Nutritional factors affecting α-amylase production by *Aspergillus versicolor* and *Aspergillus terreus*: Effect of nitrogen source of growth medium

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Abstract: *Aspergillus versicolor and Aspergillus terreus* isolated from deteriorated rice using potato dextrose agar grew at 25°C and expressed α-amylase activity in a growth medium with starch as carbon source and various nitrogen sources. The varied nitrogen sources were potassium nitrate, sodium nitrate, tryptone, peptone, ammonium chloride, ammonium sulphate, glycine and urea. Ammonium sulphate was best inducer of α-amylase by *Aspergillus versicolor* whereas, glycine was best inducer by *Aspergillus terreus*.
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1. Introduction

Members of the genus Aspergillus are filamentous cosmopolitan and ubiquitous fungi commonly isolated from soil, plant debris and indoor air environment (Streets, 1969). While the teleomorphic state exists for species in this group, some are accepted to be mitosporic without any known sexual spore (Dutta, 2007). Some members produce the hydrolytic enzyme amylase (Ladokun and Adejuwon, 2011) and carcinogenic toxins in infected food causing harm to humans and animals (Willey *et al*., 2008). Amylase is induced during fungal growth on a suitable substrate (Adejuwon, 2011).

In the present investigation, we examined inductivity of α-amylases in the phytopathogens *Aspergillus versicolor* and *Aspergillus terreus* isolated from deteriorated rice grown in a defined medium with varying source of nitrogen. The enzymes were compared. Although α-amylase are of industrial importance, the process of growth and enzyme induction may be developed and engaged in biological warfare and bioterrorism.

2. Materials and Methods

2.1 Source and Identification of Isolates

The isolates, *Aspergillus versicolor* and *Aspergillus terreus*, for this research were from deteriorated rice and 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 (Hanlin, 1990). Each isolate was cultured on Potato Dextrose agar plates.

2.2 Culture Conditions and Inocula

The isolates were subcultured on Potato Dextrose agar slants in MacCatney bottles. Each fungus was again subcultured into slants in test tubes of the same medium and incubated at 25°C. Ninety-six hr-old cultures were used in this investigation. Modifying the method of Adejuwon and Olutiola (2007), cultures were grown in a defined medium of the following 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) in 500ml of distilled water. The carbon source used was starch (soluble). The nitrogen source was varied as: potassium nitrate, sodium nitrate, ammonium sulphate, ammonium chloride, tryptone, peptone, glycine and urea . Conical flasks (250 ml) containing 100 ml growth medium were 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 Enzyme Assay

2.3.1 α-Amylase

α-Amylase activity was determined using the method of Pfueller and Elliott (1969) . The reaction mixtures consisted of 2 ml of 0.05% (w/v) starch in 0.2 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 10 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 enzyme activity was defined as the amount of enzyme which produced 0.01 percent 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 mg protein.

2.4 Ammonium Sulphate Fractionation

The crude enzymes, on the tenth of activity were treated with ammonium sulphate (analytical grade) at 90% saturation. Precipitation was allowed to continue at 4°C for 24 h. The mixtures were centrifuged at 6,000g for 30 minutes at 4°C using a cold centrifuge (Optima LE-80K Ultracentrifuge, Beckman, USA) at the Central Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria. The supernatant were discarded. The precipitate was redissolved in 0.2 M 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.

2.5 Dialysis

With a multiple dialyser, and using acetylated dialysis tubings (Visking dialysis tubings, Sigma) (Whitaker *et al.* 1963) the enzymes were dialysed under several changes of 0.2 M citrate phosphate buffer, pH 6.0 at 4°C for 24 h. Thereafter we assayed for α-amylase activity using the method of Pfueller and Elliott (1969). Protein content was determined using the method of Lowry *et al*. (1951).

3. Results

Aspergillus versicolor and *Aspergillus terreus* grew and expressed amylase activity in the modified growth medium used in this present investigation. Ammonium sulphate, glycine, potassium nitrate, sodium nitrate, ammonium chloride, urea, tryptone and peptone as source of nitrogen for growth in the medium, with starch as carbon source induced enzyme activity. α-Amylase activity expressed by the tenth day of incubation is represented in Table 1.

Table 1: Effect of nitrogen source on activity of α-amylase produced by fungi

Nitrogen source	Isolate	Specific activity (Units/mg Protein)
Ammonium sulphate	*Aspergillus versicolor*	3.28 ± 0.18
	Aspergillus terreus	0.05 ± 0.01
Glycine	*Aspergillus versicolor*	0.38 ± 0.13
	Aspergillus terreus	2.48 ± 0.03
Potassium nitrate	*Aspergillus versicolor*	0.50 ± 0.01
	Aspergillus terreus	1.30 ± 0.10
Ammonium chloride	*Aspergillus versicolor*	3.00 ± 0.25
	Aspergillus terreus	0.25 ± 0.05
Peptone	*Aspergillus versicolor*	0.25 ± 0.03
	Aspergillus terreus	1.50 ± 0.05
Sodium nitrate	*Aspergillus versicolor*	0.38 ± 0.03
	Aspergillus terreus	1.48 ± 0.02
Tryptone	*Aspergillus versicolor*	0.25 ± 0.01
	Aspergillus terreus	0.20 ± 0.05
Urea	*Aspergillus versicolor*	0.15 ± 0.01
	Aspergillus terreus	2.32 ± 0.03

Each value represents the mean of three replicates with standard error

4. Discussion

In this investigation, by the tenth day of incubation, at 25°C, α-amylase was produced varyingly by *Aspergillus versicolor* and *Aspergillus terreus* with the nitrogen sources of growth employed, with starch as carbon source. Ammonium sulphate, ammonium chloride, sodium nitrate and tryptone as nitrogen sources of growth were better inducers of α-amylase in *Aspergillus versicolor* than in *Aspergillus terreus*. However, glycine, potassium nitrate, peptone and urea better induced α-amylase in *Aspergillus terreus* than in *Aspergillus versicolor*. Adejuwon (2010) reported the induction of amylase similarly by *Aspergillus niger* isolated from citrus fruit. Ammonium sulphate induced highest activity on the tenth day in *Aspergillus versicolor* where as, glycine

induced highest activity in *Aspergillus terreus* by the tenth day.

From the results of this study, it is suggested that the isolates, *Aspergillus versicolor* and *Aspergillus terreus*, can be explored as biomarkers of industrial production of α-amylase. The possibility of also exploring them as means in biological warfare and in bioterrorism should not be ignored.

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