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Full Length Research Paper

Partial Purification of α-amylase Expressed by a Tropical Mutant Strain of *Aspergillus niger* IFE 08

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A mutant of *Aspergillus niger* induced by ultraviolet radiation of a strain of a tropical wild type (*Aspergillus niger* IFE 08) expressed α-amylase activity in a defined medium with starch as carbon source and ammonium chloride as nitrogen source of growth and development. The enzyme was subjected to partial purification by ammonium sulphate precipitation followed by dialysis. Specific activity of the enzyme increased 1.71 fold while recovery was 29.9% after dialysis.

Keywords: *Aspergillus niger*, tropical, mutant strain, partial purification, α-amylase

Introduction

α-Amylases (endoamylase; α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) require Ca^{2+} for their structural integrity and catalysis (Robyt, 1984). Microbial sources of α-amylases include the fungi (Wu *et al*., 2004; Rahardjo *et al*., 2005; Najafi and Kembhavi, 2005). In a recent investigation, it was reported that a strain *Aspergillus niger* IFE 04 expressed significantly active α-amylase in a defined medium with starch as carbon source and ammonium chloride as nitrogen source of growth (Adejuwon *et al*, 2015). In this study, a mutant of a tropical strain *Aspergillus niger* IFE 08 was cultured in a same defined medium (Adejuwon *et al.*, 2015) also with ammonium chloride as nitrogen source and starch as carbon source for fungal growth. It was capable of expression of α-amylase. This enzyme expressed by the mutant was partially purified by ammonium sulphate precipitation and dialysis. Observations are herein reported.

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Materials and Methods

Source and Identification of Isolate (Wild Type *Aspergillus niger* IFE 08)

The tropical wild type strain of *Aspergillus niger* (IFE 08) used in this study 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 isolated from a diseased yam (*Dioscorea alata*) tuber. The isolate 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 (Cannon and Kirk, 2007).

Identity was further confirmed by Genetic DNA sequencing using Polymerase Chain Reaction (PCR) at the Bioscience Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Induction of Mutation in Wild Type Strain *Aspergillus niger* IFE 08

The wild type strain *Aspergillus niger* (IFE 08) after identification was subjected to mutation by culturing repeatedly on potato dextrose agar at 30^{o}C and then continual exposure to ultra violet radiation at 10^{o}C for a period of thirty-six months. Discerned phenotypic change induced by mutation was a blackish to brownish pigmentation on potato dextrose agar. Genetic (nuDNA) analyses of the wild type and mutant strains were determined to be different. G + C ratio and DNA relatedness performed by Genomic DNA buffer Set and Genomic – tip 500/G (QIAGEN) were determined in the two strains to be different.

Culture Conditions and Inoculum (Mutant)

The mutant strain of the wild type isolate was cultured and maintained on potato dextrose agar plates and slants. The mutant strain was further sub-cultured into test tube slants of potato dextrose agar and incubated at 20^{o}C. Ninety-six hr-old culture of the mutant strain was used as inoculum. Using the modified method of Olutiola and Ayres (1973), culture (mutant) was grown in a defined medium composed of: $MgSO_4 \cdot 7H_20$ (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 $FeSO_4 \cdot 7H_20$ (1 mg) with starch (10 g) as carbon source and ammonium chloride (9.9 g) as nitrogen source (Sigma) in 1 litre of distilled water. Conical flasks (250 ml) containing 50 ml growth medium were inoculated with 1 ml of an aqueous spore suspension containing approximately $5x10^6$ spores per ml of isolate. Experimental flasks contained inoculated sterilized medium while control flasks contained un-inoculated sterilized medium. Fungal spores (mutant) were counted using the Neubauer counting chamber (Olutiola *et al.*, 1991). Experimental and control flasks were incubated without shaking at 25^{o}C (Adejuwon and Ladokun, 2015).

Extraction of α-Amylase

Optimum α-amylase activity (Pfueller and Elliott, 1969) was expressed by the mutant on the eighth day. The contents of flask on this day of optimum α-amylase activity were filtered through glass fibre filter paper (Whatman GF/A) at 20^{o}C. Protein content of the filtrates was thereafter determined (Lowry *et al.* 1951).

Assay for α- Amylase

α-Amylase activity was assayed using the method of Pfueller and Elliott (1969). Reaction mixtures were 2 ml of 0.2% (w/v) soluble starch in 0.02 M citrate phosphate buffer (pH 6.0) as substrate and 0.5 ml of enzyme. Controls were only 2 ml of the prepared substrate. The contents of both experimental and control tubes were incubated at 35^{o}C for 20 min. Reaction in each tube was terminated with 3 ml of 1 N HCl. 0.5 ml enzyme was then added to the control tubes. 2ml mixture from each set of experimentals and controls was transferred into new sets of test tubes. 3ml 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 α-amylase, which produced 0.1% reduction in the intensity of the blue colour of starch-iodine complex under conditions of the assay. Specific activity was calculated as enzyme units per milligram protein.

Ammonium Sulphate Precipitation

On the day of optimum α-amylase activity, the crude enzyme was treated with ammonium sulphate (analytical grade, BDH) at 40-90% saturation. Precipitation was at 4^{o}C for 24 h. Centrifugation followed and was at 6,000g for 30 minutes at 4^{o}C using a high speed cold centrifuge (Optima LE-80K Ultracentrifuge, Beckman, USA). Thereafter the supernatant was discarded. Protein precipitate was reconstituted in 0.02 M citrate phosphate buffer (pH 6.0). The protein content of the enzyme precipitate was determined by the method of Lowry *et al.* (1951). α-Amylase activity was determined by the method of Pfueller and Elliott (1969).

Dialysis

Using a multiple dialyzer and acetylated dialysis tubings (Visking dialysis tubings, Sigma) (Whitaker *et al.* 1963), the ammonium sulphate precipitated enzyme was dialysed under several changes of 0.02 M citrate phosphate buffer (pH 6.0) at 4^{o}C for 24 h. Thereafter, the protein content of the dialysed enzyme was determined using the method of Lowry *et al.* (1951). α-Amylase activity was determined using the method of Pfueller and Elliott (1969).

Results

The mutant of isolate strain *Aspergillus niger* (IFE 04) expressed α-amylase activity within a period of eight days in the defined medium at 25^{o}C. The purification steps are presented in Table 1. Optimum activity of the crude was

Table 1: Partial purification of α-amylase obtained from a mutant strain of *Aspergillus niger* IFE 08

Fraction	Total Activity (Units)	Total Protein (mg)	Specific Activity (Units/mg protein)	Yield (%)	Purification fold
Crude extract	3437	22.4	153.4	100	1
90% $(NH_4)_2SO_4$ Precipitation	1027	3.9	262.3	29.9	1.71

recorded as approximately 154 Units/mg protein. Total activity of crude extract was recorded as 3,437 Units with total protein being 22.4mg. On dialysis subsequent to ammonium sulphate precipitation of crude enzyme, total activity reduced to 1,027 Units with total protein also reducing to 3.9mg. However, specific activity had increased from approximately 154 Units/mg protein to approximately 263 Units/mg protein. After ammonium sulphate precipitation and dialysis of the crude enzyme, specific activity of the crude had increased 1.71 fold while recovery was 29.9%.

Discussion

In this study, the mutant of strain *Aspergillus niger* IFE 08 isolated from yam (*Dioscorea alata*) expressed α-amylase activity in the defined medium composed of starch as carbon source and ammonium chloride as nitrogen source of fungal growth and development. In an earlier investigation, Vahidi *et al.* (2005) reported that starch, arginine and ammonium chloride were good sources of carbon and nitrogen in medium for α-amylase production by *Mucor spp.*; the effect of nitrogen concentration compared to starch concentration on amylase production was insignificant. In their much earlier investigation Olutiola and Cole (1977) reported nitrate, ammonium and asparagine as good sources of nitrogen for growth and sporulation of *Aspergillus sydowi*. Suresh *et al*. (1999) have reported the production of a starch hydrolyzing enzyme by a UV-induced mutant strain of *Aspergillus niger*.

Activity of the crude α-amylase expressed by the mutant *A.niger* was recorded as approximately 154 Units/mg protein. This was the optimum activity expressed by the isolate in the defined medium and was at day eight of inoculation of medium. The enzyme was expressed at 25^{o}C. Studies by Yuen *et al.* (1998) on wood decay fungi (Ascomycetes and Hyphomycetes) isolated from submerged wood collected in tropic and sub-tropic fresh water revealed optimum temperatures of 20^{o}C and 25^{o}C for growth and amylase production. However, a α-amylase produced by *Bacillus sp* isolated from soil sample was optimally active at 75-80^{o}C (Sajedi *et al*., 2005). Also, α-amylase from *Bacillus licheniformis* is able to hydrolyse soluble starch within a temperature range of 60-75^{o}C (Rodriguez *et al*., 2006).

In conclusion, the mutant *Aspergillus niger* used in our investigation was capable of production of α-amylase at 25^{o}C with starch as carbon source and ammonium chloride as nitrogen source in our defined medium for fungal growth. It is either that the ultra-violet radiation and consecutive culturing of the wild type strain altered the fungal gene to effect inductive or constitutive expression of α-amylase or that the gene coding for this enzyme was unaltered by mutation (A need for further investigation). However previous studies have shown other strains of *Aspergillus niger* as capable of expression of α-amylase (Literature cited in Introduction and Discussion). Inspite of the implications and dangers to health *Aspergillus niger* may pose, the industrial application of this innovative for α-amylase production is encourage in the tropics and even the temperate zones of the world.

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