

*Full Length Research Paper*

# Production and characterization of amylase produced by *Bacillus megaterium* isolated from a local yam peel dumpsite in Minna, Niger State

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Ten grams (10 g) of soil obtained from a local yam peel dumpsite in Minna, Niger State was analyzed. The bacterial isolates identified were *Bacillus megaterium* and *Staphylococcus aureus*. *Bacillus megaterium* was used in the present study for the production of amylase enzyme as it recorded the largest zone of activity. Amylase activity was determined using DNS method. Highest yield of amylase by *B. megaterium* was obtained after 48 h of incubation. The optimum temperature for the activity of amylase produced was obtained at 60 °C with a concentration of 0.55 mg/ml. Optimum pH was obtained at pH 7.0 with a concentration of 0.67 mg/ml. The results showed that *B. megaterium* is a good producer of extracellular amylase at high temperatures which could be an indication that amylase produced would be thermostable.

**Key words:** *Bacillus megaterium*, yam peel dump site, amylase, optimum activity.

## INTRODUCTION

Amylases are hydrolases that function by the breakdown or hydrolysis of starch into reducing fermentable sugars, mainly maltose and reducing non fermentable or slowly fermentable dextrans. Numerous microorganisms like *Saccharomyces capsularia*, *Bacillus coagulans*, *Bacillus* sp. HOP-40, and *Bacillus megaterium* 16 M, have been used for a -amylase production by solid state fermentation (SSF) using agro-industrial residues (Modi et al., 1994). Amylases are important enzymes used in the food industry for hydrolysis of starch to yield glucose syrups amylase –rich flour and proper formation of dextrin during baking. Also they are employed in textile industry for removal of starch sizing. In the production of detergents, amylases are used to dissolve starches from fabrics (Burtis and Ashwood, 1999). Amylases constitute a class of industrial enzymes, which alone form approximately 25% of the enzyme market covering many industrial processes such as sugar, textile, paper, brewing, distilling industries and pharmaceuticals (Mamo

et al., 1999; Pandey et al., 2000; Oudjeriouat et al., 2003). Starch degrading enzymes like amylase have received great deal of attention because of their perceived technological significance and economic benefits. Evidences of amylase in yeast, moulds and bacteria have been reported and their properties documented (Buzzini and Martini, 2002; Oyeleke and Oduwole, 2009). Studies on bacteria amylase especially in the developing countries have concentrated mainly on *Bacillus* spp probably because of the simple nature and nutritional requirements of this organisms (Omemu et al., 2005; Ajayi and Fagade, 2006; Oyeleke and Oduwole 2009).

Amylases are important enzymes employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents. Starch degrading enzymes like amylase have received great deal of attention because of their perceived technological significance and economic benefits. Members of the genus *Bacillus* are heterogeneous and they are very versatile in their adaptability to the environment. There are various factors that influence the nature of their metabolic processes and

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enzymes produced (Ajayi and Fagade, 2006; Oyeleke and Oduwole 2009). A great deal of attention is being given to thermophilic and extremely thermophilic microorganisms and their enzymes (Ajayi and Fagade, 2006; Oyeleke and Oduwole, 2009). *Bacillus* species produce a large variety of extra cellular enzymes, such as amylases, which have significant industrial importance (Cordeiro et al., 2003). In the same vein, bacterial enzymes are known to possess more thermostability than fungal amylases (Eke and Oguntimhin, 1992). Therefore, the objectives of the study were to isolate and identify amylolytic bacteria from local yam peel waste dumpsites, perform partial characterization of the enzyme production and its properties with regard to the effect of temperature and pH, and to maximize the reserve of huge foreign exchange spent on the importation of amylase enzyme.

## MATERIALS AND METHODS

### Isolation of bacterial strains from soil sample

One gram of the soil sample was weighed into 9 ml of sterile distilled water and then placed into a water bath and maintained at 90°C for 1h. Serial dilution of  $10^{-5}$  of 1 ml of the mixture was introduced into a sterile Petri dish using the pour plate method into cool molten nutrient agar which had been fortified with 1% starch. The poured plate was incubated at 37°C for 24 h. Different bacterial strains obtained as typical colonies were sub-cultured and thereafter purified several times on fresh nutrient agar plates. The isolated pure strains were screened for the production of extracellular amylase production using starch agar as described by Bertrand et al. (2004). The pure cultures were streaked at the center of the sterile starch agar plates and the plates were incubated at 37°C for 24 h. After incubation, 1% iodine solution was over-layered on the agar plates and observation was made to note the substrate utilized zone around the colony. The strain that formed a better zone was taken for further study. Based on Bergey's manual of systematic bacteriology (Garrity et al., 2001), the isolated strain was identified as *B. megaterium*.

### Inoculum preparation

A two day actively growing cultures of the test isolates were maintained as stock cultures in starch agar slants and was grown at room temperature for 5 days and stored at 4°C for regular subculturing. The media used had the following composition (in g /L): Starch 1.0, Peptone 6.0,  $KCl_2 \cdot 4H_2O$  0.5 and  $MgSO_4 \cdot 7H_2O$  0.5, served as inoculation media for all the experiments. The pure culture was inoculated into sterile inoculation medium and was incubated at 37°C on a rotary shaker over night. The fresh over night culture was used as an inoculum for the growth study and the production of enzyme.

### Comparative determination of growth

Two hundred and fifty milliliter (250 ml) of sterile starch medium broth was prepared and 2% of inoculum was aseptically added in to the medium and incubated at 37°C in shaking incubator. Culture growth was determined by JENWAY, 6305 spectrophotometer at

540 nm.

### Extraction of enzyme from bacteria (recovery of amylase)

The selected strains of isolates were propagated at 37°C for 24 h in 50 ml of 8% (w/v) of starch medium placed in 250 ml Erlenmeyer flasks and placed in a shaker incubator operated at 120 rpm at 30°C. The extracellular enzyme solutions were obtained by centrifugation at 5000 rpm for 20 min using a high speed centrifuge. The supernatant obtained was collected and used as enzyme source and used in enzyme assays.

### Demonstration of enzyme activity

Amylase activity was assayed as described by Ramakrishna et al. (1982) by pipetting 0.5 ml of culture extract "enzyme" into test tubes and 1 ml of 1% soluble starch in citrate phosphate buffer having a pH of 6.4. The reducing sugars liberated were estimated by the 3, 5 – dinitrosalicylic acid (DNSA) method Bertrand et al. (2004). The reaction mixture was incubated in a water bath at 40°C for 30 min. A blank consisting of 1 ml of soluble starch in citrate – phosphate buffer (pH 6.4) was also incubated in a water bath at the same temperature and time with the other test tubes. The reaction was terminated by adding 1 ml of DNSA reagent in each test tube and then immersing the tubes in a boiling water bath for 5 min after which they were allowed to cool and 5 ml of distilled water was added. The absorbance for all the test tubes was measured at 540 nm with spectrophotometer (JENWAY, 6305).

### Effect of temperature on enzyme activity

The optimal temperature for activity was determined by assaying activity of the enzyme at different temperature ranges of 30, 40, 50, 60, 70, 80, 90 and 100°C for 48 h. Samples were taken at regular intervals and analyzed for amylase activity.

### Effect of pH on enzyme activity

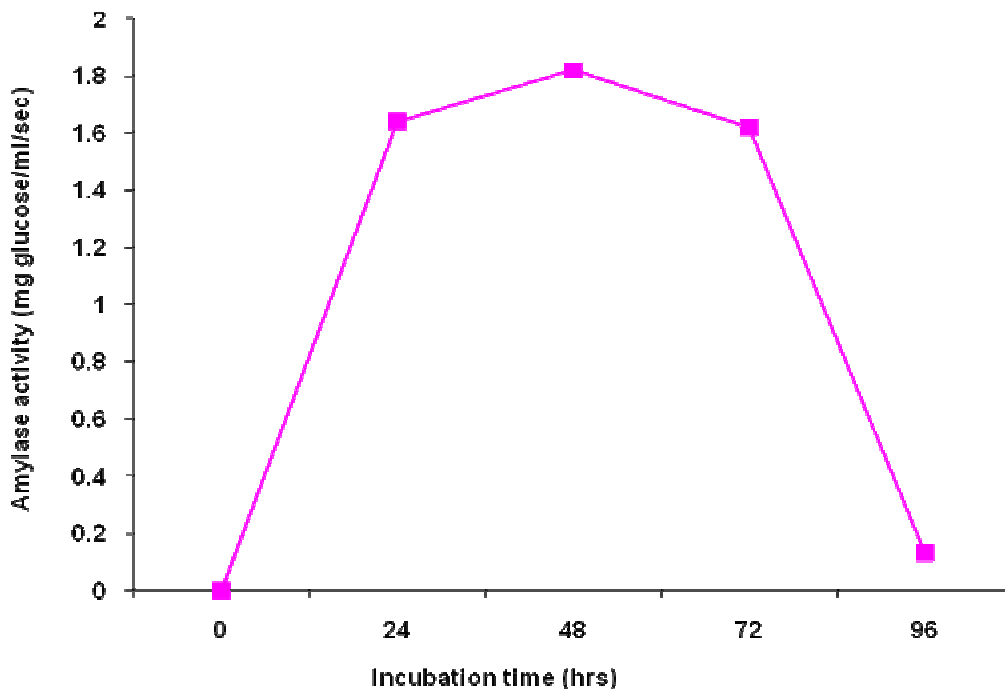
The optimum pH for enzyme (amylase) activity was determined by running the assay activity between pH ranges of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10. The enzyme activity for each pH was determined using the method of Bertrand et al. (2004). Adjustments of the pH were done by addition of hydrochloric acid (0.1N) and 0.1N sodium hydroxide to achieve acidity and alkalinity respectively. The flasks were incubated at 37°C for 48 h. Samples were taken at regular intervals and analyzed for amylase activity.

### Effect of incubation period on amylase production

The present study was carried out at different incubation periods such as 72, 96, 120 and 144 h, after which assay was determined by Dinitrosalicylic method (Bertrand et al., 2004).

## RESULTS

Three isolates that had creamy colonies and were all catalase positive, gram positive, rod shape; hydrolyze starch (blue blackish colour) and utilized glucose. The



**Figure 1.** Effect of incubation period on the yield of amylase enzyme produced by *B. megaterium*.

bacterial isolates were identified and characterized as *B. megaterium* and *Staphylococcus aureus*. *B. megaterium* recorded the largest zone of clearance and was selected for further studies.

The effect of incubation period on the yield of amylase enzyme is shown in Figure 1. *B. megaterium* had amylase activity of 1.64 mg/ml at 24 h, this was followed by an increase in amylase activity at 48 h (1.82 mg/ml), which recorded maximum amylase activity. After this hour, there was a decline in amylase activity at 72 h (1.62 mg/ml) and 96 h (0.13 mg/ml).

The effect of temperature on activity of amylase produced by *B. megaterium* (Figure 2). At 30°C, *B. megaterium* had amylase activity of 0.47 mg/ml. There was an increase in amylase activity as the temperature reached 40°C, this was followed by a sharp decrease in amylase activity at 50°C (0.39 mg/ml). Further increase in temperature led to on sharp increase in amylase activity at 60°C (0.55 mg/ml) which recorded maximum activity. Further increase in temperature after this led to a decrease in amylase activity.

The effect of pH on the activity of amylase produced by *B. megaterium* (Figure 3). At pH 4, *B. megaterium* had amylase activity of 0.46 mg/ml. As the pH increased to pH 5, there was a slight decrease in activity which was then followed by a rise in amylase activity as pH approached 6. Maximum activity was recorded at pH 7 with an activity of 0.57 mg/ml. Further increase in pH led to a gradual decrease in amylase activity.

## DISCUSSION

The production and characterization of amylase produced by *B. megaterium* isolated from a local yam peel dumpsite in Minna was examined. The result reveals that *Bacillus* spp forms one of the major soil saprophytes as reported by Olajuyigbe et al. (2005) who identified twenty five bacterial isolates from soil of which nine were identified as *Bacillus* species. The occurrence of amylolytic organisms in soil agrees with earlier reports of Rehena (1989), Adebisi and Akinyanju (1998) and Omemu et al. (2005), that soil is known to be a repository of amylase.

The effect of incubation period on growth (Figure 1) of *B. megaterium* reveals that it had a maximum growth of 1.410 nm after 48 h upon incubation in starch medium in the shaker incubator. This reflects that *B. megaterium* metabolize the nutrients in the culture medium. The effect of incubation period on amylase production revealed that the highest enzyme production period for *B. megaterium* was at 48 h (Figure 2) with maximum amylase activity of 1.82 mg/ml. Activity was lost gradually after this hour. Similar reports were observed by Nehra et al. (2004); Oyeleke and Oduwole (2009). Increase in incubation period resulted in decrease in the production of amylase by *B. megaterium*. This may be due to the fact that after maximum production of amylase enzyme (maximum incubation time), there was production of other by-products and a depletion of nutrients. These by-products

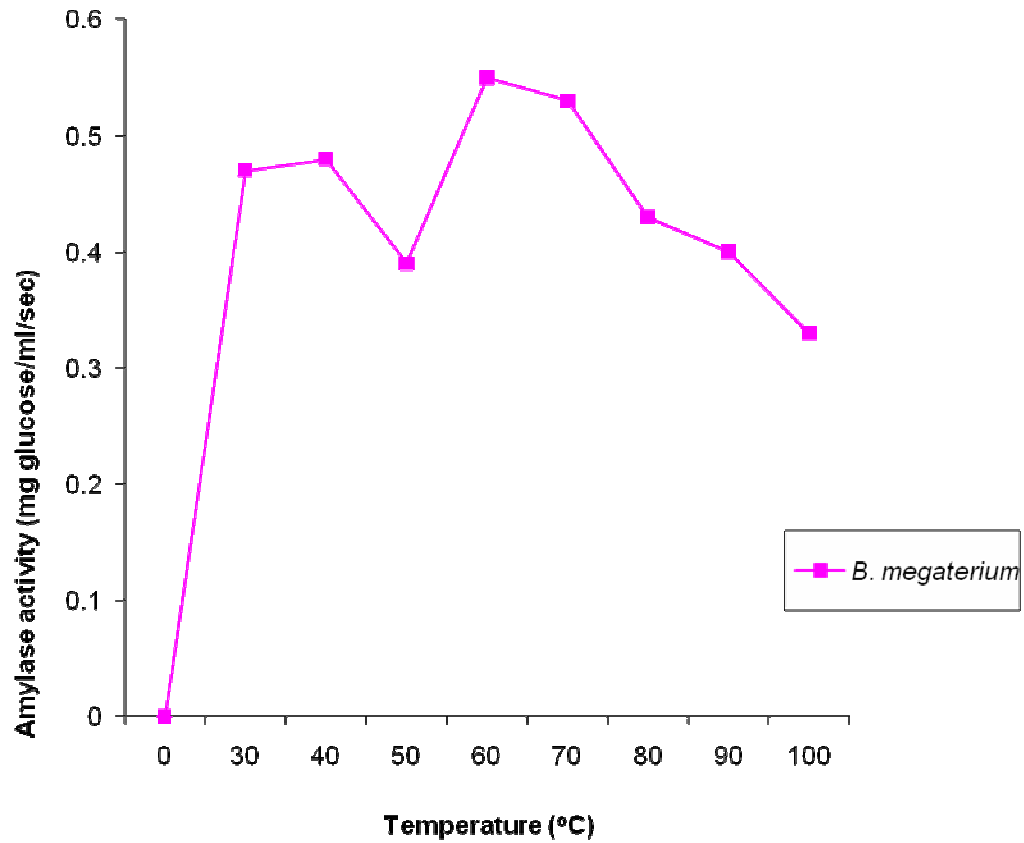


Figure 2. Effect of temperature on activity of amylase produced by baaterial isolates.

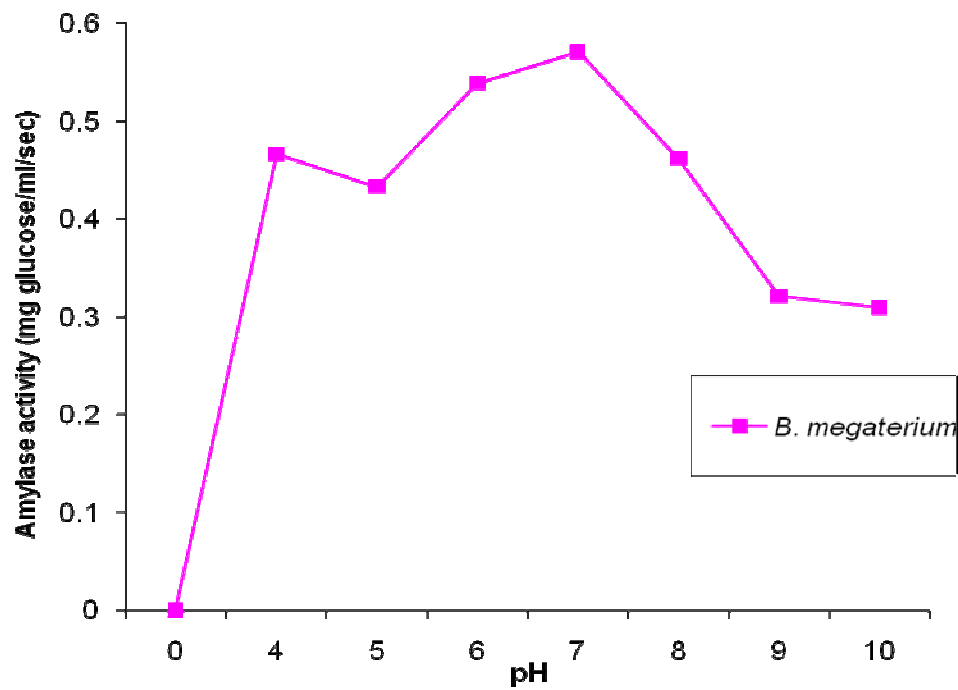


Figure 3. Effect of pH on the activity of amylase produced by *B. megaterium*.

inhibited the growth of the organisms and hence, enzyme formation (Ali, 1992; Gupta et al., 2008).

Temperature and pH are the most important factors, which markedly influence enzyme activity. *B. megaterium* had an enzyme activity of 0.47 mg/ml at 30°C. As the temperature increased to 40°C, the enzyme activity increased (0.48 mg/ml). This was followed by a sharp decrease in enzyme activity at 50°C. Further increase in temperature was followed by an increase in enzyme activity. Optimum amylase activity was recorded at 60°C, which reached a peak (0.55 mg/ml). Further increase in temperature beyond this led to a decrease in amylase activity. This agrees with the report of Oyeleke and Oduwole (2009); Daniel et al. (2010), that during isomerisation, temperature is preferably maintained within the range 20 – 90°C and the best activity is obtained with 50 – 75°C.

Maximum amylase yield (0.67 mg/ml/min) was achieved at pH 7 by *B. megaterium* although, pH 4 -10 supported amylase production (Figure 3). The results suggest that maximum amylase activity was observed at neutral pH. But considerable amount of activity (0.46 – 0.31 mg/ml/min) was obtained at alkaline pH showing the wide application nature of the amylase enzyme identified. This result is in agreement with Oyeleke and Oduwole (2009) and Daniel et al. (2010) who stated in their report that most bacterial enzymes function between a pH range of 6 and 8. The results suggest that there is stimulation of enzymes at a neutral pH. Similar observations were made by Olajuyigbe and Ajele (2005) who recorded optimum pH of 8.0 for *Bacillus* species. The study revealed the potential of agricultural wastes' capability to produce amylase by *B. megaterium*. It can be concluded that these isolate can be industrially exploited for the synthesis of amylase enzyme.

## REFERENCES

- Adebiyi CAB, Akinyanju JA (1998). Thermophilic amylase production from the soil. *Niger. J. Sc. Technol.*, 11(1): 30-38.
- Ajayi AO, Fagade OE (2006). Growth pattern and structural nature of amylases produced by some *Bacillus* species in starchy substrates. *Afr. J. Biotechnol.*, 5(5): 440-444.
- Ali GAO (1992). Formation of protease by *Aspergillus fumigatus* and *Penicillium* spp. *J. King Saud Univ. Sc.*, 4(2): 127.
- Bertrand TF, Fredric T, Robert N (2004). Production and partial characterization of a thermostable amylase from *Ascomycetes* yeast strain isolated from starchy soil. McGraw-Hill Inc., New York, pp. 53-55.
- Burtis CA, Ashwood ER (1999). *Tietz Textbook of Clinical Chemistry* (3<sup>rd</sup> edn). W.B. Saunders Company, California, pp. 689-698.
- Buzzini P, Martini A (2002). Extracellular enzymatic activity profiles in yeast and yeast – like strains isolated from tropical environments. *J. Appl. Microbiol.*, 93: 1020-1025.
- Cordeiro CAM, Martinas MLL, Lucaino A (2003). Production and properties of alpha amylase from thermophilic *Bacillus* specie. *Braz. J. Microbiol.*, 33: 1-3.
- Daniel RM, Peterson ME, Danson MJ (2010). The molecular basis of the effect of temperature on enzyme activity. *Biochem. J.*, 425(2): 353-360.
- Garrity GM, Brenner DG, Krieg NR, Staley JT (2001). *Bergey's Manual of Systematic Bacteriology*, second edition, Springer, New York.
- Gupta A, Gupta VK, Modi DR, Yadava LP (2008). Production and characterization of  $\alpha$ - amylase from *Aspergillus niger*. *J. Biotechnol.*, 7: 551-556.
- Mamo G, Gashe BA, Gessesse A (1999). A highly thermostable amylase from a newly isolated thermophilic *Bacillus* sp.WN11. *J. Appl. Microbiol.*, 86: 557-560.
- Modi HA, Patel KC, Ray RM (1994). In *Solid State Fermentation* (ed. Pandey, A.), Wiley Eastern Publishers, New Delhi, pp. 137-141.
- Nehra KS, Singh A, Sharma J, Kumar R, Dhillon S (2004). Production and Characterization of alkaline protease from *Aspergillus* species and its compatibility with commercial detergents. *Asian J. Microbiol. Biotechnol. Environ. Sc.*, 6: 67-72.
- Olajuyigbe FM, Ajele TO (2005). Production dynamics of extracellular protease from *Bacillus* species. *Afr. J. Biotechnol.*, 4(8): 776-779.
- Omemu AM, Akpan I, Bankole MO, Teniola OD (2005). Hydrolysis of raw tuber starches by amylase of *Aspergillus niger* AMO7 isolated from the soil. *Afr. J. Biotechnol.*, 4(2): 342-344.
- Oudjeriouat N, Moreau Y, Santimone M, Svensson B, Marchis-Mouren G, Desseaux V (2003). On the mechanism of amylase: Acarbose and cyclodextrin inhibition of barley amylase isozymes. *Eur. J. Biochem. FEBS.* 270: 3871-3879.
- Oyeleke SB, Oduwole AA (2009). Production of amylase by bacteria isolated from a cassava waste dumpsite in Minna, Niger State, Nigeria. *Afr. J. Microbiol. Res.*, 3(4): 143-146.
- Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000). Advances in microbial amylases. *J. Biotechnol. Appl. Biochem.*, 31: 135-152.
- Rehena F, Ventaksubbiah L, Naud K (1989). Preliminary studies on the production of thermostable  $\alpha$  – amylase by mesophilic strain of *Bacillus licheniformis*. *Chem. Microbiol. Technol. Leberism*, 12: 8-13.