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Starch Hydrolysis and α -Amylase Activity of *Aspergillus* and *Chaetomium*

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Abstract: Starch hydrolyzing and α -amylase producing micro-fungi were isolated from soil. Among the isolated fungal species *Aspergillus fumigatus* and *Chaetomium globosum* were found prominent starch hydrolyzing and amylase producing fungi. Maximum starch hydrolysis was recorded in 6 to 7 days old culture for both *A. fumigatus* and *C. globosum*. However, α -amylase activity was found higher in 9 to 11 days old culture. Starch hydrolysis, α -amylase and protease activity of *A. fumigatus* was found higher as compare to *C. globosum*. At higher temperature (45°C) also performance of *A. fumigatus* was better than *C. globosum*. As *Aspergillus fumigatus* was found to be better thermotolerant, it can therefore be utilize in starch processing industry as well as for amylase production at high temperature level.

Key words: α -amylase, *Aspergillus fumigatus* protease, starch hydrolysis, *Chaetomium globosum*

INTRODUCTION

Starch-degrading enzymes are gaining more importance among the industrial enzymes because of the importance of starch, sugar and other products in modern biotechnology era (Prakasham *et al.*, 2007). Microbial amylase has replaced chemical hydrolysis in starch processing industry (Gupta *et al.*, 2003). They are also potential candidates in the medicinal, clinical and fine chemical industries (Becks *et al.*, 1995; Gupta *et al.*, 2003). Industrial demand for these enzymes is limited with specific applications as in the food industry, where fungal amylases are preferred over other microbial sources mainly because of their more acceptable GRAS (Generally regarded as safe) status (Prakasham *et al.*, 2007). Fungi are therefore attracting increasing attention as sources of amylolytic enzymes suitable for the industrial conversion of starch into maltose or glucose (Mishra and Maheshwari, 1996). However, there have been few reports about the control of extracellular α -amylase production by fungi. Also, conflicting results have been reported with respect to the suggested mechanism of amylases synthesis control in fungi (Nahas and Waldemarin, 2002).

Aspergillus fumigatus is a thermotolerant fungus with a world wide distribution (Domsch *et al.*, 1980). Key to the success of *A. fumigatus*, in the environment as a degrader of plant polymers and its ability to secrete a broad range of hydrolases, enabling it to mobilize and assimilate carbon and nitrogen from a wide array of polymeric materials of plant and animal origin (Robson *et al.*, 2005). *Chaetomium* sp. are important agents in the decomposition of plant matter reaching the soil. Good sporulation is obtained where carbon source is provided in an insoluble form such as starch or cellulose and little soluble carbohydrate is added (Domsch *et al.*, 1980). They can also tolerate high temperature

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and show enzymatic activity in the temperature range of 45-60°C (Maheshwari *et al.*, 2000). Present study deals with the measurement of starch hydrolysis and α -amylase production by two fungal species isolated from soil.

MATERIALS AND METHODS

To isolate starch hydrolyzing and amylase producing fungi, soil samples were collected and screened out from Botanical Garden of Rajiv Gandhi University, Itanagar, India, during January to July 2007. Isolation was carried out by serial dilution plate method using Rose Bengal agar media supplemented with 1% soluble starch. A selection of amylase producing fungi was done on the basis of transparent zone created due to hydrolysis of starch by staining the culture plate with iodine solution (containing 0.3% iodine and 0.6% KI). Subsequently, the amylase positive fungal colonies were pure cultured in potato dextrose agar media and identified.

Identification of fungal species was done with the help of manuals of Domsch *et al.* (1980) and Barnett and Hunter (1972). Two highly starch hydrolyzing fungi were identified as *Aspergillus fumigatus* and *Chaetomium globosum*. One copy of both the fungal species is preserved by dry preservation at -80°C in PDA media containing 20% glycerol and fresh cultures are maintained for different biochemical and molecular evaluations. The minimal medium for amylase production contained: 0.14% KH_2PO_4 , 1% NH_4NO_3 , 0.05% KCl, 0.01% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 2% Starch (pH 6.5). Erlenmeyer flask (100 mL) containing 50 mL of medium was inoculated with approximately one loopfull of inoculum. After 5 days of growth, the culture was examined and diluted with the required quantity of autoclaved distilled water so as to get approximately 3×10^6 numbers of spores per mL of the culture. One milliliter of the culture was added per 50 mL of medium and incubated at 30°C temperature for 11 days with continuous shaking to avoid clumping of mycelium.

Cultured fungal medium was filtered using Whatman filter paper No. 42. The supernatant containing crude enzyme extract was considered for further analysis. Amount of starch hydrolyzed to simple sugar by fungal species was assayed based on the decrease in iodine staining of starch. One milliliter each of crude and heat killed enzyme extract was taken for each sample. To it 1 mL of 1% soluble starch in citrate-phosphate buffer (pH 6.5) was added. Tubes were incubated in water bath at 45°C for 30 min. The reaction was stopped by adding 1 mL of 0.5N HCl. One milliliter of the above acidified solution was added to 1 mL of 0.5N HCl. To 1 mL of the above solution 1 mL iodine solution was added and then 20 mL distilled water was added and finally colour intensity was measured at 660 nm using spectrophotometer (Systronic, 106). An enzyme unit was defined as the amount of enzyme reducing 1 μg of starch under assay condition.

α -amylase activity was measured by using dinitro-salicylic acid as described by Mamo and Gessesse (1997). One milliliter each of crude and heat killed enzyme extract was taken for each sample. To it 1 mL of 1% soluble starch in citrate-phosphate buffer (pH 6.5) was added. Tubes were incubated in water bath at 45°C for 30 min. The reaction ceased after 30 min of incubation by adding 2 mL of Di-Nitro Salicylic (DNS) reagent. Colour intensity was determined at 540 nm using spectrophotometer (Systronic, 106). The optical density value of killed samples give an idea of reducing sugar on diurnal basis, whereas the value recorded by incubation non-killed enzyme extract of different growth intervals in starch solution (as substrate) for 30 min minus the value of killed samples gives an idea of reducing sugar produced in 30 min of incubation period. Maltose calibration curve was used to convert colour to reducing sugar equivalent. An enzyme unit was defined as amount of α -amylase releasing 1 μg maltose or maltose equivalent from the substrate under assay condition. To assay the protease activity protein in the culture filtrate was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. A unit of protease activity was defined as amount of protein releasing 1 μg tyrosine from BSA under assay conditions. For measurement of biomass cells were harvested by filtration through pre weight Whatman No. 42 filter paper and dried to a constant weight at 110°C for 24 h. The biomass was then determined as mg/50 mL culture.

RESULTS AND DISCUSSION

Diurnal activity on starch hydrolysis by *A. fumigatus* and *C. globosum* was measured at different days interval growing culture at 30°C. Among the two fungal species, *A. fumigatus* was found to hydrolyse higher amount of starch in culture than *C. globosum* (Fig. 1). Higher hydrolysis was recorded in six and seven days old culture for both the fungal species but after that activity was found to declined. In a separate experiment activity of enzyme secreted by respective fungal species on different days interval was measured using starch as substrate with a known amount of crude enzyme extract incubating at 45°C for 30 min. Higher activity (8.6×10^2 U mL⁻¹) was recorded for enzyme secreted by *A. fumigatus* and lower in case of *C. globosum*. Maximum activity was recorded with enzyme extract of 6th day old culture for both fungal species. A reduction in activity was noticed with enzyme extract of 7th days onwards old culture (Fig. 2).

Maximum production of α -amylase (19.0×10^2 U mL⁻¹) was shown by *A. fumigatus* in 9 day old culture. It was noticed that from 5th to 9th days of incubation there was steady increase in production of α -amylase which begin to decline only after 9 day old culture (Fig. 3). In *C. globosum* also, higher production of α -amylase was recorded in 9 day old culture but production was not consistent. Among the two fungal species, higher activity of α -amylase production was found in *A. fumigatus*. On measuring the α -amylase activity of crude enzyme extract produced by fungal species in different days old culture it was found that 6 and 9 days old culture extracts have higher activity in case of *A. fumigatus* but for *C. globosum* activity was maximum with extract of 8 day old culture (Fig. 4).

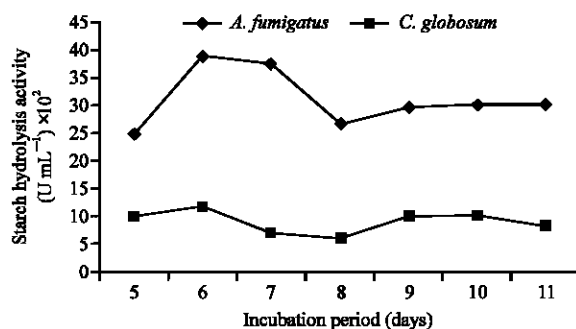


Fig. 1: Starch hydrolysis activity of fungal species in culture extract at different days interval

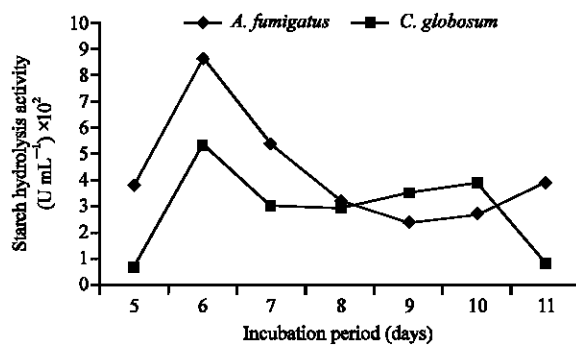


Fig. 2: Starch hydrolysis activity of crude enzyme extracts of fungal species per 30 min at 45°C

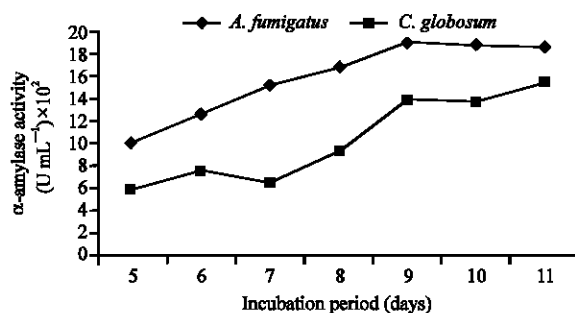
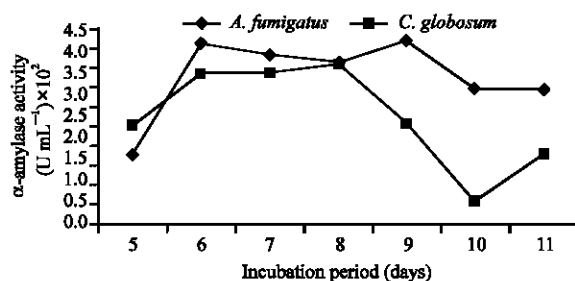
Fig. 3: α -amylase activity of fungal species in culture extract at different days intervalFig. 4: α -amylase activity of crude enzyme extracts of fungal species per 30 min at 45°C

Table 1: Protease activity and biomass of fungal species grown at 30°C

Incubation period (in days)	Protease activity		Biomass content	
	<i>A. fumigatus</i>	<i>C. globosum</i>	<i>A. fumigatus</i>	<i>C. globosum</i>
5	45	30	75	107
6	98	28	84	117
7	91	43	88	125
8	109	46	90	139
9	114	55	109	161
10	176	214	114	163
11	250	247	110	155

There was consistent increase in protease activity and higher was found with 11 day old culture for both the species. Similar to protease activity there was continuous increase in biomass of both fungal species and maximum biomass of fungal species was found in 11 days old culture (Table 1). Significant positive correlation was found between the biomass and protease activity in both the strains ($p = 0.02$ and 0.05 , respectively). α -amylase activity of both filtrate and crude enzyme extract showed significant correlation with the biomass and protease activity for both fungal species (Table 2).

A. fumigatus was found to reduce more sugar per unit time as compared to *C. globosum* under the assay conditions. Present study showed faster amylase production (from 6-9 days of incubation) as compare to the report given by Goto *et al.* (1998) and Moreira *et al.* (2001). Goto *et al.* (1998) reported maximum production of α -amylase and glucoamylase in *Aspergillus fumigatus* after 8-10-days of cultures at 37°C whereas Moreira *et al.* (2001) reported maximum activity from 10-14 days of incubation at 30°C. They reported maximum amylase production in 1% synthetic maltose (α -MG) followed by 1% starch and lowest in maltose as carbon source. Higher amylase activity ($388 \pm 9 \text{ U g}^{-1}$) was also recorded in *Thermomyces langunosus* (a thermophilic fungus) using 1% soluble starch as

Table 2: Karl Pearson correlation coefficient among starch hydrolysis and α -amylase activity of fungal species with protease activity and biomass content

Factors	Correlation with protease activity	Correlation with biomass
Starch hydrolysis activity of <i>A. fumigatus</i> in culture extract	0.006 (p = NS)	-0.115 (p = NS*)
Starch hydrolysis activity of <i>A. fumigatus</i> crude enzyme extract per 30 min at 45°C	-0.109 (p = NS)	-0.421 (p = NS)
α -amylase activity of <i>A. fumigatus</i> in culture extract	0.731 (p = 0.05)	0.964 (p = 0.001)
α -amylase of <i>A. fumigatus</i> crude enzyme extract per 30 min at 45°C	0.135 (p = NS)	0.313 (p = NS)
Protease activity of <i>A. fumigatus</i>	-	0.803 (p = 0.02)
Starch hydrolysis activity of <i>C. globosum</i> in culture extract	-0.011 (p = NS)	-0.074 (p = NS)
Starch hydrolysis activity of <i>C. globosum</i> crude enzyme extract per 30 min at 45°C	-0.235 (p = NS)	0.112 (p = NS)
α -amylase activity of <i>C. globosum</i> in culture extract	0.791 (p = 0.02)	0.940 (p = 0.001)
α -amylase of <i>C. globosum</i> crude enzyme extract per 30 min at 45°C	-0.173 (p = NS)	-0.214 (p = NS)
Protease activity of <i>C. globosum</i>	-	0.689 (p = 0.05)

*NS: Not Significant

carbon source (Kunamneri *et al.*, 2005). In contrast present study showed almost similar activity using 2% soluble starch as carbon source for both fungal species. Silva and Peralta (1998) reported liberation of 161 μ mol glucose equivalents per minute per mg protein, respectively by *Aspergillus fumigatus* cultured at 40°C. Starch hydrolysis activity in *C. globosum* was found maximum from 6 days old culture while α -amylase activity was higher in 9-11 days old cultures. Chen *et al.* (2005) reported 2.39×10^3 U of glucoamylase enzyme production in *Chaetomium thermophilum* after 7 days of incubation at 50°C. Higher amylase activity by *A. fumigatus* could be due to its fast growth rate as compared to *C. globosum*.

It can be concluded from the above study that *A. fumigatus* had higher amylase activity as compared to *C. globosum* at both 30 and 45°C of temperature and therefore it can be useful for amylase production though maximum amylase production was obtained at higher temperature level. *A. fumigatus* is a naturally thermotolerant species that can grow at temperatures up to 55°C and survive temperatures of up to 70°C. It is ubiquitous in the environment, frequently found on dead organic material and its conidia are among the most abundant in the atmosphere (Mullins *et al.*, 1984). *Aspergillus fumigatus* may therefore have wide scale application in starch processing industry on account of their thermo stability and ability to degrade raw starch.

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