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Screening of some Fungal Isolates for their Potentialities for Uricase Production and Optimization of the Factors Affecting the Production

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ABSTRACT

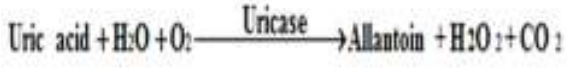
The main objective of this study was screening of some fungal strains for their ability to produce uricase and selecting the most potent isolates for medically important uricase production. Twenty-five fungal strains were screened for their potentialities for uricase production by uricase plate assay method on uric acid medium. After incubation, the formation of clear zone around the fungal colony indicated its potentiality for uricase production. Among the tested strains of fungi using the plate assay method, *Emericella quadrilineata, Aspergillus welwitschiae*, *Aspergillus carenus* and *Aspergillus flavus* were the most active fungal strains which were able to produce uricase. These fungal strains were tested for uricase production under submerged fermentation conditions. The highest uricase activity in liquid medium containing uric acid was obtained from *Emericella quadrilineata* (23.38 U/mL), *Aspergillus welwitschiae* (19.87 U/mL), *Aspergillus carenus* (16.37 U/mL) and *Aspergillus flavus* (10.91 U/mL).The optimum conditions for maximum *Emericella quadrilineata* and *Aspergillus welwitschiae* uricase production were at temperature of 30^{o}C, uric acid concentration of 3 g /L and pH 6.5.

Keywords: Uricase production, uric acid**,** plate assay method**,** submerged fermentation**.**

INTRODUCTION

Uric acid is closely related to the purine bases. Normally, the levels found in tissues and the bloods are derived from both the breaking of old cells and from the degradation of purine containing foods in normal diets especially red meats and organ meats (such as liver and kidneys), shellfish and alcohol. When uric acid crystals accumulate or precipitate in blood (due to human uricase absence, hyperuricemia occurred) can promote gout (painful metabolic abnormality diseases) (Nakagawa *et al*., 2006). Increasing uric acid amount in biological fluids can lead to chronic kidney failure; Lesch-Nyhan syndrome and some organic acidemias (Burtic and Ashwood, 1994)

Uricase [oxygen oxidoreductase, EC 1.7.3.3], is the enzyme which catalyzes the uric acid oxidation to more soluble allantoin, hydrogen peroxide and carbon dioxide (Brogard *et al*., 1972) and playing role in nitrogen metabolism.



Microorganisms, animals and higher plants are capable of producing uricase on its own, but humans cannot produce uricase because of the mutation in the fifth exon of uricase gene. So human being has to depend on the other easily available sources like microbes. uricase can be use as diagnostic reagent for uric acid measuring in blood and other biological fluids which consider significant application for uricase (Adamek*et al*., 1989). Uricase is useful for urate determination in clinical analysis by coupling it with a 4-amino-antipyrine-peroxidase system (Gochman and Schmitz 1971). Uricase used as a protein drug for toxic urate accumulation reduction (Colloc'h *et al*., 1997). Also it can be used as an additive in c formulations hair coloring agents (Nakagawa *et al*., 1995), it used as a therapeutic enzyme for gout and hyperuricemia treatment (Ganson *et al*., 2006). Immobilized Uricase may be used as a uric acid biosensor (Arslan, 2008).

The aim of the present work was screening of some fungal strains for their ability to produce uricase and selecting the most potent isolates for uricase production and also for optimization of the factors affecting the production.

MATERIALS AND METHODS

Fungal strains

Twenty-five fungal strains were kindly provided by Dr. Ehab Ali Metwally Hassan (Botany Department, Faculty of Science, Mansoura University) to test their ability for uricase production. All fungal strains were cultured on plates containing potato dextrose agar medium (PDA) of the next composition (g/L): 200 infusion potatoes, 20 dextrose and 15 agar. Plates were inoculated with fungal strains and incubated for 7 days at 28±2.0oC to get fresh fungal strains. Also fungal strains were maintained on PDA slants and stored at 4oC for further studies.

Potentiality of fungal strains for uricase production

The fungal strains were cultured on uric acid production medium which was prepared using the next components (g/L): sucrose 20, uric acid 3, di-potassium hydrogen phosphate 1, magnesium sulphate heptahydrate 0.5, sodium chloride 0.5, ferrous sulphate 0.01 and agar 15 (Abdel-Fattah and Abo-Hamed, 2002).Then incubated at 30^{o}C, pH 6.5 for five days. The formation of clear zone around the colonies considered as the production of uricase measuring. Finally, uricase production fungal strains were confirmed under submerged fermentation conditions.

Submerged-fermentation

Five discs from five days old cultures which have the ability to produce uricase were transferred to 250 mL Erlenmeyer conical flask which contained 50 mL of liquid uric acid medium (g/L): sucrose 20, K_2HPO_4 1, NaCl 0.5, $MgSO_4$. 7 H_2O 0.5, $FeSO_4$. 7 H_2O 0.01, uric acid 3, distilled water up to 1L, pH 6.8 (Abdel-Fattah and Abo-Hamed, 2002)**.**The flasks were incubated at 30^{o}C for five days. Later than the incubation, the myceliums of the isolates were collected by centrifugation at 8000 rpm for 10 minutes. Then, the cell free supernatant was used for determination of the enzymatic activity.

Uricase assay

According to Adamek *et al.* (1989) procedures**,** uricase activity was assayed**.** Two mL of a solution containing 10 Mg/mL uric acid (in borate buffer 0.2 M, pH 8.5), 0.8 mL of distilled water and 0.1 mL of crude enzyme were incubated at 37°C. After 30 min of incubation, additing 0.2 mL of 0.1 M potassium cyanide solution to the reaction mixture for the enzyme reaction stopping. In blank, adding a solution of potassium cyanide before the enzyme. The

absorbances of all samples were spectrophotometrically measured at 293 nm. The difference between the absorbance of the sample and blank was corresponding to the decrease in uric acid concentration.

Factors affecting uricase production:

Optimization of various factors that affecting the production of uricase and evaluating the fungal strains activity for production of uricase. The effect of temperature on uricase production was tested as culturing the isolates in Fifty mL of sterilized liquid uric acid medium in 250 mL Erlenmeyer flask, and the medium was incubated at different values of temperature (25, 30, 35, 40 and 45^{o}C). After the incubation, the uricase activity was measured to determine the optimum temperature. The fungal strains cultivated on fermentation mediaum at different values of pH (6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) for studying the best pH for uricase production and incubated at the optimum temperature. Uric acid is consider the substrate which applied in the fermentation medium, the medium was supplemented with different concentrations (1, 2, 3, 4 and 5 g/L) for determination of optimal concentration of uric acid for uricase production.

RESULTS

Potentiality of strains for uricase production

The potentialities of some fungal strains to produce uricase were detected by plate assy method. After cultivation the fungal strains on uric acid medium, the inoculated dishes were incubated at 30°C for five days. The clear zone appearance around the fungal colony disc indicating its potentiality to produce uricase. The results in Table 1 indicates that *Aspergillus welwitschiae*, *Emericella quadrilineata, Aspergillus carenus* and *Aspergillus flavus* were the most active strains that able to produce uricase enzyme, followed by *Aspergillus peyronelii* and *Aspergillus terrus* which also have low ability for uricase production. Fig. 1 showing the potentiality of *Asperagillus welwitschiae*, *Emericella quadrilineata* to produce uricase as detected by plate screening assay as a clear zone formation around fungal colony.

Table 1. The potentiality of twenty- five fungal strains for uricase production by plate method.

No.	Fungal strains	Uricase production
1	*Emericella quadrilineata*	++ve
2	*Aspergillus welwitschiae*	++ve
3	*Cunninghamella achinulata*	-ve
4	*Mucor circinelloids*	-ve
5	*Penicillum janthinellum*	-ve
6	*Aspergillus peyronelii*	+ve
7	*Penicillum citrinum*	-ve
8	*Aspergillus sclerotiorum*	-ve
9	*Rhizopus azygosporus*	-ve
10	*Aspergillus japonicas*	-ve
11	*Penicillium fellutanum*	-ve
12	*Aspergillus terrus*	+ve
13	*Aspergillus flavus*	++ve
14	*Aspergillus petrakii*	-ve
15	*Trichoderma koningi*	-ve
16	*Aspergillus pseudoniger*	-ve
17	*Aspergillus carenus*	++ve
18	*Aspergillus tamarrii*	-ve
19	*Aspergillus oryzae*	-ve
20	*Emericella nidulans*	-ve
21	*Aspergillus ustus*	-ve
22	*Penicillum wortmannii*	-ve
23	*Fusarium poae*	-ve
24	*Fusarium solani*	-ve
25	*Fusarium tabacinum*	-ve

++ve = high ability for uricase production,
+ve = low abilityof uricase production
-ve = absence of uricase production.

Fig. 1. Uricase activity of A) *Emericella quadrilineata* and B) *Aspergillus welwitschiae* detected by plate assay production of the enzyme indicated by clear zone formation in the medium surrounding the colony.

Submerged-fermentation

The ability of active fungal strains to produce uricase was confirmed under submerged fermentation conditions. The results indicates that, under submerged fermentation conditions, the most active fungal strains producing uricase were *Emericella quadrilineata* (23.38 U/mL), *Aspergillus welwitschiae* (19.87 U/mL), *Aspergillus carenus* (16.37 U/mL) and *Aspergillus flavus* (10.91 U/mL). The most potent strains which are *Emericella quadrilineata*, *Aspergillus welwitschiae* showed in Fig. 2.

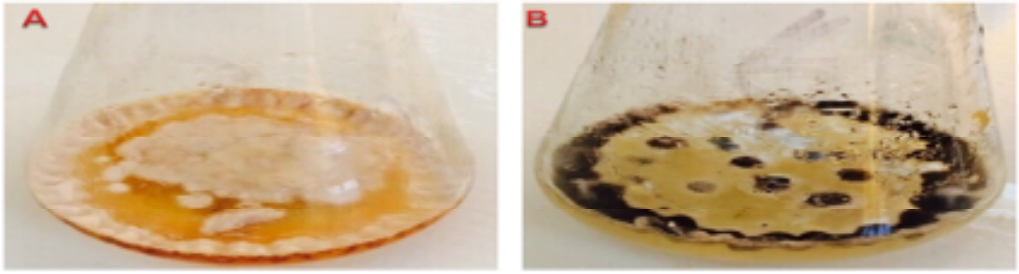


Fig. 2. A. *Emericella quadrilineata* and B) *Aspergillus welwitschiae* growth during the production of uricase in flask after inoculation and incubation at 30 °C for 5 days.

Optimization of factors affecting uricase production

Substrate concentrations

Uric acid is considering the main substrate used for the production of uricase. So, this experiment was conducted for selection of the favorable uric acid concentration for uricase production by *Aspergillus welwitschiae* and *Emericella quadrilineata*. The results showed in Fig. 3 illustrated that the maximum *Emericella quadrilineata* uricase production (24.7 U/ mL) showed when uric acid concentration was 3 g /L. This mean the optimum uric acid concentration for highest uricase production by *Emericella quadrilineata* was 3 g /L. Also, Fig. 3 presented the optimal uric acid concentration for maximum *Aspergillus welwitschiae* uricase production (20.93 U/mg) was 3 g /L. Gradually decreasing in uricase production was occurred when the concentration of uric acid increased.

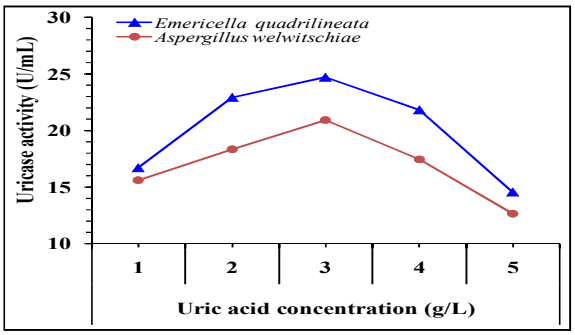


Fig. 3. Effect of different uric acid concentrations on uricase production by *Emericella quadrilineata* and *Aspergillus welwitschiae*.

pH Values

This experiment was carried out for determination of the optimum value of pH for the production of uricase by *Aspergillus welwitschiae* and *Emericella quadrilineata* . Fig. 4 presented the effect of different pH values on *Emericella quadrilineata* uricase production. as the pH value increased, the uricase activity increased till reached the highest (25.47 U/mL) at pH 6.5. Fig. 4 illustrated that the optimum pH for maximum uricase production by *Aspergillus welwitschiae* (21.83 U/ mL) was also at 6.5. But uricase activity was regularly decreased by increasing the value of pH.

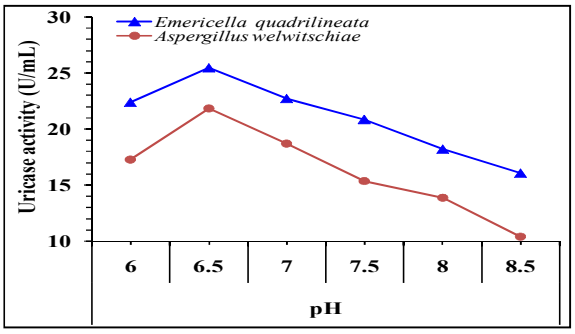


Fig. 4. Effect of different pH on uricase production by *Emericella quadrilineata* and *Aspergillus welwitschiae*.

Temperature

The present experiment was carried out for selecting the optimal temperature for uricase production by *Aspergillus welwitschiae* and *Emericella quadrilineata* . The results in Fig. 5 explained that uricase production by *Emericella quadrilineata* increased by increasing temperature till reached the highest (26.38 U/ mL) at 30°C. Also, Fig. 5 shown the optimum temperature for maximum production of uricase by *Aspergillus welwitschiae* (22.58 U/mg) was obtained at 30°C. After that, by increasing the temperature, gradually decreasing in uricase activity was occurred.

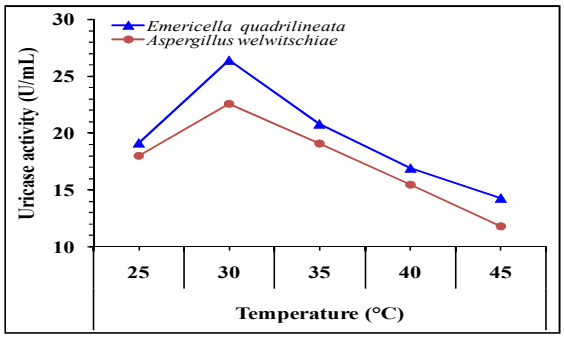


Fig. 5. Effect of different temperatures on uricase production by *Emericella quadrilineata* and *Aspergillus welwitschiae*.

DISCUSSION

Screening of some fungal strains for their potentialities to produce uricase and optimization of some fermentation conditions for maximum *Emericella quadrilineata* and *Aspergillus welwitschiae* uricase production were the main purposes of this study. The results indicate that *Emericella quadrilineata* uricase activity was found to be 23.38 U/mL, *Aspergillus welwitschiae* was 19.87 U/mL under submerged fermentation in liquid uric acid medium. Yazdi *et al.* (2006) showed that *Mucor hiemalis* uricase activity was (1.25 U/mL). While *Gliocladium viride* MTCC3835 was found to produce high activity uricase (63.14 U/mL) (Nanda *et al*., 2012). Geweely and Nawar (2011) illustrated that the maximum *Aspergillus niger* uricase production was 47.40 U/mL. Also, Mabrouk *et al.* (2010) found the most activity of uricase produced by *Gliomastix gueg* (NRC 1A) was 275.98 U/mL.

The optimum fermentation conditions (uric acid concentration, pH and temperature) for uricase production by *Emericella quadrilineata* and *Aspergillus welwitschiae* were examined. Uric acid is considered as nitrogen source in the medium used for the production of uricase**.** In the present study, the optimum concentration of uric acid for *Emericella quadrilineata* and *Aspergillus welwitschiae* uricase production was 3 g/L. This result is in agreement with the result of Hatijah and Ruhayu (2013) who shown the optimum uric acid concentration for maximum uricase production by *Aspergillus flavus* was 2 g/L. While Yazdi *et al.* (2006) proved that the optimal uric acid concentration for maximum *Mucor hiemalis* uricase production is 7.0 g/L.

Also, temperature is considering one of the factors affecting uricase production. Our results presented the optimal temperature for *Aspergillus welwitschiae* and *Emericella quadrilineata* uricase production was 30°C. This result is agreement with Yazdi *et al.* (2006) who proved that 30°C was the optimal temperture to produce uricase by *Mucor hiemalis* and Abdel-Fattah and Abo-Hamed (2002) who showed that the optimal temperature for the production

of uricase was 30°C. Also, the results of Allam and El-Zainy (1969) showed that 28°C was optimal temperture for uricase production by *Penicillium chrysogenum* and *Fusarium moniliforme* .

The effect of different values of pH on uricase production was carried out. Our result clarified that the optimal pH for uricase production by *Aspergillus welwitschiae* and *Emericella quadrilineata* was 6.5. This result is agreed with those obtained by Yazdi *et al*.(2006) who illustrated that the optimum pH was 6.0 for the production of uricase by *Mucor hiemalis*. Also, Tohamy and Shindia (2001) showed that the pH 6.0 was the optimal for *Asperagillus flavus* uricase production. On the other hand, Mabrouk *et al.* (2010) found that the maximum activity of *Gliomastix gueg* uricase was detected at 9.0 and Ammar *et al*. (1988) showed that the optimal pH to produce uricase by *Aspergillus. flavus* S.79 was 9.2.

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فحص بعض العزلات الفطرية لإمكانياتها على انتاج انزيم اليوريكاز وتاثير العوامل المختلفة على انتاجه

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الهدف الرئيسى من هذه الدراسة هو تقييم قدرة السلالات الفطرية المتاحة على إنتاج إنزيم اليوريكاز وإختيار العزلة الاكثر فاعلية لانتاج الانزيم. يعتبر حمض اليوريك المادة أو الركيزة الأساسية لانتاج إنزيم اليوريكاز. ولذا تم فحص خمس وعشرون سلالة فطرية للكشف عن قدرتهم على إنتاج إنزيم اليوريكاز باستخدام حمض اليوريك. ولقد كانت أكثر السلالات نشاطا ولها القدرة على إنتاج إنزيم اليوريكاز هى: *Aspergillus welwitschiae*، *Emericella quadrilineata, Aspergillus carenus*، *Aspergillus peyronelii* ، *Aspergillus terrus.* يهدف هذا البحث أيضا لدراسة العوامل المؤثرة على إنتاج إنزيم اليوريكاز وتقيم نشاط السلالاتين الفطرية التى لهما قدرة كبيره على إنتاج إنزيم اليوريكاز وهما *Aspergillus welwitschiae*، *Emericella quadrilineata* تحت تاثير هذه العوامل والتى تشمل: تاثير درجات الحرارة المختلفة؛ تاثير قيمة الحموضة على إنتاج إنزيم اليوريكاز وأخيرا تأثير تركيز حمض اليوريك على إنتاج إنزيم اليوريكاز.