Optimization of Uricase by *Aspergillus terreus* NRC 7

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**Abstract:** Nine different fungal strains were screened for uricase production. From these strains *Aspergillus terreus* NRC7 proved to be the best potent for uricase production. Optimization of uricase showed that pH 6 was the optimum for uricase production in the basal medium containing sucrose as a sole carbon source, uric acid as a nitrogen source, cysteine HCl, cystine and glutamic acid enhanced uricase production. The optimum conditions for high crude uricase enzyme activity were 30µg of uric acid substrate (167.7U/ml) at 10µl of enzyme concentration at 30°C (172.8 U/ml) after 10 min of the reaction time.

**Key words:** Uricase - Fungi - Optimization - Crude enzyme

**INTRODUCTION**

Uricase is an enzyme belonging to the class of the oxido-reductases and catalyses the oxidation of uric acid to allantoin and thus plays an important role in the purine degradation pathway. Uricase in its conjugated form can be used as a therapeutic enzyme for the treatment of hyperuricemia and gout Nancy *et al.* [1]. It is also used as a clinical reagent for the determination of blood and serum uric acid concentrations Ademek *et al.* [2]. Some microorganisms have been used to produce uricase [3]. Uricase production ability by microorganisms and some physio-chemical parameters were tested to optimize uricase productivity [4]. Thus uricase turns out to be a very important therapeutic enzyme which is immensely required in its purest form with high activity. Microorganisms, animals and higher plants are capable of producing uricase on its own, but human being cannot produce uricase because of the mutation in the uricase gene. So human being has to depend on the other easily available source like microbes. Five different fungal strains belonging to *Gliocladium* and *Gliomastix* species were initially screened for their uricase producing capability, among which *Gliocladium viride* MTCC 3835 was identified to produce highly active uricase [5]. Screening of microbes for uricase production and estimation of uricase. This was achieved by utilizing the fact that uric acid dissolves on being acted upon by uricase [6]. Two strains of *Aspergillus niger* were tested for uricase producing ability and optimize the culture conditions for maximum uricase production [7].

The main purpose of this research was to screen the available local fungal strains for uricase producing and to optimize the conditions for maximum uricase activity.

**MATERIALS AND METHODS**

**Microorganisms:** Nine different fungal strains were tested for their ability to produce uricase enzyme. All fungal strains used were brought from the culture collection of the Chemistry of Natural and Microbial Products Department at National Research Center, Dokki, Giza, Egypt. These fungal strains were as follows: *Aspergillus flavus* NRC 25, *Aspergillus oryzae* NRC 34, *Aspergillus penicillioides* NRC 8, *Aspergillus terreus* NRC 7, *Blastomyces dermatitides* NRC 11, *Mucor plumbeus* NRC 6, *Penicillium corylophilum* NRC 14, *Penicillium echinatum* NRC 18 and *Penicillium paraphergal* NRC 37. The cultures were maintained on Czapek Dox agar medium.

**Production Medium:** The purchased fungal strains were cultivated on the production medium containing uric acid as an inducing agent in the medium. The basal medium was prepared using the following contents (g/l): uric acid (1.0), K$_2$HPO$_4$ (1.0), MgSO$_4$ (0.5), NaCl...
(0.5), FeSO₄ (0.01) and sucrose (20.0), the pH was adjusted at 6.5 and autoclaved at 121°C for 15 minutes [8].

**Fermentation for Uricase Production:** Two discs (6 mm in diameter) from 7 days old cultures were transferred to 250 ml Erlenmeyer conical flasks each containing 50 ml fermentation medium. The inoculated flasks were incubated on a rotary incubator shaker at 150 rpm for 7 days at 30°C after which the mycelium of each isolate was collected by centrifugation at 5000, 6000 rpm for 15 min at 4°C. The cell free supernatant was used as a crude enzyme for further determinations.

**Uricase Assay:** Uricase activity was measured according to the procedure described by Adamek et al. [2]. Two ml of a solution containing uric acid (10 mg/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 were added to the mixture to stop the enzyme reaction. The absorbance of all samples was measured at 293 nm. The difference between the absorbance of the sample and standard was 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 1µmol uric acid to allantoin per min at 30°C.

**Factors Affecting Uricase Production:** Optimization of different factors to evaluate maximum production and catalytic activity of crude uricase produced by the fungal strain were studied.

**pH Values:** The selected fungal isolate cultivated in fermentation medium at different pH values (4, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) determination of uricase enzyme was detected.

**Carbon Sources:** This experiment was designed to select the favorable carbon source for uricase production; sucrose was replaced by 1% of each lactose, dextrose, fructose and glucose as a source of carbon.

**Nitrogen Sources:** Uric acid was supplemented by 0.5% of ammonium sulphate, casein, peptone, tryptophan, sodium nitrate.

**Different Inhibitors and Activators:** Different metals such as sodium cyanide, calcium chloride, zinc sulphate, magnesium sulphate, magnesium chloride, copper sulphate, mercuric chloride cysteine HCl and cystine have been added to culture medium at a concentration equimolar to the concentration to the basal medium.

**Vitamins:** Four different vitamins were used in this experiment to produce a maximum quantity of uricase enzyme, these vitamins were ascorbic acid, tryptophane, glutamic acid, folic acid were supplemented to the medium at a concentration of 1.0 g/l.

**Optimization of Crude Enzyme Substrate Concentrations:** The substrate (uric acid) was applied into the reaction mixture at different concentrations (5, 10, 20, 30, 40 and 50 mg/ml) of borate buffer pH 8.5. Determination of enzyme activity was calculated as mentioned.

**Enzyme Concentration:** The crude enzyme was added to the reaction mixture at different concentrations (10, 20, 30, 40, 50 and 60 µ/ml).

**Temperatures of the Reaction:** The crude enzyme reaction mixture was incubated at different temperature (25, 30, 35, 40, 45 and 50°C).

**Different Reaction Times:** The crude uricase enzyme was incubated at different times of reaction (10, 20, 30, 40 and 50 min) to study the best time for reaction mixture.

**RESULTS AND DISCUSSION**

**Production of Uricase by Different Fungal Strains:**
The results presented in Table 1 showed that of the cultures investigation Aspergillus terreus NRC 7 (7 days old cultures) was the most potent and produced in shaken cultures the highest uricase enzyme (123.2 U/ml) followed by Aspergillus flavus NRC 25 (119.2 U/ml) and Aspergillus penicilloides NRC 8 (117. 8 U/ml) while Penicillium corylophilum NRC14 produced the least amount of uricase (70.90 U/ml). Uricase, an enzyme produced by several fungal strains, Atalla et al. [9] produced uricase enzyme from nineteen fungal strains, Gliomastix gueg (NRC 1A) has proved to be the most active producers of uricase enzyme (275.98 U/ml) which is higher than the activity reported with Yazdi et al. [10], who found that Mucor hiemalis produced (1.25U/ml) while Gloeocladium viride MTCC3835 was found to produce high activity uricase (63.14U/ml) [5]. From the obtained results Aspergillus terreus NRC 7 was selected for the subsequent experiments.
Effect of Different pH Values on Uricase Production:
The present study was carried out to observe the effect of different pH values (4, 4.5, 5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) on uricase activity. The results in Fig. 1 illustrated that the highest uricase activity was attained at pH 6.0 produced (140.1 U/ml) and gradually decreasing in uricase activity were shown when increasing the pH. This result is in agreement with those obtained by Yazdi et al. [10] and Tohamy and Shindia [11], who found that the pH 6.0 was optimum for uricase production from *A. flavus* and *Mucor hiemalis*. This result was in disagreement with those reported by Ali and Ibrahim [7], who found that pH 9 was optimum for uricase production (5.5 U/ml) from *Aspergillus niger* (Tom).

Effect of Different Carbon Sources on Uricase Production:
This experiment was designed to select the favourable carbon source for uricase production. The results in Fig. 2 showed that the highest amount of uricase enzyme (140.1 U/ml) was produced in the medium containing sucrose as a sole carbon source followed by dextrose (120 U/ml), the least amount of uricase (88.7 U/ml) produced when fructose was the carbon source. These results are in agreement with those obtained by Abd El Fattah and Abo-Hamed [8], who reported that *Aspergillus flavus* produced the highest amount of uricase in the medium containing sucrose. The effect of various carbon sources on the formation of uricase by microorganisms was studied by Lotfy [12] produced (1.25 U/ml) by *Basillus thermocatenulatus* and Ali and Ibrahim [7] (2.0 U/ml) from *Aspergillus niger* (Tom).

Effect of Different Nitrogen Sources on Uricase Production:
The present experiment was conducted to test the suitability of different nitrogen sources for uricase production. The results presented in Fig. 3 showed that the highest amount of uricase (140.1 U/ml) was attained when the fermentation medium contained uric acid. These results were coincided with Abd El Fattah and Abo-Hamed [8], who produced uricase in medium containing uric acid as a sole nitrogen source. Ali and Ibrahim [7] found that casein was the best nitrogen source for uricase production by *Aspergillus niger* (Tom) produce (4.86 U/ml).
Effect of Different Inhibitors and Activators on Uricase Production: Fig. 4 illustrated that cysteine HCl and cystine enhanced the uricase activity to 150 & 168 %, respectively where as magnesium chloride, calcium chloride metal ions reduced the enzyme activity to 60.1 % and 36.4%, respectively. Inhibition of uricase activity was noticed with Co$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ and enhanced with Cu$^{2+}$ and Ca$^{2+}$ [13], while an other study conducted the effect of metal ions on uricase activity produced from Mycobacterium sp. strain ZZJ4-1 that Mn$^{2+}$, Fe$^{3+}$, Zn$^{2+}$ and Ca$^{2+}$ had no inhibitory activity on uricase enzyme [14]. Yazdi et al. [10] found that Cu$^{2+}$, Ca$^{2+}$ increased the enzyme production by 20% and 15% respectively, also they found that Cd$^{2+}$ inhibit enzyme production by about 85%. Saeed et al. [15] also reported that the inhibition of uricase activity was noticed with Co$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ and enhanced with Cu$^{2+}$ and Ca$^{2+}$.

Effect of Different Vitamins on Uricase Production: Results in Fig. 5 showed that glutamic acid (1.0 g/l) gave the highest effect on uricase production by the selected organism. Also, tryptophan and folic acid appeared to stimulate uricase production but at a lesser extent. Such findings were obtained by Abd El Fattah and Abo Hamed [8], who found that the production of uricase was hardly affected by the incorporatio of most studied vitamins irrespective of the fungus. However, the addition of vitamin C, molasses and vitamin B12 slightly stimulated this process in Aspergillus terreus, Aspergillus flavus and Trichoderma spp. respectively. They also demonstrated that addition of nicotinic acid, folic acid and riboflavin exhibited various inhibitory effects against uricase production. Atalla et al. [9] found that folic acid and ascorbic acid had high stimulatory effect on uricase production by Glomastic gueg (NRC1A)(1180.02 U /ml, 1169.87 U /ml), respectively.

Some Properties of the Crude Uricase Preparation from Aspergillus terreus NRC 7: Effect of Different Substrate Concentrations on Crude Uricase Activity: The effect of substrate (uric acid) concentration (5, 10, 20, 30, 40 and 50 µg/ml) with 1ml enzyme was studied. The results in Fig. 6 demonstrated that, 30µg/ml of uric acid substrate gave high uricase activity (167.7U/ml). On the other hand, higher concentration above 30 µg/ml led to decrease in the activity. These results were disagreement with Yazdi et al. [10], who found that Mucor hiemalis gave higher uricase activity at 10µg. Also, Saeed et al [15], who found that 20 µg/ml of uric acid (substrate) was enough to be used in the reaction mixture.
Effect of Different Enzyme Concentrations on Crude Uricase Activity: The effect of different enzyme concentrations (10, 20, 30, 40 and 50µl) was studied. The relation between enzyme concentration and enzyme activity was recorded. The results presented in Fig. 7 indicated that the enzyme activity of uricase decreased with increasing the enzyme concentration above 10µl. These results are concomitant with those reported by Saeed et al. [15], who found that 10µl (20 U/ml) of the enzyme per reaction was enough to be used in the reaction mixture.

Effect of Different Temperature of the Reaction on Crude Uricase: The effect of incubation temperature ranged from 30-50°C on the activity of uricase in the crude enzyme is shown in Fig. 8. It was noticed from the results that crude uricase activity exhibited maximum activity (172.8 U/ml) at 30°C and over 50°C the activity decreased. These results are in agreement with those reported by Abd El Fattah and Abo Hamed [8] and Yazdi et al. [10], who found that the optimum temperature for uricase activity was 30°C.

Effect of Different Reaction Time on Crude Uricase Enzyme: The effect of reaction time on the activity of uricase enzyme is shown in Fig. 9, the crude enzyme reached to its maximum activity as described in materials and methods. The results demonstrated that the maximum uricase activity (172.8 U/ml) was detected after 10 min. Uricase activity decrease with increasing the reaction time and dropped to (154.7U/ml) after 30 min and to (149.4 U/ml) after 50 min of the reaction time. This result was coincided with Yazdi et al. [10] who found that the optimum reaction time for uricase activity was after 10 min.

CONCLUSION

The present study indicated that Aspergillus terreus NRC7 was the suitable for uricase production at pH 6 in the basal medium containing sucrose as a sole carbon source, uric acid as a nitrogen source, cysteine HCl, cystine and glutamic acid as additives for increasing uricase production. The optimum conditions for 10µl of crude uricase activity were 30µg/ml of uric acid as a substrate (167.7U/ml) at 30°C (172.8 U/ml) after 10 min of the reaction time.

REFERENCES