Insight Bacteriology 2 (1): 1-2, 2013
ISSN 2044-8961 / DOI: 10.5567/BACTERIOL-IK.2013.1.2

A *Bacillus* spp. A Probable Source of Thermophilic α-Amylaseproduction at Industrial Scale

Idress Hamad Attitalla
Department of Microbiology, Faculty of Sciences, Omar Al-Mukhtar University,
Box 919, Al-Bayda, Libya

Enzymes are catalytic proteins which in a biological system increases the rate of anabolic or catabolic reactions. Enzymes due to their non toxic, easily biodegradable properties are used in industries to replace the various toxic chemicals which were previously used to produce industrial products (Hasan *et al*., 2010). For example nowadays, in place of synthetic compounds detergent industry extensively uses several protease, amylases, lipases and cellulases enzymes due to their reliable and efficient stain removing activities. Among these enzymes, α-amylase is an industrially important and highly demanded enzyme; it mediates the breakdown of starch into different valuable compounds e.g. maltose, glucose and fructose (Van der Maarel *et al*., 2002). Due to high usage of starch its use has increased in different industries e.g., baking, detergents etc. In textile industry it is used to remove excessive starch from the fabric (Ahmed and Kolisis, 2011), starch is used as a thickener to print the fabric (Teli *et al*., 2009). Moreover, its use in baking industry improves the bread quality by giving it a smooth texture and delays aging (Zeng *et al*., 2011). Thus α-amylase has gained a significant importance in textile, baking and detergent industry, therefore its large quantities are required to fulfill the industrial demands. But for proper application in industry, enzymes able to endure high temperatures are preferred (Soares *et al*., 2011). To fulfill its demands, it is obtained from different sources e.g. fungi and bacteria; its production is affected by the genetics of organism used and the environmental conditions (Esfahanibolandbalaie *et al*., 2008; Kiran and Chandra, 2008). These environmental factors are temperature, pH and the concentration of growth nutrients. Moreover the enzymes of specific favorable behavioral conditions (based upon its temperature, pH stability and reactivity) are obtained from the organisms of respective growth environment (Arikan, 2008). As α-amylase obtained from a thermophilic (high temperature) bacteria showed 70% stability at 60°C with pH range of 10.0-11.0 and it showed resistance against many chelators. Therefore to obtain α-amylase of high quality (able to endure high temperature) and activity, bacteria may act as an efficient organism.

Bacillus species are mostly studied to produce large quantity of α-amylase with high thermostability (Joshi, 2011). Recently, Ahmed and Ibrahim (2011) identified *Bacillus licheniformis* as a potent producer of a thermostable α-amylase, from the soils of Khartoum State. They isolated 270 strains of *Bacillus* out of which four showed significant production of amylase. Among these 4 strains, strain number 2 cleared highest diameter of starch solution zone with highest amylolytic activity. This strain then subjected to morphological and biochemical examination for identification and named as *B. licheniformis*. It was a spore forming rod shaped gram+ve bacteria, which metabolized nitrogen, starch, urea and citrate. It showed anaerobic as well as aerobic growth and produced several carbohydrates (e.g., glucose, mannose, xylose, sucrose etc.) in the process of fermentation. Its ability to metabolize starch might be due to α-amylase presence. In Horikoshi II growth medium it showed maximum growth between 24-84 h of cultivation, in this time its highest growth and highest amylolytic activity was at 36 h. After this for about two days its enzymatic and growth activity was low, which later got peak at 72 h. Hence, both activities showed a similar time pattern and can be said that α-amylase production by *B. licheniformis* was associated with its growth. But the α-amylase might not be the major enzyme in *B. licheniformis* and to verify its dominant presence the 3,5-Dinitrosalicylic acid (DNS) test was done. In DNS test *B. licheniformis* crude enzyme showed the similar time pattern which was followed by growth and amylolytic activities. Furthermore, it showed maximum activity with 1% concentration of substrate and High-Performance Liquid Chromatography (HPLC) revealed the presence of glucose and maltose as starch catalytic products. These carbohydrates confirm the presence of α-amylase as its dominant enzyme. Thus *B. licheniformis* was majorly producing a thermostable α-amylase which can tolerate high temperature (50-90 °C). It showed huge optimal pH range o f 6-9 and within this range its maximum value was at pH 9. The optimum temperature and pH for its activity were 70 °C and 10, respectively. Hence α-amylase

produced by *B. licheniformis* was a thermostable, alkaline enzyme which could be suitable for high temperature reactions of industries.

Enzymes are an important part of biological system and nowadays they are playing a remarkable role in industries. Like many other enzymes, α-amylase is a commercially required enzyme which catalyses the breakdown of starch and produces small carbohydrates e.g. glucose. It helps in removing stains so, is used in detergents and is also used in baking industry due to its good effects on bread texture. Whereas in textile its catabolic quality helps in removing waste starch from fabric, hence it has considerable importance in many industries. Some experiments on bacteria highlighted them as its major producer, which can produce α-amylase possessing highly thermostable and alkaline pH stable characteristics. According to Ahmed and Ibrahim (2011), *B. licheniformis* strain out of total 270 examined *Bacillus* species was its efficient producer, which was confirmed by HPLC and DNS analysis. Its optimum temperature was 70°C and was highly active at pH 9. It was also active at even higher temperature (90°C) and pH 10. Thus *B. licheniformis* can be used to produce large quantities of an alkaline and thermostable industrially required α-amylase enzyme.

REFERENCES

Ahmed, A.A. and H.M. Ibrahim, 2011. A potential new isolate for the production of a thermostable extracellular α-amylase. J. Bacteriol. Res., 3: 129-137.

Ahmed, H.E. and F.N. Kolisis, 2011. An investigation into the removal of starch paste adhesives from historical textiles by using the enzyme α-amylase. J. Cult. Heritage, 12: 169-179.

Esfahanibolandbalaie, Z., K. Rostami and S.S. Mirdamadi, 2008. Some studies of α-amylase production using *Aspergillus oryzae*. Pak. J. Biol. Sci., 11: 2553-2559.

Hasan, F., A.A. Shah, S. Javed and A. Hameed, 2010. Enzymes used in detergents: Lipases. Afr. J. Biotechnol., 9: 4836-4844.

Joshi, B.H., 2011. A novel thermostable alkaline α-amylase from *Bacillus circulans* PN5: Biochemical characterization and production. Asian J. Biotechnol., 3: 58-67.

Kiran, K.K. and T.S. Chandra, 2008. Production of surfactant and detergent-stable, halophilic and alkalitolerant alpha-amylase by a moderately halophilic *Bacillus* sp. Strain TSCVKK. Applied Microbiol. Biotechnol., 77: 1023-1031.

Soares, J.C., P.R. Moreira, A.C. Queiroga, J. Morgado, F.X. Malcata and M.E. Pintado, 2011. Application of immobilized enzyme technologies for the textile industry: A review. Biocatal. Biotrans form., 29: 223-237.

Teli, M.D., P. Rohera, J. Sheikh and R. Singhal, 2009. Use of *Amaranthus* (Rajgeera) starch vis-a-vis wheat starch in printing of vat dyes. Carbohydr. Polym., 76: 460-463.

Van der Maarel, M.J.E.C., B. van der Veen, J.C.M. Uitdehaag, H. Leemhuis and L. Dijkhuizen, 2002. Properties and applications of starch converting enzymes of the α-amylase family. J. Biotechnol., 94: 137-155.

Zeng, J., H.Y. Gao, L. Jin, Z.P. Zhang, H.R. Zhang, 2011. Texture of wheat bread improved by á-amylase and glucose oxidase. Adv. Mater. Res., 236-238: 35-38.