The Effect of Carbon Source of Growth on α-Amylase Production by a Tropical Isolate *Penicillium rubrum*

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Abstract: Background: In a recent investigation, a tropical isolate *Penicillium rubrum* was reported to express relative substantial α-amylase activity with starch as carbon source and urea, tryptone or peptone as nitrogen source of growth. **Materials and methods:** In the present investigation, a defined growth medium with potassium nitrate as nitrogen source was inoculated with spore suspensions of approximately $6x10^5$ spores per ml of a same tropical strain *Penicillium rubrum.* The carbon source of growth was varied and was separately starch, maltose, sucrose, lactose, glucose and galactose. Bread as sole carbon source was also inoculated with the same spore suspension of the isolate. Incubation was at 25°C. Extracellular proteins produced in medium during the process of growth were analysed for α-amylase activity. **Results:** Protein extracts in medium inoculated with the tropical *Penicillium rubrum* exhibited α-amylase activity with all the carbon compounds used in the study. Maximum expression was with starch and was 252 units/mg protein on the 6th day of inoculation of medium. Delayed expressions were observed with sucrose, lactose, glucose and galactose, suggestive of slight enzyme repression. **Conclusion:** Potassium nitrate is a good source of nitrogen when starch is carbon source of growth for expression of α-amylase in the tropical strain of *Penicillium rubrum*. Sucrose seems not to be a good source of carbon with potassium nitrate as nitrogen source of growth for substantial amylase expression in *Penicillium rubrum* at 25°C.
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Key words: *Penicillium rubrum*, α-amylase, growth medium, carbon source

1. Introduction

Penicillim is known for its importance in the production of the antibiotic penicillin and griseofluvin (Tortora *et al.*, 2004). However, most species of the fungus are common contaminants of storage products most especially in the tropics (Brock and Madigan, 1991) as observed in Nigeria, West Africa.

It was recently demonstrated that a tropical strain of *Penicillium rubrum* produced α-amylases in a defined medium with starch as carbon source and certain nitrogen compounds (Adejuwon *et al.*, 2015). In this current investigation, potassium nitrate as nitrogen source of the same medium; and also bread as carbon source and growth medium were inoculated with spore suspensions of this tropical strain of *Penicillium rubrum*. The effects of varied carbon compounds on α-amylase production are herein reported.

2. Materials and Methods

2.1 Isolate Source and Identification

The isolate *Penicillium rubrum* (PEN 03) used for this research investigation was a tropical strain and a part of the culture collection of Professor Patrick O. Olutiola in the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria, West Africa. The isolate was identified at the Seed Health Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Mycological techniques contained in the illustrated Handbook of Fungi were used in identification of the isolate (Hanlin, 1990).

2.1.1 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 25°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 potassium nitrate as nitrogen source (9.9 g) and a carbon (10 g) source (Sigma) in 1 litre of distilled water. The carbon source used was varied. The carbon sources were independently starch, maltose, sucrose, lactose, glucose and galactose. Conical flasks (250 ml) containing 100 ml growth medium were inoculated with 1 ml of an aqueous spore suspension containing approximately $6x10^5$ spores per ml of isolate. Spores were counted using the Neubauer counting chamber (Olutiola *et al.*, 1991; Adejuwon, 2011). Experimental and control flasks were incubated without shaking at 25^0C (Olutiola and Nwaogwugwu, 1982). Protein

content of the inoculated medium was determined using the Lowry *et al,* (1951) method.

2.2 α-Amylase Assay

The method used in the determination of α-amylase in this investigation was that 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°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 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 expressed as α-amylase units per mg protein.

3. Results

All the carbon compounds used in this study supported α-amylase expression in *Penicillium rubrum*. Potassium nitrate was the nitrogen source of growth (Table 1).

With starch as carbon source, α-amylase activity was nil at day one. Activity was expressed as 49 units/mg protein at day two. α-Amylase activity increased steadily with optimum expressed as 252 units/mg protein on day six, thereafter a decline was observed.

When maltose was carbon source, α-amylase activity was 22 units/mg protein at day one. Activity increased daily with optimum expressed as 73 units/mg protein at day seven after which there was a decline.

With sucrose as carbon source, α-amylase activity was nil at the 1st and 2nd days of inoculation of medium. Activity was 1 unit/mg protein at day three, 10 units/mg protein at day nine and an optimum 19 units/mg protein at day ten.

When lactose was carbon source, α-amylase activity was nil at days one and two. It was 58 units/mg protein on day three. Activity rose steadily to an optimum 192 units/mg protein on day seven after which a decline was observed.

With glucose as carbon source, α-amylase activity was 140 units/mg protein at day one but activity was nil at day two. Activity was detected again at day three and was 34 units/mg protein. Activity declined to 7 units/mg protein at day four but was 27 units/mg protein at day five. There was a decline to 6 units/mg protein at day six after which there was a steady rise in activity reaching 38 units/mg protein at day ten.

With galactose as carbon source, α-amylase activity was nil at the 1st and 2nd days of inoculation of the defined growth medium. Activity was 1 unit/mg protein at the 3rd day. It rose and was an optimum 26 units/mg protein at day eight. A drastic decline to 6 units/mg protein expressed at days nine and ten was observed.

When bread was carbon and growth source, α-amylase activity expressed by our tropical strain of *Penicillium rubrum* was 23 units/mg protein at day one of inoculation of medium. An optimum 143 units/mg protein at day two was observed. Activity declined steadily thereafter to a 30 units/mg protein at day ten.

Table 1: Effect of carbon source on amylase activity produced by *Penicillium rubrum*

	Days										
Carbon Source	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Bread	23	143	95	84	63	50	50	46	45	30	
Starch	0	49	49	137	177	252	54	53	53	52	
Maltose	22	51	51	52	53	62	73	57	53	50	
Sucrose	0	0	1	2	3	4	4	6	10	19	
Lactose	0	0	58	78	104	129	192	77	68	65	
Glucose	140	0	34	7	27	6	10	11	28	38	
Galactose	0	0	1	4	9	9	18	26	6	6	

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

4. Discussion

In the tropical strain of *Penicillium* rubrum used in this study, delayed expressions of α-amylase activity were observed when starch, sucrose, lactose and galactose were independently carbon source of growth with potassium nitrate as nitrogen source in a defined growth medium used in an earlier investigation (Adejuwon *et al*., 2015). The possibility

of repression in activity is considerable with sucrose, lactose and galactose since expression seem delayed even at the 2nd day of inoculation of medium. Glucose as carbon source was most interesting with an unexpected activity expressed as 140 units/mg protein at 24hr inoculation of medium. A repressed activity to a nil was observed at 48hr inoculation thereafter activity was again detected. The observed repression might be a feedback inhibition but with the possibility of glucose playing a role in the expression of this enzyme suggestive at the genetic level. This is evidenced with the detection of activity at 24hr. inoculation of medium. Activity is expected to be detected at 48hr but was however nil. If so at the genetic level then glucose might actually be signaling on promotor/regulatory genes of the fungus. Reports from earlier studies claim potassium nitrate as a good nitrogen source for expression of α-amylase in *Penicillium rubrum* (Adejuwon *et al*., 2015).

Sucrose seems not to be a good carbon source when potassium nitrate is nitrogen source of growth for production of α-amylase by this tropical strain *Penicillium rubrum* at 25°C.

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