

## Studies on uricase induction in certain bacteria

Essam A. Azab<sup>1\*</sup>, Magda M. Ali<sup>2</sup> and Mervat F. Fareed<sup>3</sup>

1. Section Microbiology, Botany Department, Faculty of Science, Tanta University, Tanta, Egypt  
[a\\_essam@gawab.com](mailto:a_essam@gawab.com) – [essam@agr.kyushu-u.ac.jp](mailto:essam@agr.kyushu-u.ac.jp) – [azab\\_essam@hotmail.com](mailto:azab_essam@hotmail.com)
2. Biology Department, Faculty of Education in Kafr El-Sheikh, Tanta University, Kafr El-Sheikh, Egypt.
3. Home Economic Department, Faculty of Specific Education, Tanta University, Tanta, Egypt.

### ABSTRACT

Three strains of *Proteus vulgaris* and two *Streptomyces* species were screened for inducible uricase formation. *P. vulgaris* (1753 and B-317-C), *Streptomyces graminofaciens* and *S. albidoflavus* showed inducible uricase activity, but *P. vulgaris* U7 did not show activity under the experimental conditions tested. Different amounts of constitutive and induced uricase were obtained by the four organisms using different culture media. The enzyme was induced in the producing organisms by different concentrations of different inducers, and uric acid was the most potent inducer. Using the optimal concentration of uric acid as inducer, the conditions of uricase induction in the test organisms were optimized. In *P. vulgaris* strains (1753 and B-317-C), the incubation temperature of 37 °C, initial pH of culture media of 7 and agitation rate of 180 rpm, showed the highest level of uricase induction. In the two *Streptomyces* species, the uricase induction was optimized at 28 °C incubation temperature and pH 7. The agitation rate of 200 and 220 rpm showed the highest induction activity in *Streptomyces graminofaciens* and *S. albidoflavus*, respectively. The highest levels of induced uricase were obtained at induction times of 140 min, 140 min, 42 h and 36 h in *P. vulgaris* 1753, B-317-C, *Streptomyces graminofaciens* and *S. albidoflavus*, respectively. The uricase was present as cell-bound enzyme in the producing organisms and no activity was recorded in the culture supernatants.

**KEYWORDS:** Uric acid, *P. vulgaris*, *Streptomyces* species, concentration dependence, time course

### 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 diagnoses of gout since urate accumulation is a causative factor of gout in humans (Nishiya *et al.* 2002). Uricase is useful for enzymatic determination of urate in clinical analysis by coupling it with a 4-amino-antipyrine-peroxidase system (Gochman & Schmitz 1971). Uricase can be also used as a protein drug to reduce toxic urate accumulation (Colloc'h *et al.* 1997).

Uricase has been purified from various sources, and its properties investigated (Vogels & Van der Drift 1976). Several reports demonstrate that uricase is produced by bacteria such as *Micrococcus* and *Brevebacterium* (Kida & Kuniyama 1966), *Streptomyces* (Watanabe & Fukumoto 1970; Watanabe *et al.* 1969), *Bacillus pasteurii* (Christians & Kaltwasser 1986), *Proteus mirabilis* (Rando *et al.* 1990) and *E. coli* (Nakagawa *et al.* 1996). Ammar *et al.* (1987) stated that an Egyptian soil actinomycete identified as *Streptomyces albosriseolus* potentially produced uricase.

\* Address For Correspondence

*Bacillus fastidious* grows well only in media containing uric acid, allantoin or allantoate, as the main or sole source of carbon, nitrogen and energy. High levels of uricase were found in crude extract of cells grown on uric acid (Kaltwasser 1971; Bongaerts & Vogels 1976). Kaltwasser (1968; 1969) observed an increase of uricase activity in *Alcaligenes eutrophus* (*Hydrogenomonas* H16), *Pseudomonas aeruginosa* and *Micrococcus denitrificans* after transfer into media containing uric acid. It has been reported by Rouf & Lompfrey (1968) that the ability of microorganisms to degrade uric acid and to use it for growth is an inducible property of various bacteria. Uricases of *Bacillus fastidious* (Kaltwasser 1971; Bongaerts & Vogels 1976) and of *Aspergillus nidulans* (Scazzocchio & Darlington 1968) were reported to be induced by uric acid. Tanaka *et al.* (1977) demonstrated that, in addition to uric acid, xanthine, guanine, adenine and hypoxanthine were also effective for inducing uricase in *Candida tropicalis*. In *Streptomyces* sp., Watanabe & Fukumoto (1970) reported that uricase could be induced. Rouf & Lompfrey (1968) reported that the lag in the disappearance of uric acid with washed cells, grown in  $(\text{NH}_4)_2\text{SO}_4$  medium, of *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Serratia marcescens* (MC75) showed that the ability of these organisms to decompose uric acid was not a constitutive property, but was induced.

The present investigation aimed to study the pattern of uricase induction in some *Proteus vulgaris* strains (eubacteria) and two actinomycetes, namely *Streptomyces graminofaciens* and *S. albidoflavus*.

## MATERIALS AND METHODS

*Proteus vulgaris* U7, *P. vulgaris* B-317-C and *P. vulgaris* 1753 were kindly provided by Professor H. H. Martin (Institute of Microbiology, Technische Hochschule, Darmstadt, Germany). *Streptomyces graminofaciens* was isolated from cultivated Egyptian soil, and *S. albidoflavus* was isolated from a fodder sample of chicken collected from Kafr El-Sheikh governorate, Egypt. Both species of *Streptomyces* were isolated and identified by Ali (1997).

In this study, six culture media were used for the *P. vulgaris* strains. Medium 1 was nutrient broth (Oxoid, England). Medium 2 was glucose tryptone broth (g/l): tryptone, 10 g; yeast extract, 1 g; glucose, 10 g; pH 7.2 (Agate & Bhat 1964). Medium 3 was tryptone soya broth (g/l): tryptone, 5 g; soya extract, 10 g; glucose, 2 g; pH 7.2 (Gavin 1957). Medium 4 (g/l):  $\text{KH}_2\text{PO}_4$ , 2.72 g;  $\text{NaHPO}_4$ , 5 g; glucose, 10 g;  $\text{NH}_4\text{NO}_3$ , 0.5 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 g;  $\text{Ca}(\text{NO}_3)_2$ , 0.1 g and then 1 ml trace salt ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 1 g;  $\text{H}_3\text{BO}_3$ , 0.1 g;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 1 g;  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.25 g;  $\text{NaMoO}_4$ , 0.25 g/ 100 ml distilled water) was added (Babu *et al.* 1995). Medium 5 (g/l): glucose, 10 g;  $(\text{NH}_4)_2\text{SO}_4$ , 1 g;  $\text{K}_2\text{HPO}_4$ , 2.5 g;  $\text{KH}_2\text{PO}_4$ , 5 g;  $\text{CaCl}_2$ , 0.05 g;  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.02 g;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.0005 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 g, pH 7.2 (adapted from Rouf & Lompfrey 1968). Medium 6 (g/l): peptone, 10 g; yeast extract, 3 g; and NaCl, 7.5 g; pH 7.4. For preparation of the plates 20 g/l agar was added (Bachrach 1957).

For the two *Streptomyces* species five different media were used. Medium I (g/l): Peptone 20.0 g; glucose 30.0 g;  $\text{KH}_2\text{PO}_4$ , 1.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g; NaCl, 0.5 g; pH 7 (Watanabe & Fukumoto 1970). Medium II was starch nitrate medium (g/l): starch, 20.0 g;  $\text{KNO}_3$ , 2.0 g;  $\text{K}_2\text{HPO}_4$ , 1.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g; NaCl, 0.5 g;  $\text{CaCO}_3$ , 3.0 g;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g; 1 ml trace element solution ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g;  $\text{MnCl}_2 \cdot 7\text{H}_2\text{O}$ , 0.1 g;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g, distilled water up to 100 ml); pH 7 (Shiriling & Gottlieb 1966). Medium III (g/l): Na propionate, 4.0 g;  $\text{K}_2\text{HPO}_4$ , 0.5 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.001g; Glycerol, 5.0 g, pH 7.5 (from Ouhdouch 1989). Medium IV was glycerol-asparagine medium (g/l): L-asparagine, 1.0 g;

glycerol, 10.0 g; K<sub>2</sub>HPO<sub>4</sub>, 1.0 g; trace element solution ( FeSO<sub>4</sub> . 7 H<sub>2</sub>O, 0.1 g; MnCl<sub>2</sub>. 7H<sub>2</sub>O, 0.1 g; ZnSO<sub>4</sub> . 7 H<sub>2</sub>O, 0.1 g, distilled water up to 100 ml), 1 ml; pH 7 (adapted from Pridham & Lyons 1961). Medium V (g/l): glycerol, 30.0 g; NaCl, 5.0 g; K<sub>2</sub>HPO<sub>4</sub>, 1.0 g; MgSO<sub>4</sub>. 7 H<sub>2</sub>O, 0.2 g; CaCl<sub>2</sub>, 0.1 g; pH 7. For plates 20.0 g agar was added (adapted from Sugisaki *et al.* 1970).

The test organisms were inoculated onto plates containing the appropriate media (medium 6 for *P. vulgaris* strains and medium V for *Streptomyces* species) with 0.2 % (w/v) uric acid for *P. vulgaris* strains and 0.5 % for *Streptomyces* species. The appearance of clear zones around the colonies after overnight incubation at 37 °C for *P. vulgaris* strains and after 48 h at 30 °C for *Streptomyces* species constitute uricase-positive results. This experiment was carried out in triplicates.

Tests for the specific and nonspecific induction of uricase varied according to the test organism. In *Proteus vulgaris* strains, overnight cultures of *P. vulgaris* strains were prepared in the six previously described media at 37 °C and agitation rate of 150 rpm (Model OSFT-LS-R, Refrigerated). The overnight cultures were diluted 1:20 in the same prewarmed fresh media and incubated at 37 °C for two hours at 150 rpm. Two sets for each organism were performed. To one set, uric acid (as inducer) was added to a final concentration of 0.1 g/l and the other set was used for nonspecific induction (without uric acid) and the incubation was allowed to continue for two hours more under the same conditions. 50 ml portions of the induced and non-induced cultures were mixed rapidly with 1.5 mg chloramphenicol (final concentration of 50 µg/ml) and centrifuged at 6000 rpm for 30 min at 4 °C. The supernatants were kept at – 20 °C and the cell sediments washed twice with 0.05 M potassium-sodium-phosphate buffer pH 7 and resuspended in 0.8 ml of buffer containing DNase activated with MgCl<sub>2</sub> and 0.2 ml of 50 mM EDTA containing 0.5 mg/ml lysozyme, incubated at 37 °C for 30 min and frozen at -20 °C.

In *Streptomyces* species, dense spores (from two plates) were suspended in 5 ml of 10 % (v/v) sterilized glycerol and served as a source of inoculum. Two sets of 50-ml portions of the five media described for *Streptomyces* were inoculated with 100 µl of glycerol stock inoculum and incubated at 30 °C for 24 h at an agitation rate of 150 rpm. One set of these cultures were induced with uric acid to a final concentration of 0.5 g/l and the other served as nonspecific induction set (control): the two sets were then incubated for 24 h more under the same conditions. Each culture was mixed rapidly with 1.5 mg chloramphenicol, cooled in ice-water bath and then the mycelia separated by centrifugation. The supernatants were kept at -20 °C and the separated mycelia washed twice with 10 ml K-Na- phosphate buffer and resuspended in 1 ml buffer containing DNase and 0.5 ml 50 mM EDTA containing 0.5 mg/ml lysozyme and incubated at 37 °C for 30 min and frozen at -20 °C.

The proteins of the culture supernatants (of *Proteus* and *Streptomyces*) were precipitated with 80 % ammonium sulfate and the precipitates were collected and redissolved in buffer and kept at – 20 °C.

To prepare cell free extracts, 4 ml ice-cold buffer was added to the thawed cells and mycelia. The cells of the *P. vulgaris* strains were sonicated by using the microtip of the sonicator (Model UP 200S) at 100 % amplification and 10 pulses each of 10 seconds with an interruption of 30 seconds for cooling in an ice-water bath. The mycelia of the two *Streptomyces* species were sonicated by 7 pulses each of 30 seconds and 30 seconds for cooling. The cell debris was removed by centrifugation (Sigma, laboratory cooling centrifuge, 1K1S) at 15000 rpm for 1 h at 4 °C, and the clear supernatants were stored at -20 °C until needed.

To determine uricase activity, to 2 ml of 59.5  $\mu\text{M}$  uric acid ( $10 \mu\text{g ml}^{-1}$  buffer) as a substrate, 0.3 ml distilled water and 0.5 ml crude enzyme were added and mixed well and then incubated at 30 °C for 5 min. To the mixture, 0.2 ml of 0.1 N KCN was added and mixed well to stop the reaction. For a control, the KCN was added and mixed well before the addition of crude enzyme, then the absorbance of test and control was measured at 293 nm (Itaya *et al.* 1967).

The protein content of the enzyme crude extract was estimated according to the method described by Bradford (1976) with bovine serum albumin as standard.

Using the appropriate medium for each organism (medium 6 for *P. vulgaris* strains, medium I for *S. graminofaciens* and medium V for *S. albidoflavus*), uricase induction was carried out using different concentrations of uric acid, allantoin, xanthine and guanine. The crude enzyme was extracted from each organism and the activities were assayed.

To study the effect of incubation temperature, the tested organisms were grown and induced with uric acid for uricase production at various incubation temperatures (15, 20, 25, 28, 30, 35, 37, 40, and 45 °C). At the end of incubation period, the crude enzyme was extracted and assayed at different temperature. To study the effect of pH, the initial pH values of the appropriate medium (medium 6 for *P. vulgaris* strains, medium I for *Streptomyces graminofaciens* and medium V for *S. albidoflavus*) were adjusted to different pH values using 2 N HCl and 2 N NaOH. The organisms were grown and induced for uricase production with uric acid and the enzyme activities measured at different pH values. To study the effect of agitation rate, the tested organisms were grown and induced in the appropriate medium adjusted to the optimal pH at the convenient temperature but at various agitation rates (0.0, 50, 80, 100, 120, 150, 200, and 220 rpm). The induced enzyme was extracted and the activities were measured at the various agitation rates.

The time-course of uricase induction was studied using the tested microorganisms grown in the appropriate media adjusted to the optimal pH at 37 °C for 2 h for *P. vulgaris* strains and at 28 °C for 24 h for *Streptomyces* species. The optimal concentrations of uric acid were added to the culture and the growths were allowed to continue. The induced cells were harvested at various time intervals after the addition of inducer. The enzyme crude extracts of the induced cells were prepared and the activities were measured.

## RESULTS

The plate assay method indicated that both *Proteus vulgaris* 1753 and B-317-C produced uricase enzyme, recording clear zones (due to uric acid degradation) of 1.4 and 1.8 cm after 1 day of incubation and 1.9 and 2.4 cm after 2 days, respectively. Little increase of diameters of clear zones was obtained after 5 days (Table 1). Larger amounts of uricase seemed to be produced by the strain B-317-C. For *P. vulgaris* U7, no clear zones were recorded, which might indicate that no uricase was produced by this wild type strain of *P. vulgaris* under the experimental condition used.

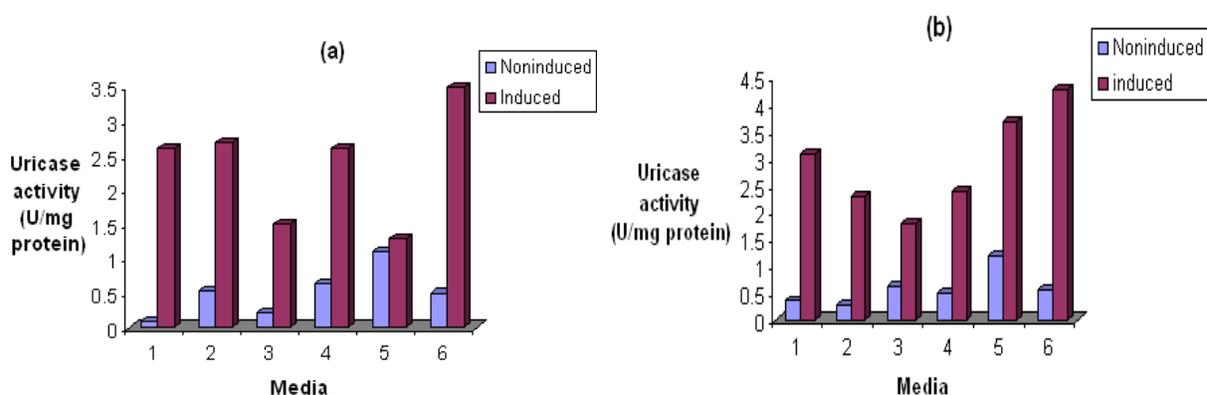
Both *Streptomyces graminofaciens* and *S. albidoflavus* showed uricase formation. A clear zone of 1 cm was recorded for *S. graminofaciens* after two days, increasing to 2.2 cm after seven days. Larger clear zones were obtained around the colonies of *S. albidoflavus* (Table 1).

Table 1: Detection of uricase formation by *P. vulgaris* strains and *Streptomyces* species (mean  $\pm$  SEM)

Organisms	Clear zones (cm)				
	1 day	2 days	5 days	7 days	9 days
<i>P. vulgaris</i> 1753	1.4 $\pm$ 0.01	1.9 $\pm$ 0.01	2.0 $\pm$ 0.07	2.0 $\pm$ 0.06	-
<i>P. vulgaris</i> B-317-C	1.8 $\pm$ 0.03	2.4 $\pm$ 0.3	2.5 $\pm$ 0.07	2.5 $\pm$ 0.2	-
<i>P. vulgaris</i> U7	0.0	0.0	0.0	0.0	-
<i>S. graminofaciens</i>	0.4 $\pm$ 0.002	1.0 $\pm$ 0.02	1.8 $\pm$ 0.1	2.2 $\pm$ 0.07	2.3 $\pm$ 0.1
<i>S. albidoflavus</i>	0.6 $\pm$ 0.002	1.5 $\pm$ 0.03	2.3 $\pm$ 0.07	2.7 $\pm$ 0.36	2.7 $\pm$ 0.011

The various culture media were used to study the nonspecific induction of uricase, i.e. the amounts of enzyme produced constitutively. The same media were used to induce uricase using 0.1 g/l uric acid for *P. vulgaris* strains and 0.5 g/l for *Streptomyces* species (specific induction). For *P. vulgaris* strains 1753 and B-317-C, medium 5 supported the highest amounts of uricase production constitutively (Figure 1). Different amounts of the enzyme were produced using different culture media by the two strains. The lowest amounts of constitutive uricase were induced using media 1 and 2 in *P. vulgaris* 1753 and B-317-C, respectively. Medium 6 together with 0.1 g/l uric acid as inducer caused the highest amounts of induced uricase in both strains (Figure 1). As in *P. vulgaris* strains, different amounts of uricase were produced constitutively using different culture media by the two *Streptomyces* species. In *S. graminofaciens* (Figure 2a) the highest amount of induced enzyme was obtained using medium I and 0.5 g/l uric acid. Medium V supported the highest amounts of both constitutive and induced uricase in *S. albidoflavus* (Figure 2b). In the presence of the same inducer concentrations (0.1 g/l for *Proteus* and 0.5 g/l for *Streptomyces*), different amounts of induced uricase were obtained using different culture media by the two *P. vulgaris* strains and the two species of *Streptomyces*.

Medium 6 which supported the highest levels of the induced uricase in *P. vulgaris* strains, and medium I for *S. graminofaciens* and medium V for *S. albidoflavus* were used for further induction experiments.

Figure 1: Nonspecific and specific induction of uricase in *P. vulgaris* in (a) 1753 and (b) B-317-C.

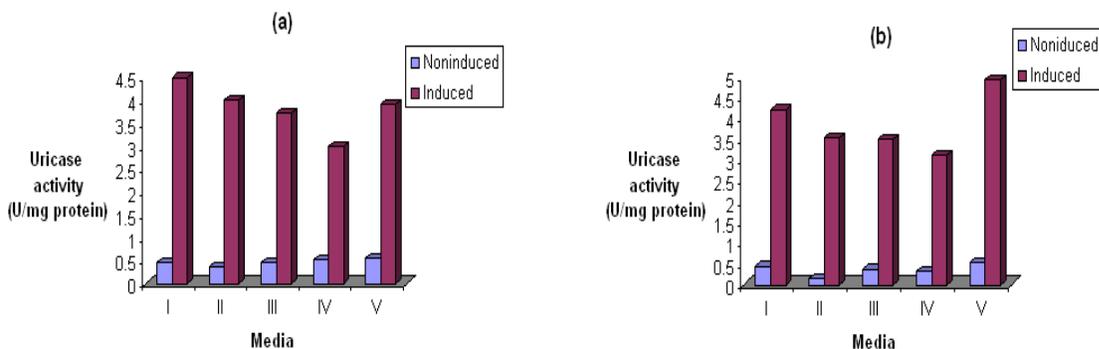


Figure 2: Nonspecific and specific induction of uricase in (a) *Streptomyces graminofaciens* and (b) *S. albidoflavus*.

The induction of uricase in *P. vulgaris* strains by different concentrations (0.0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 g/l) of uric acid, allantoin, xanthine and guanine was studied in *P. vulgaris* strains 1357 and B-317-C, using medium 6. In both strains, for all inducers used, the amounts of induced enzyme increased with increasing inducer concentration up to certain point, and then decreased (Figure 3). Compared to other concentrations and other inducers, the highest amounts of uricase were obtained using 0.2 g/l and 0.15 g/l uric acid in *P. vulgaris* 1357 and B-317-C, respectively. 0.1 g/l of allantoin induced higher amounts of uricase in *P. vulgaris* 1357 and B-317-C compared to the other concentrations. In both strains, the highest amounts of induced enzyme were obtained using 0.15 g/l xanthine of the concentrations tested. The highest levels of uricase induction were recorded using 0.15 g/l guanine in *P. vulgaris* 1357 and 0.1/g l in *P. vulgaris* B-317-C. For both *Proteus vulgaris* strains, uric acid was the most potent inducer for uricase, and *P. vulgaris* B-317-C produced higher amounts of the enzyme compared to the other strain (Figure 3 a & b).

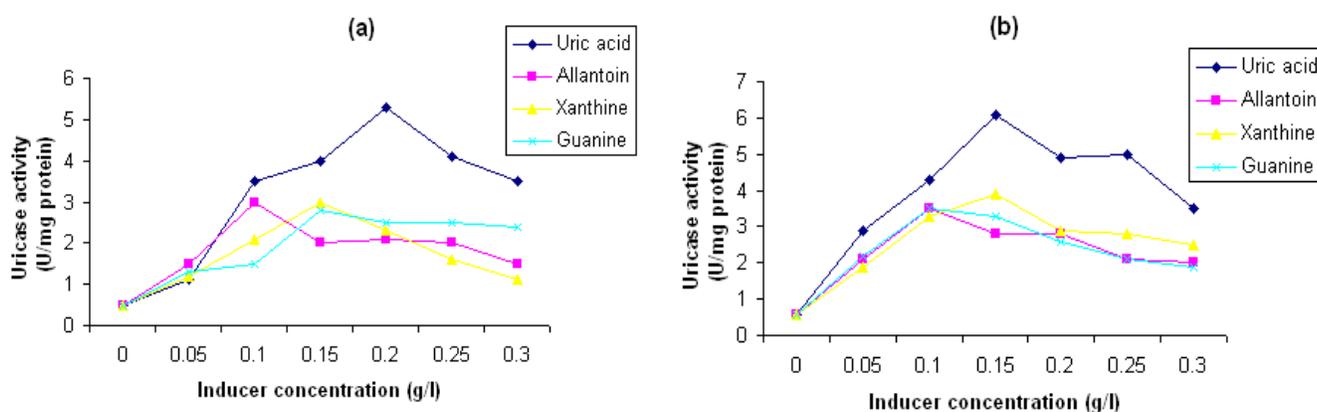


Figure 3: Concentration dependence of uricase induction by different inducers in *P. vulgaris* (a) 1357; (b) B-317-C.

In *Streptomyces* species, concentrations of 0.0, 0.25, 0.5, 0.75, 1.0 and 2.0 g/l of uric acid, allantoin, xanthine and guanine were used to induce uricase using medium I for *S. graminofaciens* and medium V for *S. albidoflavus* (Figure 4 a & b). The highest amounts of uricase were induced in *S. graminofaciens* and *S. albidoflavus* using 1 g/l uric acid as inducer, compared to other concentrations and other inducers. Compared to other concentrations of

allantoin and xanthine, 0.75 g/l induced the highest levels of uricase in *S. graminofaciens*, and guanine recorded the lowest induction potency (Figure 4 a). In *S. albidoflavus*, 1 g/l of uric acid induced the highest level of uricase, 13.7 times greater than the control. Allantoin at concentration of 0.5 g/l induced the highest level of uricase (7.9 times the control) compared to the other concentrations of this inducer. Lower inducing potencies were obtained using xanthine and guanine in this species (Figure 4 b).

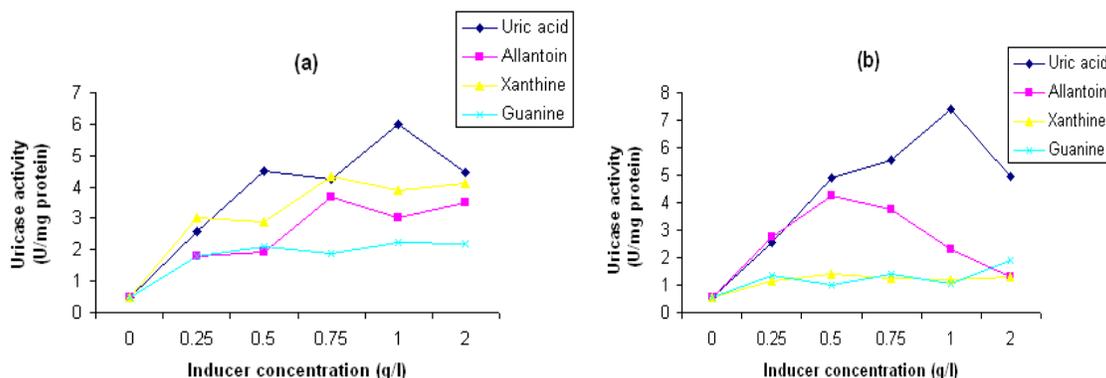


Figure 4: Concentration dependence of uricase induction by different inducers in (a) *Streptomyces graminofaciens* and (b) *S. albidoflavus*.

The effect of incubation temperature, initial pH of the culture media and agitation rate on uricase induction in the test organisms was investigated. The optimal concentrations of the potent inducer, uric acid (0.2 g/l for *P. vulgaris* 1357 and 0.15 g/l for B-317-C, and 1.0 g/l for both species of *Streptomyces*) were used for induction.

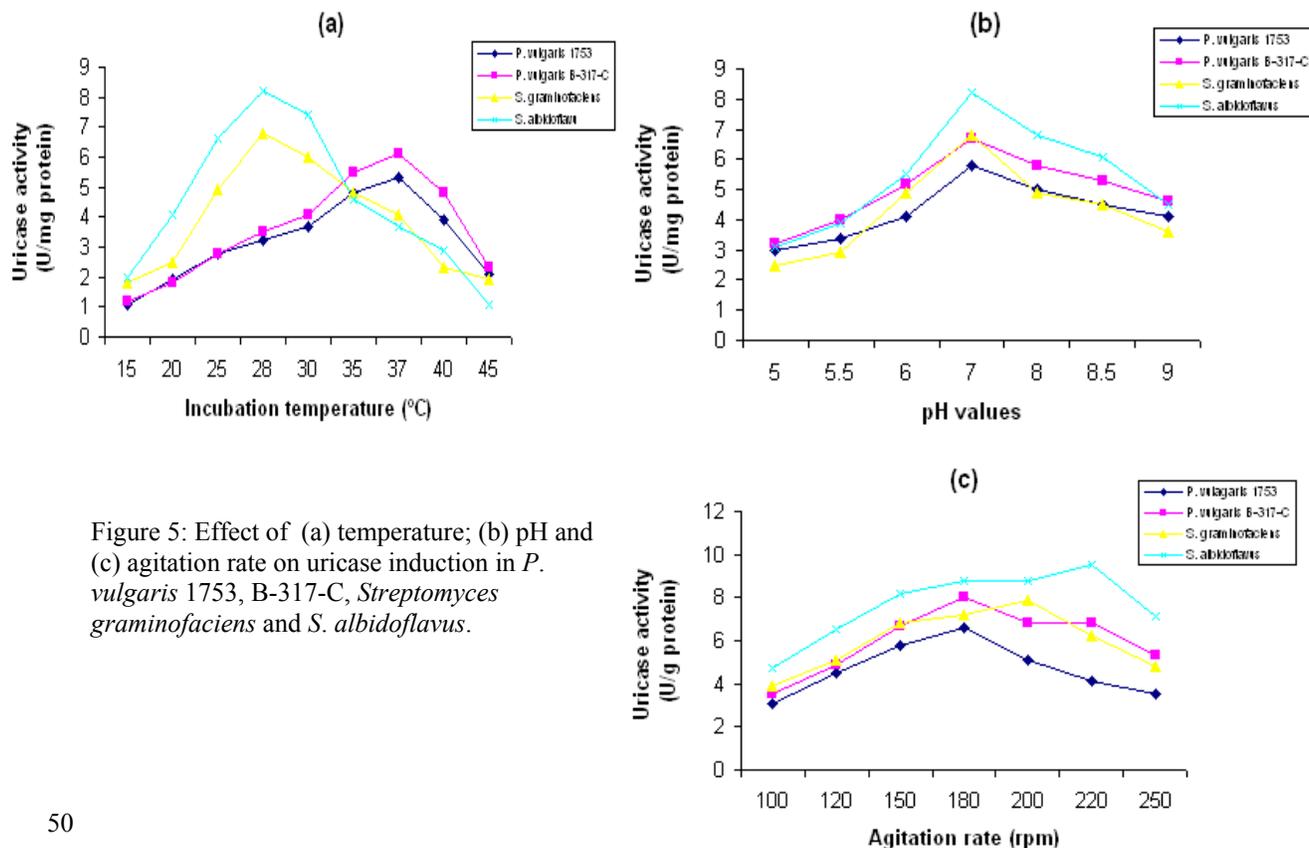


Figure 5: Effect of (a) temperature; (b) pH and (c) agitation rate on uricase induction in *P. vulgaris* 1753, B-317-C, *Streptomyces graminofaciens* and *S. albidoflavus*.

For the all organisms tested, the amounts of induced uricase increased with increasing incubation temperature up to a maximum, and then decreased. In *P. vulgaris* 1753 and B-317-C, the highest amounts were obtained at 37 °C (Figure 5a), and 28 °C was the best incubation temperature for uricase induction in both *Streptomyces* species.

Neutral pH was the optimal initial pH of the culture media at which the highest levels of induction were obtained in the all organisms tested and around neutrality the amount of induced uricase decreased (Figure 5 b).

Uricase induction was markedly affected by agitation rate in both *P. vulgaris* strains and *Streptomyces*. Generally the amounts of induced uricase increased by aeration up to 180 rpm (*P. vulgaris* strains), 200 rpm (*Streptomyces graminofaciens*) and 220 rpm (*S. albidoflavus*), and then decreased (Figure 5 c).

In *P. vulgaris* strains, the amount of induced enzyme was determined at different time intervals after the addition of the inducer (uric acid, 0.2 g/l for strain 1753 and 0.15 g/l for B-317-C) in medium 6 at 37 °C, pH 7 and 180 rpm agitation rate. In both strains the amount of induced uricase increased with time, reaching a maximum at 140 min after the addition of inducer, and then decreased with time (Figure 6a).

We also studied the time course of induction in *Streptomyces* species under optimized conditions for uricase induction (incubation temperature of 28 C at pH 7 and 1 g/l uric acid for both species, and in medium I at agitation rate of 200 rpm for *Streptomyces graminofaciens*, and in medium V at 220 rpm for *S. albidoflavus*). The induction level of uricase in *Streptomyces graminofaciens* peaked 42 h after induction, whilst the equivalent figure for *Streptomyces albidoflavus* (Figure 6b) was 36 h.

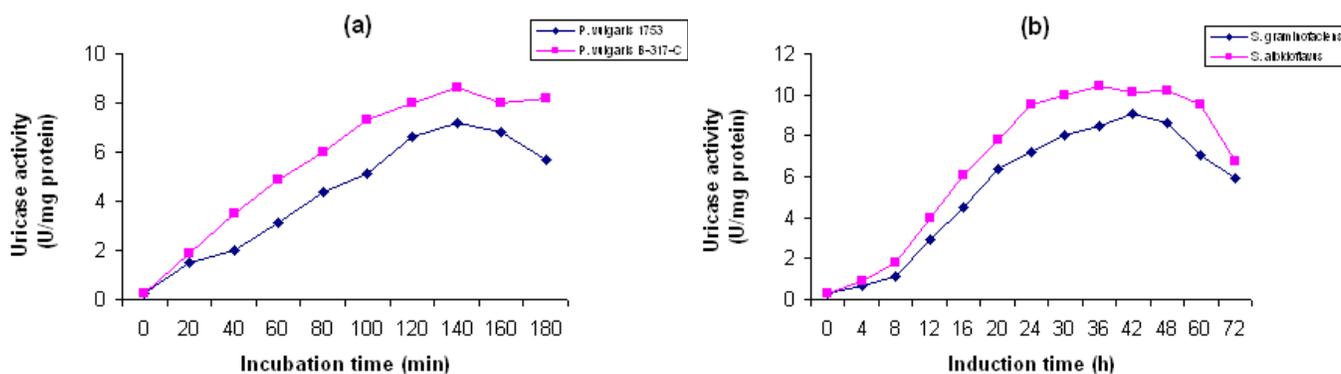


Figure 6: Time course of uricase induction (a) in *P. vulgaris* 1753 and B-317-C, and (b) in *Streptomyces graminofaciens* and *S. albidoflavus*.

## DISCUSSION

Increasing attention has been directed toward using intracellular enzymes for industrial, analytical and medical purposes. Uricase (urate oxidase) is a promising enzyme that may be

applicable to the analysis of serum or urinary uric acid, because of its high specificity toward this acid (Tanaka *et al.* 1977). Uricase is an enzyme participating in the purine breakdown pathway, catalyzing the oxidation of uric acid in the presence of oxygen to allantoin and hydrogen peroxide (Fraisse *et al.* 2002). Three strains of *P. vulgaris* and two species of *Streptomyces* were used in this investigation to study the induction pattern of uricase in these different organisms, and to optimize the conditions required for highest enzyme production. Out of the three strains tested, *P. vulgaris* 1753 and B-317-C showed uricase activity. The wild type strain, *P. vulgaris* U7 did not produce constitutive or inducible uricase. Using the same strains (*P. vulgaris* 1753, B-317-C and U7) but for another enzyme ( $\beta$ -lactamase), Azab (1992) reported that *P. vulgaris* 1753 and B-317-C produced inducible  $\beta$ -lactamase, whereas *P. vulgaris* U7 did not. After he had transformed the  $\beta$ -lactamase regulatory gene (*ampR* and *ampC*) to *P. vulgaris* U7, the transformant produced inducible  $\beta$ -lactamase. In our case, the situation might be the same and the wild type strain, *P. vulgaris* U7 may lack the regulatory gene of uricase induction.

Both *Streptomyces graminofaciens* and *Streptomyces albidoflavus* produced inducible uricase. Induced formation of uricase by *Streptomyces* sp. resting cells was also reported by Watanabe & Fukumoto (1970). Using different culture media for growing the four uricase-positive organisms tested, different amounts of constitutive uricase were induced (nonspecific induction). The presence of cyclic molecules other than uric acid and its derivatives (which might have inducing activity) in the culture media may explain the large discrepancies in the amounts of uricase depending on the culture media used. In accordance to our results, Bongaerts *et al.* (1978) stated that in various organisms, uricase synthesis is regulated by the components of the growth medium. Although medium 5 for *P. vulgaris* strains and medium V for *Streptomyces graminofaciens* supported the highest amounts of uricase constitutively, the greatest amounts of inducible uricase were obtained using other culture media.

Out of the tested inducers, uric acid was the most potent inducer in all the uricase-positive organisms. In *P. vulgaris* 1753, approximately the same amounts of uricase (2.8 to 3 U/mg protein) were induced using 0.1 g/l allantoin and 0.15 g/l xanthine or guanine. In *P. vulgaris* B-317-C, allantoin and guanine showed lower levels of induction. Guanine was a poor uricase inducer in *Streptomyces graminofaciens*, while xanthine and allantoin were intermediate inducers. Both xanthine and guanine were poor and allantoin an intermediate inducer in *Streptomyces albidoflavus*. Uricase induction in the all test organisms by uric acid, allantoin, xanthine and guanine seemed to be a concentration-dependent process. Watanabe & Fukumoto (1970) concluded that the presence of hypoxanthine or xanthine was required with urate in the medium for the complete induction of uricase by the growing cells of a strain of *Streptomyces* sp.

The conditions of uricase induction using the optimal concentration of uric acid as the best inducer were optimized. In *P. vulgaris* 1753 and B317-C, the optimal incubation temperature for uricase induction was 37 °C, and in *Streptomyces graminofaciens* and *Streptomyces albidoflavus* it was 28 °C. The optimal pH of the culture media for uricase induction was 7 for the all test organisms. In accordance to our findings, Watanabe *et al.* (1972) reported that urate uptake activity by a strain of *Streptomyces* sp. was optimal at around 30 °C and at a pH near neutrality. Uricase induction was also markedly affected by aeration in all the test organisms. It has been reported that appropriate aeration stimulates uricase formation by *Candida tropicalis* (Tanaka *et al.* 1977). Uricase induction was also greatly affected by the induction time.

*P. vulgaris* B-317-C produced higher amounts of constitutive and induced uricase, compared to the other strain. *Streptomyces albidoflavus* (isolated from fodder of chicken)

produced higher levels of constitutive and induced uricase activity than that of *Streptomyces graminofaciens* (isolated from cultivated soil). No uricase activity was detected in the culture supernatants and the uricase enzyme induced in all test organisms was cell-bound enzyme.

## REFERENCES

- Agate A & Bhat JY (1964) Microflora associated with the rhizosphere of *Calotropis gigantea*. *Journal of the Indian Institute of Science* 46: 1-10.
- Ali M. M. 1997. Potency of selected actinomycetes for certain antibiotic production. Ph.D. Thesis, Section Microbiology, Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.
- Ammar MS, Elwan SH & EL-Shahed AS (1987) Uricolytic *Streptomyces albogriseolus* from an Egyptian soil. 1. Taxonomy and uricase production and properties. *Egyptian Journal of Microbiology* 22 (2): 261-279.
- Azab EA (1992) Potency of structurally different  $\beta$ -lactam antibiotics as inducers of  $\beta$ -lactamase in gram-negative bacteria. Ph.D. Thesis, Section Microbiology, Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.
- Babu KS, Ajith Kumar PV & Kunli AAM (1995) Mineralization of phenol and its derivatives by *Pseudomonas* sp. Strain CP4. *The World Journal of Microbiology and Biotechnology* 11: 661-664.
- Bachrach U (1957) The aerobic breakdown of uric acid by certain *Pseudomonas*. *Journal of General Microbiology* 17: 1-11.
- Bongaerts GPA & Vogels GD (1976) Uric acid degradation by *Bacillus fastidiosus* strains. *Journal of Bacteriology* 125: 689-697.
- Bongaerts GPA, Uitzetter J, Brouns R & Vogels GD (1978) Uricase of *Bacillus fastidiosus* properties and regulation of synthesis. *Biochemica et Biophysica Acta* 527: 348-358.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.
- Christians S & Kaltwasser H (1986) Nickel-content of urease from *Bacillus pasteurii*. *Archives of Microbiology* 145: 51-55.
- Colloc'h N, el Hajji M, Bachet B, L'Hermite G, Schiltz M, Prange T, Castro B & Mornon JP (1997) Crystal structure of the protein drug urate oxidase-inhibitor complex at 2.05 Å resolution. *Nature Structural Biology* 4: 947-952.
- Fraisse L, Bonnet MC, Farcy JP, Agut C, Dersigny D & Bayol A (2002) A colorimetric 96-well microtiter plate assay for determination of urate oxidase activity and its kinetic parameters. *Analytical Biochemistry* 309: 173-179.
- Gavin JJ (1957) Analytical Microbiology, II. The diffusion method. *Applied Microbiology* 5: 25-35.
- Gochman N & Schmitz MJ (1971) Automated determination of uric acid, with use of a urease-peroxidase system. *Clinical Chemistry* 17: 1154-1159.
- Itaya K, Yakamoto T & Fukumoto J (1967) Studies on yeast uricase. Part I. Purification and some properties of yeast uricase. *Agricultural and Biological Chemistry* 31:1256-1264.
- Kaltwasser H (1968) Inductive formation of particle-bound uricase in *Hydrogenomonas* H16 and other aerobic bacteria. *Archives of Microbiology* 60: 160-171.
- Kaltwasser H (1969) Uric acid degradation and biosynthesis of the enzymes uricase, glyoxylate carboligase and urease. in *Hydrogenomonas* H16. II. Effect of uric acid, fructose and nitrogen deficiency on enzyme formation. *Archives of Microbiology* 65: 288-302.
- Kalwasser H (1971) Studies on the physiology of *Bacillus fastidiosus*. *Journal of Bacteriology* 107: 780-786.
- Kida J & Kunihiya M (1966) Studies on bacterial uricase (I) Isolation of uricase Producing bacteria and some cultural conditions for production. *Journal of Fermentation Technology* 44: 789-796.
- Nakagawa S, Ishino S & Teshiba S (1996) Construction of catalase deficient *Escherichia coli* strains of the production of uricase. *Bioscience Biotechnology and Biochemistry* 60: 415-420.
- Nishiya Y, Hibi T & Oda J (2002) A purification method of the diagnostic enzyme *Bacillus* uricase using magnetic beads and non-specific protease. *Protein Expression and Purification* 25(25): 426-429.
- Ouhdouch Y (1989) Bactéries actinomycètes rares producteur d'antifongiques criblage, sélection et étude taxonomique d'une souche active (purification de l'antifongique elobore). These Docteur de Uni de Nancy I, U. E. R. Alimentation et Nutrition, Nancy, France.

- Pridham TG & Lyons JA (1961) *Streptomyces albus* (Rossi Dorla) Waksman Et. Henricii Taxonomic study of strains labeled *Streptomyces albus*. *Journal of Bacteriology* 81: 431-441.
- Rando D, Steglitz U, Morsdorf G & Kaltwasser H (1990) Nickel availability and urease expression in *Proteus mirabilis*. *Archives of Microbiology* 154: 428-432.
- Rouf MA & Lomprey TF (1968) Degradation of uric acid by certain aerobic bacteria. *Journal of Bacteriology* 96: 617-622.
- Scazzocchio C & Darlington AJ (1968) The induction and repression of the enzymes of purine breakdown in *Aspergillus nidulans*. *Biochemica et Biophysica Acta* 166: 557- 568.
- Shirling EB & Gottlieb P (1966) Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology* 16: 313-340.
- Sugisaki Z, Watanabe N, Koyama H, Kobayashi T & Iguch N (1972) Uricase. *Chemical Abstracts* 77: 124796y.
- Tanaka A, Yamamura M, Kawamoto S & Fukui S (1977) Production of uricase by *Candida tropicalis* using n-alkane as substrate. *Applied and Environmental Microbiology* 34: 342-346.
- Vogels GD & Van der Drift C (1976) Degradation of purines and pyrimidines by microorganisms. *Bacteriological Review* 40: 403-468.
- Watanabe Y & Fukumoto J (1970) Studies on the formation of uricase by *Streptomyces*. Part II. The induced formation of uricase by resting cells. *Agricultural and Biological Chemistry* 34: 1625-1632.
- Watanabe Y, Yano M. & Fukumoto J (1969) Studies on the formation of uricase by *Streptomyces*. I. The effect of purine bases on the induced formation of uricase by the cultured cells. *Agricultural and Biological Chemistry* 33: 1282- 1290.
- Watanabe Y, Yano M. & Fukumoto J (1972) Studies on the formation of uricase by *Streptomyces*. Part IV. The uptake of urate by the cells. *Agricultural and Biological Chemistry* 36 (1): 1-10.

### الملخص العربي

#### دراسات على إحداث إنزيم اليوريكيز في بكتيرات مختارة

.	-	-	-	-	-	-1
.	-	-	-	-	-	-2
.	-	-	-	-	-	-3