Advances in Bioscience and Bioengineering
ISSN 2201-8336
Volume 3, Number 1, 2015, 12-19

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

Adekunle Odunayo Adejuwon[1], Anthonia Olufunke Oluduro[2], Femi Kayode Agboola[2] and Patrick Ojo Olutiola[3]

[1]Department of Microbiology, Faculty of Information Technology and Applied Sciences, Lead City University, Ibadan, Nigeria

[2]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria

[3]Department of Biological Sciences, Bowen University, Iwo, Nigeria

Corresponding author: Adekunle Odunayo Adejuwon, Department of Microbiology, Faculty of Information Technology and Applied Sciences, Lead City University, Ibadan, Nigeria

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 $5x10^5$ 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

Agriculture (IITA), Ibadan, Nigeria, with the aid of the illustrated Handbook of Fungi (Hanlin, 1990).

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^{o}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: $MgSO_4.7H_20$ (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 $FeSO_4.7H_20$ (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 $5x10^5$ 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^{0}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^{o}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^{o}C for 24 h. Mixtures were centrifuged at 6,000 g for 30 minutes at 4^{o}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*

Nitrogen source	**Days**		
	8	**9**	**10**
Ammonium chloride	56	16	16
Urea	377	395	433
Potassium nitrate	401	343	130
Ammonium sulphate	19	18	0
Glycine	310	352	344
Sodium nitrate	135	172	454
Tryptone	5	5	5
Peptone	271	546	488

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

Acknowledgements

This research was supported by the year 2007 Small Grant Award of the British Mycological Society with Dr. Adekunle Odunayo Adejuwon as recipient.

References

[1] Adejuwon, A.O. (2011). Nutritional factors affecting α-amylase production in *Lasiodiplodia theobromae* Pat. *Biotechnology, Bioinformatics & Bioengineering* 1(1): 131-135.

[2] Adejuwon, A.O., Abe, O.E., Bamkefa, B.A., Ajayi, A.A., Awojobi, K.O., Adejuwon, M.A. and Ologbosere, O. (2013). α-Amylases by strains of *Candida albicans* and *Fusarium sp.*: Expression and characterization. *Report and Opinion* 5(6): 10-17.

[3] Bohinski, R.C. (1983). *Modern Concepts in Biochemistry*. 4th Edition. Allyn and Bacon Inc., Boston, London, Sydney, Toronto. 531pp.

[4] Hanlin, R.T. (1990). *Illustrated Genera of Ascomycetes*. American Phytopathological Society Press, St. Paul, Minnesota. 263pp.

[5] Hashim, S.O., Delgado, O.D, Martinez, M.A., Kaul, R.H., Mulaa, F.J. & Mattiasson, B. (2005). Alkaline active maltohexaose-forming α-amylase from *Bacillus halodurans* LBK 34. *Enzyme and Microbial Technology*, 36(1): 139-146.

[6] Ladokun, O.A. and Adejuwon, A.O. (2011). *Amylase from Aspergillus fumigatus Associated with Deterioration of Rice* (*Oryzae sativa*). Conference on International Research on Food Security, National Resource Management and Rural Development, Tropentag 2011, University of Bonn, Bonn, Germany, October 5-7, 2011.

[7] Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193: 265-275.

[8] Olutiola, P.O. & Ayres, P.G. (1973). Utilization of carbohydrates by *Rhynchosporium secalis*.I. Growth and sporulation on glucose, galactose and galacturonic acid. *Physiologia Plantarum*, 29: 92-96.

[9] Olutiola, P.O. & Nwaogwugwu, R.I. (1982). Growth, sporulation and production of maltase and proteolytic enzymes in *Aspergillus aculeatus. Transactions of the British Mycological Society* 78(1): 105-113.

[10] Olutiola, P.O., Famurewa, O. & Sonntag, H.G. (1991). *An Introduction to General Microbiology*: *A Practical Approach.* Heidelberger Verlagsanstalt und Druckerei GmbH, Heidelberg, Federal Republic of Germany. 267pp.

[11] Pfueller, S.L. & Elliott, W.H. (1969). The extracellular α-amylase of *Bacillus stearothemophilus. Journal of Biological Chemistry* 244: 48-54.

[12] Reddy, N.S, Nimmagadda, A. and Sambasiva Rao, K.R.S. (2003). An overview of the microbial α-amylase family. *African Journal of Biotechnology* 2(12): 645-648.

[13] Tortora, G.J., Funke, B.R. and Case, C.L. (1998). *Microbiology*: *An Introduction*. Menlopark, California,

Benjamin/Cunnings Publishers. 810pp.

[14] Tortora, G.J., Funke, B.R. and Case, C.L. (2004). *Microbiology*: *An Introduction.* Pearson Education Inc. 898pp.

[15] Whitaker, D.R., Hanson, K.R. & Datta, P.K. (1963). Improved procedure for purification and characterization of Myrothecium cellulase. *Canadian Journal of Microbiology and Physiology* 41: 671-696.