E3 Journal of Medical Research Vol. 1(7). pp. 091-094, August, 2012
Available online @ http://www.e3journals.org
ISSN 2276-9900 © E3 Journals 2012

Full length research paper

Production of amylase from *Aspergillus niger* using a defined synthetic growth medium and also rice (*Oryza sativa*) as growth substrate

Adekunle Odunayo Adejuwon [1*], Olanike Olajide[2] and Olabisi Adewole[2]

[1]Department of Microbiology,Faculty of Information Technology and Applied Sciences,Lead City University,Ibadan,Nigeria.
[2]Nike Ventures,Yawiri, Akobo-Ojurin,Ibadan,Nigeria.

Accepted June 6, 2012

Aspergillus niger **grew in both a growth medium with rice as carbon and growth source and in a defined synthetic medium with varying carbon and nitrogen sources at 25°C producing amylase. Optimum amylase activity in rice was expressed on the eighth day of incubation as 0.58 Units. In the synthetic growth medium with starch as carbon source and tryptone as nitrogen source, optimum amylase activity was expressed on the seventh day as 0.47 Units; with ammonium chloride as nitrogen source and maltose as carbon source of growth, optimum amylase activity was expressed on the ninth day as 3.525 Units. This investigation suggests a means of production of amylase for industrial purposes.**

Keywords: *Aspergillus* niger; Amylase; synthetic growth medium; rice

INTRODUCTION

The genus *Aspergillus* includes over 185 species and about 20 species have so far been reported as causing opportunistic infections in man (Tortora *et al*., 2004; Prescott *et al*., 2005). Among these species, *A. fumigatus* is the most commonly isolated species followed by *A. flavus* and *A. niger. A. clavatus, A. glancus, A. oryzae, A. terreus, A. ustus* and *A. versicolor* are among the other species less commonly isolated as opportunistic pathogens (Brock DT, Madigan MT 1991; Brock *et al*., 1994). Food infected by *A. flavus* may be carcinogenic to humans and animals (Willey *et al*., 2008). *A. flavus* is a saprophyte of grains. It produces mycotoxins in infected food (Streets RB 1969). Infection of peanuts (*Arachis hypogaea*) seeds by *A. flavus* and *A. parasiticus* is a serious problem that can result in aflatoxin contamination in the seed (Liang *et al*., 2005). *A. flavus* produces aflatoxins B, G and cyclopiazonic acid CPA (Novas MV, Cabral D 2002).

*Corresponding Author Email:adejuwon_ao@yahoo.com; Tel: +2348069781680

Beta-1,3-Glucanase activity in peanut seed is induced by infection with *A. flavus* (Liang *et al*., 2005).Rice (*Oryza sativa*) is a monocotyledonous cereal which belongs to the Grass family Gramineae or Poaceae (Stern et al., 2003). With over 7,000 varieties of rice, its pericarp and embryo contain 70-80% starch, 7% proteins, 1.5% oils, some vitamins (mostly A, B and C) and some essential minerals (Dutta AC 2007). According to Sizer and Whitney (2000), rice contains fibre and the vitamin folate and provides 80% of the calories consumed by humans world-wide (Stern et al., 2003).The present study was designed to produce amylase from *Aspergillus niger* using rice as substrate and using a defined synthetic growth medium.

MATERIALS AND METHODS

Sources and identification of isolate

The isolate of *A. niger* for this research was from deteriorated rice and identified using techniques contained in the illustrated Handbook of fungi (Alexopoulos CJ 1962). The identification was done by

observing cultural and morphological characteristics. The isolate was cultured on Potato Dextrose agar. The nature of growth, rate of growth, colony colour and sporulation patterns were carefully observed. Sporulating mature cultures were used in microscopic examination. Fungal samples were taken from advancing margins and centres of the growth regions with the aid of sterile inoculating needle. The samples were smeared on glass slides and stained with lactophenol cotton blue. After placing the cover slips, macroscopic and microscopic morphological characteristics like arrangement and shape of spores, type of sporangia, type of hyphae, presence or absence of septa on hyphae were examined under the high power objective of a compound binocular microscope.

Culture conditions and preparation of inocula

The isolate was subcultured and maintained on Potato Dextrose agar plates and slants. The fungus was further subcultured into test tubes of the same medium and incubated at 25^{o}C. Ninety-six-hour-old culture of *A. niger* was used as inoculum. The culture was grown in a defined medium of the underlisted composition: $MgSO_4.7H_20$, K_2HPO_4, KH_2PO_4, L-cysteine, biotin, thiamine and $FeSO_4.7H_20$ with added carbon and nitrogen sources (Sigma). Conical flasks (250 ml) containing 50 ml growth medium was inoculated with 1 ml of an aqueous spore suspension containing approximately $5x10^4$ spores per ml of the isolate. Experimental and control flasks were incubated without shaking at 25^{0}C.

Carbon and Nitrogen sources

Certain carbon and nitrogen sources of the growth medium were used in this investigation. When the nitrogen source of growth was tryptone, the carbon source employed was starch. However, when the carbon source of growth was maltose, the nitrogen source employed was ammonium chloride.

Rice as a source of carbon and growth medium

Rice (Caprice) was bought at the main market, Bodija, Ibadan, Nigeria. The rice was added to distilled water (1% w/v) and autoclaved at 15 lb/in^2 at 121^{o}C. Conical flasks (250 ml) containing 100 ml of the rice medium was inoculated with 1 ml of an aqueous spore suspension containing approximately $5x10^4$ spores per ml of the isolate.Experimental and control flasks were incubated without shaking at 25^{0}C.

On a daily basis, the contents of each flask were filtered through glass fiber filter paper (Whatman GF/A). The protein content of the filtrates was determined using the method of Lowry *et al.* (1951).The filtrates were analysed for amylase activity using the modified method of Xiao *et al.* (2006). The filtrates were used as crude preparation.

Enzyme Assay

Amylase

Amylase activity was determined using the modified method of Xiao *et al.* (2006). The reaction mixtures consisted of 2ml of 0.1% (w/v) starch (Sigma) in 0.2M citrate phosphate buffer, pH 6.0 as substrate and 0.5ml of enzyme. These were the experimentals. The controls consisted of only 2 ml of the prepared substrate. The contents of both experimental and control tubes were incubated at 35^{o}C for 30 mins. The reactions were terminated with 3 ml of 1N HCl. Enzyme (0.5ml) was added to the contents of each control. 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 0.1N HCl were added into the contents of each test tube after which 0.1ml of iodine solution was added. Optical density readings were taken spectrophotometrically at 620 nm. Enzyme activity was defined in units. One unit of enzyme activity was defined as the amount of enzyme which produced 0.1% reduction in the blue colour of the starch-iodine complex.

RESULTS

Amylase activities of *Aspergillus niger* on growth medium

Rice as carbon and growth source

Amylase activity was detected on the growth medium used in this study. When rice was carbon and growth medium, amylase activity was optimum on the eighth day and expressed as 0.58 Units (Table 1).

Starch as carbon source and tryptone as nitrogen source

When starch was carbon source with tryptone as nitrogen source, amylase activity was optimum on the seventh day and was expressed as 0.47 Units (Table 2).

Ammonium chloride as nitrogen source and maltose as carbon source

When the nitrogen source was ammonium chloride with maltose as carbon source, amylase activity was optimum

Table 1: Effect of rice as carbon and growth source on amylase activity produced by *A. niger*

Carbon source		Days 1	2	3	4	5	6	7	8	9	10
Rice	Activity (Units)	0.00	0.00	0.025	0.065	0.01	0.325	0.32	0.58	0.535	0.54
	Protein (OD 600nm)	0.27	0.20	0.25	0.275	0.295	0.30	0.345	0.32	0.35	0.455

Table 2: Effect of starch as carbon source and tryptone as nitrogen source on amylase activity produced by *A. niger*

Carbon source		Days 1	2	3	4	5	6	7	8	9	10
Starch	Activity (Units)	0.00	0.00	0.00	0.00	0.00	0.01	0.47	0.395	0.34	0.45
	Protein (OD 600nm)	0.14	0.265	0.165	0.165	0.185	0.155	0.15	0.235	0.21	0.25

Table 3: Effect of ammonium chloride as nitrogen source and maltose as carbon source on amylase activity produced by *A. niger*

Nitrogen Source		Days 8	9	10
Ammonium chloride	Activity (Units)	3.4	3.525	3.05
	Protein (OD 600nm)	0.325	0.55	0.55

on the ninth day and expressed as 3.525 Units (Table 3).

DISCUSSION

Amylase was produced by *A. niger* in growth medium containing rice as carbon and growth source at 25^{O}C. Production of activity was optimum on the eighth day. However, activity was optimum on the sixth day with starch as carbon source and tryptone as nitrogen source. With ammonium chloride as nitrogen source and maltose as carbon source, amylase activity was optimum on the ninth day. Conditions of growth of the organism seemed viable for amylase production. Enzymes occur in every living cell, hence in all microorganisms. Each single strain of organism produces a large number of enzymes, hydrolyzing, oxidizing or reducing, and metabolic in nature (Prescott *et al*., 2005). Bacterial α-amylases are produced at a much wider range of temperature. *Bacillus amyloliquefaciens*, *B. subtilis*, *B. licheniformis* and *B. stearothermophilus* are among the most commonly used *Bacillus* sp. reported to produce α- amylase at temperatures 37-60°C (Mielenz, 1983). A cold active α-amylase from Antarctic psychrophile *Alteromonas haloplanktis* was reported to exhibit maximum α-amylase production at 4 °C (Feller *et al*., 1998). The influence of temperature on amylase production is related to the growth of the organism. Hence, the optimum temperature depends on whether the culture is mesophilic, thermophilic or psycrophilic. Among the fungi, most amylase production studies have been done with mesophilic fungi within the temperature range of 25–37 °C (Francis *et al*., 2003).

The carbon sources (starch and maltose) and the

nitrogen sources (tryptone and ammonium chloride) employed in this investigation, incorporated into the growth medium, induced amylase production by *A. niger*. Bread, starch and maltose, sucrose, lactose, glucose and galactose as carbon sources with potassium nitrate as nitrogen source supported growth and α-amylase production by *Lasiodiplodia theobromae* (Adejuwon, 2011b).An extremely important use for fungal amylases is in conversion of partially acid hydrolyzed starch to sweet syrups. Acid hydrolysis is a random action whereas enzymic hydrolysis is a patterned one. By proper control of the type and proportion of enzymes used (α-amylase, amyloglucosidase, maltase) syrups of almost any desired proportions of glucose, maltose, and dextrins may be produced. Cold active amylases are mostly extra cellular and are highly influenced by nutritional and physicochemical factors such as temperature, agitation, pH, nitrogen source, carbon source, inducers, inorganic sources and dissolved oxygen.To meet the demand of industries, low-cost medium is required for the production of α-amylase. Both solid state fermentation (SSF) and submerged fermentation (SmF) could be used for the production of α-amylases, although traditionally these have been obtained from submerged cultures because of ease of handling and greater control of environmental factors such as temperature and pH. SSF has been used for long to convert moist agricultural polymeric substrates such as wheat, rice, soy, cassava, etc. into fermented food products including industrial enzymes (Pandey *et al*., 1995). Adejuwon (2010) reported the production of amylase by *A. niger* isolated from citrus fruit. *A. flavus* Linn. associated with the green mould rot of yam produced a β-amylase in a synthetic growth medium with optimum activity at pH 5.0 (Adejuwon, 2011a). *Penicillium* species isolated from apple fruit produces amylase stimulated by $Ca^{++,}$ $Mg^{++,}$ Na^{+} and K^{+} (Adejuwon, 2011c).

Conclusively, amylase can be produced for industrial purpose from *A. niger* grown in rice and the defined synthetic growth medium with starch as carbon source and tryptone as nitrogen source and also with ammonium chloride as nitrogen source and maltose as carbon source.

REFERENCES

Adejuwon AO (2010). Synthetic production of amylase from *Aspergillus niger* isolated from citrus fruit. *African Journal of Basic and Applied Sciences* 2(5-6): 158-160.

Adejuwon AO (2011a). β-amylase associated with the green mould rot of *Dioscorea rotunda*. *Insight Biochemistry* 1(1): 1-4.

Adejuwon AO (2011b). Nutritional factors affecting the production of α-amylase in *Lasiodiplodia theobromae* Pat. *Biotechnology, Bioinformatics and Bioengineering* 1(1): 131-135.

Adejuwon AO (2011c). Synthetic production of amylase from *Penicillium species* isolated from citrus fruit. *World Applied Sciences Journal* 13(3): 415-418.

Alexopoulos CJ (1962). *Introductory Mycology*. John Wiley and Sons Inc., New York, London. Pp 263-273.

Brock DT, Madigan MT (1991). *Biology of Microorganisms*. Prentice-Hall International Inc. 900pp.

Brock DT, Madigan MT, Martinko JM, Parker J (1994). *Biology of Microorganisms*. Prentice-Hall, Inc. Eaglewood Cliffs, New Jersey. Pp 528-530.

Dutta AC (2007). *Botany for Degree Students*. Oxford University Press, New Delhi. 708pp.

Feller G, Bussy OL, Gerday C (1998). Expression of psychrophilic genes in mesophilic hosts; Assessment of the folding state of a recombinant α-amylase. *Appl. Environ. Microbiol*. 64: 1163–1165.

Francis F, Sabu A, Nampoothiri KM, Ramachandran S, Ghosh S, Szakacs G, Pandey A (2003). Use of response surface methodology for optimizing process parameters for the production of α-amylase by *Aspergillus oryzae*, *Biochem. Eng. J*. 15: 107–115.

Liang XQ, Holbrook CC, Lynch RE, Guo BZ (2005). Beta-1,3-glucanase activity in peanut seed (*Arachis hypogaea*) is induced by inoculation with *Aspergillus flavus* and copurifies with a conglutin-like protein. *Phytopathology* 95: 506-511.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193:265-275.

Mielenz JR (1983). *Bacillus stearothermophilus* contains a plasmid-borne gene for α-amylase. *Proc. Natl. Acad. Sci*. 80: 5975–5979.

Novas MV, Cabral D (2002). Association of mycotoxin and sclerotia production with compatibility groups in *Aspergillus flavus* from peanut in Argentina. *Plant Disease* 86: 215-219.

Pandey A, Ashakumary L, Selvakumar P (1995). Copra waste- A novel substrate for solid-state fermentation. *Biores. Technol*. 51: 217–220.

Prescott LM, Harley JR, Klein DA (2005). *Microbiology*. McGraw Hill, New York. 992pp.

Sizer FS, Whitney EN (2000). *Nutrition*: *Concepts and Controversies*. Wadsworth, Thomson Learning, London. 567pp.

Stern KR, Jansky S, Bidlack JE (2003). *Introductory Plant Biology*. McGraw Hill Higher Education, New York. 624pp.

Streets RB (1969). *Diseases of the Cultivated Plants of the Southwest*. The University of Arizona Press.

Tortora GJ, Funke BR, Case CL (2004). *Microbiology*: *An Introduction*. Pearson Education Inc. 898pp.

Willey JM, Linda MS, Woolverton CJ (2008). *Prescott, Harley and Klein's Microbiology*. McGraw Hill Companies Inc. 1088pp.

Xiao Z, Storms R, Tsang A (2006). A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Analytical Biochemistry* 351(1): 146-148.