# Exploration of Indian food for isolation of thermostable amylase producer

1. **Anupama Pathak***

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

2. **Joice Gavali**

3. **Mayuri Sarsar**

4. **Aparna Paikrao**

**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**

An organism having ability to produce extracellular salt stable and thermostable amylase was isolated from mango pickle. Identification of the bacterium was done based upon morphological and biochemical tests as *Bacillus megaterium.* Further this strain was screened

for the amylolytic activity in presence of salt using starch agar plates with 11 % NaCl and results indicated that it was amylase producer. It has produced 1.63 U/ml of polyextremophilic amylase. Highest catalytic activity of the enzyme was recorded at temperature of 60°C and pH 9 and enzyme remained stable with 11% salt concentration.

## 1. Introduction

Amylase (E.C.3.2.11) is an enzyme which belongs to the group of hydrolase and which catalyzes starch into sugars. It is also known as Glycoside hydrolyses. Amylases are classified into α-amylase, β- amylase and Glucoamylase. Due to its wide areas of potential applications in various fields such as pharmaceuticals, brewing, detergent, sugar production,

baking, textile, waste water treatment and paper manufacturing, it occupies 25 % of the enzyme market (Pathak et al 2014;Gavali and Pathak 2015).

The organisms which can tolerate more than one extreme like high salt concentration and temperature are called as Polyextremophiles. Production of enzyme from such organism is major field of investigation as these enzymes are stable at more than one harsh condition (Pathak AP and Rathod MG 2014; Satyanarayna et al 2005)

We therefore aimed at exploration of polyextremophiles for production and characterization of amylase enzyme.

**Keywords**: amylase, Polyextremophiles, mango pickle, *Bacillus megaterium.*

## 2. Materials and Methods

### 2.1. Isolation and screening of amylolytic polyextremophiles

Homemade mango pickle was collected. Aqueous Suspension was prepared by adding 1 gm of pickle sample in 100 ml sterile distilled water. Sample was filtered and 100 µl of filtrate was spread on Nutrient Agar plates containing 11% W/V NaCl and incubated at 40$^{o}$C for 24 h. Incubated plates were observed and isolated colony was selected for further experiments. Selected isolate was inoculated on starch agar plates containing 11% W/V NaCl to confirm alkaline amylase production. Efficient amylase producer was selected and subjected for its morphological and biochemical characterization (Pathak et al 2015; Pathak AP and Rathod MG 2015; Rathod MG and Pathak AP 2014; Hingole SS and Pathak AP 2013; Pathak AP and Sardar AG 2012).

### 2.2. Identification of isolate

The selected isolate was subjected for gram staining and motility. Carbohydrate utilization profile of isolate was studied by inoculating pure culture in basal nutrient medium in which additional carbohydrates such as sucrose, fructose, maltose and lactose were used. IMViC test and Catalase test were also carried out (Gavali JT and Pathak AP 2016; Polkade et al 2015; Sharma et al 2015; Sonalkar et al 2015; Sharma et al 2009).

**2.3. Production and Extraction of amylase**

1 ml of 24 h active culture of isolate was inoculated into 500 ml amylase production medium containing Starch 2.5 g, Yeast Extract 2.5 g, $(NH_4)_2SO_4$ 1.2 g, $MgSO_4.7H_2O$ 0.1 g, $KH_2PO_4$ 1.5 g, $CaCl_2.2H_2O$ 0.125g and NaCl 25g. The production was carried out at 40$^oC$ for 48h at 120 rpm in orbital shaking incubator. The mycelium was removed and whole fermented broth was filtered through Whatman filter paper 41µm.The filtrate was then used to determine enzyme assay (Pathak AP and Rathod MG 2016; Pathak et al 2014; Khairnar et al 2012).

**2.4. Enzyme Assay**

Amylase activity was estimated by the analysis of reducing sugar released during hydrolysis of 1% (w/v) starch in 0.1 M sodium citrate buffer by the Dinitrosalicylic 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 condition (Pathak AP and Rathod MG 2016; Pathak et al 2014; Khairnar et al 2012).

**2.5. Characterization of crude Amylase**

**2.5.1. Effect of pH and temperature on catalytic activity of crude amylase**

Enzyme activity was determined at various pH and temperature values ranging from 3 to 9 and 10 to 60$^oC$ respectively. The optimum pH and temperature for catalytic activity of crude amylase was determined.

**3. Results and Discussion**

### 3.1. Isolation, screening and identification

One efficient polyextremophilic amylase producer was isolated from homemade mango pickle. The isolate showed luxuriant growth at 11 % NaCl concentration. The selected isolate was studied for their morphological and biochemical characteristics. According to Bergey's Manual of Systematic Bacteriology, the isolate was identified as *Bacillus megaterium.*

Results of morphological characters, staining features and biochemical characters are given in table 1.

**Table 1: Morphological, Microscopic, Biochemical and Physiological Characterization of isolate**

| **Colony characteristics** | **Isolate** |
|---|---|
| Shape | Round |
| Size(mm) | 0.1 |
| Color | Milky white |
| Elevation | Flat |
| Margin | Regular |
| Surface | Smooth |
| Consistency | Sticky |
| **Microscopic Features** | |
| Cell Shape | Cocci |
| Cell size (Micron) | 2.0 |
| Cell Motility | Motile |
| Grams nature | +ve |
| Sporulation | Spore former |
| **IMViC Test** | |
| Indole test | - |

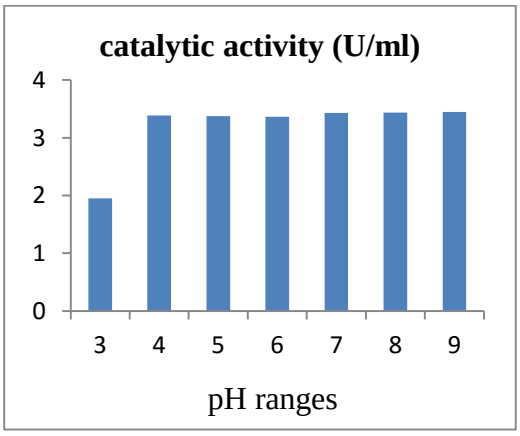
| | |
|---|---|
| Methyl red test | + |
| VP test | - |
| Citrate utilization test | + |
| **Sugar utilization pattern** | |
| Maltose | - |
| Fructose | + |
| Sucrose | - |
| Lactose | + |
| **Enzyme profile** | |
| Catalase | + |
| Amylase | + |
| Protease | + |

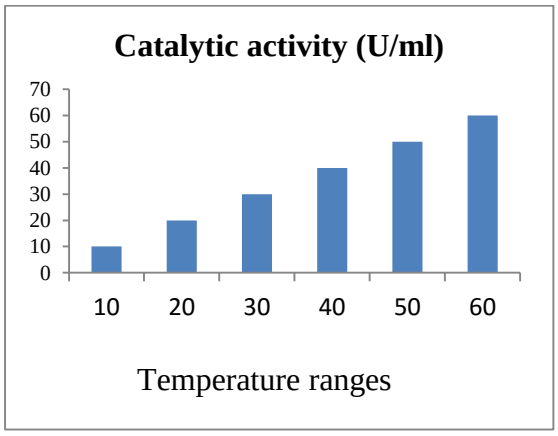
### 3.2. Production of salt and high temperature stable amylase

*Bacillus megaterium* produced 1.63 U/ml of salt and high temperature stable amylase after 48 h of production period.

### 3.3. Characterization of crude salt and high temperature stable amylase

Optimum catalytic activity of crude amylase derived from *Bacillus megaterium* was recorded at pH 9 and temperature 60$^{o}$C.



## 4. Conclusion

Microbial enzymes are abundant and ubiquitous in the halophillic environment, where they serve essential functions that promote microbial survival. Therefore exploration of these enzymes could be of great significant in various biotechnological applications. This amylase stable at high salt and high temperature can be used in several processes where high salt concentrations are present.

## 5. Acknowledgment

Authors are thankful to Hon'ble Vice Chancellor, S.R.T.M. University, Nanded for providing infrastructure and necessary facilities.

## 6. References

1. 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.
2. Gavali, J., & Pathak, A. (2015). 17. Isolation and Identification of Cold Active Amylase Producer From Gastro Intestinal Tract Of Channa Striata.*Current Trends in Aquaculture*, *53*.
3. 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.
4. Satyanarayana, T., Raghukumar, C., & Shivaji, S. (2005). Extremophilic microbes: Diversity and perspectives. *Current science*, *89*(1), 78-90p.
5. Pathak A.P., Gavali J., Gunjakar P (2015) Isolation and Characterization of Psychrophilic Carotenoid producers from gastrointestinal tract of *Rohtee vigorsi*. IJAPBC 4(4): 821-824 ISSN 227-4688 IF 4.976

6. 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
7. 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
8. Pathak A.P., Lodge N., Gavali J., Rathod M.G., (2015) Isolation and characterization of cold-active protease producer from ice factory samples. Int. J. Adv. Pharm. Biol. Chem. 4(4) 751-754. ISSN 227-4688 IF 4.976
9. 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
10. Pathak A.P.and Gavali J.T. (2015) Isolation and identification of carotenoid producer from gastrointestinal tract of *Rohtee vigorsi*. International journal of advanced research in basic and applied sciences. Current trends in aquaculture UGC and SRTMUN sponsored.
11. Pathak, A. P., & Rathod, M. G. (2015). Cultivable bacterial diversity of terrestrial thermal spring of Unkeshwar, India. *J. Biochem. Tech.*, *5*(4), 814-818.
12. Rathod, M. G., & Pathak, A. P. (2014). Isolation and Identification of Alkaline Protease Producer from Selected Alkaline Habitat. *International Journal of Innovative Biological Research*, *3*(1), 1-6.
13. 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.

14. 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)
15. Pathak, A. P., & Sardar, A. G. (2012). Isolation and characterization of carotenoid producing Haloarchaea from solar saltern of Mulund, Mumbai, India. *Indian J. Nat. Prod. Resour*, *3*, 483-488.
16. Gavali, J., & p Pathak, A. (2016). Isolation and Identification of Cold Active protease producing Psychrophilic Bacterium. *Journal of Cell and Life Sciences*, *2*(1), 1-4.
17. Polkade, A.V., Ramana, V.V., Joshi, A.A., Pardeshi, L. & Shouche, Y.S., *Rufibacter immobilis* sp. nov., a novel strain isolated from high altitude saline Lake, *Int. J. Syst. Evol. Microbiol*., 65(2015) 1592-1597.
18. 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.
19. 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.
20. Sharma, A., Shouche, Y.S., Kumar, B. & Kulkarni, G., Characterization and identification of *Geobacillus* spp. isolated from Soldhar hot spring site of Garhwal Himalaya, India, *J. Basic Microbiol.*, 49(2009) 187-194.
21. 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.

22. Pathak AP, Rathod MG (2016) Taxonomic assessment of thermostable amylase producer from Unkeshwar hot spring Nanded. Journal of cell and life science (in press).
23. 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.
24. 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.
25. Khairnar, R. S., Mahabole, M. P., & Pathak, A. P. (2012). Nanoactivator mediated modifications in thermostable amylase from Bacillus licheniformis.Indian J Biochem Biophy.