# Production of amylase by *Penicillium* sp. using solid state fermentation method and inexpensive agricultural residues

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## Abstract

Industrially important amylase producer was isolated from soil samples of S.R.T.M. University campus in Nanded district of Maharashtra and identified based on its morphological and microscopic characters as *Penicillium* sp. AFS3. Production of amylase was carried out by using wheat and soybean husks in solid state fermentation. 81.80 U/mL production of amylase was recorded after 96 h. AFS3 amylase has shown remarkable catalytic efficiency at pH range 4-6 and temperature range 30-50 $^{o}$C as well. Therefore amylase from *Penicillium* sp. AFS3 can be used in different biotechnological industries where hydrolysis of starch is carried out at low pH and elevated range of temperature.

**Keywords:** acid amylase, *Penicillium* sp., soybean husk, wheat husk, amylase activity

## 1. Introduction

Amylases are classified into acidic, neutral and alkaline amylases based on their optimal pH for activity. Amylases have industrially important applications in detergent, agricultural, starch liquefaction, saccharification, textile, paper, food and baking industries (Sharma and Satyanarayana, 2012).

Utilization of agro-industrial residues has become popular to achieve cost effective production of industrially important enzymes like amylases, proteases, cellulases, lipases etc. and in waste management. Some previously reported agro-industrial residues used for amylase production are wheat bran, rice bran, fruit stalks, husks of cereals and oil seeds and deoiled cakes of coconut, sesame, groundnut, palm kernel and olive. At present agro-industrial residues are considered to be good substrates for the enzyme production in solid state fermentation (SSF). Fungi are suitable microorganisms for SSF according to the theoretical concept of water activity. Moreover fungal biomass is easy to separate from fermented media. Some advantages of SSF over submerged fermentation are superior productivity, simpler technique, lower capital investment, lower energy requirement, less water input and output, better product recovery and lack of foam build up (Sharma and Satyanarayana, 2012; Sharma, 2013; Sharma and Satyanarayana, 2013; Sharma *et al*., 2015).

Therefore, in present investigation we have selected agricultural residues for production of amylase in SSF from a newly isolated efficient amylase producing fungi.

## 2. Materials and Methods

### 2.1. Isolation, screening and identification

Soil samples were collected from S.R.T.M. University campus and mixed in equal proportion to form a composite soil sample. pH of composite soil sample was recorded. Aliquots of diluted soil samples were spread on yeast extract peptone dextrose (YEPD) agar, potato dextrose agar and Czapek Dox agar supplemented with streptomycin at 10 mg/mL concentration to suppress the growth of bacteria and plates were incubated at 30 $^{o}$C for 5 days in an incubator (Kumar make, Mumbai). Morphologically distinct colonies were isolated and further spot inoculated on starch agar (pH 7) plates to screen for extracellular amylase production. These plates were incubated at the same temperature and incubation period. After incubation, Grams iodine solution was poured on the surface of agar and the plates were

observed either for presence or absence of zone of clearances around the grown cultures. The efficient amylase producer fungi was selected based on the size of zone formed. Efficient amylase producer was further identified by recognition of its morphological and microscopic features (Sharma *et al*., 2009; Khairnar *et al*., 2012; Kolekar *et al*., 2013; Visagie *et al*., 2014; Sardar and Pathak, 2014; Dastager *et al*., 2015; Gavali and Pathak, 2015; Pathak and Rathod, 2015; Polkade *et al*., 2015; Pathak et al., 2015a,b,c; Pathak *et al.*, 2016).

**2.2. Production and extraction of amylase**

Fresh culture of selected isolate was wetted by adding 10 mL of nutrient salt solution ($KH_2PO_4$ 3 g/L, ammonium nitrate 5 g/L, NaCl 1 g/L and $MgSO_4$ 0.5 g/L). The spores were scratched and 5 mL of homogeneous spore suspension was inoculated in fermentation medium prepared in a Petri dish containing each 10 g of powdered form of wheat and soybean husks (pH adjusted to 7). The spore suspension was mixed thoroughly in medium to form cake. Solid state fermentation was carried out at 30 $^{o}$C for 96 h. After incubation, the pieces of cake were transferred in a conical flask and 150 mL of distilled water was added. The flask was shaken in orbital shaking incubator (Remi make, Mumbai) for 2 h at 200 rpm. The contents from the flask were filtered and centrifuged at 10,000 rpm and 4 $^{o}$C for 10 min. The supernatant was used as source of crude amylase from the selected isolate (Rathod and Pathak, 2014a).

**2.3. Qualitative amylase assay**

Crude amylase (1 mL) was added to the test tube containing 1 mL of 1 % starch solution prepared in 0.2 M phosphate buffer (pH 7.0). This mixture was incubated at 30 °C for 10 min. The produced reducing sugar was measured using the dinitrosalicylic acid method (Ghorbel *et al.*, 2009). One unit activity of amylase was defined as the amount of enzyme

required to liberate 1 µmol $min^{-1}$ $mL^{-1}$ of reducing sugar expressed as maltose equivalent, under the assay conditions (Pathak and Sardar, 2014).

### 2.4. Characterization of partially purified amylase

#### 2.4.1. Effect of temperature on catalytic activity of amylase

Catalytic activities of crude amylase from the selected isolate were recorded at temperature range 20-60 $^{o}C$ with an interval of 10 $^{o}C$ under standard assay conditions as described previously (Bhunia *et al*., 2013; Pathak and Rathod, 2014).

#### 2.4.2. Effect of starch concentration on catalytic activity of amylase

Catalytic activities of crude amylase from the selected isolate were recorded by performing assay at starch concentrations 2-20 mg/mL at pre-determined temperature (Pathak *et al*., 2014a, b; Rathod and Pathak, 2014b; Pathak and Rathod, 2016).

#### 2.4.3. Effect of pH on catalytic activity of amylase

Catalytic activities of crude amylase from the selected isolate were recorded by performing assay at pH range 4-8 with an interval of 1 pH unit by using 0.1 M citrate buffer and phosphate buffer at pre-determined temperature and starch concentration (Pathak and Sardar, 2012, 2014; Pathak and Rathod, 2013; Sonalkar *et al*., 2015).

## 3. Results and Discussion

### 3.1. Isolation, screening and identification of amylase producer

pH of composite soil sample was recorded as 7.3. Two fungal colonies were appeared on yeast extract peptone dextrose (YEPD) agar and designated as AFS1 and AFS2. Eight fungal colonies were appeared on potato dextrose agar and designated as AFS3 to AFS10. Five fungal colonies were appeared on Czapek Dox agar and designated as AFS11 to AFS15. After screening, AFS3 has shown luxuriant growth and a largest zone of clearance on starch agar (Fig. 1). Therefore AFS3 was selected for its identification and production of amylase. AFS3 was identified as *Penicillium* sp. by comparing its morphological and microscopic

characteristics with the standard description as given in A manual of soil fungi, by Gilman (1957), Industrial mycology by Onions *et al.* (1981) and Compendium of soil fungi by Domsch *et al*. (1980).

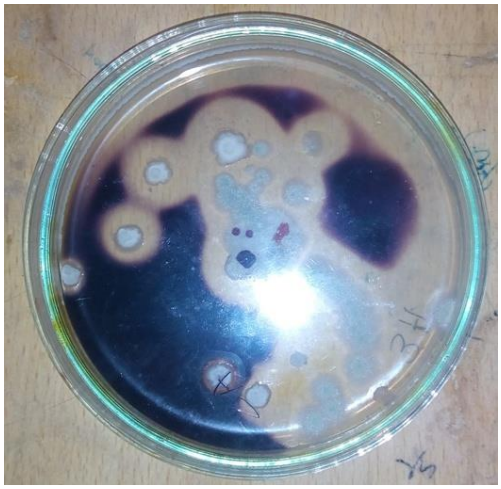

Fig. 1: Screening of AFS3 isolate for amylase production

### 3.2. Production of amylase

Production of crude amylase from *Penicillium* sp. AFS3 was recorded 81.80 U/mL after 96 h of incubation period.

### 3.3. Characterization of crude amylase

#### 3.3.1. Effect of temperature on amylase activity

Crude amylase from *Penicillium* sp. AFS3 exhibited maximum catalytic efficiency at 40 $^{o}$C (84.72 U/mL) temperature followed by at 30 $^{o}$C (81.80 U/mL), 50 $^{o}$C (78.88 U/mL), 60 $^{o}$C (75.95 U/mL) and 20 $^{o}$C (49.66 U/mL) (Fig. 2).

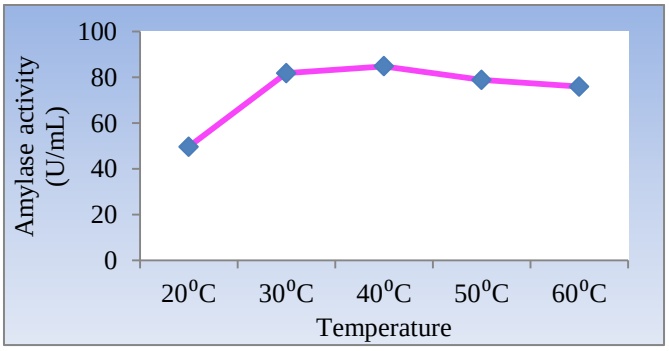


Fig. 2: Effect of temperature on catalytic activity of *Penicillium* sp. AFS3 amylase

### 3.3.2. Effect of different starch concentrations on amylase activity

Crude amylase from *Penicillium* sp. AFS3 exhibited maximum catalytic efficiency at 18 mg/mL starch concentration (292.14 U/mL) followed by at 16 mg/mL (233.71 U/mL), 14 mg/mL (178.21 U/mL), 12 mg/mL (87.64 U/mL), 20 mg/mL (84.72 U/mL), 10 mg/mL (35.05 U/mL), 8 mg/mL (29.21 U/mL), 6 mg/mL (17.52 U/mL), 4 mg/mL (14.60 U/mL) and 2 mg/mL (5.84 U/mL) (Fig. 3).

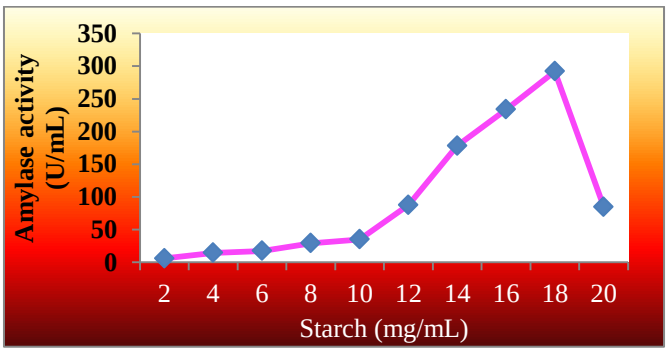


Fig. 3: Effect of different concentration of starch on catalytic activity of *Penicillium* sp. AFS3 amylase

### 3.3.3. Effect of pH on amylase activity

Crude amylase from *Penicillium* sp. AFS3 exhibited maximum catalytic efficiency at pH 5 (934.87 U/mL) followed by at pH 4 (904.65 U/mL), pH 6 (262.93 U/mL), pH 7 (233.71 U/mL) and pH 8 (175.28 U/mL) (Fig. 4).

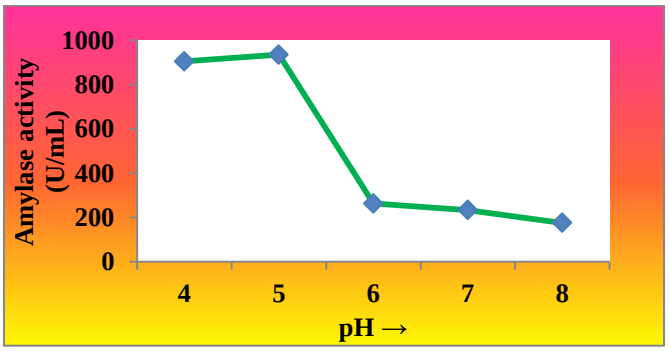


Fig. 4: Effect of different pH on catalytic activity of *Penicillium* sp. AFS3 amylase

## 4. Conclusions

Efficient amylase producer was isolated and identified as *Penicillium* sp. AFS3. Remarkable production of amylase (81.80 U/mL) was recorded by using soybean and wheat husks. *Penicillium* sp. AFS3 amylase exhibited maximum catalytic efficiency at pH 5, 40 $^{o}$C and 18 mg/mL starch concentration. Therefore amylase from *Penicillium* sp. AFS3 can be used for starch liquefaction where the native pH of starch slurry is 3.2-4.5 in different biotechnological industries.

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## 6. References

Bhunia, B., Basak, B., Bhattacharya, P., & Dey, A. (2013). Process engineering studies to investigate the effect of temperature and pH on kinetic parameters of alkaline protease production. *Journal of bioscience and bioengineering*, *115*(1), 86-89.

Dastager, S. G., Mawlankar, R., Mual, P., Verma, A., Krishnamurthi, S., Joseph, N., & Shouche, Y. S. (2015). *Bacillus encimensis* sp. nov. isolated from marine sediment. *International journal of systematic and evolutionary microbiology*, *65*(5), 1421-1425.

Domsch, H.K., Anderson, T.H. and Gams, W. 1980. Compendium of Soil Fungi. Volume I. Academic Press, A subsidiary of Harcourt Brace Jovanovich, Publishers, London.

Gavali, J., & Pathak, A. (2015). Isolation and Identification Of Cold Active Amylase Producer From Gastro Intestinal Tract Of *Channa Striata*. *Current Trends in Aquaculture*, *53*.

Gilman, J.C. 1957. A Manual of Soil Fungi. (Second Indian Reprint), Oxford and IBH Publishing Co. New Delhi. India.

Ghorbel, R. E., Maktouf, S., Massoud, E. B., Bejar, S., & Chaabouni, S. E. (2009). New thermostable amylase from *Bacillus cohnii* US147 with a broad pH applicability. *Applied biochemistry and biotechnology*, *157*(1), 50-60.

Khairnar, R. S., Mahabole, M. P., & Pathak, A. P. (2012). Nanoactivator mediated modifications in thermostable amylase from *Bacillus licheniformis*. *Indian J Biochem. Biophy*.49, 468-471.

Kolekar, Y. M., Pawar, S. P., Adav, S. S., Zheng, L., Li, W., Shouche, Y. S., Dastager, S. G., Kodam, K. M. (2013). *Alishewanella solinquinati* sp. nov., isolated from soil contaminated with textile dyes. *Current microbiology*, 67(4), 454-459.

Pathak A. P., Parware S. U., Rathod M. G. (2016). Isolation and identification of industrially important salt stable amylase producer. *International Journal for Research in Biology and Pharmacy Research* (in press).

Pathak A. P., Lohagave A. G., Rathod M. G. (2015a). 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.

Pathak A.P., Jethaliya C.S., Sarsar M.S., Jadhav S.R. (2015b) Isolation and characterisation of potential amylase producing strain from the agriculture waste. *Int. J. Adv. Pharm. Biol. Chem*, 4(4): 829-832.

Pathak, A.P., Lodge, N., Gavali, J.T., & Rathod, M.G. (2015c). Isolation and characterization of cold-active protease producer from ice factory samples. *Int. J. Adv. Pharm. Biol. Chem*, *4*, 751-754.

Pathak, A. P., & Rathod, M. G. (2013). Production and characterization of alkaline protease by *Bacillus pasteurii*: a Lonar Soda Lake Isolate. *Innovative Research in Chemistry*, *1*(1), 22-26.

Pathak, A. P., & Rathod, M. G. (2014). Exploration of Unkeshwar hot springs in Maharashtra for thermostable amylase producer. *Res. Rev. Biosci*, *8*(7), 269-276.

Pathak, A. P., & Rathod, M. G. (2015). Cultivable bacterial diversity of terrestrial thermal spring of Unkeshwar, India. *Journal of Biochemical Technology*, *5*(4), 814-818.

Pathak, A. P., & Sardar, A. G. (2012). Isolation and characterization of carotenoid producing Haloarchaea from solar saltern of Mulund, Mumbai, India. *Indian J. Natural Products Resources*, *3*, 483-488.

Pathak, A. P., & Sardar, A. G. (2014). Isolation and characterization of salt stable protease producing archaea from marine solar saltern of Mulund, Mumbai. *Indian J. Mar. Sci*, *43*(3), 412-417.

Pathak, A. P., Rathod, M. G., & Rampurkar, V. V. (2014a). An eco-friendly approach for thermostable amylase production using *Bacillus firmus* APP6: a hot spring isolate, Asiatic J. *Biotech. Res*, *4*(4), 101-105.

Pathak, A. P., Sardar, A. G., & Janaj, P. C. (2014b). Exploring the salted fish for alkaline protease producing bacteria. *Indian J. Mar. Sci.*, *43*, 10 (in press).

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.

Polkade, A. V., Ramana, V. V., Joshi, A., Pardesi, L., & Shouche, Y. S. (2015). *Rufibacter immobilis* sp. nov., isolated from a high-altitude saline lake. *International journal of systematic and evolutionary microbiology*, *65*(5), 1592-1597.

Rathod, M. G., & Pathak, A. P. (2014a). Isolation and identification of alkaline protease producer from selected alkaline habitat. *International Journal of Innovative Biological Research*, *3*(1), 1-6.

Rathod, M. G., & Pathak, A. P. (2014b). Wealth from waste: Optimized alkaline protease production from agro-industrial residues by *Bacillus alcalophilus* LW8 and its biotechnological applications. *Journal of Taibah University for Science*, *8*(4), 307-314.

Sardar, A. G., & Pathak, A. P. (2014). Exploring the microbiota of solar saltern of Mulund, Mumbai, India. *Indian J. Mar. Sci., 43*, 634-641.

Sharma, A., Satyanarayana, T. (2013). Microbial acid-stable amylases: Characteristics, genetic engineering and applications. *Process Biochemistry*, *48*, 201‑211.

Sharma, A., Satyanarayana, T. (2012). Production of Acid-Stable and High-Maltose-Forming α-Amylase of *Bacillus acidicola* by Solid-State Fermentation and Immobilized Cells and Its Applicability in Baking. *Appl Biochem Biotechnol*, *168*, 1025‑1034.

Sharma, A., Pandey, 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. *Journal of basic microbiology*, *49*(2), 187-194.

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. *Bioresource technology*, *196*, 518-524.

Sharma, N. K. (2013). Chemical composition of oilseed cakes and deoiled cakes in Nepal. *Online Journal of Animal and Feed Research (OJAFR)*, *3*(1), 74-76.

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.

Onions, A.H.S., Allsopp, D. and Eggins, H.O.W. 1981. Smith's Introduction to Industrial Mycology (Seventh Edition), Edward Arnold Publishing Co.

Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G., Seifert, K.A., Varga, J., Yaguchi, T. & Samson, R. A. (2014). Identification and nomenclature of the genus *Penicillium*. *Studies in mycology*, *78*, 343-371.