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Laboratory Simulation of Cellulose Degradation by Soil *Aspergilli*

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Abestract: In this research sixtythree isolates of *Aspergillus* spp. isolated from cultural soils were assayed for cellulose degradation ability. Spores suspension from all isolates grown on PDA was prepared. One ml spore suspension were used for inoculation of flasks containing 100 ml of minimal broth medium with no carbon source except 1% carboxy methyl cellulose. Glucose production assays started from third day after inoculation and repeated each 2 days up to 27th day. Reaction of glucose and related reagent were colorimetric measured at 525 nm *via* spectrophotometer. Isolates of the same species had no significance difference but the ability of different species were noticeable. species of *A. niger* and *A. niveus* had highest and *A. fumigatus*, *A. auricomus, A. carneus, A. caespitosus, A. parasiticus* species least ability for *in vitro* cellulose degradation. Glucose levels elevated up to 17th and 18th day for *A. niger* and *A. niveus* and had decreases in future days. Its obvious that soil *Aspergilli* have high cellulose degradation ability.

Key wordes: *Aspergillus* spp · CMC · Spectrophotometer

INTRODUCTION

Cellulases are a group of hydrolytic enzymes capable of hydrolyzing cellulose to smaller sugar components like glucose units. Cellulolytic enzymes play an important role in natural biodegradation processes where plant lignocellulosic material are efficiently degraded by cellulolytic fungi and bacteria. In industry, celluloytic enzymes have found novel applications in the production and processing of chemicals, foods and manufactured goods such as paper, rayon and cellophane. Cellulases, for instance have been extensively utilized for extraction of valuable components from plant cells, improvement of nutritional values of animal feed and the preparation of plant protoplasts in genetic research [1]. Generally, fungi produces three major types of cellulolytic enzymes: endoglucanase, exoglucanase and cellobiohydrolase [2]. These enzymes are extracellular and inductive in nature [3]. The ability to produce cellulase are widespread among fungi and this has became the subject of extensive investigation. This study focused mainly on soil fungi isolated from different regions of Iran for only one high lighted biochemical process were tested in laboratory conditions.

MATERIALS AND METHODS

Samples and Isolation of Fungi: Soil samples were taken from cultural soils of different parts of Iran. The soil samples were mixed with sterile distilled water and a serial dilution were prepared. From the dilutions, 0.5ml volumes were pipetted onto potato dextrose agar (PDA) and incubated at 30°C for three days. Fungi was isolated from the mixed isolates from each plate and subcultured on PDA. Subculturing was continued until a pure isolate was obtained from single spore cultures.

Fungi Identification: All isolated fungi were subjected to morphological and microscopic examination. All species were identified according to Klich [4].

Culture Medium: A broth culture medium containing 10 g Carboxy Methyl Cellulose (CMC), 0.05 g $FeSO_4$, 7 H_2O, 0.25 g $MnSO_4$, H_2O, 0.25 g $COCl_2$, 0.25 g $ZnSO_4$, 0.25 g $(NH_4)_2SO_4$, 2 g KH_2PO_4, 0.25 g $MgSO_4$, 7 H_2O, 0.4 g $CaCl_2$, 0.3 g urea, 0.2 ml Tween 80 per liter for cellulose degradation experiments. Fifty ml of broth medium has been distributed between 250 ml Erlenmeyer flasks and then autoclaved at 121°C for 20 minutes.

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Inoculation and Sampling: Each flask inoculated with spore suspension then flasks treated at 25°C for 27 days. Sampling was started three days after inoculation and repeated each two days for sugar assays.

Sugar Assay: Five hundred µl of broth medium in each clean test tube were subjected for released sugar assays. Released sugars concentrations were determined using Arsenate-Molybdate reagent [5].

RESULTS AND DISCUSSION

Cellulose is world's most abundant organic substance [6] and comprises a major storage form of photosynthesized glucose. It is the major component of biomass energy [7]. Because a large proportion of vegetation added to soil is cellulose therefore, decomposition of cellulose has a special significance in the biological cycle of carbon [8]. In industry, these enzymes have found novel application in production and processing of chemicals, food and manufactured goods such as paper, rayon etc. and extraction of valuable components from plants and improvement of nutritional values of animal feed [9]. Fungi are well known agents of decomposition of organic matter in general and of cellulosic substrate in particular as reported by Lynd *et al.* [10].

Screening of different genera, species and even isolates is the first step for finding acceptable enzyme producer isolates. Macris [11] in surveys on some genera like *Trichoderma*, *Fusarium*, *Aspergillus*, *Phanerocheate*, *Chrysosporium* and *Sclerotium* showed some differences in their cellulase activity. Members of the fungal genus *Trichoderma* and *Aspergillus* have been extensively studied, particularly due to their ability to secrete cellulose-degrading enzymes. The most common and most potent cellulase producers are *Trichoderma ressei, T. koningii, Fusarium* sp., *Aspergillus* and *Penicillium* sp., [12]. In the present work cellulase activity of a sharp saprophytic soil inhabitant fungi and some allied species are examined.

Results obtained during this study showed that all of *Aspergillus* isolates have ability of cellulose degradation (Table 1). The results showed that there is no significant difference in cellulase activity between isolates belonging to one species; however, significant statistical variations are detectable between different *Aspergillus* species. Among treated species *A. niger* and *A. niveus* have the highest of released sugar and isolates belonging to *A. fumigatus, A. auricomus, A. carneus, A. caespitosus* and *A. parasiticus* showed the lowest potential of glucose level production.

Table 1: Screening and measurement of cellulase activity by *Aspergillus* spp

Name of isolates	Number of isolates	Percentage(%)	Sugar assay
Aspergillus niger	4	6.34	0.932
A. niveus	2	3.17	1.022
A. sclecotriorum	1	1.58	0.731
A. vestus	3	4.76	0.679
A. candidus	6	9.52	0.489
A. awamori	2	3.17	0.430
A. terreus	16	25.39	0.296
A. flavus	6	9.52	0.245
A. parasiticus	1	1.58	0.111
A. caespitosus	2	3.17	0.183
A. carneus	6	9.52	0.113
A. auricomus	3	4.76	0.194
A. fumigatus	11	17.45	0.094
Total	63	100	

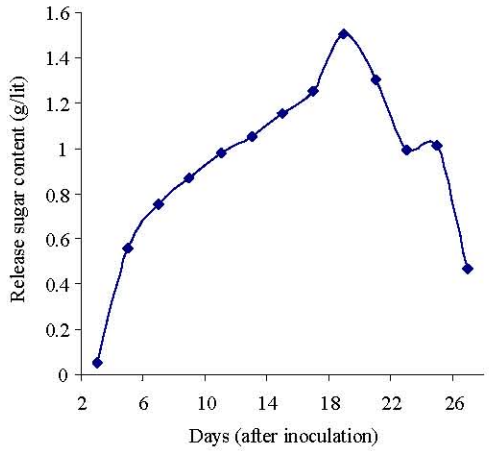

Fig. 1: Variations in released sugars from *A. niger* isolates during sampling days

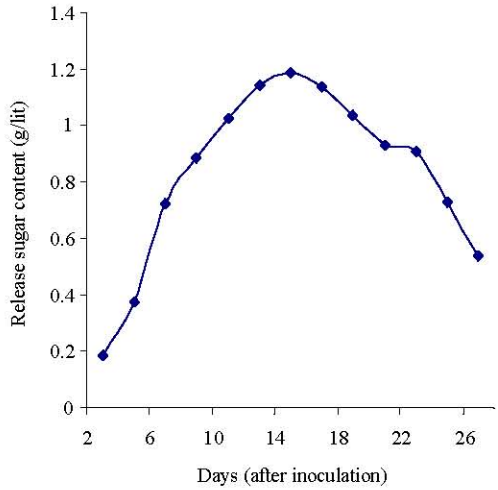

Fig. 2: Variations in released sugars from *A. niveus* isolates during sampling days

Sazci *et al.* [13] in surveys on cellulose activity in different genera reported that *Trichoderm harzianum* and *A. niger* showed highest and *Trichothecium roseum, Trichoderm reesei, A. ochraceus* and *P. italicum* exhibited lower activity against CMC.

Moreover Jahangeer *et al.* [14] measured cellulase activity of 115 isolated fungal strains. They found that *Trichoderma, Aspergillus* and *Fusarium* strains had highest activity. Also their results showed that between different *Aspergillus* species, *A. niveus* had the highest cellulase activity.

In high sugar producer isolates, glucose levels in *Aspergillus niger* isolates have been increased until 18 days post inoculation, however and then have been decreased until day 22th and then showed no variation until day 27th(Fig.1).

Aspergillus niveus isolates have produced their highest amount of released sugar in 17 days after inoculation, however, the trend will be then dropped down until day 27th (Fig. 2).

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