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

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Abstract: Background: *Penicillium rubrum* contains rubratoxin associated with jaundice and convulsions in man. In the tropics, this fungus is a common contaminant of cereal grains. **Materials and Methods:** A defined medium with starch as carbon source and varied nitrogen source was inoculated with spore suspensions of approximately $3x10^5$ spores per ml of a tropical strain of *Penicillium rubrum.* Incubation was at 30°C. **Results:** Extracellular α-amylase was expressed within a period of ten days in the inoculated defined medium. Ammonium chloride, urea, potassium nitrate, ammonium sulphate, glycine, sodium nitrate, tryptone and peptone as nitrogen source supported good expression of amylase by *Penicillium rubrum.* However, Urea, tryptone and peptone were best nitrogen sources. **Conclusion:** Starch as carbon source of growth with urea, tryptone or peptone as nitrogen source will support good expression of α-amylase activity by *Penicillium rubrum* within ten days.
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1. Introduction

Penicillium rubrum belongs to the taxonomic Division Eumycota, Subdivision, Eumycotina, Class Ascomycetes, Order Eurotiales and Family Trichocomaceae (Hanlin, 1990). It infects grains and grain products in the tropics producing rubratoxin B and rubratoxin A (dihydrorubratoxin B) causing kidney and liver damage in infected hosts (El-banna *et al.*, 1987). Rubratoxin B has been observed to be teratogenic and fetotoxin in mice (El-banna *et al.*, 1987).

α-Amylases are hydrolytic enzymes requiring calcium ions for stability (Aiyer, 2005). They are produced by a variety of microorganisms especially fungi (Adejuwon, 2011; Adejuwon *et al.*, 2012; Ajayi *et al.*, 2014).

In this current investigation, a defined medium with starch as carbon source was inoculated with spore suspensions of a tropical strain of *Penicillium rubrum.* This was with a view to understanding the varying effects of different nitrogen compounds as part of the growth medium on the fungus' expression of α-amylase.

2. Materials and Methods

2.1 Source and Identification of Isolate

The tropical strain of the isolate *Penicillium rubrum* (PEN 03) used in this investigation was part of the culture collection of Professor Patrick 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. Techniques contained in an illustrated Handbook of Fungi (Hanlin, 1990) were used in identification.

2.2 Culture conditions and inoculum

The isolate *Penicillium rubrum* (PEN 03) 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 30°C. Ninety-six-hr-old culture was used in this investigation. 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 50 ml growth medium were inoculated with 1 ml of an aqueous spore suspension containing approximately $3x10^5$ 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 30^0C (Olutiola and Nwaogwugwu, 1982).

2.2.1 α-Amylase assay

α-Amylase activity was determined using the method of Pfueller and Elliott (1969) 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 670 nm. One unit of α-amylase activity was arbitrarily defined as the amount of α-amylase 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 calculated as α-amylase units per mg protein.

3. Results

The results of this investigation are represented in Table 1. As observed, starch as carbon source in our defined medium with ammonium chloride as nitrogen source supported production of α-amylase by *Penicillium rubrum.* Activity was 56 units/mg protein on the eighth day, rising to an optimum 66 units/mg protein on the ninth day and then declining to 60 units/mg protein on the tenth day. Urea as nitrogen source supported the expression of α-amylase by *Penicillium rubrum.* Activity was 135 units/mg protein on day eight, an optimum 231 units/mg protein on day nine and a decline to 228 units/mg protein on day ten. When potassium nitrate was nitrogen source, α-amylase expression by *Penicillium rubrum* was 43 units/mg protein on day eight. Activity increased steadily. It was 58 units/mg protein on day nine and 82 units/mg protein on day ten. With ammonium sulphate was nitrogen source, α-amylase activity was nil at day eight. It was 56 units/mg protein at nine but 43 units/mg protein at day ten. With glycine, activity was 55 units/mg protein at day eight. It rose steadily to 67 units/mg protein on day nine and 95 units/mg protein on day ten. With sodium nitrate as nitrogen source, α-amylase activity again rose steadily. It was 39 units/mg protein at day eight, 74 units/mg protein at day nine and 94 units/mg protein at day ten. When tryptone was nitrogen source, α-amylase activity declined steadily. It was 178 units per mg protein at day eight 146 units/mg protein at day nine and 134 units/mg protein at day ten. With peptone as nitrogen source of growth α-amylase activity was 155 units/mg protein on day eight, 173 units/mg protein on day nine and 158 units/mg protein on day ten.

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

Nitrogen source	Days		
	8	9	10
Ammonium chloride	56	66	60
Urea	135	231	228
Potassium nitrate	43	58	82
Ammonium sulphate	0	56	43
Glycine	55	67	95
Sodium nitrate	39	74	94
Tryptone	178	146	134
Peptone	155	173	158

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

4. Discussion

All the nitrogen compounds used in this current investigation with starch as carbon source supported production of α-amylase by our tropical strain of *Penicillium rubrum* suggestive of constitutive expression of the enzyme in the fungus. However, urea, tryptone and peptone separately as nitrogen source were best supports at days eight to ten. According to Malhotra *et al.* (2000), tryptone and starch were best sources of nitrogen and carbon for production of a calcium independent α-amylase by an extremely thermophilic strain of *Bacillus thermooleovorans.* Also peptone was a good source of organic nitrogen for production of α-amylase by *Bacillus licheniformis* (Aiyer, 2004).

α-Amylases are important enzymes with tremendous applications in the industrial sector. In Nigeria, West Africa, they are imported in tons resulting in cumulating national debts. The need for indigenous production of this enzyme is important and the resourceful avenue for production is available. The industrial production of α-amylase is encouraged in the tropics especially Nigeria, West Africa. This can be facilitated by exploring the constitutive expression of the enzyme as demonstrated in our strain of *Penicillium rubrum.*

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