Expression of α-Amylase by a Tropical Strain of *Penicillium citrinum*: Effect of Nitrogen Source of Growth

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Abstract: Background: *Penicillium citrinum* is an anamorph mesophilic fungus capable of producing tanzawaic acid A, mevastatin, quinocitrinine A, quinocitrinine B and nephrotoxic citrinin. In the tropics, this fungus is found on mouldy citrus fruits and usually contaminates tropical spices and cereals. **Materials and Methods:** A defined medium with starch as carbon source and varied nitrogen source was inoculated with spore suspensions of approximately $5x10^5$ spores per ml of a one hundred and twenty-hour old culture of a tropical strain of *Penicillium citrinum* plated on potato dextrose agar. **Results:** Extracellular α-amylase was expressed within a period of ten days in the inoculated defined medium. Urea, ammonium chloride, ammonium sulphate and sodium nitrate were good nitrogen sources of growth and α-amylase production in *Penicillium citrinum.* **Conclusion:** Starch as carbon source of growth with urea, ammonium chloride or ammonium sulphate as nitrogen source will support good expression of α-amylase activity by *Penicillium citrinum.* Optimum expression is day dependent.
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Key words: *Penicillium citrinum*, defined medium, α-amylase

1. Introduction

Penicillium citrinum belongs to the taxonomic Division Eumycota, Subdivision, Eumycotina, Class Ascomycetes, Order Eurotiales and Family Trichocomaceae (Alexopoulos, 1962). It is a mould found in the tropics on citrus fruits (Streets, 1969). It is a common contaminant of cereals and tropical spices (Fiqueroa *et al.*, 2009; Hashem and Alamn, 2010; Houbraken *et al.*, 2010).

α-Amylases are hydrolytic enzymes relevant in textiles, breweries and detergents (Aiyer, 2005). They are mostly imported into Nigeria, West Africa for industrial purposes.

In this present investigation, a defined medium with starch as carbon source was inoculated with a strain of *Penicillium citrinum* with a view to understanding the effect of varying nitrogen compounds on the expression of α-amylase by the fungus.

2. Materials and Methods

2.1 Isolate source and identification

The isolate, *Penicillium citrinum* for this research study was part of the culture collection of Professor P.O. Olutiola formerly of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. It 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 (Hanlin, 1990).

2.2 Culture conditions and inoculum

The isolate was cultured and maintained on Potato Dextrose agar slants and plates. The fungus was subcultured into test tubes of the same medium and incubated at 25°C. One hundred and twenty-hr-old culture was used in this study. According to the modified 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 FeSO4.7H20 (1 mg) with starch as carbon source (10 g) and a nitrogen (9.9 g) sources (Sigma) in 1 litre of distilled water. The nitrogen source used was varied. They were ammonium sulphate, ammonium chloride, glycine,

urea, potassium nitrate, tryptone, peptone and sodium nitrate. Conical flasks (250 ml) containing 100 ml growth medium were 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). Experimental and control flasks were incubated without shaking at 25^0C (Olutiola and Nwaogwugwu, 1982).

2.2.1 α-Amylase assay

The method of Pfueller and Elliott (1969) was used to determine α-amylase activity in this investigation. 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°C for 30 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 620 nm. 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 as enzyme units per mg protein.

3. Results

From the results of this investigation, as observed (Table 1), starch as carbon source of the defined medium and ammonium chloride as nitrogen source was effective in stimulating the production of α-amylase in *Penicillium citrinum* with optimum activity expressed on the eighth day as 51 units/mg protein. Activity declined steadily on days nine and ten and were 34 and 22 units/mg protein respectively. Urea as nitrogen source supported the expression of α-amylase by *Penicillium citrinum.* Activity was also optimum on the eighth day of inoculation of medium and was 58 units/mg protein. A decline followed on the ninth and tenth days. Activity was nil on the tenth. With potassium nitrate as nitrogen source, α-amylase expression by *Penicillium citrinum* increased from day eight to day nine of inoculation of medium. At day eight, activity was 5 units/mg protein. It was 16 units/mg protein at day nine. Activity declined to 14 units/mg protein at day ten. When ammonium sulphate was nitrogen source, α-amylase activity was nil at days eight and nine. Activity was substantial at day ten and was to 53 units/mg protein. With glycine, activity was 24 units/mg protein at day eight. However it was nil at days nine and ten. With sodium nitrate as nitrogen source, α-amylase activity was 45 units/mg protein at day eight. It rose to 51 units/mg protein at day nine and decline to 41 units/mg protein at day ten. When tryptone was nitrogen source, α-amylase activity was 5 units per mg protein at day eight, rising to 18 units/mg protein at day nine, but however declining to 16 units/mg protein at day ten. With peptone as nitrogen source of growth, α-amylase activity rose steadily from days eight to ten. It was10 units/mg protein on day eight, 13 units/mg protein on day nine and 54 units/mg protein on day ten.

Table 1: Effect of nitrogen source on α-amylase activity produced by *Penicillium citrinum*

Nitrogen source	Days		
	8	9	10
Ammonium chloride	51	34	22
Urea	58	13	0
Potassium nitrate	5	16	14
Ammonium sulphate	0	0	53
Glycine	24	0	0
Sodium nitrate	45	51	41
Tryptone	5	18	16
Peptone	10	13	54

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

*Readings of results of activity were presented from day eight to ten because of relative observable comparability

4. Discussion

Urea, ammonium sulphate, ammomium chloride and sodium nitrate best enhanced α-amylase produced by *Penicillium citrinum* at days nine to ten of inoculation of our defined medium. Declines in activity were observed from days eight to ten for ammonium chloride, urea and sodium nitrate. The ammonium and sodium salts used in this study seem good enhancers of α-amylase in *Penicillium citrinum*. In a much earlier investigation, sodium nitrate supported best growth and ammonium tartrate supported best sporulation of *Aspergillus tamarii* Kita (Olutiola, 1973). According to Vahidi *et al.* (2005), ammonium chloride was a good nitrogen source in growth medium for amylase production by *Mucor spp.* However, reports of Hassan and Karim (2012) seem to support urea as a good nitrogen source for alpha amylase production by *Bacillus subtilis* with ammonium sulphate and sodium nitrate being less effective nitrogen sources for α-amylase production by their strain of *Bacillus subtilis.*

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