Effect of Irradiation on Uricase Produced by Two Strains of *Asperigillus niger*

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Abstract: The main purpose of this research is to screen the available local fungal strains for their uricase producing ability, to select the most potent isolate and also to optimize the culture conditions for maximum uricase production. Two strains of *Aspergillus niger*; *A. niger* (Thom) Thom & Raper 1945 which is thermotolerant and *A. niger* van Tieghem 1867 which is mesophilic were used during this study. Uric acid medium was the best for uricase production (2.14 and 2.45 U/ml); sucrose and starch were the best carbon sources (2.1 & 2.77 U / ml) while casein and peptone were the best nitrogen source (4.86 and 3.16 U/ml) ; alkaline medium (pH 9.0 & 8.0) were the best for the experimental fungi (4.42 and 5.27 U/ml); higher temperatures (45º & 35ºC) were the best the experimental fungi (4.65 and 5.11 U/ml) . Low dose of Gamma radiation increased the production of uricase (0.5 & 0.3 KGy) gave the best results for uricase production (11.82 and 12.32 U/ml).
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1. Introduction

Uricase or ureate oxidase (EC 1.7.3.3), an enzyme catalyzing the oxidation of uric acid to allantion and plays an important role in purine metabolism **(Wu *et al*., 1994).** This enzyme is widely present in most vertebrates except humans **(Schiavon *et al.,* 2000**). Various natural sources were discovered such as bacteria **(Mansour *et al.,* 1996)**, fungi **(Farley and Santosa, 2002)** and eukarayotic cells **(Montabini *et al.,* 1997).** The first important application discovered for uricase was in clinical biochemistry as a diagnostic reagent for measurement of uric acid in blood and other biological fluids **(Adamek *et al.,* 1989)**. The enzyme has been reviewed by **(Mahler, 1963)**. Gout is a painful disorder, characterized by uricemia, recurrent attacks of acute arthritis, deposition of sodium urate in and around joints, and in many cases, formation of uric acid calculi **(Lee *et al*., 1988).** Uricase was originally isolated from mammalian organisms. Recently interest was concentrated on microbial preparations from various fungi, yeast and bacteria. The microbial enzyme is inducible and therefore, the presence of uric acid or some other inducer in the medium is necessary for enzyme production **(Adamek *et al.,* 1989)**. Although several microbial sources of uricase have been proposed for this clinical indication, only one has actually been used commercially under the trade mark of uricozyme and is isolated and purified from *Aspergillus flavus*.

In various microorganisms uricase synthesis is regulated by components of the growth medium and the ability to degrade uric acid and to use it for growth is an inducible property of these microorganisms **(Vander Drift and Vogels, 1975).** Moreover, it was suggested that uricase formation might be controlled by a repression in which a metabolite derived from both the nitrogen and carbon sources may participate **(Bongaerts *et al*., 1977).**

Several investigators **(Yazdi *et al*., 2006; Lotfy, 2008)** studied the optimal temperature and pH for the production of uricase by microorganisms. The effect of various carbon and nitrogen sources on the formation of uricase by microorganisms was studied by several authors **(Azab *et al*., 2005; Zhou *et al.,* 2005; Yazdi *et al*., 2006; Lotfy, 2008 and Geweely & Nawar, 2011).**

Gamma radiations are high energy radiations emitted from certain radioactive isotopes such as Cobalt 60. Gamma radiations have been shown to exert their lethal effect on microorganisms through two types of action characterized as "direct and indirect action" **(Lea, 1955).**

1. Direct action: in which the ionizing events occurs within the microorganisms in or near the genetic apparatus or some other vital structure directly damaging it so that subsequent division is impossible.
2. Indirect action: in which the ionizing event may occur either outside the microorganisms in the extracellular water environment or within the organism in the intercellular fluids creating active chemical entities which act on the microbe causing its death **(Lea, 1955).**

The lethal effect of ionizing radiation on fungi has been studied by many investigators **(Stotozky and Mortensen, 1959; Ingram, 1975; Rowley *et al.,* 1978)**. Also, the effect of ionizing radiation on growth as well as biochemical and biological activities of fungi has been established by

many authors **(Kim *et al*., 1968; Salama *et al*., 1977; El-Zawahry *et al.,* 1983; Abo-El-Khair, 1986; Shinidia, 1986; Chacharkar *et al*., 1996).** On the other hand, the effect of gamma radiation dose on fungal enzyme production was also studied by many workers **(Macris, 1983; El-Zawahry and Mostafa, 1991; Kumakura, 1993; Ito and Nessa, 1996; Shimokawa *et al*., 2007).** They found more or less as our results.

2.Materials and Methods

Organisms

Five isolates of our lab collection of fungi were tested for uricase production. They are ***Aspergillus niger*** (Thom) Thom & Raper 1945*;* ***Absidia corymbifera*** (Cohn) Saco & Trotter 1912 *;* ***Myceliophthora ferrugsii*** (Klopotek) Oorschot 1977*;* ***Aspergillus niger*** van Tieghem 1867 and ***Acremonium strictum*** W. Gams 1971 (Table I). Two strains of *Aspergillus niger*; (Thom) Thom & Raper 1945 and van Tieghem 1867 were used during this study.

Media

Three different media were used for uricase production. Yeast extract sucrose medium: Sucrose 150.0 g; yeast extract 20.0 g. **(Davis *et al*., 1966)**. Czapek- Dox- medium: $NaNO_3$ 2.0 g; K_2HPO_4 1.0 g; KCI 0.5 g; $MgSO_4$ 0.5 g; $FeSO_4.7H_2O$ 0.01 g and sucrose 20.0 g. **(Huang and Ling, 1973).** Uric acid medium: uric acid 1.0 g; K_2HPO_4 1.0 g; $MgSO_4$ 0.5 g; NaCl 0.5 g; $FeSO_4$ 0.01 g and sucrose 20.0 g. **(Abdel Fattah and Abo hamed, 2002)**. Ingredient of each medium was dissolved in 1 liter distilled water and pH was adjusted to 6.5-7.0.

Enzyme assay

Uricase activity was measured according to the procedure described by **Adamek *et al*. (1989).** To 2 ml of a solution containing uric acid (10µg per ml of borate buffer 0.2 M, pH 8.5), 0.8 ml of water and 0.1 mL of crude enzyme at 25 °C were added. After 10 min, 0.2 mL of 0.1 M potassium cyanide solution was added to the mixture to stop the enzyme reaction. In the reference sample, the solution of potassium cyanide was added to the mixture before the addition of the crude enzyme. The absorbance of both samples was measured at 293 nm. The difference between absorbance of the sample and reference is equivalent to the decrease in uric acid during the enzyme reaction. One unit of uricase enzyme was equal to the amount of enzyme which converts 1 µmol of uric acid to allantoin per min at 30 °C.

Protein determination

The protein content of the purified enzyme was measured by UV absorbance at 280 nm **(Markwell *et al*., 1978)** using bovine serum albumin as a standard.

Preparation of fungal cultures for radiation

The source of radiation was Cobalt-60 Indian gamma cell located at National Center for Radiation Research and Technology (Nasr City, Cairo) to whom the author is greatly indebted. The dose rate was 13.30 KGy/Sec at the time of experiment. There are two methods were used in this investigation; the first by exposing of spore suspensions of each fungus from seven days old fungal cultures containing yeast-starch agar medium to different radiation doses (0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0 and 5.0 KGy). The other method by direct exposing of the fungal slants of each fungus from seven days old fungal cultures to different radiation doses (0.1, 0.3, 0.5, 0.7, 0.9, 1.0, 2.0 and 3.0).

3. Results and Discussion

Five isolates namely: *Aspergillus niger* (Thom) Thom & Raper 1945*; Absidia corymbifera* ; *Myceliophthora ferrugisa; Aspergillus niger* van Tieghem 1867 and *Acremonium strictum* were investigated for the production of uricase Table (I) and only two of them; *Aspergillus niger* (Thom) Thom & Raper 1945 and *Aspergillus niger* van Tieghem 1867 were chosen for this study due to the high amount of uricase they produced. Yeast extract sucrose medium, Czapek- Dox- medium and Uric acid medium were investigated for production of uricase by the experimental fungi. The results (Fig.1) showed that uric acid medium was the best for uricase production, it gave 2.139 and 2.456 U/ml for *Aspergillus niger* (Thom) Thom & Raper 1945 and *A niger* van Tieghem 1867 respectively. Other authors pointed to the presence of uric acid or some other inducer in the medium is necessary for enzyme production **(Adamek *et al.,* 1989)**.

Sucrose was omitted from uric acid medium and replaced by 1% of each glucose, fructose, lactose, maltose, starch, cellulose and sucrose. The experimental fungi were investigated to produce uricase using these sources. The results (Fig.2) showed that sucrose was the best carbon source for *Aspergillus niger* (Thom) Thom & Raper 1945 giving 2.1 U / ml while starch was the best for *Aspergillus niger* van Tieghem 1867 giving 2.77 U/ml . The effect of various carbon sources on the formation of uricase by microorganisms was studied by several authors **(Azab *et al*., 2005; Zhou *et al.,* 2005; Yazdi *et al*., 2006; Lotfy, 2008).**

Uric acid was omitted from uric acid medium and supplemented by 0.5% of $NaNO_3$, KNO_3, $(NH_4)_2SO_4$, NH_4Cl, peptone and casein as a sole sources of nitrogen. Results (Fig.3) showed that casein was the best nitrogen source *Aspergillus niger* (Thom) Thom & Raper 1945 giving 4.86 U/ml while

peptone was the best nitrogen source *Aspergillus niger* van Tieghem 1867 giving 3.16 U/ml. The effect of various nitrogen sources on the formation of uricase by microorganisms was studied by several authors **(Azab *et al*., 2005; Zhou *et al.*, 2005; Yazdi *et al*., 2006; Lotfy, 2008).**

Several investigators **(Yazdi *et al*., 2006; Lotfy, 2008)** studied the optimal temperature and pH for the production of uricase by microorganisms. Results (Fig.4) showed that pH 9.0 was the best for the production of uricase by *Aspergillus niger* (Thom) Thom & Raper 1945 giving 4.42 U/ml while pH 8.0 was the best for the production of uricase by *Aspergillus niger* van Tieghem 1867 giving 5.27 U/ml . On the other hand the best temperature (Fig.5) for uricase by *Aspergillus niger* (Thom) Thom & Raper 1945 was 45ºC giving 4.65 U/ml, while the best temperature for *Aspergillus niger* van Tieghem 1867 was 35ºC giving 5.11 U/ml.

The effect of gamma radiation dose on fungal enzyme production was also studied by many workers **(Macris, 1983; El-Zawahry and Mostafa, 1991; Kumakura, 1993; Ito and Nessa, 1996; Shimokawa *et al*., 2007).** We tried here to study the effect of different gamma radiation doses on the production of uricase by the experimental fungi. The results (Fig.6) showed that low dose of gamma radiation increased the production of uricase where 0.5 KGy gave 11.82 U/ml with *Aspergillus niger* (Thom) Thom & Raper 1945 while 0.3 KGy gave 12.32 U/ml with *Aspergillus niger* van Tieghem 1867

Table (I): Uricase production by five isolates of fungi

Fungi	Uricase activity (U/ml)
Aspergillus niger (Thom) Thom & Raper 1945	**1.985**
Absidia corymbifera (Cohn) Saco & Trotter 1912	**0.632**
Myceliophthora ferrugsii (Klopotek) Oorschot 1977	**0.583**
Aspergillus niger van Tieghem 1867	**1.574**
Acremonium strictum W. Gams 1971	**0.482**

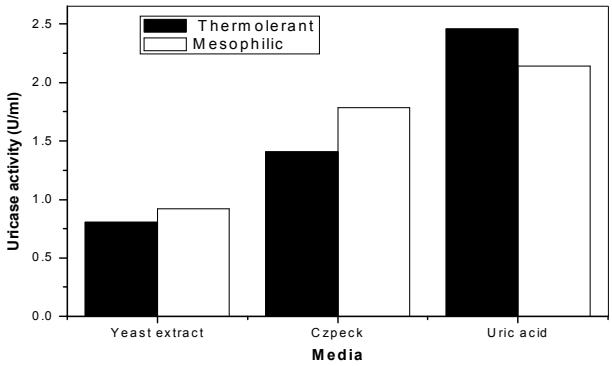

Fig. (1): Effect of different media on uricase production by the experimental fungi

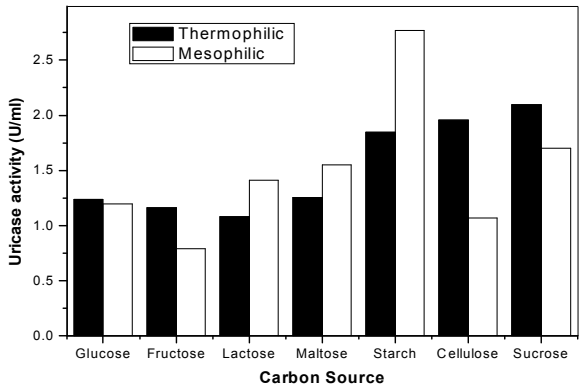

Fig. (2): Effect of different carbon sources on uricase production by the experimental fungi

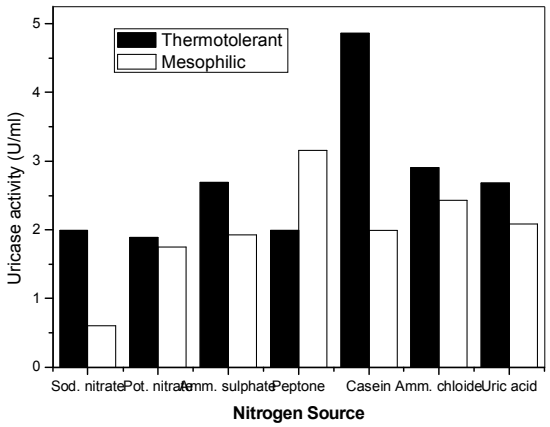

Fig.(3): Effect of different nitrogen sources on uricase production by the experimental fungi

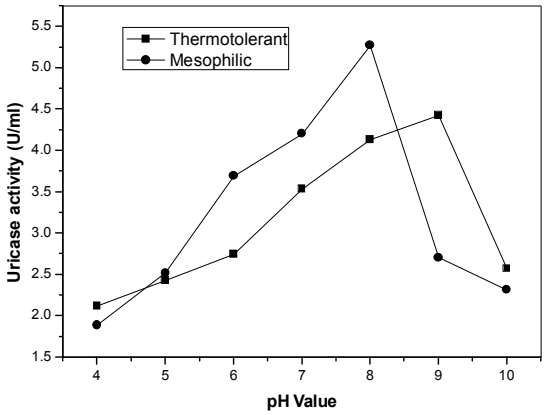

Fig.(4): Effect of different pH values on uricase production by the experimental fungi

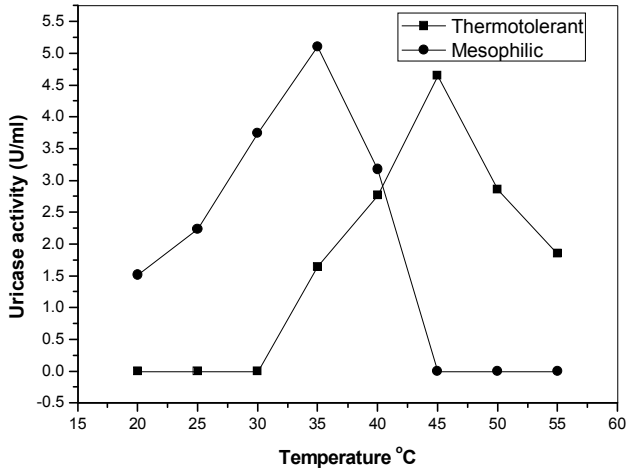

Fig.(5): Effect of temperature on uricase production by the experimental fungi

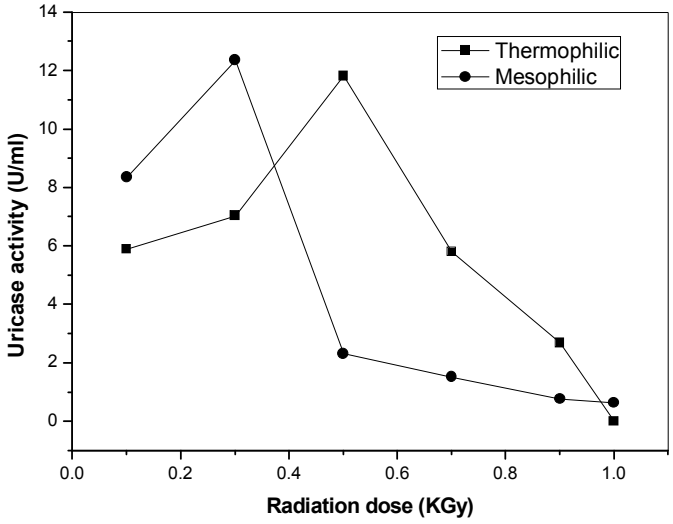

Fig. (6): Effect of radiation dose on uricase production by the experimental fungi

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