

Production, Optimization, Purification and Properties of Uricase Isolated From Some Fungal Flora in Saudi Arabian Soil

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Abstract: The present study deals with production, extraction and purification of the intracellular and extracellular fungal uricase. The fungal flora will be isolated from soil were (*Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Fusarium moniliforme*). Optimization of some nutritional and physical factors in the basal medium in order to intensify the production of extracellular and intracellular uricases will be carried out. 0.1% uric acid, 0.2% sodium phosphate were higher inducer for *A. niger* uricases. The enzymes will be purified to homogeneity from the most uricase producing organism (*A. niger*) by salting out with ammonium sulphate, dialysis and passage through chromatography resins (Sephadex G-200 column, Sephadex G-100 and Diethylaminoethyl cellulose column) and test for purity by simple polyacrylamide gel electrophoresis technique. Three extracellular uricase UI, UII and UIII and one intracellular uricase UIV were obtained with specific activities 105.9, 81.25, 101.96 and 9.66, respectively. The molecular weight of *A. niger* extracellular uricase isoenzymes UI, UII, UIII and UIV were 39.70, 30.50, 55.30 and 18 KDa., respectively. Studying factors affecting the activity of the purified uricase enzyme will be determined.

INTRODUCTION

Uricase catalyzes the oxidative opening of the purine ring of urate to yield allantoin, carbon dioxide, and hydrogen peroxide. Determining the urate concentration in blood and urine is effective for the diagnosis of gout as urate accumulation is a causative factor of gout in humans. The enzyme is useful for enzymatic determination of urate in clinical analysis by coupling with 4-aminoantipyrine-peroxidase system (Abdel-Fattah and Hesham, 2005). Uricase can be also used as a protein drug to reduce the toxic urate accumulation (Schiavon *et al.*, 2000). Decomposition of uric acid by microorganisms regarding their identities, occurrence and uricolytic activities of different microorganisms isolated from soil was studied by (Desouky, 1981). Fortunately, microbial uricase have been obtained in the purified form by many researchers (Bongaerts *et al.*, 1978 and Wang *et al.*, 1980). The method for the determination of uric acid in blood serum by its reaction with uricase was described by (Galbán *et al.*, 2001). Some microorganisms (Nobutoshi *et al.*, 2000; Hunag and Wu, 2004 and Ishikawa *et al.*, 2004) have been used to produce uricase. Uricase production ability by microorganisms and some physicochemical parameters were tested to optimize uricase productivity was studied by (Xue *et al.*, 2005).

MATERIAL AND METHODS

Sources of Isolation and Fungal Isolation:

The microorganisms recorded in this study were isolated from soil samples collected from Al-mesk lake soil and agricultural soil- Jeddah distinct- Saudi Arabia. The basal medium consisting of 0.3% uric acid, 0.2% K_2HPO_4 , 0.05% KH_2PO_4 , 0.01% $MgSO_4 \cdot 7H_2O$, 0.01% NaCl and 0.01% $CaCl_2$ and 1.0% (v/v) trace elements solution containing of 5.2% $ZnSO_4$, 5.0% $FeSO_4 \cdot 7H_2O$, 5.0% $CuSO_4 \cdot 7H_2O$ and 0.05% $MnSO_4 \cdot H_2O$.

Assay of Uricase Enzyme for Isolated Fungal Isolates:

Uricase activity was measured spectrophotometrically according to Mahler, 1970 by the disappearance of uric acid, which detected by the decrease in absorbance at 293 nm. The assay mixture contained 0.1 ml of enzyme solution in 0.1 M borate buffer pH 9.0 and 0.12 mM uric acid in a final volume of 3.0 ml. Incubation was carried out at 30 °C for 30 min. The reaction was terminated by the addition of 200 ml of 0.1 N KCN. The absorbance was measured at 293 nm. As a control, the solution of KCN was added to the substrate before the addition of the enzyme solution. One unit of enzyme was defined as the amount of enzyme necessary to transform 1 mmol of uric acid into allantoin in 1 min at 30 °C.

Determination of Total Dry Matter (TDM) and Linear Growth for all Isolated Fungal Species:

The mycelium was dried at 80 °C till constant weight to get the dry weight. Known volumes of uric acid medium were sterilized by autoclaving, then adjust the pH at 7 (Khodair *et al.*, 1990). Aliquots of about 15 ml of this medium were dispersed into sterile petri-dishes, (9cm diameter) triplicate plate. Each dish was inoculated at its center with a fungal disc (1 cm diameter) cut from the colony margin of 2-4 day old cultures growing on

uric acid medium. The plates were incubated for 24 hour intervals, after which colony diameters (in cm) were measured (mean of two diameters at right angles to each other) (Kuthubutheen and Pugh, 1978).

Selection of Microorganism for Assay of Uricase Activity:

All isolated species were assayed for their uricase activity on uric acid medium. The fungal species which showed high extracellular and intracellular uricase activities was selected for the next experiments.

Effect of Different Nitrogen Sources and Concentration on Induction of Uricase Enzyme:

The liquid medium containing 0.3% uric acid was used as control. The following nitrogen sources were used: (ammonium chloride, glycine, pepton and sodium nitrate). The following nitrogen concentrations were added to the medium (%) 0.1, 0.3, 0.5, 0.7 and 0.9.

Effect of Different Phosphate Sources and Concentration on Induction of Uricase Enzyme:

The medium containing the optimum source and concentration of the chosen nitrogen source with 0.2% dipotassium hydrogen phosphate was used as control. The following phosphate sources: (sodium phosphate, magnesium phosphate and ammonium phosphate) were added. The following phosphate concentrations (0.1%, 0.2% (control), 0.4% and 0.6%) was used.

Effect of PH and Temperature:

The medium containing the optimum source and concentration of the chosen nutritional factors was used as control. The following buffer solutions were used: - citrate buffer solution for pHs 4, 6; boric acid-borax buffer solution for pH 8; carbonate bicarbonate buffer solution for pH 10 and glycine NaCl-NaOH buffer solution for pHs (10.9, 11.4), each at 0.05M concentration. The triplicate flasks of liquid medium containing the optimum source and concentration of the chosen nutritional factors and optimum pH were used. Incubation was carried out at the following temperatures; 20 °C, 27 °C, 35 °C, 40 °C, and 60 °C, respectively for 10 days.

Purification and Molecular Weight of Uricase Enzymes:

The enzyme solution was treated with ammonium sulfate by raising the concentration of ammonium sulfate to 80% (Segel, 1968). Each precipitate was dissolved in 10 ml 0.05M citrate buffer pH 6.2, placed in a dialysis bag and dialyzed against distilled water in a refrigerator for 48 hours, until the enzyme solution inside the bag becomes free from sulfate. The dialyzed enzyme sample was then incorporated into the column for elution. The protein content of each fraction was determined according to (Bradford, 1976). Column chromatography by combination of basic anion exchange DEAE- Sephadex and gel filtration was recorded by Anand *et al.*, 1990 and Ganju *et al.*, 1989. The dialysed enzyme was applied in small amount of buffer pH 6.2 to Sephadex G-200 column, followed by Sephadex G-100 and finally DEAE-Sephadex. Sodium dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) discontinuous system was used according to Laemmli, 1970.

Characterization of the Purified Extracellular and Intracellular Uricase Enzymes:

In this experiment, identical reaction mixtures were prepared. These mixtures were incubated at the following temperatures: - 5 °C, 20 °C, 27 °C, 35 °C, 40 °C, 60 °C and 80 °C for 5 minutes. To study the effect of different pH values ranging from 4- 11.4 on extracellular and intracellular uricase activities. The effect of substrate concentration on the purified extracellular and intracellular uricase enzymes activities was studied using different substrate concentrations ranging from (0.4-10.3 mg/ml) in the reaction mixture. The effect of extracellular and intracellular uricase enzymes with protein concentration ranging from 5.17-60 µg protein/ml on the extracellular and intracellular uricase enzyme activities were investigated.

RESULTS AND DISCUSSIONS

The Micro Floral Picture:

Table 1 includes the total counts and numbers of isolated fungal species from both Al-mesk lake soil and agricultural soil sources. A total of 49 fungal colonies were isolated from Al-mesk soil lake representing three fungal species, on the other hand the total count of agriculture soil fungi was 51 colonies which constituted four fungal species. In the present study, *Aspergillus niger* and *Rhizopus stolonifer* were the most dominant species with high occurrence in the two sources of isolation (Al-mesk soil lake and agriculture soil). The low occurrence was representing by *Fusarium moniliforme* which isolated only from agriculture soil. Yazdi *et al.*, 2006 stated that one hundred and sixty-five strains of microorganisms with the ability to grow in a medium containing uric acid as a major source of nitrogen were isolated from soil samples. Among them, a zygomycete fungus with well-developed columellae was recognized to produce high levels of uricase enzyme in a short time. Screening of microorganisms with uricase activity was achieved by Lehejckova, *et al.*, 1986.

Assay of Extra and Intracellular Uricase Enzymes of Isolated Fungi:

Table 2 includes the assay of extracellular and intracellular uricase enzyme activities on uric acid as a substrate for the isolated fungal species. The maximum significant value of extracellular and intracellular uricase enzymes was shown in the culture filtrate of *Aspergillus niger* (47.40 and 12.90 unit/ml), respectively whereas the minimum value could be detected with *Fusarium moniliforme* (2.40 and 0.77 unit/ml). The regulation of uricase enzyme from the purine catabolic pathway in any member of the Zygomycetes was recorded by Farley and Santosa, 2002 and also Leplatois, *et al.*, 1993 stated that *Aspergillus* uricase accumulates intracellularly. Uricase production and properties was recorded by Ammar, *et al.*, 1988.

Table 1: Frequency of occurrence of fungal genera isolated from both Al-mesk lake soil and agriculture soil (0-5 Low, 6-12 Moderate, 13-20 High).

| Fungal species | Al-mesk lake soil | | Agriculture soil | |
|--------------------------------|-------------------|-------------------------|------------------|-------------------------|
| | Count | Frequency of occurrence | Count | Frequency of occurrence |
| <i>Aspergillus niger</i> | 20 | High | 20 | High |
| <i>Fusarium moniliforme</i> | - | - | 2 | Low |
| <i>Penicillium chrysogenum</i> | 9 | Moderate | 9 | Moderate |
| <i>Rhizopus stolonifer</i> | 20 | High | 20 | High |
| Total count | 49 | | 51 | |
| Number of species | 3 | | 4 | |

Table 2: Assay of extracellular and intracellular uricase activities on uric acid as a substrate for isolated fungal species.

| pH | Extracellular uricase (unit/ml) | Intracellular uricase (unit/ml) |
|-------------|---------------------------------|---------------------------------|
| 4 | 35.40 | 9.40 |
| 6 | 43.90 | 9.80 |
| 8 (Control) | 45.10 | 12.10 |
| 10 | 38 | 11 |
| 10.9 | 36.07 | 10.50 |
| 11.4 | 30.60 | 9.60 |
| (LSD) | 17.2 | 1.12 |

Table 3: Effect of pH values on the production of uricase enzymes produced by *A. niger* after 10 days growth**Determination of Total Dry Matter (TDM) and Linear Growth for all Isolated Fungal Species:**

Figure 1 includes the dry weight gain of the isolated fungal species. The data show that *Penicillium*

| Fungal species | Extracellular uricase (Unit/ml) | Intracellular uricase (Unit/ml) |
|--------------------------------|---------------------------------|---------------------------------|
| <i>Aspergillus niger</i> | 47.40 | 12.90 |
| <i>Fusarium moniliforme</i> | 2.40 | 0.77 |
| <i>Penicillium chrysogenum</i> | 8.10 | 2.30 |
| <i>Rhizopus stolonifer</i> | 26.70 | 8.60 |
| LSD | 16.2 | 3.6 |

chrysogenum followed by *Aspergillus niger* showed a significant rise in dry weight gain (0.23 and 0.21 g/100ml), respectively. Oppositely *Fusarium moniliforme* recorded the lowest dry weight gain (0.08 g/100ml). Three uricolytic filamentous fungi and factors influencing the assay and productivity of crude uricase was investigated by Kiadó 2005. *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium chrysogenum* gave the highest linear growth (5.94, 5.61 and 5.44 cm, respectively), while significant decrease was shown by *Fusarium moniliforme* with the lowest value (4.22 cm) Figure 2.

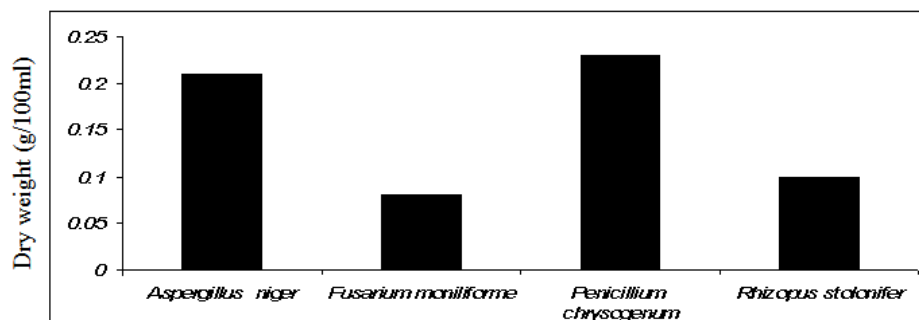
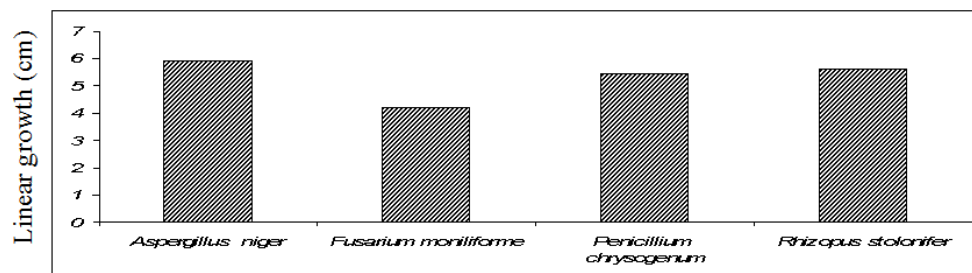
Table 4: Effect of temperature on the production of uricase enzymes produced by *A. niger* after 10 days growth

| Temperature (°C) | Extracellular uricase (unit/ml) | Intracellular uricase (unit/ml) |
|------------------|---------------------------------|---------------------------------|
| 20 | 37.20 | 12.60 |
| 27 (control) | 177.60 | 24.80 |
| 35 | 163.20 | 21.60 |
| 40 | 155.10 | 15.70 |
| 60 | 110.30 | 11.80 |
| (LSD) | 31.2 | 1.18 |

Table 5: Purification scheme of *A. niger* extracellular and intracellular uricase.

| Purification steps | Total volume (ml) | | Total protein (mg) | | Total activity (Units) | | Specific activity | | Purification fold | | Recovery (%) | |
|---|-------------------|------|--------------------|-------|------------------------|-------|-------------------|------|-------------------|------|--------------|------|
| | Ext. | Int. | Ext. | Int. | Ext. | Int. | Ext. | Int. | Ext. | Int. | Ext. | Int. |
| Crude extract | 1300 | 400 | 1701 | 650.0 | 10105 | 2523 | 5.94 | 3.88 | - | - | 100 | 100 |
| (NH ₄) ₂ SO ₄ | 32 | 20 | 1245 | 240.4 | 8976 | 1697 | 7.21 | 7.06 | 1.21 | 1.8 | 88.83 | 67.2 |
| Dialyses | 8.8 | 4.7 | 501 | 250.0 | 6077 | 1259 | 12.13 | 5.04 | 2.04 | 1.2 | 60.14 | 49.9 |
| Sephadex G-200 | 5 | 5 | 62 | 101 | 2600 | 625.0 | 41.94 | 6.19 | 7.06 | 1.5 | 25.73 | 24.8 |
| Sephadex G-100 | 5 | | | | | | | | | | | |
| Peak A | | | 14 | 35 | 1100 | 286.0 | 78.57 | 8.18 | 13.2 | 2.1 | 10.89 | 11.3 |
| Peak B | | | 10 | | 745 | | 74.50 | | 12.5 | | 7.37 | |
| Peak C | | | 8 | | 1200 | | 150.00 | | 25.2 | | 11.88 | |
| DEAE-Sephadex | 5 | 2 | | | | | | | | | | |
| Peak A | | | 10.6 | 22.2 | 1123 | 214.0 | 105.90 | 9.66 | 17.8 | 2.4 | 11.11 | 8.50 |
| Peak B | | | 8 | | 650 | | 81.25 | | 13.6 | | 6.43 | |
| Peak C | | | 7.11 | | 725 | | 101.96 | | 17.1 | | 7.17 | |

EXT.= EXTRACELLULAR URICASE, INT.= INTRACELLULAR URICASE

**Fig. 1:** Dry weight gain (g/100 ml) for the selected fungal cultures (LSD= 0.12).**Fig. 2:** Linear growth (colony diameter) for the selected fungal cultures (LSD=1.2).**Selection of Most Potent Isolates Producing Uricase:**

A. niger was the highest producer for extracellular and intracellular uricase enzyme (47.40 and 12.90 unit/ml), accompanied with high occurrence on both tested soil samples (Al-mesk lake soil and agriculture soil), so it was used for the following experiments. Production of uricase enzyme from *Aspergillus niger* as a beneficial source of uricase enzyme and determination of some factors affecting the activity was achieved by Ertan and Aksz, 2000.

Optimization of Culture Medium:

Figure 3 shows the effect of different nitrogen sources on the production of uricase enzymes produced by *A. niger* after 10 days growth. When uric acid was used as a nitrogen source, extracellular and intracellular uricase was significantly highly produced (45.66 and 9.46 unit/ml), respectively. Accordingly, uric acid was proved to be the best nitrogen source for maximum uricase production by *A. niger* and it was used as proper substrates in further experiments. The obtained results was in accordance with that stated by Ramana, and Sastry, 1993 who suggested that uric acid complexes as growth substrates and inducers of uricase enzyme activity. Also Yazdi *et al.*, 2006 optimized composition of the medium consisted of uric acid for maximum production of uricase.

Figure 4 shows the effect of different concentrations of uric acid on the production of uricase enzymes produced by *A. niger*. The highest yields of extracellular and intracellular uricase activities were obtained at 0.1% uric acid concentration. Accordingly, the nitrogen sources and concentrations at which the highest activity was obtained (0.1% uric acid) were used in the following experiments. Our results were in agreement with that obtained by Lotfy, 2008 who said that microbial uricase was a uric acid inducible enzyme and the maximum activity was observed in the presence of uric acid. In general, uricase production was far more enhanced by using the organic nitrogen than the inorganic nitrogen. It was possible that organic nitrogen may contain most kinds of amino acids for the growth of bacterium that could be metabolized directly by cells, consequently promoting the uricase production. Wang, and Marzluf, 1979 demonstrated nitrogen regulation of uricase synthesis. Figure 5 reveals the effect of different phosphate sources on the production of uricase enzymes produced by *A. niger* after 10 days growth. Sodium phosphate was the best phosphate source used by *A. niger*, where the maximum significant extra- and intracellular uricase activity was 33 and 9 unit/ml, respectively. A significant decrease in extra and intracellular uricase when using the rest of phosphate sources was evident. Liu, and Li, 1989 demonstrated the culture conditions for uricase productivity. Figure 6 includes the increase in the concentration of sodium phosphate up to 0.2% resulted in a significant increase in extra- and intracellular uricase activities of *A. niger* (reaches to 52.63 and 6.68 unit/ml, respectively), so this concentration was used in further studies. A significant decrease in uricase activities was shown with other concentrations. Induction and synthesis of uricase was estimated by Nahm and Marzluf, 1987. The data in Table 3 reveal the effect of pH values on the production of uricase enzymes by *A. niger* after 10 days growth. The gradual increase in pH value of the culture medium of *A. niger* from 4-8 was accompanied with an increase in extra and intracellular uricases to reach its maximum yield at pH 8 (45.10, 12.10 unit/ml, respectively). Table 4 presents the maximum extra- and intracellular uricase activities of *A. niger* (177.60, 24.8 unit/ml) were recorded at 27 °C. Yazdi *et al.*, 2006 stated that the optimum pH and temperature for uricase production in the optimized medium were pH 6 and 30 °C, respectively.

Purification of Extra- and Intracellular Uricases of *A. Niger*:

The specific activities of uricases in the crude extract of filtrate and mat were 5.94 and 3.88, respectively, (Table 5). Column chromatography of extra- and intracellular uricases was applied to Sephadex G-200 followed by Sephadex G-100. From the elution profile in Figure 7 and Table 7, it can be seen that *A. niger* extracellular uricase was eluted from the column in the three peaks of extracellular uricase activity. In case of intracellular *A. niger* uricase, it can be found that it was eluted from Sephadex G-100 column in one peak designated as A in the fractions 32-40. The active fractions of the three peaks of extracellular uricase and the one active peak of intracellular uricase were pooled. The lyophilized fraction for each of extracellular uricase UI, UII and UIII and intracellular UIV from Sephadex G-100 column were applied to DEAE- Sephadex column (Figure 12). Farley and Santosa, (2002) stated that uricase activity was increased from 10 to 40 fold under derepression conditions and was induced by exogenous uric acid (60 to 78 fold). Purification of microbial uricase was estimated by Adamek, *et al.*, 1989. Demnerova *et al.*, 1986 Purified uricase by different strains of microorganisms. Purification and characterization of uricase was detected by Wang and Marzluf, 1980.

Estimation of the Molecular Weight of the Three Pure Extracellular Uricase Isoenzymes (UI, UII and UIII) and Intracellular Uricase Isoenzyme (UIV) Produced by *A. Niger*:

The estimated molecular weight of *A. niger* extracellular uricase isoenzymes UI, UII and UIII were 39.70, 30.50 and 55.30 KDa., respectively. In case of intracellular uricase isoenzyme UIV, the molecular weight was 18 KDa (plate 1).

Characterization of Pure *A. Niger* Uricases UI, UII, UIII and UIV:

Purification and properties of uricase were recorded by Liu, *et al.*, 1994. The results in Figures 13 show that the optimum temperature of extracellular UI, UIII and UIII was at 35 °C, while the intracellular uricase UIV gave its maximum activity (37.1 unit/ml) at 27 °C. Figure 14 reveals that the extracellular and intracellular uricases UI, UII, UIII and UIV reach to its maximal value (34.0, 42.1, 38.7 and 35.0 unit/ml) at pH 8, respectively. The activity of the purified extra and intracellular uricases was measured with increasing concentrations of standard uric acid substrate ranging from 1-8 mg/ml. A hyperbolic relationship between the activity of UI, UII, UIII and UIV and uric acid concentration was obtained up to 5.0 mg/ml, above this concentration, the enzymes showed nonsignificant changes (Figure 15). Isolation and some properties of uricase were detected by Kinsella, 1985. Figure 16 shows that there is a direct relation between the activity of extracellular uricases UI, UII, UIII and the quantity of protein. Linear significant increase in enzyme activity was recorded up to 20 µg enzyme protein, after this concentration a nonsignificant difference was shown. Concerning intracellular uricase UIV, the maximum value of enzyme activity was found at 40 µg enzyme protein. High cell density cultivation and high recombinant protein production of uricase was estimated by Nakagawa, *et al.*, 1995.

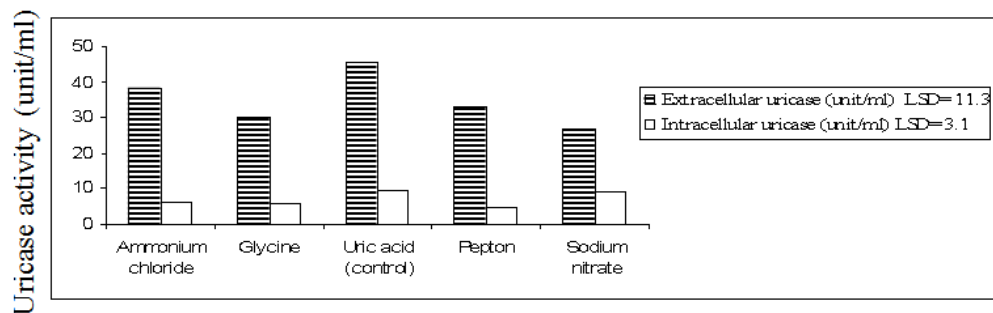


Fig. 3: Effect of different nitrogen sources on the production of *A. niger* uricase.

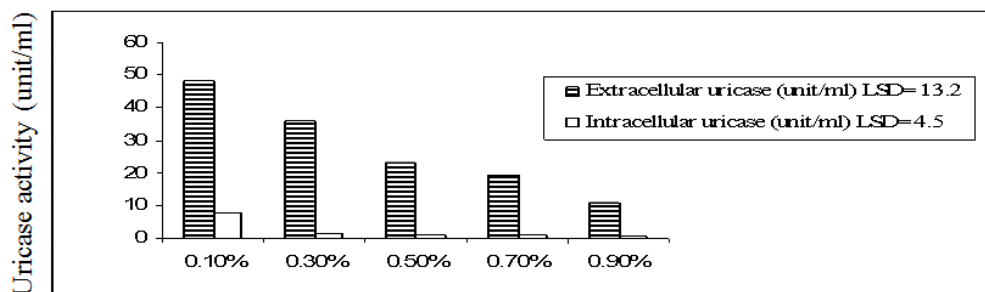


Fig. 4: Effect of different concentration of uric acid on the production of *A. niger* uricase.

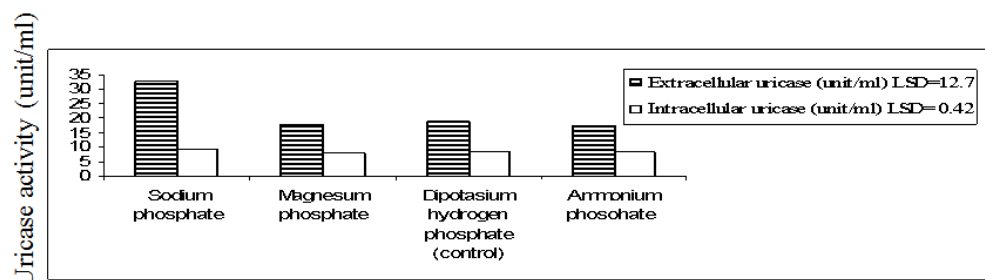


Fig. 5: Effect of different phosphate sources on the production of *A. niger* uricase.

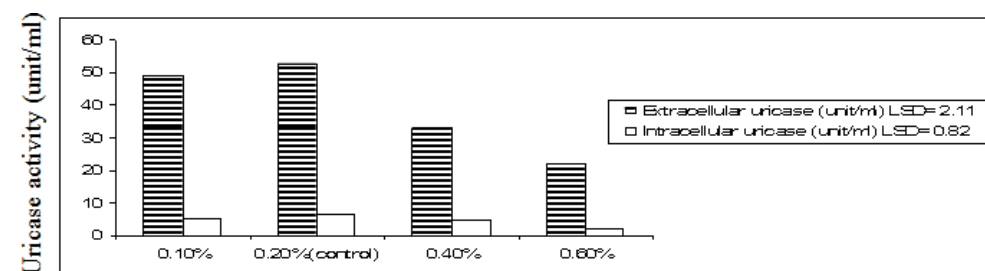


Fig. 6: Effect of different concentration of sodium phosphate on the production of *A. niger* uricase.

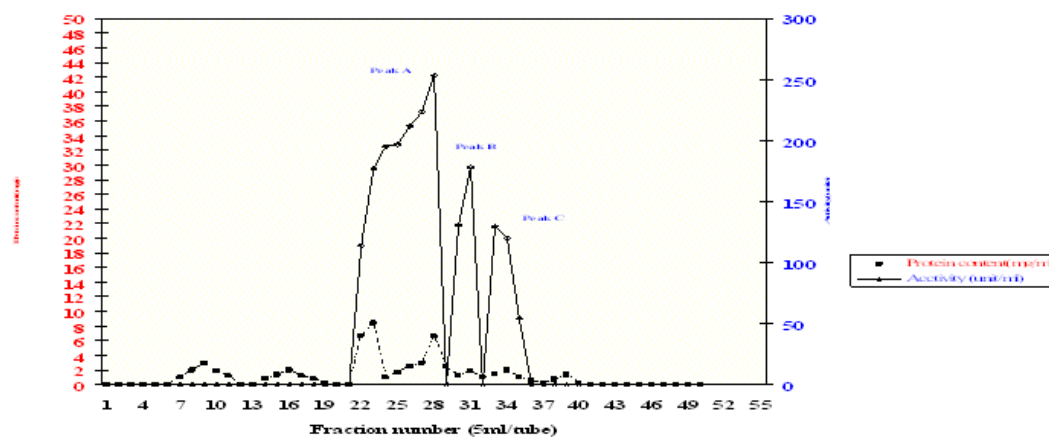


Fig. 7: Typical elution profile for the behaviour of *A. niger* extracellular uricase on Sephadex G-100.

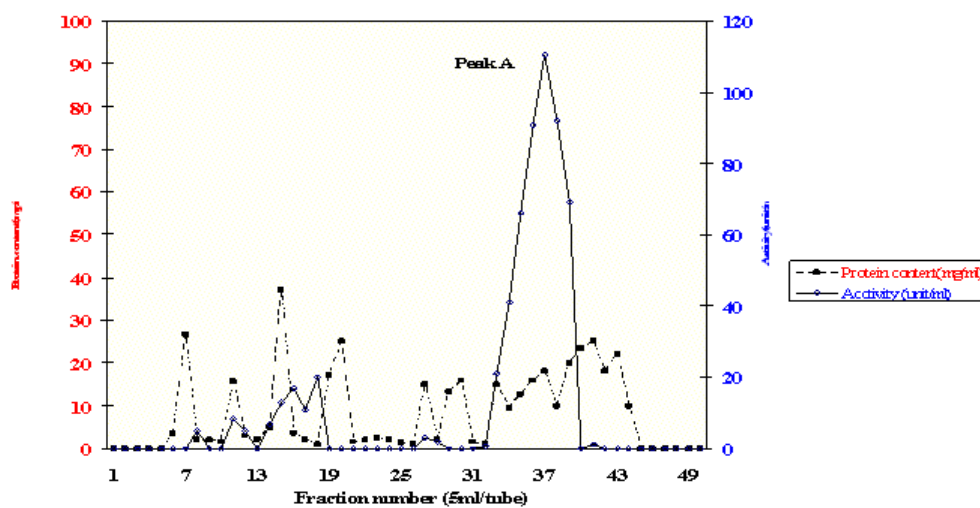


Fig. 8: Typical elution profile for the behaviour of *A. niger* intracellular uricase on Sephadex G-100.

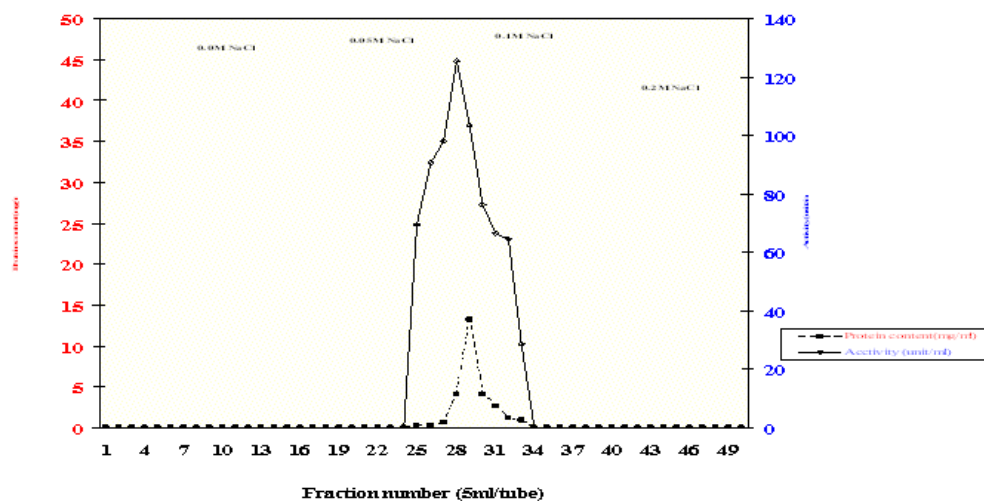


Fig. 9: Typical elution profile for the behaviour of *A. niger* extracellular uricase peak A on DEAE-Sephadex.

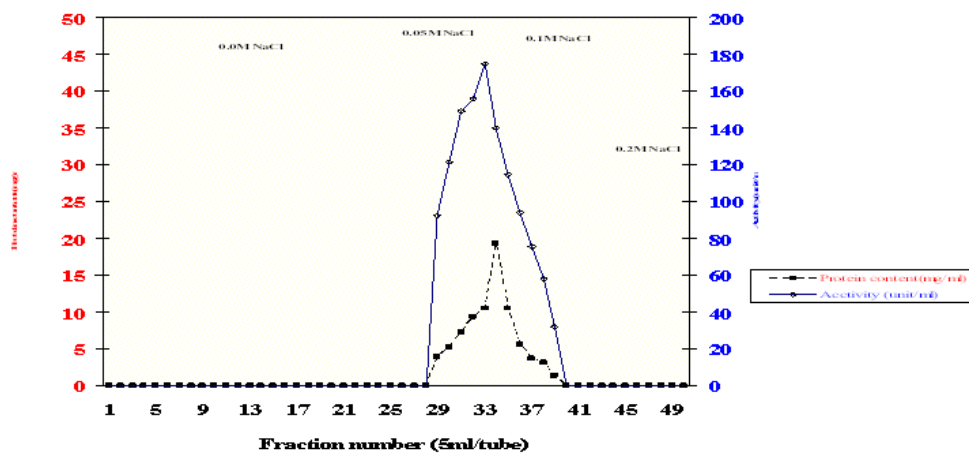


Fig. 10: Typical elution profile for the behaviour of *A. niger* extracellular uricase peak Bon on DEAE-Sephadex.

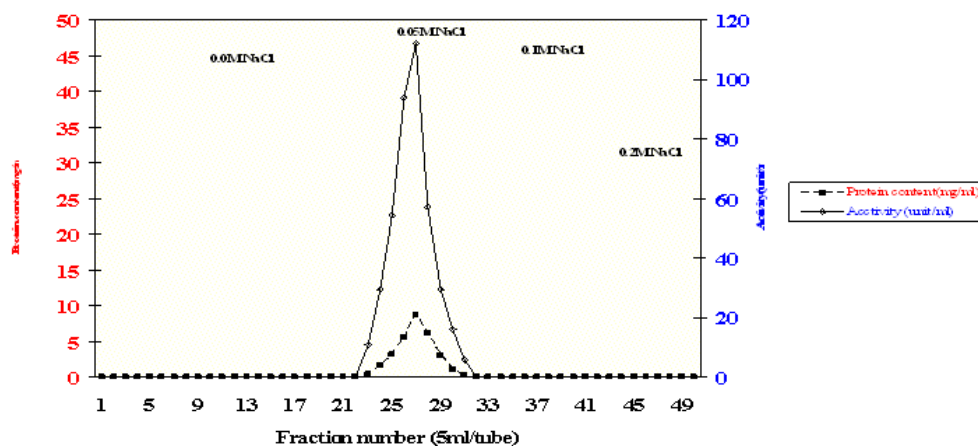


Fig. 11: Typical elution profile for the behaviour of *A. niger* extracellular uricase peak C on DEAE-Sephadex.

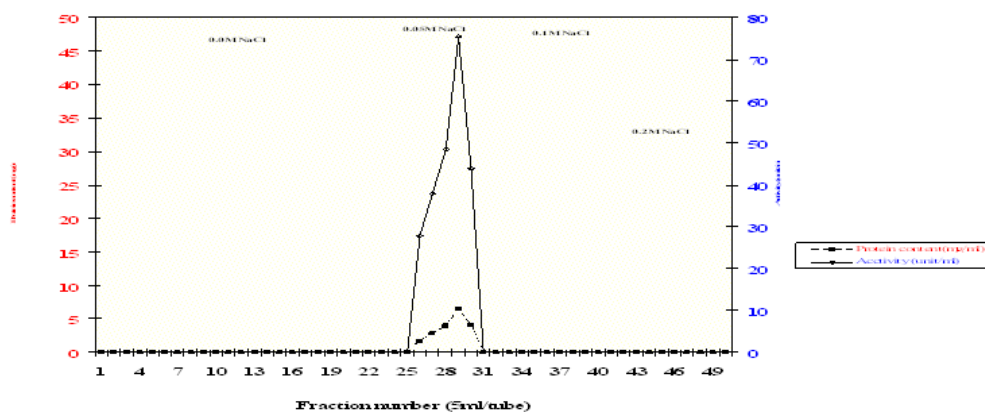


Fig. 12: Typical elution profile for the behaviour of *A. niger* extracellular uricase UTV peak A on DEAE-Sephadex.

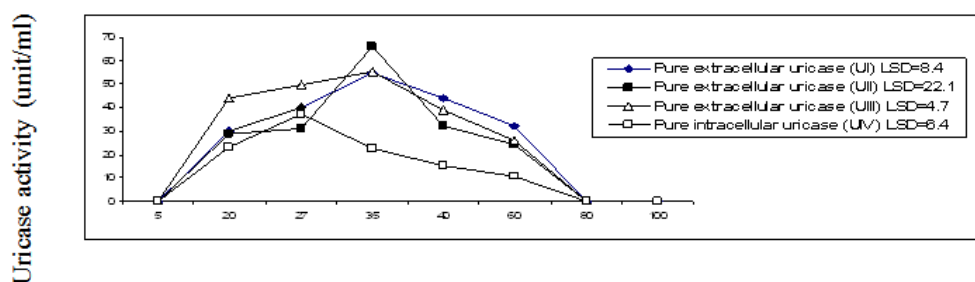


Fig. 13: Effect of temperature on the activity of the purified *A. niger* extracellular and intracellular uricase.

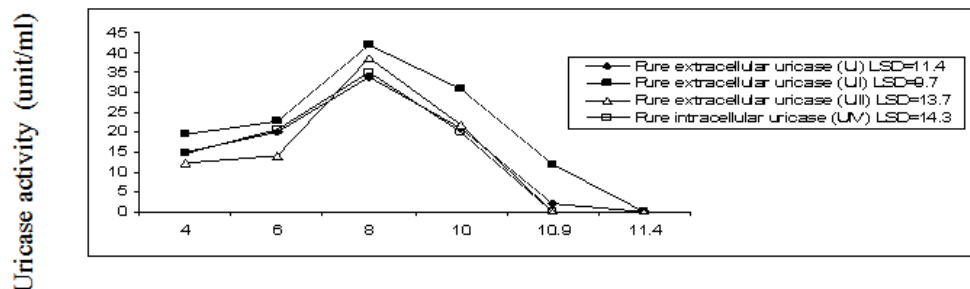


Fig. 14: Effect of initial pH values on the activity of the purified *A. niger* extracellular and intracellular uricase.

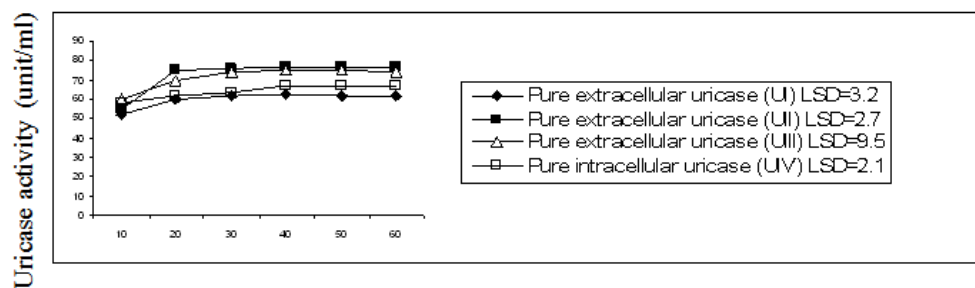


Fig. 15: Effect of substrate concentrations on the activity of purified *A. niger* extracellular and intracellular uricase.

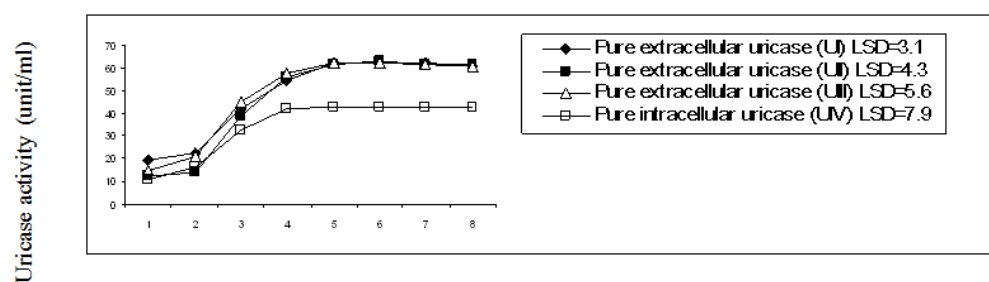


Fig. 16: Effect of protein concentration on the activity of the purified *A. niger* extracellular and intracellular uricase.

Conclusions And Recommendations:

In conclusion, the present study indicates that the isoenzyme UI produced from the *Aspergillus niger* is the most preferable isoenzyme due to its highest specific activity (105.9). So the isozyme UI will be useful for enzymatic determination of urate in blood and urine in clinical analysis and it will be effective for the diagnosis of gout as urate accumulation which is a causative factor of gout in humans (Abdel-Fattah and Hesham, 2005).

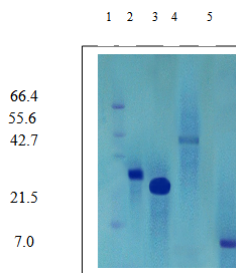


Plate 1: SDS-PAGE of *A. niger* purified uricase. Lane 1: Marker proteins; lane 2, 3, 4: Extracellular uricase (UI, UII, UIII); lane 5: Intracellular uricase (UIV).

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REFERENCES

- Abdel-Fattah, R. and M. Hesham, 2005. Improved production of *Pseudomonas aeruginosa* uricase by optimization of process parameters through statistical experimental designs, *Process Biochem.*, 40: 1707–1714.
- Adamek, Y., B. Kralova, M. Suchova, O. Valentova and K. Demnerova, 1989. Purification of microbial uricase, *J. Chrom.*, 497: 268 – 275.
- Ammar, M.S., S.H. Elwan and E.M. Desouky, 1988. Purification and some properties of uricase from *Aspergillus flavus*, S-79. *Egypt. J. Microbiol.*, 23: 88-98.
- Anand, L., S. Krishnamurthy and P.J. Vithayathil, 1990. Purification and properties of xylanase from the thermophilic fungus, *Humicola lanuginosa* (Griffon and Maublanc) Bunce. *Archives of Biochem. and Biophys.*, 276: 546-553.
- Barnett, H.L., 1960. Illustrated genera of imperfect fungi. Burgess Publishing company, Minneapolis, pp: 225.
- Barnett, H.L. and B.B. Hunter, 1972. Illustrated genera of imperfect fungi. Burgess Publishing company, Minneapolis, pp: 241.
- Bongaerts, G.P.A., J. Uizetter, R. Brouns and G.D. Vogels, 1978. Uricase of *Bacillus fastidiosus*. Properties and regulation of synthesis. *Biochem. Biophys. Acta.*, 2: 348-58.
- Bradford, M.M., 1976. Photometric methods for protein determination. *Anal. Biochem.*, 72: 248-254.
- Cooney, D.G. and R. Emerson, 1964. Thermophilic fungi: An account of their biology, activities, and classification. Free man W. H. Publ. Co., San Fransisco. 188.
- Crisan, E.V., 1964. Isolation and culture of thermophilic fungi. *Contrib. Boyce thompson Inst.*, 22: 291-301.
- Crisan, E.V., 1959. The isolation and identification of thermophilic fungi. M. Sc. Thesis, Purdue University, Lafayette, Indian. C. f., Ainsthworth and Sussman, 1968.
- Demnerova, K., B. Kralova, R. Lehejokova, V. Adamek, 1986. Purification of uricase by different strains. *of Streptomyces*, *Biotech. Lett.*, 8(8): 577-578.
- Desouky, E.M., 1981. Microbiological and biochemical studies on certain uric acid decomposing microorganisms. Ph.D. Thesis, Botany Dept. Faculty of science, Al-Azhar University, Cairo, Egypt.
- Ertan, F. and E. Aksz, 2000. *Aspergillus niger* Öden .rikaz Enziminin .retilmes i ve Aktiviteye Etkili Bazı Fakt.rlerin Belirlenmesi. *Turk J Biol.*, 24, Ek Sayı, 11-23.
- Farley, P. and S. Santosa, 2002. Regulation of expression of the *Rhizopus oryzae* uricase and urease enzymes. *Can J Microbiol.*, 48(12): 1104-8 12619824.
- Galbán, J., Y. Andreu, M. Almenara, S. Marcos and J. Castillo, 2001. Direct determination of uric acid in serum by a fluorometric-enzymatic method based on uricase. *Talanta*, 54: 847-854.
- Ganju, R.K., P.J. Vithayathil and S.K. Murthy, 1989. Purification and characterization of two xylanases from *Chaetomium thermophile* var. *coprophile*. *Can. J. of Microbiol.*, 35: 836-842.
- Gilman, J.C., 1957. A manual of soil fungi. The lowstate college press. Ames, Iowa, U.S.A. pp: 450.
- Kendrick, B., 1971. Taxonomy of Fungi Imperfecti. Toronto University, Canda.
- Khodair, A.A., A.S. Ramadani and A.M. Aggab, 1990. Occurrence and density of alkalophilic bacteria and fungi in saline soils of Makkah District, Saudi Arabia. *Arab. Gulf. J. Scient. Res.*, 3: 119-132.

- Kiadó, A., 2005. Bioconversion of poultry wastes I - Factors influencing the assay and productivity of crude uricase by three uricolytic filamentous fungi. *Acta Microbiologica et Immunologica Hungarica*, 49(4): 445-454.
- Kinsella, J.E., B. German and J. Shetty, 1985. Uricase from fish liver: Isolation and some properties. *Comp. Biochem. Physiol.*, 82 B: 621 - 624.
- Koyama, N., 1989. Sodium dependant of the stability under alkaline conditions of the membrane of an alkaloophilic *Bacillus federation* of European Microbiological Societes. *Microbiology Letters*, 61: 7-10.
- Kuthubutheen, A.J. and G.J.F. Pugh, 1978. Effects of fungicides on physiology of phylloplane fungi. *Trans. Br. Mycol. Soc.*, 71: 261-269.
- Laemmli, U., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)*, 227: 680-685.
- Lehejckova, R., K. Demnerova, B. Kralova, 1986. Screening of microorganisms with uricase activity. *Biotech. Lett.*, 8(5): 341 - 342.
- Leplatois, P., B. Douarin, G. Loison, 1993. High-level production of a peroxisomal enzyme: *Aspergillus flavus* uricase accumulates intracellularly and is active in *Saccharomyces cerevisiae*, *Gene*, 122(1): 139-145.
- Liu, J. and G. Li., 1989. Culture conditions for uricase of *Candida utilis*. *Acta Microbiol. Sinica*, 29(1): 45-50.
- Liu, J., G. Li, H. Liu and X. Zhou, 1994. Purification and properties of uricase *Candida sp.* and its application of uric acid analysis in serum, *Appl. Biochem. Biotechnol.*, 47(1): 57 - 63.
- Mahler, J. L., 1970. A new bacterial uricase for uric acid determination. *Anal Biochem.*, 38: 65-84.
- Moubasher, A.H., 1993. Soil Fungi in Qatar and other Arab Countries. Scientific and Applied Research Center, University of Qatar. (pp: 566).
- Michael, L.G. and S.H. Michael, 1989. A radiochemical high-performance liquid chromatographic assay for urate in human plasma, *Anal Biochem.*, 176: 290-293.
- Nahm, B.H., G.A. Marzluf, 1987. Induction and de novo synthesis of uricase a nitrogen - regulated enzyme in *Neurospora crassa*, *J. Bacteriol.*, 169(5): 1943-1948.
- Nakagawa, S., H. Oda and H. Anazawa, 1995. High cell - density cultivation and high recombinant protein production of *Escherichia coli* strain expressing uricase, *Bio. Biotech. Biochem.*, 59(12) 2263 -2267.
- Ramana, V.V. and K.S. Sastry, 1993. Chromium- uric acid complexes as growth substrates and inducers of uricase in *Neurospora crassa*, *J. Ünorg. Biochem.*, 50(2): 107-117.
- Raper, K.B. and D.L. Fenell, 1965. The genus *Aspergillus*. Williams Comany Baltimore, U.S.A., pp: 876.
- Samson, R.A., 1979. A compilation of the *Aspergilli* described since 1965. *Studies in Mycol.*, 18: 1-39.
- Segel, I.H., 1968. Biochemical calculations. John Wiley and Sons New York Chichester. Brisbane. Toronto Singapore Edition, 2: 370.
- Somkuti, G.A. and F.J. Babel, 1968. Purification and properties of *Mucor pusillus* acid protease. *J. Bacteriol.*, 95: 1407-1414.
- Walfridsson, M., M. Anderlund, X. Bao and B. Hahn-Hägerdal, 1997. Expression of different levels of enzymes from the *Pichia stipitis* XYL1 and XYL2 genes in *Saccharomyces cerevisiae* and its effects on product formation during xylose utilization. *Appl. Microbiol. Biotechnol.*, 48: 218-224.
- Wang, L.C. and G.A. Marzluf, 1979. Nitrogen regulation of uricase synthesis in *Neurospora crassa*, *Mol.Genet.*, 176: 385-392.
- Wang, L.C. and G.A. Marzluf, 1980. Purification and characterization of uricase, a nitrogen regulated enzyme from *Neurospora crassa*, *Biochem. Biophys.*, 201: 185-193.
- Wang, L.L., C. Wen and A.M. George, 1980. Purification and characterization of uricase. *Arch. Biochem. Biophys.*, 1: 185- 193.
- Yazdi, M.T., G. Zarrini, E. Mohit, M.A. Faramarzi, N. Setayesh, N. Sedighi and F.A. Mohseni, 2006. *Mucor hiemalis*: a new source for uricase production. *World Journal of Microbiology and Biotechnology*, 22: 325-330.
- Xue, Z., M. Xiao, S. Gui, L. Xia and K. Guoa, 2005. Isolation of a thermostable uricase-producing bacterium and study on its enzyme production conditions. *Process Biochemistry*, Article in Press.