Expression of α-Amylase by *Aspergillus niger*: Effect of Nitrogen Source of Growth Medium

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Abstract: Background: *Aspergillus niger* is capable of growth on suitable substrates with the elaboration of enzymes to sustain development. In the tropics, this fungus is a common contaminant of most storage products, rendering them unsuitable for their primary purpose. **Materials and Methods:** A defined medium with starch as carbon source and varied nitrogen source was inoculated with spore suspensions of approximately $5x10^5$ spores per ml of *Aspergillus niger*. **Results:** Extracellular α-amylase was expressed within a period of ten days in the inoculated defined medium. Ammonium chloride was able to induce highest activity, expressed as 1,268 units/mg protein on the eighth day of inoculation of medium. Least activity were with tryptone and peptone with optimum activities expressed as 10 units/mg protein on the ninth day and 9 units/mg protein on the tenth day respectively. **Conclusion:** Starch as carbon source of growth with ammonium chloride as nitrogen source are good supports for production of α-amylase by *Aspergillus niger*. However, repression of activity is possible after the eighth day dependent on the growth medium constituents and fungal metabolites.
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1. Introduction

Aspergillus niger belongs to the taxonomic Division Eumycota, Subdivision, Eumycotina, Class Ascomycetes, Order Eurotiales and Family Trichocomaceae (Alexopoulos, 1962). It can be isolated from the soil, plant debris and the air (Streets, 1969). It is a common cause of pulmonary aspergillosis with sometimes high mortality rates (Hildebrand Jr. *et al.*, 1990; Ito and Lyons, 2002).

α-Amylases are hydrolytic and capable of bond cleavage in starch at the α-1,4 glycosidic linkages (Lehninger, 1982). They are of industrial significance and could be of microbial source (Aiyer, 2005). Microbial α-amylases could be metalloenzymes with calcium at their active sites (Bordbar *et al.*, 2005).

In this current investigation, a defined medium was inoculated with a strain of *Aspergillus niger* with a view to assessing the effect of different nitrogen sources of the defined medium on α-amylase expression by the fungus.

2. Materials and Methods

2.1 Isolate source and identification

The isolate, *Aspergillus niger* for this research was part of a culture collection of Professor Patrick O. Olutiola formerly of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. It was identified at the Seed Health Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, using techniques contained in the illustrated Handbook of Fungi (Hanlin, 1990).

2.2 Culture conditions and inoculum

The isolate was subcultured and maintained on Potato Dextrose agar plates and slants. The fungus was further subcultured into test tubes of the same medium and incubated at 25ºC. Ninety-six-hr-old culture was used in this investigation. According to the modified method of Olutiola and Ayres (1973), culture was grown in a defined medium of the following composition: $MgSO_4.7H_20$ (0.1 g), K_2HPO_4 (2 g), KH_2PO_4 (0.5 g), L-cysteine (0.1 g), biotin (0.005 mg), thiamine (0.005 mg) and FeSO4.7H20 (1 mg) with starch as carbon source (10 g) and a nitrogen (9.9 g) sources (Sigma) in 1 litre of

distilled water. The nitrogen source used was varied. They were ammonium sulphate, ammonium chloride, glycine, urea, potassium nitrate, tryptone, peptone and sodium nitrate. Conical flasks (250 ml) containing 100 ml growth medium were inoculated with 1 ml of an aqueous spore suspension containing approximately $5x10^5$ spores per ml of isolate. Spores were counted using the Neubauer counting chamber (Olutiola *et al.*, 1991). Experimental and control flasks were incubated without shaking at 25^0C (Olutiola and Nwaogwugwu, 1982).

2.2.1 α-Amylase assay

α-Amylase activity was determined using the method of Pfueller and Elliott (1969) . The reaction mixtures consisted of 2 ml of 0.2% (w/v) starch in 0.02 M citrate phosphate buffer, pH 6.0 as substrate and 0.5 ml of enzyme. Controls consisted of only 2 ml of the prepared substrate. The contents of both experimental and control tubes were incubated at 35ºC for 20 min. The reaction in each tube was terminated with 3 ml of 1 N HCl. Enzyme (0.5 ml) was then added to the control tube. Two millilitre of the mixture from each of the sets of experimentals and controls was transferred into new sets of clean test tubes. Three millilitre of 0.1 N HCl was added into the contents of each test tube after which 0.1 ml of iodine solution was added. Optical density readings were taken at 670 nm. One unit of enzyme activity was defined as the amount of enzyme which produced 0.1 percent reduction in the intensity of the blue colour of starch-iodine complex under conditions of the assay. Specific activity was expressed as enzyme units per mg protein.

3. Results Analysis

From the results of our investigation, as observed on Table 1, starch as carbon source of our defined medium and ammonium chloride as nitrogen source induced α-amylase activity with optimum activity on the eighth day expressed as 1,268 units/mg protein. Activity declined steadily on days nine and ten. Urea as nitrogen source supported the expression of α-amylase by *Aspergillus niger*. Activity was also optimum on the eighth day of inoculation of medium and was 51 units/mg protein. A gradual decline followed on the ninth and tenth days. With potassium nitrate as nitrogen source, α-amylase expression by *A. niger* gradually declined from day eight today ten of inoculation of medium. At day eight, activity was 48 units/mg protein. It was 86 units/mg protein at day ten. When ammonium sulphate was nitrogen source, α-amylase activity was 546 units/mg protein at day eight. Activity rose to 615 units/mg protein at day nine but declined to 582 units/mg protein at day ten. Similarly, with glycine, activity was 25 units/mg protein at day eight, 41 units/mg protein at day nine but declined to 31 units/mg protein at day ten. However, with sodium nitrate as nitrogen source, α-amylase activity was 54 units/mg protein at day eight, 57 units/mg protein at day nine and 61 units/mg protein at day ten indicating a steady increase in activity. When tryptone was nitrogen source, α-amylase activity was 6 units per mg protein at day eight, 10 units/mg protein at day nine and 8 units/mg protein at day ten. α-Amylase activity was 7 units/mg protein at day eight, 8 units/mg protein at day nine and 9 units/mg protein at day ten when peptone was nitrogen source of growth medium.

Table 1: Effect of nitrogen source on α-amylase activity produced by *Aspergillus niger*

Nitrogen source	Days		
	8	**9**	**10**
Ammonium chloride	1268	713	600
Urea	51	46	29
Potassium nitrate	48	69	86
Ammonium sulphate	546	615	582
Glycine	25	41	31
Sodium nitrate	54	57	61
Tryptone	6	10	8
Peptone	7	8	9

The measurements were the specific activity of α-amylase and the values were in units/mg protein.

4. Discussion

Of the nitrogen sources of growth medium (with starch as carbon source) engaged in this study, ammomium chloride best enhanced α-amylase activity produced by *Aspergillus niger*. This was followed by ammonium sulphate. Least activities were with tryptone and peptone. The two ammonium salts used in this investigation seem better inducers of α-amylase in *A. niger* than the other salts. According to Sarikaya and Gurgun (2000), ammonium sulphate as nitrogen source induced production of α-amylase by *Bacillus amyloliquefaciens.* Also, ammonium chloride was a good source of nitrogen in medium for production of α-amylase by *Mucor spp.* (Vahidi *et al.*, 2005). Hoewever, potassium nitrate supported more growth of *Rhynchosporium secalis* than equal quantities of nitrogen in the form of ammonium sulphate and sodium nitrate (Olutiola, 1972).

α-Amylase repression is a possibility in the present study since starch is expected to induce α-amylase production by this fungus. Repression could be as a result of products of the fungus' catabolism or simply as result of a constituent of the defined medium. However, we rule out the latter since we have a rise in activity with days of inoculation as observed with peptone, sodium nitrate and potassium nitrate. The poor expression of α-amylase activity observed with tryptone and peptone may be a relative ability to induce the enzyme in the fungus. Herein, the genetic constituent of the fungus plays a vital role.

α-Amylases are used in the industries in the production of detergents to remove stains of starch and in brewering. In Nigeria, enzymes are usually imported for these purposes. It is therefore needful to create an

avenue for the production of α-amylases in Nigeria by exploring conditions needed for fungal growth and optimum α-amylase activity by *A. niger*. Inspite of the benefits that can be derived from this exploration, the danger *A. niger* poses as biomarker in biological warfare also should not be ignored.

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