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SCREENING OF SOIL FUNGI FOR α-AMYLASE ACTIVITY

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ABSTRACT

α-Amylase is a significant enzymes employed in the starch processing industries for the hydrolysis of polysaccharides. It has a wide range of applications in many industries such as baking, brewing, wine and textile etc. Hundred filamentous fungi from various soil samples in the region of Lucknow city were isolated and screened for α-amylase synthesis. Excluding, *Aspergillus nidulans (ANid-2)* and *Aspergillus terreus (ATer-2),* all other α-amylase producing fungal strains were hyper active in the Starch Agar medium as compared to their growth in Potato Dextrose Agar medium. Species belonging to *Fusarium* and *Trichoderma* showed zero production of extra cellular α-amylase. On the other hand, out of 100, only 15 fungal strains of 2 genera viz. *Aspergillus* and *Alternaria* were found potent for α-amylase production, however, *Aspergillus niger (ANig-4)* strain was found highly efficient in the production of α-amylase. It is considered that *Aspergillus niger (ANig-4)* is a better option for the synthesis of α-amylase in the industrial sectors.

KEY WORDS: Soil fungi, α-Amylase, *Aspergillus niger, Alternaria solani*

INTRODUCTION

Amylases are hydrolases which are utilized for the breakdown or hydrolysis of starch into reducing fermentable sugars, mainly maltose and reducing non fermentable or slowly fermentable dextrins. Amylases are employed in the starch processing industries for the hydrolysis of polysaccharides (starch) into simple sugar constituents by degrading 1-4 linkage of starch [1]. Besides their use in starch saccaharification, amylases are also applied in food, baking, brewing, detergent, textile and paper industries [2].

Filamentous fungi are particularly interesting due to their easy cultivation, and high production of extracellular enzymes of large industrial potential [3]. Studies on fungal amylase particularly in the developing countries have concentrated mainly on *Rhizopus sp.,* and *Aspergillus spp.,* possibly because of their ubiquitous nature and non fastidious nutritional requirements [4]. It has been reported that while a strain of *Aspergillus niger* produced 19 types of enzymes, α-amylase was being produced by as many as 28 microbial cultures [5]. Thus, the assortment of a suitable strain for the required purpose depends upon a number of factors, in particular upon the nature of the substrate, environmental conditions etc. Optimization of growth conditions is important for best growth of fungi. The growth requirements for fungi may vary from strain to strain, although cultures of the same species and genera tend to grow best on similar media [3]. Similarly, growth responses of fungi also vary from strain to strain though they are grown on same conditions [6]. Filamentous fungi vary in pH requirements. Most common fungi grow well over the range pH 3 to 7, although some can grow at pH 2 and below e.g., *Moniliella acetoabutans, Aspergillus niger, Penicillium funicilosum* [6]. Though, many fungi particularly *Aspergilli* are known to produce different groups of enzymes but the selection of a particular strain however, remains a monotonous task, especially when commercially competent enzyme yields are to be achieved.

Therefore, the present investigation deals with the isolation and screening of local amylolytic soil fungi, for future investigation and industrial processes.

MATERIALS AND METHODS

Sample

Soil samples were collected from 4-5 cm depth with help of sterile spatula from the various locations in Lucknow city for fungal isolation and screening. Samples were stored at 4 °C for further processing.

Isolation of Fungal Cultures

Isolation of fungal colony was performed by serial dilution and spread plate method. One gram of soil sample was serially diluted in sterilized distilled water to get a concentration range from 10^{-1} to 10^{-6}. A volume of 0.1 ml of each dilution was transferred aseptically to Potato dextrose agar plates. The sample was spreaded uniformly using a glass rod. The plates were incubated at 28 °C for 72 hr.

Screening for Amylase Producing Fungi

The screening procedure for α-amylase was based on a plate culture method which uses soluble starch (1%) as the carbon source. The screening plate medium contained KH_2PO_4 (0.20%); $(NH_4)_2SO_4$

Table 1. Frequency of fungal isolates screened for their ability to possess α-amylase activity

Species	No. of Isolates	No. of α-Amylase Producers	% of α-Amylase Producers
Aspergillus niger	23	6	26.08
Aspergillus flavus	9	2	22.22
Aspergillus terreus	21	3	14.29
Aspergillus nidulans	15	2	13.33
Aspergillus fumigatus	11	1	9.09
Fusarium solani	3	0	0
Alternaria solani	12	1	8.33
	6	0	0
Total	**100**	**15**	**15**

Table 2. Screening of α-amylase producing fungi

Fungal isolates	DCZ* (cm)	FCD# on starch agar (cm)	Hydrolysis activity Index = DCZ/FCD	Colony diameter on potato dextrose agar (cm)
Aspergillus niger (ANig-1)	8.8 + 0.15	8.8 + 0.15	1.0 + 0.04	3.9 + 0.10
Aspergillus niger (ANig-2)	8.7 + 0.11	8.7 + 0.11	1.0 + 0.05	4.7 + 0.08
Aspergillus niger (ANig-3)	8.9 ± 0.14	8.9 ± 0.14	1.0 ± 0.06	7.4 ± 0.11
Aspergillus niger (ANig-4)	8.5 + 0.15	8.5 + 0.15	1.0 + 0.06	3.9 + 0.10
Aspergillus niger (ANig-5)	9.0 ± 0.00	9.0 ± 0.00	1.0 ± 0.0	7.5 ± 0.06
Aspergillus niger (ANig-6)	8.3 + 0.17	8.3 + 0.17	1.0 + 0.02	3.8 + 0.09
Aspergillus flavus (AFla-1)	8.4 ± 0.18	8.4 ± 0.18	1.0 ± 0.0	3.7 ± 0.08
Aspergillus flavus (AFla-2)	8.2 ± 0.17	8.2 ± 0.17	1.0 ± 0.05	4.2 ± 0.11
Aspergillus terreus (ATer-1)	8.6 ± 0.14	8.6 ± 0.14	1.0 ± 0.0	3.6 ± 0.07
Aspergillus terreus (ATer-2)	7.8 ± 0.12	7.8 ± 0.12	1.0 ± 0.01	8.0 ± 0.13
Aspergillus terreus (ATer-3)	7.5 + 0.12	7.5 + 0.12	1.0 + 0.02	5.8 + 0.06
Aspergillus nidulans (ANid-1)	7.5 ± 0.47	7.5 ± 0.47	1.0 ± 0.02	6.6 ± 0.08
Aspergillus nidulans (ANid-2)	6.2 ± 0.42	6.2 ± 0.42	1.0 ± 0.04	6.4 ± 0.04
Aspergillus fumigates (AFum)	8.5 ± 0.38	8.5 ± 0.38	1.0 ± 0.03	6.2 ± 0.05
Alternaria solani (ASol)	6.5 ± 0.14	6.5 ± 0.14	1.0 ± 0.07	5.9 ± 0.07

Values are mean ± standard deviation (n=3)
#DCZ = Diameter of Clear Zone
*FCD = Fungal Colony Diameter

(0.14%); $CaCl_2$ (0.03%); $MgSO_4.7H_2O$ (0.03%), Urea (0.03%), Peptone (0.10%), Trace element solution (0.01%), Triton X-100 (0.02%). Trace element solution contain in 500 ml (2.5 g $FeSO_4$, 1.0 g $CoCl_2$, 1.76 g $ZnSO_4$, 0.98 g $MnSO_4$). The pH of the medium was maintained 4.8 by using conc. HCl. The medium was sterilized by autoclaving at 121^0C and 15-17 psi for 30 min. Fungi were plated on the potato dextrose agar medium and incubated at 30 ^{o}C for 7 days. Starch degrading activities were detected as clear zones after exposure to iodine solution. Diameters of clear zones and fungal colonies were evaluated by millimeter ruler.

RESULTS AND DISCUSSION

α-Amylase is frequently used in the starch processing industries for the hydrolysis of polysaccharides. Quite a lot of microorganisms can produce α-amylase enzyme. However, fungal α-amylase has dominated use in industrial fields. In fungal sources, *Aspergillus* and *Alternaria* are significant.

A total of 15 out of 100 fungal species/strains (15%) possessed α-amylase activity (table 1). All the good fungal α-amylase producers belonged to the genus *Aspergillus* (as identified by standard microbiological procedures). Fungal isolates were identified on the basis of cultural as well as morphological characteristics [7,8]. Good over-producers of α-amylase were selected for further study.

All the 15 α-amylase producing fungal strains of *Aspergillus and Alternaria* were screened for their ability to produce α-amylase. Screening for α-amylase production from fungal isolates was carried out by starch agar plate assay. α-Amylase activity was resoluted after flooding the plates with iodine solution (0.2% I_2 / 2% KI). The ability of starch degrading activities of fungi was assessed in terms of ratio of Diameter of Clear Zone (DCZ)/Diameter of Fungal Colony (DFC). Only one strain i.e. *Aspergillus niger (ANig-4)* showed the utmost ability with 9 cm clear zone. All fungal strains showed DCZ/DFC ratio equal to one (table 2).

As a result of this study, it was determined that all the strains have ability to produce α-amylase excluding *Fusarium solani and Trichoderma viridae*. Though, the selected source i.e. Starch Agar (SA) medium, was favorable for the growth of fungi and better niche for them but the results showed the variability and potentiality among isolated fungi for α-amylases production. It was also noticed that SA medium affected the fungal growth. *Aspergillus flavus (AFla-1)* and *Aspergillus terreus (ATer-1)* showed the maximum increase in their growth in SA medium as compared to their growth in Potato Dextrose Agar (PDA) medium. Unlikely, the growth of *Aspergillus nidulans (ANid-2)* and *Aspergillus terreus (ATer-2)* was slightly reduced in SA medium (table 2). Khokhar *et al*. [3] also reported the diminished growth of *Aspergillus nidulans (ANid-2)* in SA medium in contrast with control (Malt Agar medium). There are several reports available which enumerated that the fungal isolates employed for enzyme production gave the highest detectable quantities of starch hydrolysis [9,5,10] where the selection of potent species was made by plate method. However, zonation cannot in any way be correlated quantitatively with the quantity of α-amylase produced. Thus, the isolation of fungal isolates using starch plates can only be partially selected. Therefore, the assortment of better amylolytic fungal strains can be made on biochemical basis [3]. Though the production of α-amylase has been improved significantly by the utilization of hyper-producing strains of fungi as well, efforts are still being done to find newer sources of enzymes.

CONCLUSIONS

Fifteen fungal strains exhibited α-amylase production potential. Strains of *Aspergillus flavus (AFla-1)* and *Aspergillus terreus (ATer-1)* also showed hyper growth in SA medium. These new strains may have more potential for industrial uses. It can be concluded that, these fungal strains can be industrially exploited for the synthesis of α-amylase and strain improvement studies can be carried out to enhance enzyme production.

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