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

Adekunle Odunayo Adejuwon

Department of Microbiology, Faculty of Information Technology and Applied Sciences, Lead City University, Ibadan, Nigeria Or Department of Biological Sciences (Microbiology), College of Natural and Applied Sciences, Oduduwa University, Ipetumodu, Ile-Ife, Osun State, Nigeria
ao_adejuwon@yahoo.ca, adejuwon.ao@lcu.edu.ng

Abstract: Background: Recently a tropical isolate *Penicillium citrinum* was demonstrated to express α-amylase activity with starch as carbon source and certain nitrogen compounds as growth constituents. **Materials and methods:** In this current investigation, a defined medium having the vitamins thiamine as a source of coenzyme and biotin as a source of coenzyme as well as a carrier of CO_2 but with potassium nitrate as an organic source of nitrogen for growth was inoculated with spore suspensions of approximately $6x10^5$ spores per ml of a same previous tropical strain of *Penicillium citrinum* (Adejuwon *et al*. 2015, *Report and Opinion* 7(6): 38-40.). The carbon source of growth was varied and was independently bread, starch, maltose, sucrose, lactose, glucose and galactose. Incubation was at 30°C. Extracellular proteins produced in medium during the process of growth were analysed for α-amylase activity. **Results:** The tropical strain *Penicillium citrinum* exhibited α-amylase activity with all the carbon growth compounds used in the investigation. Maximum α-amylase expression was with starch and was 527 units/mg protein observed on the 5th day of inoculation of medium. Slight delayed expression was with maltose, lactose and sucrose with activity expressed starting 2nd days for maltose and lactose but expressed starting 3rd day of inoculation of medium for sucrose. **Conclusion:** The vitamins thiamin and biotin with all the carbon compounds used in the investigation supported expression of α-amylase in *Penicillium citrinum* with potassium nitrate as nitrogen source for fungal growth at 30°C. The role of thiamine might be in a decarboxylation process in the carbohydrate catabolism with potassium nitrate suggestively playing a role in DNA regeneration. Maltose lactose or sucrose as carbon source for growth with potassium nitrate as nitrogen source seem not to support much early expression of α-amylase in *Penicillium citrinum.*
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1. Introduction

α-Amylases are hydrolytic enzymes and endoamylases capable of degradation of starch randomly along the polysaccharide fragment unlike β-amylases or exoamylases which are stepwise in degradation starting from an end of same polysaccharide fragment (Aiyer, 2005).

There have been reports of production of α-amylases by vast number of fungal cells (Adejuwon, 2013).

In this present investigation, a defined growth medium with biotin and thiamin as sources of vitamin and potassium nitrate as nitrogen source of fungal growth was inoculated with spore suspensions of *Penicillium citrinum.* The observed effect of varied carbon source for fungal growth on α-amylase activity expressed in the fungus is herein reported.

2. Materials and Methods

2.1 Source of Isolate and Its Identification

The *Penicillium citrinum* (PEN 04) used in this investigation was a tropical strain and a part of the culture collection of Professor Patrick O. Olutiola of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria, West Africa. It was isolated from decaying citrus fruit and 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 (Hanlin, 1990).

2.2 Culture Conditions and Inoculum

The isolate *Penicillium citrinum* (PEN 04) was cultured and maintained on 1% malt yeast extract-glucose agar slants and plates. The fungus was subcultured into test tubes of the same medium and incubated at 30°C. One hundred and twenty-hr-old culture was used in this investigation. According to

the modified methods of Olutiola and Ayres (1973) and Adejuwon *et al*. (2015b), culture was grown in a defined medium of the 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 $FeSO_4.7H20$ (1 mg) with potassium nitrate as nitrogen source (9.9 g) and a carbon (10 g) source (Sigma) in 1 litre of distilled water (Adejuwon *et al*., 2015b). The carbon source of fungal growth used in the investigation was varied. It was independently bread, 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. The spores were counted in a Neubauer counting chamber (Olutiola *et al.*, 1991; Adejuwon, 2011). Experimental and control flasks were incubated without shaking at 30^0C (Olutiola and Nwaogwugwu, 1982). Protein content of the inoculated medium was determined (Lowry *et al*., 1951).

2.3 Enzyme Assay

2.3.1 α-Amylase

In this investigation, α-amylase activity was determined using the method of Pfueller and Elliott (1969). The reaction mixtures consisted 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 Experimentals and controls were attendantly monitored. 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 expressed as α-amylase units per mg protein (Adejuwon *et al*., 2015b).

3. Results

3.1 Interpretation of Results

The carbon compounds used in this investigation supported α-amylase production by *Penicillium citrinum*. The nitrogen source for fungal growth was potassium nitrate. α-Amylase activity detected in growth medium with hours of incubation are presented in Table 1.

When bread as carbon source of fungal growth, α-amylase activity expressed by our tropical strain of *Penicillium citrinum* was 15 units/mg protein at day 24hr. Activity increased with days of incubation and was at optimum 118 units/mg protein at 96 hr. α-Amylase activity thereafter decreased steadily to 30 units/mg protein at 240hr.

With starch was carbon source of growth, α-amylase activity expressed by our fungal strain was 45 units/mg protein at day 24hr incubation. Activity increased with days of incubation reaching an optimum 527 units/mg protein at 120hr. Activity declined steadily thereafter to a 68 units/mg protein expressed at 216hr and 240hr incubation.

When maltose was carbon source, α-amylase activity was nil at 24hr incubation. Activity was detected at 48hr and was 8 units/mg protein. Activity increased daily with optimum expressed as 458 units/mg protein at 168hr. Thereafter there was a decline to 128 units/mg protein expressed at 216 and 240 hrs.

With sucrose as carbon source, α-amylase activity was nil at 24hr and 48hr incubation. Activity was 29 units/mg protein at 72hr incubation. Optimum activity was expressed at 168hr and was 124 units/mg protein after which there was a steady decline.

When lactose was carbon source, α-amylase activity expressed by the tropical strain *Penicillium citrinum* was nil at 24hr incubation. Activity was detected at 48hr incubation and was 7 units/mg protein. α-Amylase activity increased daily with days of incubation and was an optimum 88 units/mg protein at 192hr incubation. Activity declined to a 39 units/mg protein at 240hr.

With glucose as carbon source, α-amylase activity was 3 units/mg protein at 24hr incubation. Activity increased steadily with days of incubation with an optimum expressed as 96 units/mg protein at 168hr. Thereafter a steady decline in activity was observed falling to 62 units/mg protein at 240hr incubation.

With galactose as carbon source, α-amylase activity expressed by our strain of *Penicillium citrinum* was 3 units/mg protein at 24hr incubation. Activity increased steadily with hours of incubation and was an optimum 131 units/mg protein 168hr incubation. A decline was thereafter observed and was 60 units/mg protein at 240hr.

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

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

	Days										
Carbon Source	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Bread	15	41	40	118	47	42	37	36	34	30	
Starch	45	77	207	320	527	229	184	126	68	68	
Maltose	0	8	109	128	144	154	458	138	128	128	
Sucrose	0	0	29	52	54	66	124	115	110	62	
Lactose	0	7	9	15	15	21	58	88	47	39	
Glucose	3	14	40	47	62	91	96	69	68	62	
Galactose	3	16	18	30	43	51	131	92	65	60	

4. Discussion

The tropical strain *Penicillium citrinum* exhibited α-amylase activity with potassium nitrate as the source of nitrogen for growth and with all the carbon growth compounds used in this investigation at 30^{o}C. Maximum α-amylase expression was with starch and was 527 units/mg protein. This was observed on the 5th day of inoculation of medium. There was slight delayed α-amylase expression with maltose, lactose and sucrose as carbon source, with activity expressed starting 2nd days for maltose and lactose but 3rd day of inoculation of medium for sucrose. The vitamins thiamin and biotin used in the investigation seem to support expression of α-amylase in *Penicillium citrinum*. This is in line with previous investigation (Adejuwon *et al*., 2015b). The role of thiamine might be in a decarboxylation process in the carbohydrate catabolism with potassium nitrate suggestively playing a significant role in DNA regeneration since the basis of all protein syntheses, enzymes inclusive, is the DNA. Magnesium in the form of Mg^{2+} is necessary for actions of hexokinase in the microbial metabolism of glucose to glucose-6-phosphate as observed in the Emden Meyerhoff pathway. Herein $MgSO_4.7H_2O$ plays a vital role as a constituent of our growth medium (Adejuwon, *et al*., 2015a). However we also need to bear in mind that fungi actually produce extracellular enzymes in their degradation of substrates.

Of the carbon compounds used in our study, starch as carbon source for fungal growth with potassium nitrate as nitrogen source seem to best support α-amylase production by our strain of *Penicillium citrinum* at 30^{o}C in tropical Nigeria, West Africa. However, slight delayed expression should be envisaged with maltose, lactose and sucrose.

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Corresponding Author:
Dr. Adekunle Odunayo Adejuwon (Senior Lecturer & Sub-Dean to the Faculty),
Department of Microbiology,
Faculty of Information Technology and Applied Sciences,
Lead City University, Ibadan, Nigeria
Or Adjunct Reader/Associate Professor,
Department of Biological Sciences (Microbiology), College of Applied and Natural Sciences, Oduduwa University, Ipetumodu, Ile-Ife, Nigeria.
Telephone: +2348069781680
E-mail: ao_adejuwon@yahoo.ca, adejuwon.ao@lcu.edu.ng

References

1. Adejuwon, A., 2013. *Alpha Amylases from Fungi*: *Species of Aspergilli and Penicilli*. LAP Lambert Academic Publishing, Saarbrucken, Deutschland/Germany. 85pp. ISBN: 978-3-659-21657-2.
2. Adejuwon, A.O., 2011. Nutritional factors affecting the production of α-amylase by *Lasiodiplodia theobromae* Pat. *Biotechnology, Bioinformatics and Bioengineering* 1(1): 131-135.
3. Adejuwon, A.O., A.O. Oluduro, F.K. Agboola, P.O. Olutiola, M.J. Robbiani and S.J. Segal, 2015a. Expression of α-amylase by a tropical strain of *Penicillium rubrum*: Effect of nitrogen source of growth medium. *Report and Opinion* 7(5): 70-72.
4. Adejuwon, A.O., O.A. Ladokun, A.O. Oluduro, F.K. Agboola, P.O. Olutiola, M.J. Robbiani and S.J. Segal, 2015b. Expression of α-amylase by a tropical strain of *Penicillium citrinum*: Effect of nitrogen source of growth. *Report and Opinion* 7(6): 38-40.

5. Aiyer, P.V., 2005. Amylases and their applications. *African Journal of Biotechnology* 4(13): 1525-1529.
6. Hanlin, R.T., 1990. *Illustrated Genera of Ascomycetes*. American Phytopathological Society Press, St. Paul, Minnesota, pp: 263.
7. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193:265-275.
8. Olutiola, P.O. and P.G. Ayres, 1973. Utilization of carbohydrates by *Rhynchosporium secalis*.I. Growth and sporulation on glucose, galactose and galacturonic acid. *Physiologia Plantarum* 29: 92-96.
9. Olutiola, P.O. and R.I. Nwaogwugwu, 1982. Growth, sporulation and production of maltase and proteolytic enzymes in *Aspergillus aculeatus*. *Transactions of the British Mycological Society* 78(1): 105-113.
10. Olutiola, P.O., O. Famurewa and H.G. Sonntag, 1991. *An Introduction to General Microbiology*: *A Practical Approach*. Heidelberger Verlagsanstalt und Druckerei GmbH, Heidelberg, Federal Republic of Germany, pp: 267.
11. Pfueller, S.L. and W.H. Elliott, 1969. The extracellular α-amylase of *Bacillus stearothemophilus*. *Journal of Biological Chemistry* 244: 48-54.

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