sugar / ml enzyme / 24 h.
Av ; (B-glucosidase, saccharified Avicel SF) ; enzyme activity = mg sugar / ml enzyme / 24 h.
X; (Xylanase, hydrolysed xylan) ; enzyme activity = mg sugar/ml enzyme/ h.

RESULTS AND DISCUSSION

In order to compare the effects of media composition on the secretion of enzymes, three different media of wheat straw were used. The results of enzymes production from *A. niger* are summarized in Table (1). It revealed that amyloglucosidase activities in the three media were constant (1.4, 1.3 and 1.4 U/ml, respectively). The same quantities of alpha-amylase were detected in medium I and II (2.1 and 2.2 U/ml, respectively), but it was higher in medium III (3.1 U/ml).

Production of C₁ and Av enzymes were at the same levels of activities in medium I (2.0 and 2.1 mg sugar / ml / 24 h), medium II (2.9 and 2.4 mg sugar / ml / 24 h) and medium III (4.8 and 4.8 mg sugar / ml / 24 h), respectively. In the same time, medium III contained about two times the amounts of C₁ and Av enzymes in medium I and II. Also, medium I and II contained rather the same quantities of C_x enzyme (4.1 and 4.7 mg sugar/ml/h, respectively) and two times the amount of C_x enzyme found in medium III (8.9 mg sugar/ml/h).

Table (1):Enzymes activities in cultures filtrates of different media of wheat straw cultivated with *A. niger* for 6 days with shaking (~ 120 rpm) at 30°C.

Determination		Media		
		I	II	III
Reducing sugar	mg/ml	1.1	1.5	3.3
Alpha-amylase	U/ml	2.1	2.2	3.1
Amyloglucosidase	U/ml	1.4	1.3	1.4
C _x	mg sugar / ml / h	4.1	4.7	8.9
C ₁	mg sugar / ml / 24 h	2.0	2.9	4.8
Av	mg sugar / ml / 24 h	2.1	2.4	4.8
X	mg sugar / ml / h	7.6	14.1	16.4

The maximum activity of xylanase (X) was recorded in medium III (16.4 mg sugar/ml/h) followed by medium II and I (14.1 and 7.6 mg sugar/ml/h), respectively. Also, reducing sugars in cultures filtrate was maximum in medium III followed in medium II and I (3.3, 1.5 and 1.1 mg sugar / ml, respectively). These results are in agreement with results of Gomes *et al.* (1989) that cultivation of *Aspergillus niger* on basic mineral medium contained 10 g/L avicel produced 0.03, 1.37, 4.99 and 1.16 IU/ml of C_x, C₁, xylanase and B-glucosidase (Av), respectively, after 6 days of shaking (250 rpm) at 30°C. From these results found that *A. niger* is capable of producing the enzymes on a variety of media. The proportions of the enzymes in the three media varied and more abundant in medium III.

Results listed in Table (2) show the production of enzymes by cultivation of *A. terreus*. Data in Table (2) indicated that the amyloglucosidase activity in medium I and II was at the same levels (1.5 and 1.4 U/ml, respectively) but, it was little higher in medium III (2.0 U/ml). Alpha-amylase activity was equal in medium III and I (3.3. and 3.2 U/ml), respectively but, it was less activity in medium II (2.4 U/ml). These results are in line with Ali *et al.* (1990b) results, who found that *A. terreus* produced 1.4, 3.3 U/ml of amyloglucosidase when

Table (2): Enzymes activities in cultures filtrates of different media of wheat straw cultivated with *A. terreus* for 6 days with shaking (~ 120 rpm) at 30°C.

Determination		Media		
		I	II	III
Reducing sugar	mg/ml	ND	1.2	2.8
Alpha-amylase	U/ml	3.2	2.4	3.3
Amyloglucosidase	U/ml	1.5	1.4	2.0
C _x	mg sugar / ml / h	6.1	4.0	7.9
C ₁	mg sugar / ml / 24 h	2.5	2.3	3.9
Av	mg sugar / ml / 24 h	2.4	1.9	3.8
X	mg sugar / ml / h	9.7	12.1	14.3

ND ; not determined

grown on basal medium containing 8 mg/ml of α -methyl mannoside and sucrose, respectively, after 48 h at 38°C.

Also, data in Table (2) indicated that the highest activities of C₁ and Av enzymes were recorded in medium III (3.9 and 3.8 mg sugar/ml/24 h) followed closely by the other two media (2.5, 2.4 and 2.3 and 1.9 mg sugar/ml/24h) for C₁ and Av, respectively. Also, maximum activity of C_x enzyme was in medium III (7.9 mg sugar/ml/h) followed by medium I (6.1 mg sugar/ml/h). While, medium II contained the less quantities of C_x enzyme (4.0 mg sugar / ml / h). The levels of X enzyme activity reached a maximum in medium III followed by medium II and I (14.3, 12.1 and 9.7 mg sugar/ml/h, respectively). Also, medium III contained the higher amount of reducing sugar (2.8 mg sugar/ml). With this relation Okunev *et al.* (1981) cultivated *Aspergillus* sp. on salt medium contained 10 g wheat straw / L for 6 days with shaking (180 rpm) at 29°C, the culture filtrate contained 2.1, 0.18 and 0.0 U/ml of C₁, C_x and B-glucosidase (Av) respectively. From the results in Table (1) and (2) found that alpha-amylase and amyloglucosidase production by *A. niger* and *A. terreus* were nearly constant, since there was no remarkable increase in the production of these enzymes. *A. niger* showed high enzymes activities comparable to that of *A. terreus*. This observation was in agreement with Lakshmikant (1990) studies of five fungi and found that maximum activity of C₁, C_x and Av enzymes was maximal in *A. niger*.

Wheat straw was the most suitable for growth and activity of cellulolytic fungi. All fungi contained cellulase activity. Also, from the above mentioned results, it is observed that medium III facilitated maximum enzymes production than any other media. These results are in line with Rochi- Chui and Hnag (1990) results, who found that neutralization the medium with 10 g/L CaCO₃ greatly enhanced enzyme production from agricultural commodities. The effect of CaCO₃ may be due to its buffering capacity of the medium and increase straw hydrolysis by fungi. These results indicate the positive influence of medium composition on increasing enzymes production. The ability of microorganism for biosynthesis of enzymes must be also taken in consideration.

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إنتاج الإنزيمات من قش القمح باستخدام فطر الأسبرجلس نيجر، والاسبرجلس تيرس

سكينة أنور محمد سليمان – سمير عبد الله محمد موسى
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استخدام فطر الاسبرجلس نيجر والاسبرجلس تيرس في إنتاج إنزيمات السليوليز، الألفا- أميليز ، والأميلوجلوكوسيديز من قش القمح. وذلك باستخدام ثلاثة أنواع مختلفة من البيئات السائلة المحتوية على قش القمح وبتنمية الفطريات المستخدمة (كل على حدة) لمدة 6 أيام مع الرج (حوالى 120 لفة/ دقيقة) على 30°م ثم قدر نشاط الإنزيمات فى راشح المزارع ووجد الأتى:

- (1) أفضل المزارع المستخدمة لإنتاج الإنزيمات كانت البيئة المكونة من 10 جرام قش قمح، 10 جرام كربونات كالسيوم، 100 مليلتر ماء.
- (2) يتميز فطر الاسبرجلس تيرس بإنتاج كمية أكثر قليلاً من إنزيمات الألفا- أميليز، والأميلوجلوكوسيديز (2.00، 3.3) وحدة/ مليلتر عن فطر الاسبرجلس نيجر (1.4، 3.1) وحدة / مليلتر على الترتيب.
- (3) يتميز فطر الاسبرجلس نيجر بقدرة أعلى على إنتاج إنزيمات السليوليز وأيضاً محتوى السكر المختزل فى البيئة حيث كانت (4.8 ، 4.8) ملليجرام سكر/ مليلتر/ 24 ساعة الانزيمات الأندو جلوكانيز، B جلوكوسيديز؛ 8,9 ، 16.4 ملليجرام سكر/ ملليمتر/ ساعة من أنزيمات الأكسو- جلوكانيز، والزيلانيز على الترتيب، 3.3 ملليجرام سكر مختزل/ مليلتر..