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Amylase Activity of Saprophytic Fungi from Vegetables Wastes.

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Abstract :

Fungal Amylase is used in commercial starch modification, cleaning compounds, textile processing, fermentation pretreatment, and animal feed supplements. In present study the production of amylase by ten dominating fungal species associated with vegetable waste were tested on substrate (starch nitrate) and non substrate (glucose nitrate) medium. Amylase activity was determined by cup plate method. Among ten dominating fungi selected for the study. Fungi show maximum amylase production on the basis of zone of clearance on the cup plate method. The results showed that maximum enzyme production obtained by *Aspergillus fumigatus, Fusarium oxysporum* and *Rhizopus stolanifer* as compare to other fungi like *Aspergillus* niger A. *flavus, Alternaria alternata, Curvularia lunata, Penicillium notatum, P. spp.* and *Trichoderma viride.*

Key word : Substrate, non substrate medium, cup plate method, fungi, amylase activity, vegetable waste.

Introduction :

Amylases are a group of important enzymes which are mainly employed in the starch processing industries for the hydrolysis of polysaccharides like starch into simple sugars (Akpan et al., 1999; Damien et al., 2010)). Amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch into sugar syrups, to the production of cyclodextrins for the pharmaceutical industry . Amylases accounts for about 30% of the world's enzyme production ((Vander et al., 2002; Rita et al., 2009). 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).

The microorganisms have used for a huge importance to textile, food, baking and detergent industries and excite a large interest into the exploration of enzyme activity in microorganisms (Sivaramakrishnan *et al.*, 2006). Amylases from plants and microbial sources have been employed for centuries in brewing industry (Pal and Khanum 2010). Fungal amylases are widely used for the preparation of oriental foods (Hernandez et.al, 2006). Fungal amylases especially *Aspergillus niger* has been concentrated because of their ubiquitous nature and non fastidious nutritional requirement [Abu *et al.*, 2005]. Ikenebomeh Chickundu in the year 1997, reported that *A.niger* shows high amylolytic activity in biomass production. Submerged fermentation holds tremendous potential for the production of enzymes.

The diversity of amylases, in contrast to the specificity of their action, attracted worldwide attention in attempts to exploit their physiological and biotechnological applications (Wang et.al, 2011) Starch degrading enzymes like amylase have received great deal of attention because of their perceived technological significance and economic benefit.

The present work is undertaken for screening of amylase producing *fungi.* From vegetable waste and optimization of cultural conditions.

Materials and Methods :

Ten fungi were used that is *Aspergillus* niger *A.flavus, A. fumigatus, Alternaria alternata, Curvularia lunata, Penicillium notatum, P. spp. Rhizopus stolanifer* and *Trichoderma viride,* were used. These fungi were tested for *in-vitro* production of Amylase enzymes activity.

Production of Amylase :

Detection of amylase was also studied in the present work. The isolated fungi were grown on liquid medium containing starch 1%, KNO3 - 0.25 %, KH2PO4 - 0.1% and MgSO4.7H2O - 0.05 %. Twenty five ml of the medium was poured in 100 ml conical flasks. The flask along with medium was sterilized by autoclaving at 15 lbs pressure for 20 minutes.The autoclaved flasks were allowed to cool and isolated fungi were inoculated with 1 ml of spore suspension which was prepared from freshly growing cultures on PDA slants. The inoculated flasks were incubated at 27 ± 2 0C for 7 days. After 7 day the flasks were harvested and the contents were filtered through Whatman filter paper No. 1. The filtrate were collected in pre sterilized conical flasks and termed as crude enzyme preparation.

Amylase assay for (Cup-plate method) :

The amylase activity was determined with the help of cup late method as described by Danai (1994). In this method, 20 ml of starch nitrate medium (substrate) starch

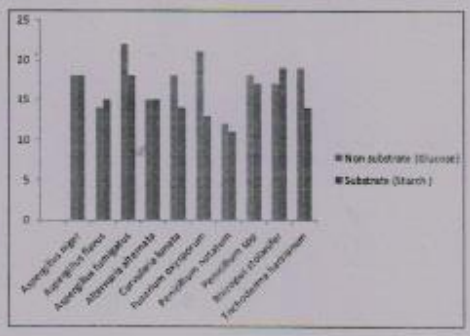
1% and agar 2% were used. The starch assay medium after sterilization was poured in each pre sterilized petriplates. The medium is allowed to cool and solid and after this a cavity was made with the help of a cork borer (8mm). The cavity was filled with 1 ml culture filtrate (crude enzyme). The plates were incubated at 27 0C for 24 hours. The plates were flooded with Lugols iodine solution as an indicator and kept it for 20 - 40 minutes. A distinct activity zones are clearly seen after removing the iodine solution with distilled water. The clear zone was resulted due to the activity of amylase and the diameter of this zone was measured and noted. Similar procedure followed for the glucose nitrate medium (non substrate).

Results and Discussion :

Table : Production of amylase on substrate and non substrate media

Fungi	Non substrate (Glucose)	Substrate (Starch)
Zone diameter in mm		
Aspergillus niger	18	18
Aspergillus flavus	14	15
Aspergillus fumigatus	22	18
Alternaria alternata	15	15
Curvularia lunata	17	14
Fusarium oxysporum	21	13
Penicillium notatum	12	11
Penicillium spp.	18	17
Rhizopus stolanifer	17	19
Trichoderma harzianum	19	14

Graph : Production of amylase by saprophytic fungi.



Detection of extracellular amylase was carried out in the same experiment and all the tested fungi secreted amylase in the medium. From the result highest amylase activity was detected in non- substrate broth medium from by *Aspergillus fumigatus* (22mm) followed by *Fusarium oxysporum* (21mm). Moderate amylase activity was detected in *Trichoderma viride*, Aspergillus niger *Penicillium species, Rhizopus stolanifer, Alternaria alternata, Aspergillus flavus and Penicillium notatum*. In substrate medium maximum amylase activity exhibited by *Rhizopus stolanifer* (19mm) followed by Aspergillus niger (18mm) and A. fumigatus (18mm). However, *Penicillium species*, *Aspergillus flavus, Alternaria alternata, Trichoderma viride Curvularia lunata, Fusarium oxysporum* and *Penicillium notatum* had a least significant amylase activity. Same result found by Kulkarni et.al, (2012) and **Sulochana et. al, (2010).**

It can concluded from table and graph, that amylase enzyme production was found to be maximum in both substrate and non substrate broth media for all tested fungi in general. *Aspergillus fumigatus* and *Fusarium oxysporum* showed highest amylase production on non substrate medium compared to the other fungi. Substrate medium also respond to high amylase production by *Rhizopus stolanifer*. Minimum production of amylase was seen in Penicillium notatum. From Vegetables waste, saprophytic fungi were highly beneficial for the production of amylase.

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