

## SCREENING OF SOME FUNGI FOR URICOLYTIC ACTIVITY

By

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### دراسة مقدرة بعض الفطريات على تحليل حمض البوليك

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في هذه الدراسة تم اختبار نشاط إنزيم اليوريكيز لعدد ٢٩ نوعاً من الفطريات ، وقد تبين أن ٢٢ نوعاً منها لها القدرة على إنتاج إنزيم اليوريكيز ووجد أن معظم الفطريات البازيدية المختبرة غير قادرة على إنتاج إنزيم اليوريكيز .

وإتضح من الدراسة أن أعلى إنتاج لإنزيم اليوريكيز تم بواسطة الفطريات الناقصة خاصة أسبر جيلس كربونيرياس ، بوتريتس فابي وأسبر جيلس سيداوي (٠,١٦ ، ٠,١٣ ، ٠,٠٩ وحدة / مل / دقيقة ) .

وقد وجد أيضاً أن كل فطريات عش الغراب المختبرة قد أعطت نشاطاً لإنزيم اليوريكيز وكان أقصاها لفطرة بليوروتس - ساجور كاجوي .

وثبت من الدراسة كذلك أنه لا توجد علاقة بين إنتاج إنزيم اليوريكيز وبين الوزن الجاف للفطريات المختبرة .

*Key Words:* Screening, Fungi, Uricase activity

#### ABSTRACT

Twenty nine species of fungi have been tested for their uricase activity. Only twenty two species of them have the ability to produce uricases.

Most of the uncapable fungi to produce uricases belong to Basidiomycotina. The highest production of uricase was achieved by *Aspergillus carbonarius*, *Botrytis fabae* and *Aspergillus sydowii* (0.16, 0.13, and 0.093 units/ml/min.).

All the cultivated mushrooms tested have uricase activity. *Pleurotus sajor-caju* has the highest uricase activity within all the cultivated mushrooms tested.

The tested fungi proved that there is no correlation between the uricase production and the mycelial dry weight.

## INTRODUCTION

It has long been known that uric acid is degraded by a number of microbial species. The uricase: urate oxidase was defined as the enzyme that hydrolyze uric acid into allantoin. The importance of microbial uricase has two benefits, firstly decomposition of the deep-litter of poultry, secondly the clinically treatment of the uremia and gout.

Uricase production by bacteria and actinomycetes have been extensively investigated. (4, 5, 6). Furthermore, yeasts have been recorded to have uricolytic activity. (8, 9, 10)

However little literatures referred to the uricase production by filamentous fungi. Brunel [2] reported that some basidiomycetes can produce uricase. Uricolytic activity was also emphasized in some species of *Penicillium* and *Aspergillus* (13). Furthermore some *Alternaria* and *Fusarium* species have been recorded to have uricolytic activity (13) In addition *Gliocladium* sp., *Neurospora* spp., *Cladosporium herbarium*, *Botrytis cinerea* and *Trichoderma koningi* have been reported to produce uricase (13, 16).

The aim of this investigation is to shed more light on the production of uricase by some selected members of Basidiomycotina and some other representative fungi.

## MATERIALS AND METHODS

### (a) Fungal cultures

Twenty nine fungal species were used for screening experiments from the following collections:

- Dr. J. N. Hedger, (Hed), U.C.W. Aberystwyth, U.K.;
  - Dr. R. B. Kemp (Ke), Edinburgh Univ., U.K.;
  - Glasshouse Crops Research Institute, U.K. (GCI),
  - American Type Culture Collection (ATCC);
  - Consultative Commet Company of Mushroom Cultivation (CCCM);
- and  
Faculty of Education, Ain Shams Univ. (Ain), Cairo, Egypt.

The cultures were routinely maintained on 3 % malt agar and Czapek-Dox agar being subcultured at 3 month intervals.

### (b) Testing procedure for uricase production

Solid Czapek-Dox medium in which sodium nitrate was replaced by uric acid on an equivalent nitrogen basis was used to test the utilization of uric acid by fungi. The medium consisted of (% w/v): sucrose, 3;  $\text{KH}_2\text{PO}_4$ , 0.1;  $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$ , 0.05;  $\text{NaNO}_3$ ,

0.2; and agar, 2.0. Uric acid was sterilized by heating at 160°C for one hour but all other ingredients of the medium were autoclaved. Six mm. diameter discs from the edge of 5-7 days cultures of the test fungi growing on 1.5% malt agar, were used to inoculate the plates centrally. Plates were incubated at 30°C or 250°C. Evidence of uricase production was taken as the appearance of clear zones on the agar around the inoculum. The clear zone diameter of each plate was measured in mm.

### (c) Measurement of the uricase activity

#### 1. Culturing and harvest of the fungal mycelia

Aliquots of 50 ml of the liquid Czapek-Dox medium were placed in 250 ml Erlenmyer flasks. The flasks were autoclaved at 15 p.s.i for 15 min. Each flask was inoculated with two 6 mm. agar discs of the tested fungus. A triplicate for each tested fungus were incubated at 30°C or 25°C. After 10 days incubation, the mycelia were harvested by filtration, washed thoroughly with distilled water and blotted dry with absorbent paper.

#### 2. Preparation of cell-free extracts

These mycelia were ground with washed cold sand in porcelain mortar and extracted with 0.05 M potassium phosphate buffer (pH7.4). The slurries were removed by centrifugation at 7000 rev./min. for 20 min. and the supernatant liquids were used as the crude enzymes preparation. All these procedures have been done in a cold condition.

#### 3. Uricase enzyme assay

The modified method of (17) and Italia et al. (18) was used. The decrease of uric acid absorbance at 293 nm was taken as an evidence of enzyme activity. In this method, 2 ml solution containing 10 of uric acid per ml of 0.2 M of borate buffer solution (pH9.0), 0.3 ml of water and 0.5 ml of each enzyme preparation were added at 30°C for 30 minutes. To stop the enzyme reaction, 0.2 ml of 0.1 N KCN blank was added and the absorption was measured. For blank preparation, 2 ml of distilled water was added instead of uric acid. For the standard preparations, KCN was added before the addition of the enzyme.

One unit of the enzyme activity is corresponding to the amount of the enzyme which oxides one mole of uric acid per one minute.

## RESULTS

### 1. Detection for uricase production

Uricase production has been detected by the formation of clear zones [19]. Clear zones began to appear in cultures accompanied with their growth after ca 72-168 h.

**Table 1**  
Uricase production by all the tested fungi

| Subdivision of  | Fungus   | Source     | Uricase Production |
|-----------------|--|------------|--------------------|
| Eumycota        |  |            |                    |
| Mastigomycotina | <i>Phytophthora palmivora</i> (Butler) Butler          | Hed        | ++                 |
|                 | <i>Cunninghamella echinulata</i> (Thaxtee) thaxtee ex, | Ain ++     |                    |
| Zygomycotina    | Blakeslee  |            |                    |
|                 | <i>Mortierella</i> sp                                  | Hed        | +++                |
|                 | <i>Rhizopus oligosporus</i> Saito                      | Hed        | +++                |
| Ascomycotina    | <i>Chaetomium globosum</i> Kunze                       | Hed        | -                  |
|                 | <i>Agaricus bisporus</i> (lang) Imbach                 | CGI        | +++                |
|                 | <i>Ceraceomyces</i> sp.                                | Hed        | +                  |
|                 | <i>Coprinus cinereus</i> (Betli)                       | Ke         | -                  |
|                 | <i>Coriolus versicolor</i> (l) Quelet                  | Hed        | -                  |
|                 | <i>Flammulina velutipes</i> (M. A. curtis) Singer      | Hed        | -                  |
| Basidiomycotina | <i>Lentinus edodes</i> (Berk) Sing                     | CGI-R 130  | -                  |
|                 | <i>Pleurotus columbinus</i> Quel. ap. Bres             | CCCM 250   | +                  |
|                 | <i>Pleurotus floridanus</i> Sing                       | ATCC 38538 | ++                 |
|                 | <i>Pleurotus ostreatus</i> (Jacq. ex.Fr) Kummer        | ATCC 38546 | +                  |
|                 | <i>Pleurotus pulmomarius</i> Fr.                       | CCCM 299   | ++                 |
|                 | <i>Pleurotus sajor caju</i> (Fr.) singer               | CCCM 290   | +++                |
|                 | <i>Schizophyllum commune</i> Fr.                       | Hed        | -                  |
|                 | <i>Serpula lacrimans</i> (Wulfen) Schroeter            | Hed        | -                  |
|                 | <i>Alternaria alternata</i> (Fr) keissler              | Hed        | +++                |
|                 | <i>Aspergillus alutaceus</i> Berh                      | Ain        | +                  |
|                 | <i>Aspergillus carbonarius</i> (Bainier) Thom          | Ain        | ++++               |
|                 | <i>Aspergillus niger</i> Van Tieghem                   | Ain        | +++                |
|                 | <i>Aspergillus terreus</i> Thom                        | Ain        | +++                |
| Deuteromycotina | <i>Aspergillus sydowii</i> (Bainier & Sartory)         |            |                    |
|                 | Thom & Church  | Ain        | ++++               |
|                 | <i>Botrytis fabae</i> Sardina                          | Hed        | ++++               |
|                 | <i>Fusarium culmorum</i> (W. G. Smith) Sacc            | Hed        | ++                 |
|                 | <i>Paecilomyces cariotii</i> Bainier                   | Ain        | ++                 |
|                 | <i>Pencillium claviforme</i> Bainier                   | Hed        | +++                |
|                 | <i>Pencillium oxalicum</i> Currie & Thom               | Ain        | +                  |

(-) No clear zone; (+) 10-20 mm; (++) 20-30 mm; (+++) 30-50 mm; (++++) 50-60 mm and (+++++) > 60 mm.

The tested fungi can be divided into five groups according to the appearance and measurement of the clear zones as shown in Table 1.

The first group (-), includes the fungi which did not produce clear zones i.e. incapable to produce uricases. This group comprises seven species; six of them belong to Basidiomycotina and the seventh is *Chaetomium globosum* (Ascomycotina). Within the Basidiomycotina *Lentinus edodes*, *Flammulina velutipe*, *Coprinus cinereus*, *Coriolus versicolor*, *Schizophyllum commune* and *Serpula lacrimans* show no uricase activities.

The second group includes the weakly positive fungi (+), three fungi of this group belong to Basidiomycotina (*Pleurotus ostreatus*, *Pleurotus columbinus* and *Ceraceomyces*) and two of Deuteromycotina, (*Aspergillus alutaceus* and *Penicillium oxalicum*).

The third group (++) includes the weakly positive fungi char-

acterized by low uricase production. These are *Pleurotus floridanus*, *Pleurotus pulmonarius* one species of Mastigomycotina (*Phytophthora palmivotra*), one species of Zygomycotina (*Cunninghamella echinulata*), and two species of Deuteromycotina (*Fusarium culmorum* and *Paecilomyces variotii*).

Eight fungi located in the fourth group (+++) which are considered to be good uricase producers and these are: two Basidiomycotina (*Agaricus bisporus* and *Pleurotus sajor-caju*), two Zygomycotina (*Rhizopus oligosporus*, *Mortierella* sp.) and four Deuteromycotina (*Aspergillus niger*, *Aspergillus terreus*, *Alternaria alternata* and (*Pencillium claviforme*).

The fifth group (+++++) includes the fungi which are considered as high uricase producers. This group included *Aspergillus carbonarius*, *Aspergillus sydowii* and *Botrytis fabae*. Accordingly we could observe that the higher producers are members of Deuteromycotina and the lower are members of Basidiomycotina

which are situated in the first, second, third and fourth groups.

## 2. Uricase activity

The species that produce clear zones were tested quantitatively for their uricase activities (Fig. 1). The three fungi included in the fifth group (high uricase producers) had the highest uricases production, they are headed by *Aspergillus carbonarius* (0.16 unit/ml/min). All *Aspergillus* spp were highly capable to produce uricase. There is only one exception, *Aspergillus alutaceus* which has low level of uricase.

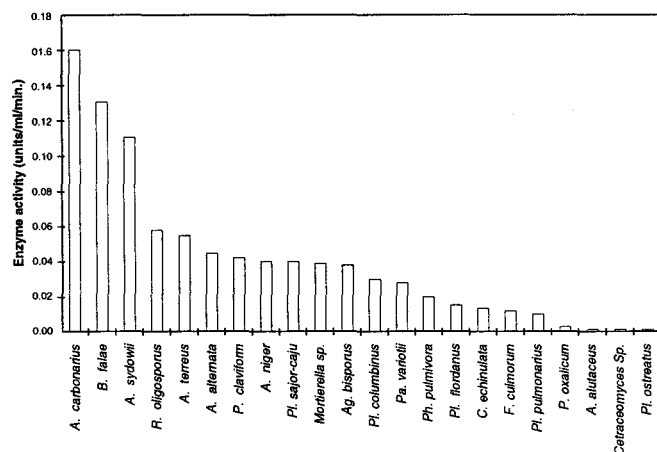


Figure 1: Comparison between the values of uricase activity of all the clear zone producing fungi in uric acid medium.

The activity level of uricases by a range of Basidiomycotina was found to be lower than that produced by the other groups of fungi. However, *Pleurotus sajor-caju* occupied the first position between all the tested Basidiomycotina (0.04 unit/ml/min.), followed by *Agaricus bisporus* (unit/ml/min.).

In accordance to the fungal groups in Table 1, enzyme activity can be added to these groups as following:

- group + (0.0010 - 0.0026 unit/ml/min.)
- group ++ (0.010 - 0.028 unit/ml/min.)
- group +++ (0.0370 - 0.0566 unit/ml/min.)
- group ++++ (0.0933 - 0.1600 unit/ml/min.)

## 3. Mycelial dry weight

The dry weight of the different fungi produced after incubation period of 168 h are shown in Fig. 2. All the uricase producing fungi can grow on Czapek-Dox medium in which sodium nitrate was replaced by uric acid as a sole source of nitrogen.

The results presented in Fig. 2 show that no correlation could be obtained between mycelial growth and enzyme activity (Fig. 1) *Aspergillus*, the weakest uricase producer (second group), sur-

passed all the tested fungi in their growth, with the exception of *Alternaria alternata* (fourth group) which was the best fungus growing in uric acid medium.

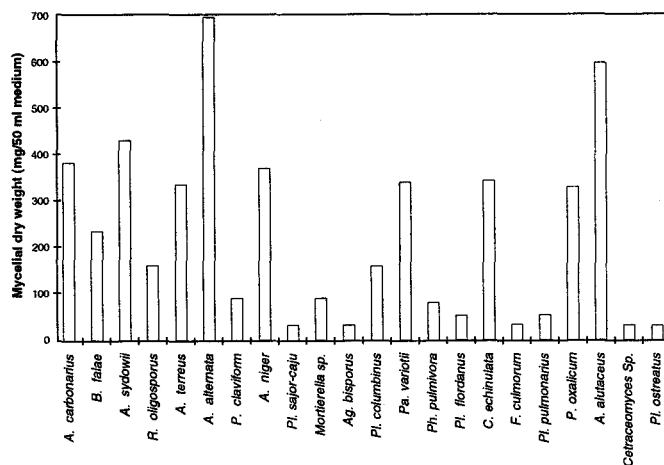


Figure 2: Comparison between the values of uricase activity of all the clear zone producing fungi in uric acid medium.

## DISCUSSION

The first aim of this work is to obtain some information about growth of higher fungi and some other fungi when cultivated on uric acid as a sole source of nitrogen.

The second aim was to measure the uricase activities of the best growing fungi in relation to their dry weights.

The higher fungi were generally low producers of uricases. About half of the Basidiomycotina tested had no ability to produce uricase. The growth of some Basidiomycotina on uric acid solid medium without producing clear zone could be attributed to their C/N ratios tolerance since they grow in straw, wood and other agricultural wastes with low N content [20, 21].

The behavioural differences of the growth of *Pleurotus* spp in presence of uric acid was controversial. *P. sajor-caju*, the best uricase producing from Basidiomycotina had the lowest dry weight when grown in uric acid. Furthermore, *Aspergillus alutaceus* and *Penicillium oxalicum* weakly positive fungi, grew well in presence of uric acid. However, there is no correlation between the growth and the enzyme activity. So, it can be speculated that uricase production is not function of cell growth as previously predicted by (5) who recorded the same results on actinomycetes.

It is well known that *Agaricus bisporus* cultivated by the addition of animal manure is added to the compost [22, 23]. The results of this investigation have also revealed that *A. bisporus* has a good uricase activity. Consequently, addition of animal manures to *Pleurotus* spp. compost can be recommended as detected by the result of this investigation, since they all can degrade uric

acid. Furthermore, animal manure would increase the nitrogen content of the compost which in turn stimulates the lignocellulose degradation by *Pleurotus* spp. (24).

Various Deuteromycotina species tested had the ability to grow on uric acid as the only source of nitrogen. It can be considered that *Aspergillus* spp. produced mostly the highest uricase activity in this study, except *A. alutaceus*. Uricase activity of nidulans (= *Emericella nidulans*) and *A. flavus* have been previously recorded [1, 25-27]. In addition, plant pathogenic Deuteromycotina and Mastigomycotina, *Botrytis fabae*, *Alternaria alternata* and *Phytophthora palmivora* were recorded to produce uricases but the former was highly producer than the later two. But *Botrytis cinerea* and *Alaternaria porri* were previously recorded to produce uricases (13).

The present results show that *Penicillium calviforme* was of a moderate uricase activity but *P. oxalicum* had a very low uricase activity. Some literatures have been done on uricases of *Penicillium* spp. Moreover, it has been recorded that *P. chrysogenum* utilized uric acid as a nitrogen source and its uricase was recorded as constitutive enzyme [13, 14].

In the present investigation the Zygomycotina species, (*Rhizopus oligosporus*) was recorded as a good uricase producer. Thus, its economic importance would be highly evaluated since it is commonly used in food production (ex. tempe).

Uricase production was also recorded, in low rate, in both *Cunninghamella echinulata* and *Mortierella* sp. Similarly, Franke *et al.* (13) recorded the uricase production by *Rhizopus nigricans*, *Cunninghamella* sp. and *Mucor* sp.

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