The effect of carbon source of growth medium on α-amylase production by strains of *Penicillium solitum* and *Aspergillus rubrum* isolated from yam (*Dioscorea alata*)

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Abstract: *Penicillium solitum* and *Aspergillus rubrum* isolated from deteriorated yam (*Dioscorea alata*) using potato dextrose agar grew and sporulated at 25°C. They expressed Ŭ-amylase activity in a synthetic growth medium with potassium nitrate as nitrogen source and certain carbon sources. The carbon sources were varied and were starch, maltose, sucrose, galactose, glucose and lactose. Rice (*Oryza sativa*) also supported fungal growth and Ŭ-amylase production. When rice was growth medium, *Aspergillus rubrum* seemed a better producer of Ŭ-amylase than *Penicillium solitum* as expressed on the tenth day of inoculation. Highest activity was expressed by *Penicillium solitum* on the tenth day of inoculation of the synthetic medium when maltose was carbon source. Least activity was expressed by *Aspergillus rubrum* when galactose was carbon source.
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1. Introduction

Several fungal species including members of Aspergilli and Penicilli are saprophytes of grains (Streets, 1969). Crop infection may lead to aflatoxin consumption in man and animals (Liang *et al.*, 2005). Novas and Cabral (2002) reported the production of aflatoxin B, G and cyclopiazonic acid by certain species of Aspergilli in infected seeds. Resistance to aflatoxin may be controlled by apistatis (Walker and White, 2001). Apart from the production of aflatoxin, several fungal species are capable of enzyme production when grown on a suitable substrate (Adejuwon *et al.*, 2006; Ladokun and Adejuwon, 2011).

Hydrolytic cleavage of yam tissues by Ŭ-amylase produced by *Lasiodiplodia theobromae* during infection may seem implicated in yam deterioration (Adejuwon, 2011). In the present study, we present the induction of Ŭ-amylase in yam (*Dioscorea alata*) phytopathogens, *Penicillium solitum* and *Aspergillus rubrum*, by a defined growth medium with a varied carbon source and rice (*Oryza sativa*). This is with a view to recommending a suitable substrate for Ŭ-amylase production.

2. Materials and Methods

2.1 Source and Identification of Fungi

Penicillium solitum and *Aspergillus rubrum* used in this research were from deteriorated yam (*Dioscorea alata*) 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).

2.2 Culture Conditions and Inocula

The isolates were cultured and maintained on Potato Dextrose agar plates. The fungi were further subcultured into test tubes of the same medium and incubated at 25°C. Ninety-six-hr-old culture of *Aspergillus rubrum* and one hundred and twenty-hr-old culture of *Penicillium solitum* were used. Based on the method of Olutiola and Ayres (1973), cultures were grown in a defined medium of the following composition: $MgSO_4.7H_20$ (0.1g), K_2HPO_4 (2g), KH_2PO_4 (0.5g), L-cysteine (0.1g), biotin (0.005mg), thiamine (0.005mg) and $FeSO_4.7H_20$ (1mg) with added carbon (10g) and nitrogen (9.9g) 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 potassium nitrate. One hundred millilitre of growth medium in conical flasks (250 ml) was inoculated with 1 ml of an aqueous spore suspension containing approximately $6x10^4$ spores per ml of isolate. Spores were counted using the Neubauer counting chamber (Olutiola *et al.*, 1991). Experimental and control flasks were incubated without shaking at 25^0C (Olutiola and Nwaogwugwu, 1982).

2.3 Rice (*Oryza sativa*) as Carbon Source

Rice (*Oryza sativa*) was bought at the Main Market, Ile-Ife, Nigeria and identified at the Herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The rice was added to distilled water (1% w/v) and autoclaved at 15Ib/in^2 (121^{o}C) for 15 minutes. 100 ml of the rice medium in conical flasks (250ml) was inoculated with 1 ml of aqueous spore suspension containing approximately 6x10^4 spores per ml of each isolate.

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 Ŭ-amylase activity using the method of Pfueller and Elliott (1969).

2.4 Enzyme and Protein Assays

2.4.1 α-Amylase

Ŭ-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^{o}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 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.

2.4.2 Protein Concentration Determination

Protein concentration was determined by the method of Lowry *et al.* (1951). The Lowry assay, a colometric protein assay, is based on the reaction of the protein with copper in alkali and the reduction of the phosphomolybdic-phosphotungstic reagent (Folinôs reagent) by the copper treated protein. Serial dilutions of bovine serum albumin was used to plot a standard graph. The unknown protein value in each test sample was intrapolated from the standard calibration graph.

2.5 Ammonium Sulphate Fractionation

On the tenth day of inoculation of medium, the crude enzymes were treated with ammonium sulphate (analytical grade) at 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. Ŭ-Amylase activity was determined using the method of Pfueller and Elliott (1969). Protein content was determined using the Lowry *et al.* (1951) method.

2.6 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^{o}C for 24 h. Ŭ-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).

3. Results

3.1 α-Amylase activities of isolates on growth media

Penicillium solitum and *Aspergillus rubrum* grew and produced Ŭ-amylase activity in the modified growth medium used in this study. The carbon sources (starch, maltose, sucrose, lactose, glucose and galactose) of growth medium, with potassium nitrate as nitrogen source and rice (*Oryza sativa*) induced activity, varyingly. Induction expressed by the tenth day of incubation is represented in Table 1.

Table 1: Effect of carbon source on Ŭ-amylase production by isolates

Carbon source	Isolate	Amylase activity (Specific Activity) (Units/mg Protein)
Rice	*Penicillium solitum*	0.43 ± 0.03
	Aspergillus rubrum	0.62 ± 0.06
Galactose	*Penicillium solitum*	0.36 ± 0.05
	Aspergillus rubrum	0.09 ± 0.04
Glucose	*Penicillium solitum*	0.44 ± 0.08
	Aspergillus rubrum	0.32 ± 0.01
Lactose	*Penicillium solitum*	0.40 ± 0.07
	Aspergillus rubrum	0.37 ± 0.08
Maltose	*Penicillium solitum*	0.76 ± 0.01
	Aspergillus rubrum	0.58 ± 0.02
Starch	*Penicillium solitum*	0.68 ± 0.03
	Aspergillus rubrum	0.60 ± 0.04
Sucrose	*Penicillium solitum*	0.59 ± 0.03
	Aspergillus rubrum	0.37 ± 0.06

Each value represents the mean of three replicates with standard error

Rice (*Oryza sativa*) supported fungal growth and Ŭ-amylase production. When rice was growth medium, *Aspergillus rubrum* seemed a better producer of Ŭ-

amylase than *Penicillium solitum* as expressed in Table 1. Highest activity was expressed by *Penicillium solitum* on the tenth day of inoculation of synthetic medium with maltose as carbon source. Least Ŭ-amylase activity was expressed by *Aspergillus rubrum* when galactose was carbon source.

4. Discussion

From the results of this investigation, starch, maltose, lactose, glucose, galactose and maltose as carbon source with potassium nitrate as nitrogen source of a defined growth medium and rice (*Oryza sativa*), induced Ŭ-amylase production by *Penicillium solitum* and *Aspergillus rubrum. Aspergillus rubrum* induced relatively more activity than *Penicillium solitum*, by the tenth day of incubation, when rice was substrate and carbon source. According to Ladokun and Adejuwon (2011), *Aspergillus fumigatus* produced amylase in rice within ten days of infection with unit activity optimum on the fifth day. Earlier investigation revealed the production of amylase by *Penicillium solitum* in a similar growth medium with varying nitrogen source (Adejuwon *et al*., 2013). Highest activity was expressed by *Penicillium solitum* on the tenth day of inoculation of medium when maltose was carbon source. Least activity was expressed by *Aspergillus rubrum* when galactose was carbon source.

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