ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF AN AMYLASE PRODUCING THERMOPHILIC BACTERIUM FROM GARDEN SOIL

Isha Kohli¹, Rakesh Tuli² and Ved Pal Singh¹*

¹Applied Microbiology and Biotechnology laboratory, Department of Botany, University of Delhi, Delhi-110 007, India. ² National Agri-Food Biotechnology Institute, Mohali, Punjab-160 071, India. * E-mail: vpsingh_biology@rediffmail.com

Abstract: A thermophilic bacterium (strain Th-3), which was able to degrade starch maximally, was isolated from the soil of Delhi University Botanical Garden. The temperature and pH optima and incubation time for the maximum growth of isolated bacterium were found to be 45°C, pH 6.0 and 24 h, respectively. In addition to amylase production, the bacterium had also shown positive results for production of protease, lipase and catalase as well as for nitrate reduction. Th-3 exhibited maximum amylolytic activity, when assayed at 45°C at pH 6.5 in the culture harvested at 24 hours of growth. The bacterium was non-pathogenic, as tested on Himedia sheep blood agar plates. The strain was sensitive to most of the antibiotics tested, except ampicillin and kanamycin to which it had shown resistance. The biochemical, microscopic and morphological features of the isolated strain indicated that it was Gram-positive, rod-shaped and closely resembled *Bacillus* species.

Keywords: Amylase, Amylolytic activity, Starch degrading enzyme, Thermophilic amylase, Thermophilic bacterium

REFERENCES

Aiba, S.; Kitai, K. and Imanaka, T. (1983). Cloning and expression of thermostable α -amylase gene from *Bacillus stearothermophilus* in *Bacillus stearothermophilus* and *Bacillus subtilis*. Applied *Environmental Microbiology*, **46**: 1059- 1065.

Aiyer, P.V. (2004). Effect of C:N ration on alpha amylase production by *Bacillus licheniformis* SPT 27. *African Journal of Biotechnology*. **3**: 519-522.

Ammor, M. S., Belén Flórez, A. and Mayo, B. (2007). Antibiotic resistance in non- enterococcal lactic acid bacteria and bifidobacteria. *Food Microbiology*, **24**: 559-570.

Asgher, M.; Asad, J.M.; Rahman, S.U. and Legge, R.L. (2007). A thermostable α-amylase from a moderately thermophilic *Bacillus subtilis* strain for starch processing. *Journal of Food Engineering*, **79**: 950-955.

Banargee, R. and Bhattacharya, B.C. (1992). Extracellular alkaline protease of newly isolated *Rhizopus oryzae. Biotechnology letter*, **14**: 301-304.

Bauer, A.W.; Kirby W.M.; Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**: 493-496.

Bernfeld, P. (1955). Enzymes of starch degradation and synthesis. *Advances in Enzymology*, **12**: 379-428. **Bose, K. and Das, D.** (1996). Thermostable α amylase production using *Bacillus lichenifornis* NRRL B1438. *Indian Journal of Experimental Biology*, **34**: 1279-1282.

Bozic, N.; Ruiz, J.; Santin, J.L. and Vujcic, Z. (2011). Optimization of the growth and α -amylase production of *Bacillus subtilis* IP 5832 in shake flask and laboratory fermenter batch cultures. *Journal of the Serbian Chemical Society*, **76**: 965-972.

Deb, P.; Talukdar, S.A.; Mohsina, K.; Sarker, P.K. and Sayem, A. (2013). Production and partial

characterization of extracellular amylase enzyme from *Bacillus amyloliquefaciens* P-001. *Springerplus*, doi:10.1186/2193-1801-2-154.

Dey, T.B. and Banerjee, R. (2012). Hyperactive αamylase production by *Aspergillus oryzae* IFO 30103 in a new bioreactor. *Letter in Applied Microbiology*, **54**: 102-107.

Gupta, R.; Gigras, P.; Mohapatra, H.; Goswami, V.K. and Chauhan, B. (2003). Microbial α -amylases: a biotechnological perspective. *Process Biochemistry*, **38**: 1599-1616.

Kathiresan, K. and Manivannan, S. (2006). αamylase production by *Penicillium fellutanum* isolated from mangrove rhizospheric soil. *African Journal of Biotechnology*, **5**: 829-832.

Mendu, D.R.; Ratnam, B.V.V.; Purnima, A. and Ayyanna, C. (2005). Affinity chromatography of αamylase from *Bacillus licheniformis. Enzyme and Microbial Technology*, **37**:712–717.

Mishra, S. and Behera, N. (2008). Amylase activity of starch degrading bacteria isolated from soil receiving kitchen wastes. *African Journal of Biotechnology*, 7: 3326-3331.

Pandey, A.; Nigam, P.; Soccol, C.R.; Soccol, V.T.; Singh, D. and Mohan, R. (2000). Review: Advances in microbial amylases. *Biotechnology and Applied Microbiology*, **31**: 135-152.

Roses, R.P. and Guerra N.P. (2009). Optimization of amylase production by *Aspergillus niger* in solid-state fermentation using sugarcane bagasse as solid support material. *World Journal of Microbiology and Biotechnology*, **25**: 1929-1939.

Samanta, A.; Bera, P.; Khatun, M.; Sinha, C.; Pal, P.; Lalee, A. and Mandal, A. (2012). An investigation on heavy metal tolerance and antibiotic resistance properties of bacterial strain *Bacillus* sp. isolated from municipal waste. *Journal of Microbiology and Biotechnology Research*, **2**: 178-189. Smitt, J.P.; Rinzema, J.; Tramper, H.; Van, M. and Knol, W. (1996). Solid state fermentation of wheat bran by *Trichoderma reesei* QMQ414. *Applied Environmental Microbiology*, **52**: 179-184.

Swain, M.R.; Kar, S.; Padmaja, G. and Ray, R.C. (2006). Partial characterization and optimization of production of extracellular α -amylase from *Bacillus subtilis* isolated from culturable cow dung microflora. *Polish Journal of Microbiology* **55**: 289-296.

Swargiari, BN. and Baruah, PK. (2013). Isolation and screening of amylolytic *penicillium* species from soil. *International Journal of Pharma and Bio Sciences*, 4: 575-581.

Vijayaraghavan, P.; Remya, C.S. and Prakash, S.G. (2011). Production of α -Amylase by *Rhizopus microsporus* using agricultural by-products in solid state fermentation. *Research Journal of Microbiology*, **6**: 366-375.