Effect of Carbon Source of Growth on $\alpha$-Amylase Production by a Strain of

*Penicillium funiculosum* Thom. Isolated from Irish Potato (*Solanum tuberosum* Linn):

Comparative Studies

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**Abstract.** Irish potato (*Solanum tuberosum* Linn.) is cultivated in the tropical middle belt of Nigeria. It is infested by a range of phytopathogens including *Penicillium funiculosum* Thom. In the present investigation, Irish potato tubers and a defined growth medium were inoculated with pure culture of *Penicillium funiculosum* Thom. The composition of the defined medium was potassium dihydrogen sulphate, magnesium sulphate, calcium sulphate, hydrated iron sulphate, manganese sulphate, copper sulphate, zinc sulphate, thiamine, biotin, sodium nitrate as nitrogen source and varied carbon sources. Proteins which exhibited $\alpha$-amylase activity were expressed and partially purified daily by ammonium sulphate fractionation followed by dialysis to remove small molecular weight impurities. The results showed that, optimum $\alpha$-amylase activity was induced by the ninth day of inoculation of the potato tubers. Only traces of the $\alpha$-amylase were observed in uninfected Irish potato tubers. Similarly, starch, glucose, lactose, galactose, glucose and maltose as carbon source in the defined medium induced $\alpha$-amylase activity varyingly. Highest value of induction was expressed when starch was carbon source followed by Irish potato, then maltose glucose, sucrose, lactose, galactose consecutively. Earliest induction was with glucose with expression starting on the second day of inoculation of the defined growth medium. Inspite of being harmful to cultivation and storage of Irish potatoes in Nigeria, West Africa, *Penicillium funiculosum* Thom. is a potential source of industrial production of $\alpha$-amylase.

**Key words:** $\alpha$-Amylase; *Penicillium funiculosum* Thom.; Irish potato (*Solanum tuberosum* Linn.)
1. Introduction

α-Amylases are starch degrading enzymes that catalyze the hydrolysis of α-1, 4-O-glycosidic bonds in polysaccharides with the retention of α-anomeric configuration in the products. Most of the α-amylases are metalloenzymes which require calcium ions (Ca\(^{2+}\)) for their activity, structural integrity and stability (Bordbar et al., 2005). Amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. These enzymes account for about 30% of the world’s enzyme production (van der Maarel et al., 2002). The α-amylase family can roughly be divided into two groups: the starch hydrolyzing enzymes and the starch modifying or transglycosylating enzymes (Satyanarayana et al., 2005).

α-Amylases are produced by bacteria and fungi (Oh et al., 2005; Hashim et al., 2005). In earlier studies, we have observed the induction of polygalacturonase and cellulase in *Penicillium funiculosum* Thom. by tomato fruits during infection (Adejuwon et al., 2006; Adejuwon et al., 2009).

Irish potato is a starchy staple food of the indigenes of the middle belt in Nigeria. In this current investigation, we envisaged the possibility of the production of hydrolytic enzymes such as α-amylases in irish potatoes infected with *Penicillium funiculosum* Thom. We engaged in this study since the pythopathogen is able to infest irish potatoes during storage, causing substantial loss to producers as observed in Jos, Nigeria and the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

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 *Penicillium funiculosum* Thom. used in this research was from deteriorated Irish potato (*Solanum tuberosum* Linn.) and identified at the Seed Health Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, with the aid of the illustrated Handbook of Fungi (Hanlin, 1990).

3.2 Culture Conditions and 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. Sixty-hr-old culture of *Penicillium funiculosum* 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: \( \text{Na}_2\text{NO}_3 \) (9.9g), \( \text{MgSO}_4\cdot7\text{H}_2\text{O} \) (0.1g), \( \text{KH}_2\text{PO}_4 \) (0.5g), \( \text{CaSO}_4 \) (0.1g), \( \text{MnSO}_4 \) (1mg), \( \text{CuSO}_4 \) (1mg), \( \text{ZnSO}_4 \) (1mg), biotin (0.005mg), thiamine (0.005mg) and \( \text{FeSO}_4\cdot7\text{H}_2\text{O} \) (1mg) with added carbon (10g) sources (Sigma) in 1 litre of distilled water. The carbon source was varied as: starch, glucose, lactose, galactose, glucose and maltose. The nitrogen source was sodium nitrate. One hundred millilitre of growth medium in conical flasks (250 ml) was inoculated with 1 ml of an aqueous spore suspension containing approximately \( 6\times10^5 \) 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).

On a daily basis, 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).
3.3 Inoculation of Irish Potato (*Solanum tuberosum* Linn.) Tubers

Irish potato (*Solanum tuberosum* Linn.) tubers were bought at the Bodija Market, Ibadan, Nigeria and identified at the Herbarium of the Department of Botany, University, Ibadan, Ibadan, Nigeria. The potatoes were surface sterilized in 3% mercuric chloride for 15 minutes. Using red heat sterilized cork borer, 3mm tissue disc was removed from each potato tuber and inoculated with tissues disc of sixty-hr-old culture of isolate grown on potato dextrose agar plates. They were kept in sterile petri dishes on sterile glass plates under bell jars with the rims of the jars sealed with vaseline to ensure air tight contact with the glass plates. This was done to avoid contamination. Controls were potato tubers inoculated with discs of sterile potato dextrose agar in plates. Incubation of both experimentals and controls was at 25°C. The potato tubers were observed daily for fungal growth and deterioration.

3.3.1 Enzyme extraction

On a daily basis, infected irish potato tubers were carefully weighed, mercerated and homogenised with cold (4°C) 0.5M NaCl in 0.02 M citrate phosphate buffer pH 6.0 to extract enzyme. The homogenate was filtered using four layers of muslin cloth and clarified using glass fibre filter paper. The protein content of the crude filtrate was determined using the method of Lowry et al. (1951). α-Amylase activity was determined using the method of Pfueller and Elliott (1969).

3.4 Enzyme and Protein Assays

3.4.1 α-Amylase

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 35°C 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 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.

3.4.2 **Protein concentration determination**

Protein concentration was determined by the method of Lowry et al. (1951). Serial dilutions of egg albumin were used to plot a standard graph. The unknown protein value in each test sample was intrapolated from the standard calibration graph.

3.5 **Ammonium Sulphate Fractionation**

On the days of optimum α-amylase activity of inoculation medium, the crude enzymes were treated with ammonium sulphate (analytical grade) at 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**

Using acetylated dialysis tubings (Visking dialysis tubings, Sigma) (Whitaker et al., 1963) and a multiple dialyser, the enzyme preparations were dialysed under several changes of 0.02M citrate phosphate buffer, pH 6.0 at 4°C for 24 h. α-Amylase activity was determined using the method of Pfueller and Elliott (1969) as described earlier. Protein content was determined using the method of Lowry et al. (1951).
4. Results and Discussion

Within ten days, the infected irish potato tubers were completely covered with greenish spores from mycelia of the isolate. Substantial α-amylase was produced during the infection process. Only traces of the enzyme were observed in uninfected irish potato tubers. This is evidenced with traces of activity at days 1 and 2 in infected potato tubers and absence of activity at days 1 of the carbon sources of inoculated growth medium (Table 1). From data, highest protein value was with sucrose as carbon source as observed on the fifth day of inoculation of growth medium. The least protein value was with galactose as observed on day 1 of inoculation of medium (Table 1). Optimum α-amylase activity was observed with: with irish potato as 279 units/mg protein on the ninth day; starch as 432 units/mg protein on the tenth day; maltose as 251 unit/mg protein on the tenth day; sucrose as 142 unit/mg protein on the ninth day; lactose as 87 unit/mg protein on the tenth day; glucose as 208 unit/mg protein on the ninth day; and galactose as 39 unit/mg protein on the tenth day (Table 2). Starch as carbon source of the growth medium induced highest α-amylase activity. The value is expressed as 432 units/mg protein and is on the tenth day of inoculation of medium (Table 2). α-Amylase induction was earliest with glucose as carbon source and expressed on day 2 of inoculation of medium as 12 units/mg protein. In Figure 1, we have the protein concentration standard curve with egg albumin as standard protein.

When rish potato tubers were infected with *Penicillium funiculosum* Thom., proteins which exhibited α-amylase activity was expressed. By the ninth day, optimum activity had been induced. Similarly, starch; glucose; lactose; galactose; glucose; and maltose in the composition of a defined growth medium induced α-amylase (Adejuwon, 2013). Starch is expected to induce α-amylase if the gene for such expression is found on the genome of such fungus. Induction by other carbon compounds used in this study apart from starch clearly suggests constitutive enzyme production since α-amylase catalyses degradation of starch to maltose and smaller dextrins. Poor induction was observed when galactose was carbon source. Inspite of *Penicillium funiculosum* Thom.
being harmful to cultivation of Irish potatoes in tropical Nigeria, the data in this study clearly suggests that α-amylase could be produced industrially using this isolate with conditions stated for production.

Table 1: Effect of carbon source on α-amylase activity produced by *Penicillium funiculosum* Thom. showing unit activity and total protein

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Activity (Units)</th>
<th>Protein (OD 600nm)</th>
<th>Activity (Units)</th>
<th>Protein (OD 600nm)</th>
<th>Activity (Units)</th>
<th>Protein (OD 600nm)</th>
<th>Activity (Units)</th>
<th>Protein (OD 600nm)</th>
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<td>0.001</td>
<td>0.20</td>
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<td>0.20</td>
<td>0.065</td>
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<td>0.10</td>
<td>0.20</td>
<td>0.325</td>
<td>0.20</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.09</td>
<td>0.00</td>
<td>0.09</td>
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<td>0.02</td>
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<td>0.02</td>
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<td>0.00</td>
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<td>0.00</td>
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Table 2: Effect of carbon source on α-amylase activity produced by *Penicillium funiculosum* Thom. showing specific activity in units/mg protein, derived from Table 1

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<th>5</th>
<th>6</th>
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<td>0</td>
<td>0</td>
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<td>96</td>
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<td>432</td>
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<tr>
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<td>0</td>
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<td>7</td>
<td>18</td>
<td>140</td>
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<td>165</td>
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<tr>
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<td>8</td>
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Fig 1: Standard curve for protein using egg albumin (Lowry et al., 1951 method)
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References


