Food and Agricultural residues: Potential substrates for amylase production

1. **Anupama P. Pathak***

   Corresponding author*: anupama.micro@rediffmail.com (9404732162)

2. **Mayuri S. Sarsar**
3. **Joice T. Gavali**
4. **Madhuri H. Shendage**

Address of institution:

School of Life Sciences (DST, FIST & UGC-SAP sponsored),

Swami Ramanand Teerth Marathwada University, Nanded 431 606, Vishnupuri, Nanded,

Maharashtra, India.

Abstract:

Selected agro residues and local fruit pulp production unit residues were used as a substrate for production of fungal amylase. The product was extracted and characterized to determine optimum pH and temperature for both production and catalytic activity.

1. Introduction:

In recent years, the potential of using microorganisms as sources of industrially relevant enzymes has stimulated our interest; specially fungi have gained much attention because of availability, high productivity and which are also amenable to genetic manipulation. Fungal amylases are used for hydrolyzing carbohydrate, protein and other constitutes of soybeans, wheat into peptides, amino acid, sugars and other low molecular weight compounds.(Khan 2011, Pathak 2016, Bedan D.2014)

Fungal amylase production by solid state fermentation has been reported to be a bit cheaper because of the enzyme extraction procedures is a ray of hope. In case of SSF the cost of the substrate also plays a key role in deciding the cost of production. Agro industrial wastes have been reported to be good substrate for the cost effective production of alpha amylases and are

thus attracting researchers for using agro industrial waste as a substrate for amylase production. (Singh S.2014)

In present study optimisation of environmental and cultural conditions for the production of amylase by four different *Aspergillus* spp. has been investigated by using mixture of different cheaper substrates.

2. Materials and Methods:

2.1 Collection and subculturing of fungi-

Collection of four different *Aspergillus* spp (*Aspergillus niger* (MTCC 1781), *Aspergillus fumigatus* (MTCC 2557), *Aspergillus oryzae* (MTCC 654), *Aspergillus paraciticus* (MTCC 411))were done 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 28^0C for 48 hours. (Johnson 2014)

2.2 Collection and pretreatment of raw material-

Four different raw material substrates (Maize, 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 and grinded in laboratory and made it powder for further use. (Pathak 2015, Saleem 2014, sharanraj 2013)

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 and sterilised it. The flasks were cooled and inoculated with *A.niger*, *A. fumigatus*, *A.oryzae*, *A. Paraciticus* respectively and incubated at 28^0C for 48 hours. (Pathak et al.2015, Rathod and Pathak 2014, Singh 2014)

2.4 Extraction and purification-

After incubation, 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.

2.5 Assay of crude amylase-

Amylase activity was estimated 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. (Rani K.2012, Pathak et al 2015, Pathak and Sardar 2014)

2.6 Effect of temperature and pH on amylase activity-

The optimum pH and temperature for catalytic activity of crude amylase was determined by incubating the reaction mixture at different pH (4, 7, and 9) and temperature (30^0-50^0C). (Pathak and Rathod 2016)

3. Results and discussion:

A.parasiticus showed maximum amylase production (38U/ml) by solid state fermentation as compare to other three cultures. Effect of temperature on enzyme activity was estimated (fig.1), *A.niger, A.oryzae and A.paraciticus* achieved its highest amylase activity at 50^0C,where as *A.fumigatus* showed maximum activity at 30^0C

Effect of pH on amylase activity is shown in fig.2, where *A.niger* showed highest activity at pH 4, *A.fumigatus* attained their maximum activity at pH9, where as *A.oryzae* and *A.paraciticus* achieved highest activity at pH 7.

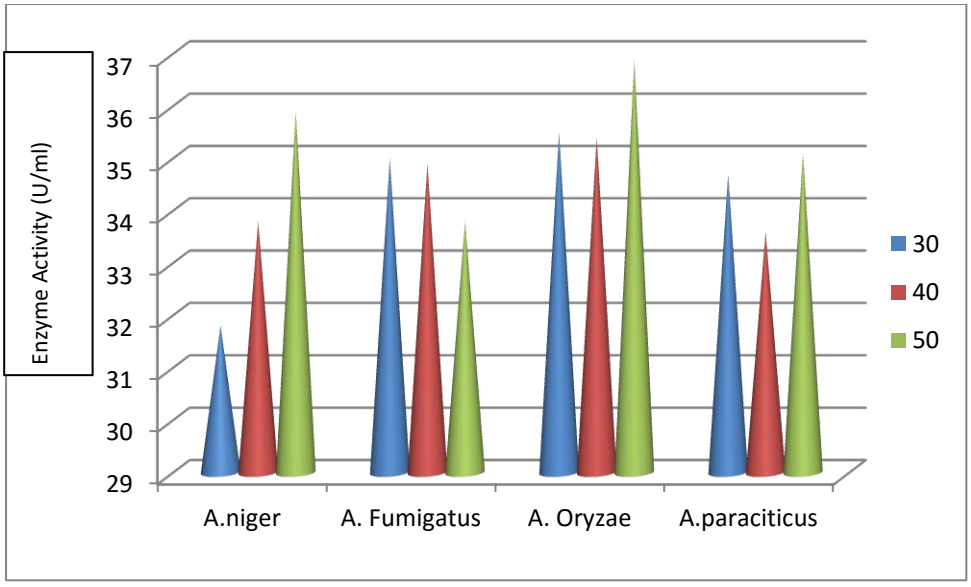

Fig.1 Effect of temperature on amylase activity

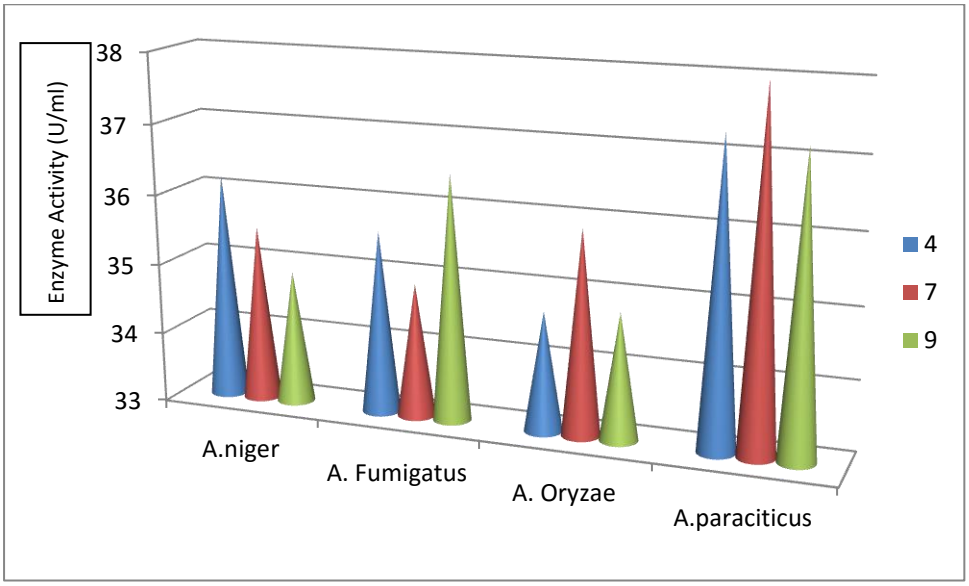

Fig.2 Effect of pH on amylase activity

Conclusion:

Among four amylase producer *A.paraciticus* showed highest amylase production (38 U/ml). The partially purified amylase showed optimum activity at 50^0 temperature and neutral pH.

Acknowledgement:

Authors are thankful to the Honorable Vice-Chancellor, S.R.T.M. University, Nanded, Maharashtra, India, for providing infrastructure and necessary facilities.

References:

1. Bedan D., Ghazi M. Aziz and Ali J. R.(2014), Optimum conditions for α- amylase production byAspergillus niger mutant isolate using solid state fermentation, Current Research in Microbiology and Biotechnology, 2,(4): 450-456
2. Hingole S.S & Pathak A.P. (2013) Report on efficient salt stable *Azospirillum* a Lonar Soda Lake isolate. Science Research Reporter, 3(2):200-203, Oct. 2013 (DOI: 10.1449)
3. Johnson F., Obeng A and Asirifi I.(2014),Amylase production by fungi isolated from Cassava processing site, Journal of Microbiology and Biotechnology Research (4),23-30
4. Khairnar, R. S., Mahabole, M. P., & Pathak, A. P. (2012). Nanoactivator mediated modifications in thermostable amylase from Bacillus licheniformis.Indian J Biochem Biophy
5. Khan J.A.and Yadav S.K (2011) Production of alpha amylases by Aspergillus niger using cheaper substrates employing solid state fermentation, International Journal of Plant Animal and Environmental Science (1), 100-108.
6. Pathak A.P, & Rathod M.G, Production and Characterization of Alkaline Protease by *Bacillus pasteurii*: a Lonar Soda Lake Isolate Innov. Res. Chem. 1(1) 22-26.
7. Pathak A.P, Rathod M.G (2016) Taxonomic assessment of thermostable amylase producer from Unkeshwar hot spring Nanded. Journal of cell and life science (in press).
8. Pathak A.P., Jethaliya C.S., Sarsar M.S., Jadhav S.R. (2015) Isolation and characterisation of potential amylase producing strain from the agriculture waste.4(4): 829-832. IJAPBC ISSN 227-4688 IF 4.976
9. Pathak A.P., Kamble G.T., Jadhav S.R., Sarsar M.S. (2015) Isolation and biochemical Characterization of potential thermostable Lipase producer from industrial effluent of oil, dairy and paper industry IJAPBC 4(4): 825-828. ISSN 227-4688 IF 4.976
10. Pathak A.P., Lohagave A.G., Rathod M.G., (2015) Exploration of paper industry effluent for isolation of efficient starchy material degrader to promote bioremediation. Int. J. Adv. Pharm. Biol. Chem. 4(4) 729-736. ISSN 227-4688 IF 4.976

11. Pathak, A. P., Sardar, A. G., & Janaj, P. C. (2014). Exploring the salted fish for salt stable amylase producing bacteria. Indian J Mar Sci,*43*, 10.
12. Pathak, A.P. & Rathod, M.G. (2016) Assessment of diverse thermostable alkaline lipase producers from Unkeshwar hot spring of Maharashtra, India. *Concept. Pure Appl. Sci. 3*(1), 1-9.
13. Polkade, A.V., Ramana, V.V., Joshi, A.A., Pardeshi, L. & Shouche, Y.S., (2015) *Rufibacter immobilis* sp. nov., a novel strain isolated from high altitude saline Lake, *Int. J. Syst. Evol. Microbiol.*, 651592-1597.
14. Rani K. (2012), Comparative study of kinetic parameters of bacterial and fungal amylases,*j.bio.innov*(1),48-57
15. Rathod, M. G., & Pathak, A. P. (2014). Wealth from waste: Optimized alkaline protease production from agro-industrial residues by Bacillus alcalophilus LW8 and its biotechnological applications. *J. Taibah Univ. Sci.*, *8*(4), 307-314.
16. Saleem, Mohsen K.H. Ebrahim (2014) Production of amylase by fungi isolated from legume seeds collectedin Almadinah Almunawwarah, Saudi Arabia. Journal of Taibah University for Science 8, 90–97.
17. Saranraj P. and Stella D. (2013) Fungal Amylase - A Review, International Journal of Microbiological Research 4 (2), 203-211
18. Sardar A.G, Pathak A.P., Exploring the microbiota of solar saltern of Mulund, Mumbai, India. Indian J Mar Sci, 43(4) 634-641
19. Sharma, A., Shouche, Y.S., Kumar, B. & Kulkarni, G., (2009) Characterization and identification of *Geobacillus* spp. isolated from Soldhar hot spring site of Garhwal Himalaya, India, *J. Basic Microbiol.*, 49187-194.
20. Sharma, B., Agrawal, R., Singhania, R. R., Satlewal, A., Mathur, A., Tuli, D., & Adsul, M. (2015). Untreated wheat straw: Potential source for diverse cellulolytic enzyme secretion by *Penicillium janthinellum* EMS-UV-8 mutant. *Bioresour. Technol.*, *196*, 518-524.
21. Singh S., et.al(2014)Production of Fungal Amylases Using Cheap, Readily Available Agriresidues, for Potential Application in Textile Industry, Hindawi Publishing CorporationBioMed Research International.
22. Sonalkar, V. V., Mawlankar, R., Ramana, V. V., Joseph, N., Shouche, Y. S., & Dastager, S. G. (2015). *Bacillus filamentosus* sp. nov., isolated from sediment sample. *Antonie van Leeuwenhoek*, *107*(2), 433-441.