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

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**Abstract:** *Aspergillus flavus* is contaminant of storage products in the tropics. A defined medium with starch as carbon source and varied nitrogen sources was inoculated with spore suspensions of approximately 5x10⁵ spores per ml of *Aspergillus flavus*. The nitrogen sources were ammonium chloride, urea, potassium nitrate, ammonium sulphate, glycine, sodium nitrate, tryptone and peptone. Extracellular α-amylase was produced by the fungus within a period of ten days in the inoculated defined medium. Peptone was able to induce highest α-amylase activity, expressed as 546 units/mg protein on the ninth day of inoculation of medium. Least activity were with tryptone and ammonium sulphate with optimum activities expressed as 5 units/mg protein on the eighth, ninth and tenth days for tryptone and nil α-amylase activity on the tenth day when ammonium sulphate was nitrogen source.

**Keywords:** α-Amylase; *Aspergillus flavus*; growth medium.
1. Introduction

α-Amylases are starch degrading enzymes usually metalloenzymes stabilized by calcium ions (Reddy et al., 2003). They are hydrolytic enzymes that yield dextrins and progressively smaller polymers composed of glucose units upon catalytic starch degradation (Bohinski, 1983). Based on their pattern of catalysis and yield of products, amylases can be categorized as: (a) alpha (α) amylase (endoamylase) (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) which hydrolyses internal α-1,4-linkages to yield maltose, maltotriose and α-dextrin (Hashim et al., 2005).

Studies have shown that several species of fungi are capable of production of α-amylases when grown on suitable substrates (Ladokun and Adejuwon, 2011; Adejuwon et al., 2013). The genetic constitution of these fungi actually determines such expression (Tortora et al., 1998). However, today, genetic manipulation has enhanced the production of varieties of enzymes in vast species of fungi (Tortora et al., 2004).

In this current investigation, a strain of Aspergillus flavus was grown in a defined medium containing starch as carbon source. The nitrogen source of growth in the medium was varied with a view to comparing the relative production of induced α-amylase by the fungus.

2. Materials and Methods

2.1 Materials

All the chemicals used in this research were of analytical grade and were purchased from Sigma Chemical Company St. Louis, MO, USA or BDH Poole, England.

3. Methods

3.1 Source and Identification of Fungi

The isolate of Aspergillus flavus used in this research was from deteriorated bread. It was identified at the Seed Health Unit of the International Institute of Tropical
3.2 Culture Conditions and Preparation of Inocula

The isolate was cultured and maintained on Potato Dextrose agar plates. The fungus was further subcultured into test tubes of the same medium and incubated at 25°C. Ninety six-hr-old culture of the strain of *Aspergillus flavus* was used in this investigation. Based on the method of Olutiola and Ayres (1973), culture was grown in a defined medium of the following composition: MgSO₄·7H₂O (0.1 g), K₂HPO₄ (2 g), KH₂PO₄ (0.5 g), L-cysteine (0.1 g), biotin (0.005 mg), thiamine (0.005 mg) and FeSO₄·7H₂O (1 mg) with added carbon (10 g) and nitrogen (9.9 g) sources (Sigma) in 1 litre of distilled water. The nitrogen source was varied as: glycine, potassium nitrate, ammonium chloride, ammonium sulphate, sodium nitrate, urea, tryptone and peptone. The carbon source was starch. One hundred millilitre of growth medium in conical flasks (250 ml) was inoculated with 1 ml of an aqueous spore suspension containing approximately 5x10⁵ spores per ml of isolate. Spores were counted using the Neubauer counting chamber (Olutiola et al., 1991). Controls were uninoculated growth medium. Experimental and control flasks were incubated without shaking at 25°C (Olutiola and Nwaogwugwu, 1982).

3.3 Enzyme Extraction

On days eight to ten of inoculation of medium (α-amylase seem significantly induced from days eight to ten in this fungus by this defined medium as observed from preliminary investigations), the contents of each flask were filtered using glass fibre filter paper (Whatman GF/A). The protein content of filtrate was determined using the Lowry et al. (1951) method. Filtrate was assayed for α-amylase activity using the method of Pfueller and Elliott (1969).
3.4 Enzyme and Protein Assays

3.4.1 α-Amylase

α-Amylase activity was determined based on 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 substrate. The contents of both experimental and control tubes were incubated at 35°C for 30 min. The reaction in each tube was terminated with 3 ml of 1 N HCl. 0.5 ml of enzyme was then added to contents of each 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 670nm. 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 in enzyme units per mg protein.

3.5 Ammonium Sulphate Fractionation

The crude enzymes on days of extraction were treated with ammonium sulphate (analytical grade) between 40- 90% saturation. Precipitation was allowed at 4°C for 24 h. Mixtures were centrifuged at 6,000 g for 30 minutes at 4°C using a cold centrifuge (Optima LE-80K Ultracentrifuge, Beckman, USA). The supernatant were discarded. The precipitate was reconstituted in 0.02M citrate phosphate buffer, pH 6.0. α-Amylase activity was determined using the method of Pfueller and Elliott (1969). Protein content was determined using the Lowry et al. (1951) method.

3.6 Dialysis

With the use of acetylated dialysis tubings (Visking dialysis tubings, Sigma) (Whitaker et al., 1963) and a multiple dialyser, the ammonium sulphate fractionated
enzyme preparations were dialysed with several changes of 0.02M citrate phosphate buffer, pH 6.0 at 4°C for 18 h. α-Amylase activity was determined based on the method of Pfueller and Elliott (1969). Protein content was analyzed using the method of Lowry et al. (1951).

4. Results and Discussion

Within eight days of inoculation of the defined medium used in this investigation α-amylase was produced by this strain of fungus. Starch supported α-amylase production. α-Amylase production seemed constitutive in this fungus since the different nitrogen sources supported enzyme production (Table 1). From data, highest α-amylase activity induction was with peptone, expressed as 546 units/mg protein on the ninth day of inoculation of medium. Least activity were with tryptone and ammonium sulphate. α-Amylase activities were expressed as 5 units/mg protein on the eighth, ninth and tenth days for tryptone; and nil on the tenth day with ammonium sulphate.

Earlier reports by Adejuwon (2011) suggest similar inductions of α-amylase in *Lasidiopodia theobromae*. Since starch induced amylase production by our strain of *Aspergillus flavus*, inspite of varying nitrogen source, we envisage α-amylase gene expression on this strain of fungus. Catabolites of enzymatic degradation might be responsible for the varying levels of activity expressed using differing sources of nitrogen. This inference is a basis for further investigations.

Inspite of being harmful to both man and animals, *Aspergillus flavus* can be engaged for industrial production of amylases in tropical Nigeria.
Table 1: Effect of nitrogen source on α-amylase activity produced by *Aspergillus flavus*

<table>
<thead>
<tr>
<th>Nitrogen source</th>
<th>Days</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>56</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Urea</td>
<td>377</td>
<td>395</td>
<td>433</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>401</td>
<td>343</td>
<td>130</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>19</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Glycine</td>
<td>310</td>
<td>352</td>
<td>344</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>135</td>
<td>172</td>
<td>454</td>
</tr>
<tr>
<td>Tryptone</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Peptone</td>
<td>271</td>
<td>546</td>
<td>488</td>
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</tbody>
</table>

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

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References


