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SCREENING OF SOME *ASPERGILLUS* SPECIES FOR THEIR CELLULASE PRODUCING ABILITY FROM THE SOIL

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ABSTRACT

Cellulose is major constituent of all plant materials. Cellulose structure is very hard and its degradation is carried out in nature by cellulase enzyme. Microorganisms are well known recognize for their enzymes producing ability. Cellulolytic enzymes are synthesized by number of fungi. Present work is based on screening of potential cellulase producing *Aspergillus* species from soil. Fifteen *Aspergillus* species were isolated from soil. They are further identified and subjected to plate assay for cellulase activity out of these five *Aspergillus* species *Aspergillus niger, A. versicolor, A. luchensis, A. terreus,* and *A. nidulans* were found to produced cellulase activity and give positive results. Intracellular and extracellular proteins were estimated from all five selected species of *Aspergillus.* Among all the five species, *Aspergillus nidulans* exhibited highest level of extracellular protein (0.682 mg/ml). Lowest level of protein was recorded in *A. terreus* (0.033 mg/ml). Quantitative estimation of cellulase was performed on intracellular and extracellular crude extract in all selected species of *Aspergillus.* Maximum and minimum intracellular cellulase activity was recorded in *Aspergillus niger* (1.87 U/L) and *A. versicolor* (1.42 U/L). The extracellular cellulase was found to be high in *A. terreus* (1.67 U/L) and lowest in *A. nidulans* (0.35 U/L).

Key Words; Cellulose, *Aspergillus,* Cellulase, Screening.

INTRODUCTION

Cellulose is the most abundant biopolymer present in the Earth. Man is using cellulose as renewable source of energy for a century (Bhat and Bhat, 1997). Cellulose is degraded by cellulase enzyme into smaller units of glucose. These enzymes are used in textiles, laundry, pulp and paper industries. Most industrial production of cellulose enzymes occurs from *Trichoderma* and *Aspergillus* species and account for 20% of world enzyme market (Bhat, 2000 and Gielkens, 1999). Present research work deals with screening of potential *Aspergillus* species for their cellulase producing capability so that species can be employed by the industries for particular enzymes production in large quantities. Work involves screening of best *Aspergillus* species which is producing industrially important enzyme in large quantity.

MATERIALS AND METHODS

Isolation of *Aspergillus* species

Different soil samples were isolated from the rotten leaves, fruits, and degraded flowers rich soil areas with an aim to isolate cellulose degrading fungi. *Aspergillus* species were isolated from soil by serial dilution method of Tate (1995). The media used for the growth of fungi were Czapeck's Dox Agar media and Potato Dextrose Agar media.

Identification of *Aspergillus* species

The isolated *Aspergillus* species were further identified from available literatures (Ellis, 1949 and Barnett, 1969) reference slides and finally by authentic authority. Pure cultures of identified *Aspergillus* species were maintained on slants of Czapeck's Dox Agar medium and stored at low temperature.

Screening of fungi for cellulase production by plate assay method

The *Aspergillus* species isolates were assayed for their potential for cellulase productions by plate assay method of (Carder, 1986) Cellulose powder was used as substrate. Carboxy methyl cellulose powder 0.5%, agar 1.7% was autoclave for 15 minutes at 121 $^{\circ}$C and 20 ml of medium was poured into petridishes. After cooling of medium it was punched with the help of gel puncher under sterilized condition. The isolated *Aspergillus* species were cultured in Czapeck's Dox broth medium. The *Aspergillus* extract and cultured broth served as extracellular enzyme source were poured in punched area of plates and incubated for 24 hrs. Plates were flooded with Congo red the clear zone exhibited shows cellulase activity.

Extraction of enzyme

Preparation of extracellular enzyme source

Aspergillus cultures inoculated into 50 ml of Czapeck's Dox broth and incubated for 5 days for growth of fungi. After harvesting mycelial mat the fungal culture broth was centrifuged at 3000 rpm for 15 minutes at room temperature supernatant served as source of extracellular enzyme.

Preparation of intracellular enzyme source.

Aspergillus species were allowed to grow in 50 ml of Czapeck's Dox broth after incubation period the mycelium were separated by filtration through Watman filter paper no 3. Mycelium was washed with cold normal saline. Fresh mycelium was weight and homogenized in 6 ml of extraction buffer (Talbot, 2005). Mycelium homogenized with sterilized sand in a pestle mortar and centrifuged at 15000 rpm at room temperature. The resulting supernatant was taken directly as the source of intracellular crude enzyme for different enzyme assay.

Protein estimation

Intracellular and extracellular proteins of the *Aspergillus* species were estimated by method of Lowry *et. al.* (1951) using crystalline bovine serum albumin as standard protein.

Quantitative estimation of cellulase

Quantitative estimation of cellulase was performed on intracellular and extracellular crude extract in all selected species of *Aspergillus* by the method of Miller (1959) using glucose 5 mg/ml as standard.

RESULTS

Isolation and identification of *Aspergillus* species

During present investigation total 15 *Aspergillus* species isolated from soil and identified are *Aspergillus awamori, A. corneus, A. flavus, A. fumigatus, A. japonicus, A. luchensis, A. nidulans, A. niger, A. niveus, A. ochraceous, A. phoensis, A. stelatus, A. terreus, A. ustus, A. versicolor.*

Plate assay method for detection of enzyme

Fifteen different isolates of *Aspergillus* species were tested for their enzymes producing capability by plate assay method. Out of fifteen *Aspergillus* five species *Aspergillus niger, A.*

versicolor, *A. luchensis*, *A. terreus*, and *A. nidulans* were found to produced cellulase activity and give positive results. Other ten species were found to give negative results hence they are not potential species for cellulase production Therefore among fifteen *Aspergillus* five species were selected for further studies **(Table 1).**

Table 1: Screening results of *Aspergillus* for cellulase activity

S.No.	Name of fungi	Results
1	*Aspergillus awamori*	_
2	*A. corneus*	_
3	*A. flavus*	_
4	*A. fumigatus*	_
5	*A. japonicus*	_
6	*A. luchensis*	+
7	*A. nidulans*	+
8	*A. niger*	+
9	*A. niveus*	_
10	*A. ochraceous*	_
11	*A. phoensis*	_
12	*A. stelatus*	_
13	*A. terreus*	+
14	*A. ustus*	_
15	*A. versicolor*	+

Quantitative estimation of protein

Intracellular and extracellular proteins were estimated from all five selected species of *Aspergillus*. Among all the five species, *Aspergillus nidulans* exhibited highest level of extracellular protein (0.682 mg/ml). Lowest level of protein was recorded in *A. terreus* (0.033 mg/ml). Other species like *A. niger* (0.576 mg/ml), *A. versicolor* (0.471 mg/ml) and *A. luchensis* (0.432 mg/ml) gave moderate results. While intracellular protein measured was *Aspergillus luchensis* (0.523 mg/ml), *A. terreus* (0.394 mg/ml), *A. niger* (0.134 mg/ml), *A. versicolor* (0.130 mg/ml) and *A. nidulans* (0.024 mg/ml). It was observed that *Aspergillus luchensis* contain highest intracellular protein **(Table 2)**.

Table 2: Protein estimation

S.No.	Name of fungi	Intracellular Concentration (mg/ml)	Extracellular Concentration (mg/ml)
1	*Aspergillus luchensis*	0.523	0.432
2	*Aspergillus terreus*	0.394	0.033
3	*Aspergillus nidulans*	0.024	0.682
4	*Aspergillus niger*	0.134	0.576
5	*Aspergillus versicolor*	0.130	0.471

Quantitative estimation of cellulase

Cellulase assay was also performed on all five species of *Aspergillus*. Intracellular cellulase activity observed are *Aspergillus niger* (1.87 U/L), *A. terreus* (1.86 U/L), *A. luchensis* (1.78 U/L), *A. nidulans* (1.47 U/L) and *A. versicolor* (1.42 U/L). Maximum and minimum cellulase activity was recorded in *Aspergillus niger* (1.87 U/L) and *A. versicolor* (1.42 U/L) **(Fig-1)**.

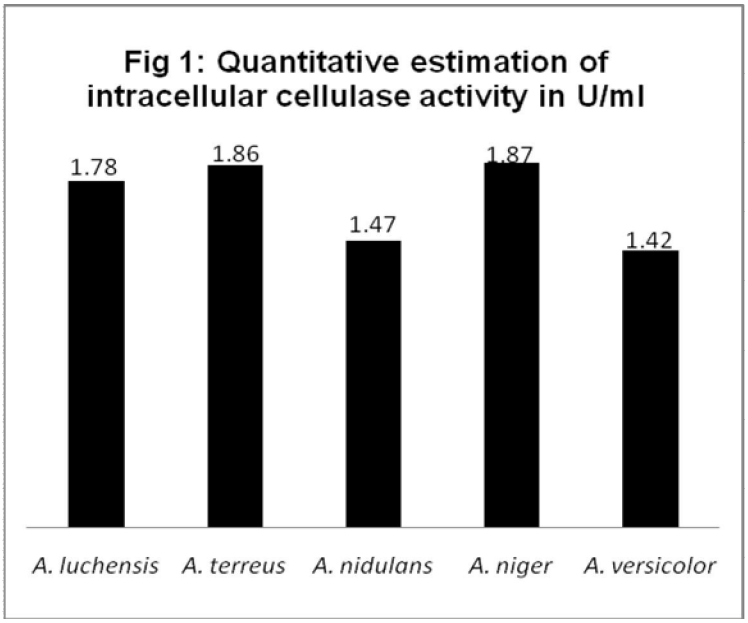

Fig 1Quantitative estimation of intracellular cellulase activity in U/L

Extracellular cellulase estimated was *Aspergillus terreus* (1.67 U/L), *A. luchensis* (1.47 U/L), *A. versicolor* (0.97 U/L), *A. niger* (0.38 U/L), *A. nidulans* (0.35 U/L). The extracellular cellulase was found to be high in *A. terreus* (1.67 U/L) and lowest in *A. nidulans* (0.35 U/L) **(Fig-2)**.

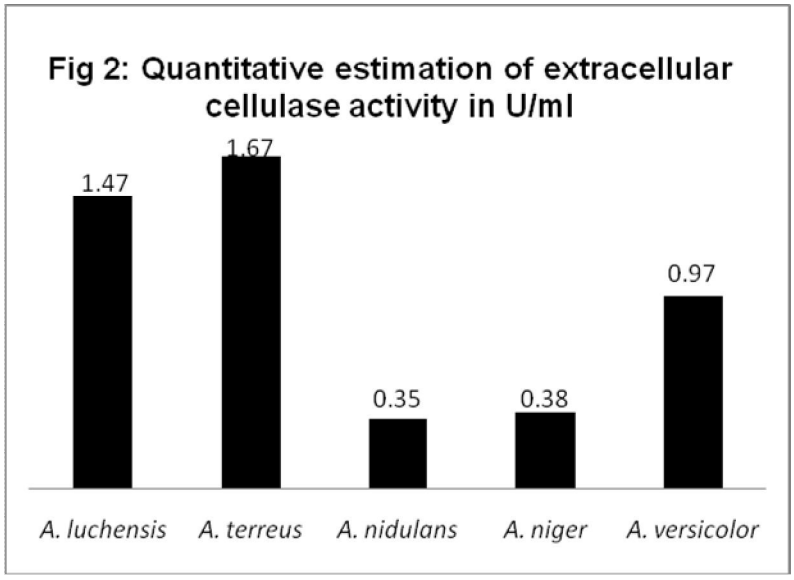

Fig 2Quantitative estimation of extracellular cellulase activity in U/L

DISCUSSION

Intracellular and extracellular proteins were estimated from all five selected species of *Aspergillus.* Among all the five species, *Aspergillus nidulans* exhibited highest level of extracellular protein. Shimizu *et.al*. (2009) also recorded protein in *Aspergillus nidulans* in high concentrations. Lowest level of protein was recorded in *Aspergillus terreus.* Other species like *Aspergillus niger, A. versicolor* and *A. luchensis* gave moderate results. The result are in line with Hombergh ven den *et.al.* (1997) who also reported highest protein concentration in *Aspergillus niger.* Intracellular protein was found to be highest in *Aspergillus luchensis* followed by *A. terreus, A. niger, A. versicolor* and *A. nidulans.* Therefore it was observed that *Aspergillus luchensis* contain highest intracellular protein.

The extracellular cellulase quantitated was found to be high in *Aspergillus terreus* as also reported by Gao *et.al*. (2008), and maximum intracellular cellulase activity is recorded in *Aspergillus niger.* The result match with Hurst *et.al.* (1977) who also reported high cellulase activity in *Aspergillus niger*.

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CONCLUSION

Five Aspergillus species were showed positive results for cellulase production extracellular

cellulase activity was found to be high in *A. terreus* and intracellular cellulase activity was recorded in *Aspergillus niger*. Hence both the species can be exploited for industrial celluase production

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