

Expression of α -Amylase by a Tropical Strain of *Aspergillus niger*: Effect of Carbon Source of Growth

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Abstract: Background: *Aspergillus niger* is an Ascomycete and a known contaminant of food especially grain products in the tropics. **Materials and methods:** In the present investigation, a defined growth medium with potassium nitrate as nitrogen source was inoculated with spore suspensions of approximately 5×10^5 spores per ml of *Aspergillus niger*. The carbon source was varied and was independently starch, maltose, sucrose, lactose, glucose and galactose. Bread as sole growth and carbon source was also inoculated with the same spore suspension of the isolate. Incubation was at 25°C. Extracellular proteins produced in medium were analysed for α -amylase activity. **Results:** The proteins produced by *Aspergillus niger* in the inoculated medium exhibited α -amylase activity. All the carbon compounds supported α -amylase expression by the fungus. Starch, maltose and bread were the most supportive. **Conclusion:** All the carbon compounds used in this investigation supported expression of α -amylase activity by *Aspergillus niger* indicative of a constitutive nature of the enzyme in the fungus. This expression in *Aspergillus niger* is most supported by starch or maltose when potassium nitrate is nitrogen source; and bread as carbon and sole growth source.

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1. Introduction

Aspergillus niger causes black mould rot of tropical fruits and vegetables (Streets, 1969). Some strains produce ochratoxin A, a potent mycotoxin and the isoflavone orobol (Figueroa *et al.*, 2009; Hashem and Alamn, 2010). The fungus belongs to the Subgenus *Circumdati* Section *Nigri* (Hanlin, 1990).

In a recent study, *Aspergillus niger* was demonstrated to produce α -amylases in a defined medium with starch as carbon source and certain nitrogen compounds (Adejuwon *et al.*, 2015).

In this present investigation, a defined medium with potassium nitrate as nitrogen source; and also bread as carbon source and growth medium was inoculated with spore suspension of a tropical strain of *Aspergillus niger* with a view to understanding the effect of varying carbon compounds on the expression of α -amylase by this strain of fungus.

2. Materials and Methods

2.1 Isolate Source and Identification

The isolate, *Aspergillus niger* (IFE 04) for this research investigation was isolated from mouldy bread by Professor Patrick O. Olutiola in the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The isolate was identified at the Seed Health Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Mycological techniques contained in the illustrated Handbook of Fungi were used in identification of the isolate (Hanlin, 1990).

2.2 Culture Conditions and Inoculum

The isolate *Aspergillus niger* (IFE 04) was cultured and maintained on Potato Dextrose agar slants and plates. The fungus was subcultured into test tubes of the same medium and incubated at 25°C. 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: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (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 FeSO₄.7H₂O (1 mg) with potassium nitrate as nitrogen source (9.9 g) and a carbon (10 g) source (Sigma) in 1 litre of distilled water. The carbon source used was varied. The carbon sources were independently starch, maltose, sucrose, lactose, glucose and galactose. Conical flasks (250 ml)

containing 100 ml growth medium were inoculated with 1 ml of an aqueous spore suspension containing approximately 5×10^5 spores per ml of isolate. Spores were counted using the Neubauer counting chamber (Olutiola *et al.*, 1991; Adejuwon, 2011). Experimental and control flasks were incubated without shaking at 25°C (Olutiola and Nwaogwugwu, 1982).

Table 1: Effect of carbon source on amylase activity produced by *Aspergillus niger*

Carbon Source	Days									
	1	2	3	4	5	6	7	8	9	10
Bread	0	0	5	14	19	48	56	263	281	191
Starch	0	0	0	0	0	9	69	98	293	435
Maltose	0	0	7	9	21	141	148	167	243	254
Sucrose	0	0	0	4	57	59	77	129	156	109
Lactose	0	0	4	9	15	16	25	26	41	95
Glucose	0	12	18	19	19	62	87	93	210	164
Galactose	0	0	0	0	0	0	2	8	13	41

2.2.1 Bread as a source of carbon

Freshly baked loaves of bread were bought at the bakery of the Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria. The bread loaf was soaked in distilled water (1% w/v), mercerated with a homogenizer and autoclaved at 15 psi (121°C) for 15 minutes. One hundred millilitre of the bread medium in conical flasks (250 ml) was inoculated with 1 ml of aqueous spore suspension containing approximately 5×10^5 spores per ml of isolate. Incubation was at 25°C.

On a daily basis, the contents of each flask were filtered through glass fibre filter paper (Whatman GF/A). The protein content of the filtrates was determined (Lowry *et al.*, 1951). The filtrates were assayed for α -amylase activity using the method of Pfueller and Elliott (1969).

2.3 α -Amylase Assay

The method used in the determination of α -amylase in this investigation was that 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 30 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 α -amylase activity was arbitrarily defined as the amount of α -amylase 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 α -amylase units per mg protein.

3. Results

Potassium nitrate was the nitrogen source in the defined medium used in this investigation. From the results of the study, as observed in Table 1, all the carbon compounds used supported α -amylase expression in *Aspergillus niger*.

When bread was carbon and growth source, α -amylase activity expressed by our tropical strain of *Aspergillus niger* was nil at day one and day two of inoculation of medium. Activity was expressed at day three (72 hr) and was 5 units/mg protein. Activity increased steadily with optimum on the 9th day and expressed as 281 units/mg protein. Activity declined to 191 units/mg protein at day ten.

With starch as carbon source, α -amylase activity was nil at days one to five. Activity was expressed as 9 units/mg protein on day six. α -Amylase activity increased steadily with optimum expressed as 435 units/mg protein on day ten.

When maltose was carbon source, α -amylase activity was nil at days one and two. It was 7 units/mg protein at day three. Activity rose steadily reaching an optimum 254 units/mg protein at day ten.

With sucrose as carbon source, α -amylase activity was nil at the 1st day to the 3rd day of inoculation of medium. Activity was 4 units/mg

protein on the 4th day, rising steadily to an optimum 156 units/mg protein on the 9th day and then declining to 109 units/mg protein on the 10th day.

When lactose was carbon source, α -amylase activity was nil at days one and two. It was 4 units/mg protein on day three, rising steadily to an optimum 95 units/mg protein on day ten.

With glucose as carbon source, α -amylase activity was nil at day one but 12 unit/mg protein at day two. Activity rose steadily reaching an optimum 210 units/mg protein at day nine and then declining to 164 units/mg protein on day ten.

Finally with galactose as carbon source, α -amylase activity was nil at 1st day of inoculation till the 6th day. α -Amylase activity was 2 units/ mg protein at the 7th day, rising to an optimum 41 units/mg protein on the 10th day.

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

4. Discussion

Starch and maltose as carbon source of growth with potassium nitrate as nitrogen source; and also bread as both carbon and sole growth source were most supportive to the expression and production of α -amylase by our tropical strain of *Aspergillus niger* at 25°C. In an earlier investigation, bread was most supportive to the production of α -amylase by *Lasiodiplodia theobromae* at 27°C (Adejuwon, 2011). In a much earlier investigation, starch, maltose, glycerol or glucose as carbon source supported extracellular production of α -amylase by *Bacillus subtilis* (Coleman, 1967).

The expression of α -amylase activity by *Aspergillus niger* with all the carbon compounds used in this study is indicative of constitutive expression of the enzyme in the fungus. The seeming delayed expression when starch was carbon source might actually depend on the concentration of our substrate relative to enzyme activity. The gene for expression of α -amylase is evidently constituted in this strain of fungus' DNA.

Galactose was least supportive to α -amylase expression by the fungus. The industrial production of α -amylase by this tropical strain of *Aspergillus niger* will not be too effective using potassium nitrate as nitrogen source with galactose as carbon source of growth. However starch or maltose as carbon source with potassium nitrate as nitrogen source and also bread as sole growth source would be a good exploration for industrial production of the enzyme by this strain of fungus but day dependent when monitoring activity.

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