

## **Amylolytic enzyme production using agricultural residue.**

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### **Abstract:**

Four different fungal cultures i.e. *Aspergillus niger* (MTCC 1781), *Aspergillus fumigatus* (MTCC 2557), *Aspergillus oryzae* (MTCC 654), *Aspergillus paraciticus* (MTCC 411) were collected from culture collection section of School of Life Sciences, SRTMU, Nanded, Maharashtra, India. Solid State Fermentation (SSF) was carried out using mixture of four different substrate namely Wheat bran, Banana peel, Pineapple peel and Pomegranate peel. After production by four fungal cultures using this substrate it was found that *A.paraciticus* showed maximum production of amylase (41U/ml) as compare to other cultures.

### **1. Introduction:**

Microorganisms have been considered as treasure of useful and diverse array of enzymes. Enzyme producing ability of such microorganism enhances their significance and economic importance. Amongst various enzymes amylases have received a great deal of attention because of their apparent technological significance and economic benefits. These are enzymes that have been found in several microorganisms like bacteria and fungi. Amongst them fungi are considered as important producer due to economical bulk production capacity and ease of manipulation. Studies on fungal amylase especially in the developing countries

have concerted mainly on members of *Aspergillus* because of the ubiquitous nature and non-fastidious nutritional requirements of the organisms.

Thus the present study was designed in the search of cheaper carbon sources for the production of amylase enzyme by different *Aspergillus* species.

## **2. Materials and Methods:**

### **2.1 Collection and subculturing of fungi-**

Four fungal cultures i.e. *Aspergillus niger* (MTCC 1781), *Aspergillus fumigatus* (MTCC 2557), *Aspergillus oryzae* (MTCC 654), *Aspergillus paraciticus* (MTCC 411) were collected from culture collection section of School of Life Sciences, SRTMU, Nanded, Maharashtra, India. These cultures were further inoculated on sterile Potato Dextrose Agar (PDA) slants and incubated at 28<sup>0</sup>C for 48 hours. (Johnson 2014, Khairnar et al 2012)

### **2.2 Collection and pretreatment of raw material-**

Raw material substrates (Wheat bran, Banana peel, Pineapple peel and Pomegranate peel) were collected from local market and juice centre of Nanded, India. Fruit waste was washed with water to remove dirt and impurities. The washed substrates were then dried in sunlight. Dried substrates were grinded in laboratory and made it powder for further use. (sharanraj 2013, Saleem 2014)

### **2.3 Media formulation and inoculation-**

Crude medium was formulated by mixing all substrates in equvi proportion. 5gm of substrate from above mixture was taken in to four different Erlenmeyer flasks, to which 15ml of Nutrient Salt Solution(NSS) (potassium dihydrogen orthophosphate 5g/L, ammonium nitrate 5g/L, sodium chloride 1g/L and magnesium sulphate 0.5 g/L) was added to adjust moisture level. These flasks were autoclaved at 121<sup>0</sup>C for 20 min at 15 psi pressure. The flasks were cooled and inoculated with *A.niger*, *A. fumigatus*, *A.oryzae*, *A. Paraciticus* respectively and incubated at 28<sup>0</sup>C for 48 hours. ( Rathod and Pathak 2014, Singh 2014)

### **2.4 Extraction and purification-**

After incubation of 48 hours, 25 ml of distilled water was added in each flask. The contents were then mixed by shaking for 30 min on rotary shaker at 200 rpm. The slurry obtained was filtered with a whatman filter paper 1 and then centrifuged at 5000 rpm for 10 min. The filtrate obtained was treated as crude enzyme. (Pathak et al 2015, Pathak and Sardar 2014)

### **2.5 Assay of crude amylase-**

Amylase activity was estimated by the analysis of reducing sugar released during hydrolysis of 1% starch (w/v) in 0.1 M phosphate buffer by dinitro-salicylic acid (DNS) method. One unit of amylase activity was defined as the amount of enzyme that releases 1mmol of reducing sugar as glucose per minute under assay conditions. (Pathak and Rathod 2016, Pathak et al 2014, Khairnar et al 2012).

### **2.6 Effect of temperature and pH on amylase activity-**

Optimum temperature for enzyme activity was estimated by incubating the reaction mixture at different temperature (30<sup>0</sup>-50<sup>0</sup>C) for 15 min. After incubation 2ml of DNSA reagent was added to stop the reaction and absorbance was measured at 420 nm by using spectrophotometer. Effect of pH on enzyme activity was studied by performing the enzyme assay at different pH (4, 7, 9 pH) using acetate buffer and phosphate buffer. Optimum pH needed for enzyme activity was determined by incubating enzyme with buffer described above for 15 min. at 30<sup>0</sup>C. After incubation enzyme activity was estimated by DNS test. (Rani K 2012, Sharma et.al 2015, Polkade et.al (2015), Pathak and Rathod 2016)

### **Results and discussion:**

Among the four fungal cultures, *A.parasiticus* showed maximum amylase production (41 U/ml) by solid state fermentation. Partially purified amylases by above all cultures were subjected to study optimum temperature and pH for amylase activity.

Effect of temperature on enzyme activity is shown in fig.1, where *A.niger*, *A.oryzae* and *A.paraciticus* achieved highest activity at 50<sup>0</sup>C where as *A.fumigatus* showed maximum activity at 30<sup>0</sup>C

Effect of pH on amylase activity is shown in fig.2, where *A.niger* and *A.fumigatus* attained their maximum activity at pH4, *A.oryzae* and *A.paraciticus* achieved highest activity at pH 7.

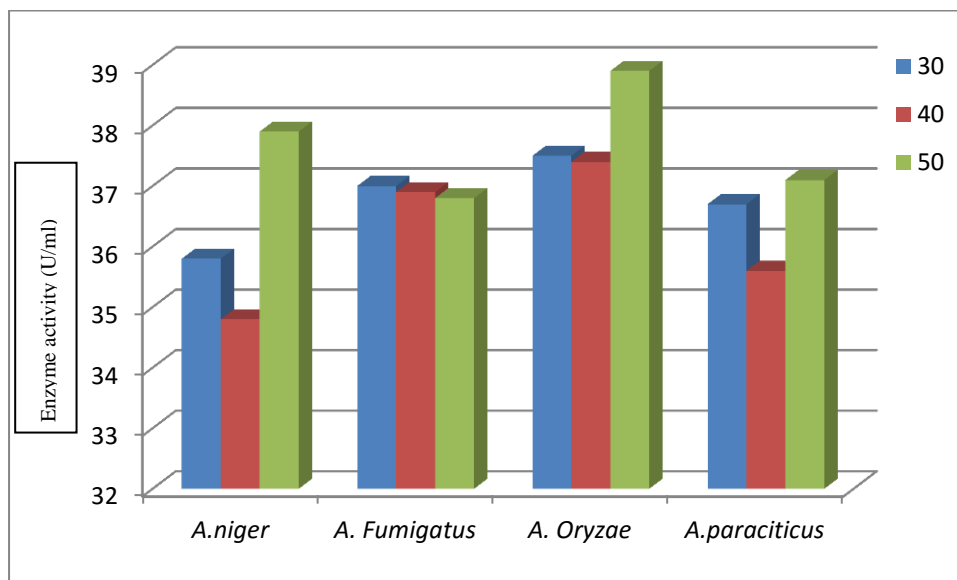


Fig.1 Effect of temperature on amylase activity

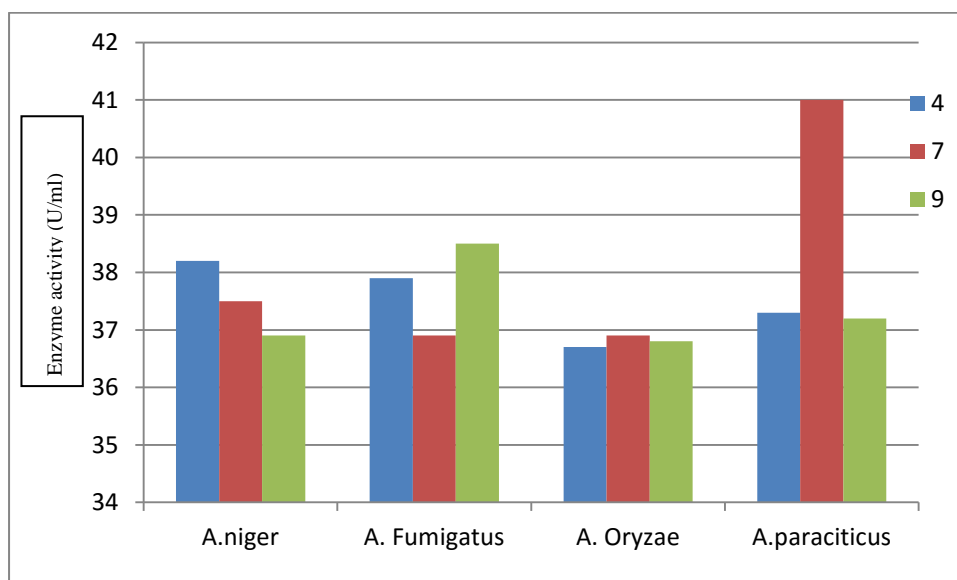


Fig.2 Effect of pH on amylase activity

### Conclusion:

Among four amylase producer *A.paraciticus* showed highest amylase production (41 U/ml). The partially purified amylase showed optimum activity at 50<sup>0</sup> temperature and neutral pH.

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