

ISOLATION AND SCREENING OF AMYLASE PRODUCING FUNGI

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ABSTRACT

The purpose of this study was to isolate and screened amylase producing fungi from different sources. A total of twenty amylase producing fungal isolates were obtained from Agricultural soil on the starch mineral agar medium. It was observed that only ten fungi isolates produced amylase, showed positive results for amylase production. The maximum amylase activity shown by yeast isolates F5 (2.6 cm) followed by F7 (2.4 cm). Preliminary morphological observations of selected fungal isolates colonies were black, green, white, creamy, yellow, reddish, powdery, flat, round, raised and shiny.

KEYWORDS: Amylase Producing Fungi, Fungal Isolates, Amylase Production

The uses of microorganisms have become a huge importance to Agricultural, food, textile, baking and detergent industries and sparked a large interest into the exploration of enzyme activity in microorganisms (Sivarama krishnan *et al.*, 2006). Amylases are among the most important enzymes which hydrolyze starch molecules to give diverse products including dextrin and progressively smaller polymers composed of glucose unit (Gupta *et al.*, 2009). These enzymes are among the most important enzymes for biotechnology with great significance, constitute a class of industrial enzymes having approximately 25% of the world enzyme market. Amylases can be obtained from several sources, such as plants, animals and microorganisms. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry. Major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and the fact that they are easily manipulated to obtain enzymes of desired characteristics (Karnwal and Nigam, 2013). Amylase can be obtained from several fungi, yeast, bacteria and actinomycetes; however, especially fungi, have gained much attention because of the availability and high productivity of fungi, which are also amenable to genetic manipulation. Many fungi had been found to be good sources of amyolytic enzymes. Many studies indicated that amylases of fungal origin are more stable than those of bacterial origin (Sanghvi *et al.*, 2011). Starch is the best substrate for production of yeast cells in a large scale due to its low price and easily available raw material in most regions of the world. Because most of yeasts from environments are safe (GRAS) compared to bacteria, interest in amyolytic yeasts has increased in recent years as their potential value for conversion of

starchy biomass to single-cell protein and ethanol has been recognized (Knox *et al.*, 2004).

MATERIALS AND METHODS

Isolation of amylase producing fungi Samples were collected from Agricultural soil. Serial dilution was made and plated on potato dextrose agar and starch mineral agar medium by spreading 0.1ml of the diluted sample. Then the plates were kept for incubation at 37°C for 3-4 days. The pure cultures were identified by their morphology and colony characteristics and sub-cultured. The isolates were maintained on PDA medium

Screening of potent amylase producing fungi by starch hydrolysis test

The selected fungal isolates were screened for amyolytic activity by starch hydrolysis test on starch agar plate. The selected fungal and yeast isolates were streaked on the starch agar plate and incubated at 37°C for 2-3 days. After incubation iodine solution was flooded with dropper for 30 seconds on the starch agar plate. Presence of blue color around the growth indicates negative result and a clear zone of hydrolysis around the growth indicates positive result. The isolates produced clear zones of hydrolysis were considered as amylase producers and were further investigated.

RESULTS AND DISCUSSION

A total of thirty five fungal isolates were obtained from Agricultural soil on PDA medium. These isolates were screened to obtain a black, green, red, yellow, whitish, powdery, and cottony whereas yeast isolates were white, off white, light yellow, greenish, reddish, shiny and gummy colonies. All the selected isolates were screened for production of amylase using

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starch plate method resulting in clear zone of starch hydrolysis in the Petri dishes after iodine treatment. Out of them, only ten fungal and ten yeast isolates were found to be positive for amylase production, as determined by

measuring the width of the clear zone (zone of hydrolysis) formed around the fungal colonies on starch agar medium. Fungal isolates F5 (2.6 cm) and F7 (2.4 cm), respectively.

Table 1: Phenotypic characteristics of isolated amylolytic fungi

S.No.	Fungal Isolates	Characteristics
1	F1	Black, powdery
2	F2	Greenish and powdery
3	F3	Whitish red and flat
4	F4	Light green and flat
5	F5	Green, scattered and flat
6	F6	White and raised
7	F7	Black, powdery
8	F8	Green and raised
9	F9	White and cottony
10	F10	Dark green and flat

Table 2: Zone diameter shown by isolated fungi on starch agar medium

S.No.	Fungal Isolates	Zone of Inhibition(c.m.)
1	F1	2.5
2	F2	2.3
3	F3	3
4	F4	1.8
5	F5	2.6
6	F6	2.0
7	F7	2.4
8	F8	1.5
9	F9	1.4
10	F10	1.9

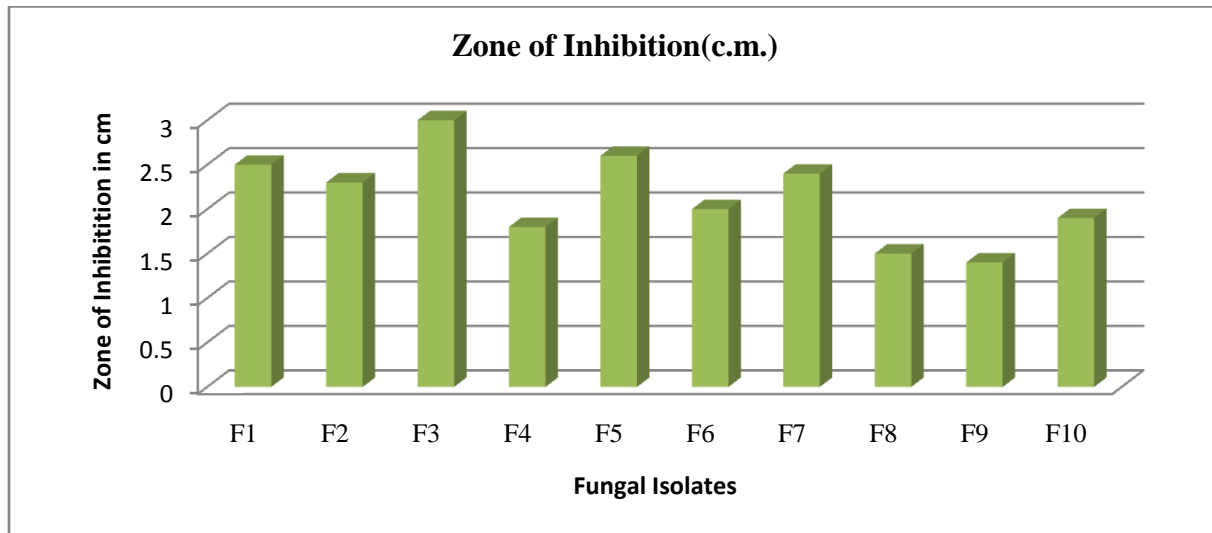


Fig.1 :Zone diameter shown by isolated fungi on starch agar medium

The Fungal isolates F5 was produced highest Zone of inhibition. Kathiresan *et al.*, (2006) reported that maximum activity was detected by *Penicillium* sp.

The maximum amylase production (185U/ml) was found with *Aspergillus fumigatus* for 6 days of incubation at 30°C (Sharma and Shukla, 2008). In conclusion twenty fungal isolated were identified for amylase production. Isolates F5 and F7 were showed highest amyolytic activity. The selected isolates can be characterized further for various useful industrial purposes.

REFERENCES

- Gupta, R., Giras, P., Mohapatra, H., Gaswami, Y.K., Chauhan, B., 2009. Microbial α - amylase: a biotechnological perspective. *Process Biochem.*, **38(11)**: 1599-1616.
- Karnwal, A. and Nigam, V., 2013. Production of amylase by isolated microorganisms and its application. *Int. J. Pharm Bio. Sci.*, **3(4)**: 354-360.
- Kathiresan, K., and Manivannan, S., 2006. α amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. *African J. Biotechnol.*,**5(10)**: 829-832.
- Knox, A.M., Preez, J.C., Kilian, S.G., 2004. Starch fermentation characteristics of *Saccharomyces cerevisiae* strains transformed with amylase genes from *Lipomyces kononenkoae* and *Saccharomyces fibuligera*. *Enzyme Microb. Technol.*, **34(5)**: 453-460.
- Sanghvi, G.V., Koyani, R.D. and Rajput, K.S. 2011. Isolation, optimization, and partial purification of amylase from *Chrysosporium asperatum* by submerged fermentation, *J. Microbiol. Biotechnol.*, **21**: 470-476.
- Sharma, D. and Shukla, A.K. 2008. Starch hydrolysis and amylase activity of *Aspergillus* and *Chaetomium*. *Asian J. Biochem.*, **3**: 284-289.
- Sivaramakrishnan, S., D. Gangadharan, K.M. Nampoothiri, C.R. Soccol and A. Pandey., 2006. Alpha amylases from microbial sources. An overview on recent developments. *Food Technol. Biotechnol.*, **44**: 173-184.

