

Purification and Optimization of Uricase Enzyme Produced by *Pseudomonas aeruginosa*

A. Anderson^{1*} and S. Vijayakumar¹

¹Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur-613503, Tamilnadu, India.

Abstract

The uricase (urate oxidase) enzyme was extracted from *Pseudomonas aeruginosa* and purified by ammonium sulphate precipitation. The molecular weight of purified uricase was determined by SDS-PAGE electrophoresis using marker proteins of known molecular weight. The optimum temperature, pH and the effect of various metal ions on uricase activity were evaluated. The results showed that 70% ammonium sulphate concentration proved high uricase activity by 40.0 U/ml than other concentration and cell free supernatant. The molecular weight of purified uricase enzyme was estimated to be 33 kDa by SDS-PAGE profile. The optimum temperature and pH was detected at 35°C and 8.5 for maximum uricase activity. Metal ions such as Co²⁺, Mn²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Cu²⁺ were reduced the enzyme activity to 33.17%, 92.18%, 72.47%, 32.73%, 64.32% and 90.10%, whereas Ca²⁺ enhanced uricase activity to 126%.

Keywords: Uricase, *Pseudomonas aeruginosa*, purification, optimization

INTRODUCTION

Uricase or urate oxidase (urate: oxygen oxidoreductase, EC 1.7.3.3) 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 [1]. Uricase enzyme is widely present in most vertebrates but is absent in humans [2]. Higher primates (apes and humans) lack functional uricase and excrete uric acid as the end product of purine degradation [3, 4]. The level of uric acid in blood increases over the normal value, can lead to gout disease [5]. Gout is characterized by persistent hyperuricemia, which results in the deposition of monosodium urate monohydrate (MSU) crystals in the joints and periarticular structures and of uric acid calculi in the urinary tract [6]. Elevated serum uric acid concentration has been associated with, a significantly increased cardiovascular risk [7], idiopathic calcium urate nephrolithiasis [8], and renal failure [9]. A high level of uric acid was related to leukemia in children [10]. Uricase is useful for enzymatic determination of urate in clinical analysis by coupling with 4-aminoantipyrine-peroxidase system [11]. Urate oxidase can be also used as protein drug to overcome severe disorders induced by uric acid accumulation [12].

Many organisms including plants and microorganisms are able to produce uricase. Uricase has been purified from leaves of *Cicer arietinum* L, *Vicia faba major* L and *Triticum aestivum* L [13]. *Proteus vulgaris* (1753 and B-317-C), *Streptomyces graminofaciens* and *Streptomyces albidoflavus* showed inducible uricase activity [14]. Several reports demonstrated that uricase is produced by bacteria such as *Micrococcus* and *Brevibacterium* [15], *Bacillus pasteurii* [16],

Proteus mirabilis [17] and *E. coli* [18]. Uricase was isolated from *Microbacterium* sp ZZJ4-1 [19], and the uricolytic activity was emphasized in *Penicillium*, *Aspergillus*, *Alternaria* and *Fusarium* spp [20]. Yeast such as *Candida tropicalis* effectively induces uricase by addition of uric acid in growth medium [21]. A study demonstrated that several fungi such as Deuteromycotina, Zygomycotina, Ascomycotina, Basidiomycotina and Mastigomycotina have an ability to produce uricase [22]. Several investigators studied the purification and characterization of uricase enzyme by microorganisms [23].

The aim of the present study was to investigate the purification and optimization of uricase for protein structure analysis and to carry out animal studies in the future for drug development. As per literature survey this could perhaps be the first report in India.

MATERIALS AND METHODS

Microorganism

The bacterial strain used in this study was previously isolated from poultry waste and was identified as *Pseudomonas aeruginosa* by specific biochemical tests [24]. The *P. aeruginosa* growth was optimized for mass level uricase production by using basal media containing several components such as 10.0g dextrose; 2.0g yeast extract; 3.0g uric acid and 5.0g NaCl. All ingredients were dissolved in 1000ml distilled water with 7.0 pH. The dextrose and yeast extract were used as a carbon and nitrogen source. The organism was allowed to grown at 35°C temperature for 36 hours incubation to obtain uricase for further analysis.

Uricase assay

The principle of enzyme measurement was as follows: uricase could catalyze the oxidation of uric acid into allantoin and H₂O₂, which was then measured by using a reaction system containing 4-aminoantipyrine, phenol and peroxidase as chromogen. In practical analysis, 0.1ml enzyme solution was incubated with a mixture of 0.6ml sodium borate buffer (pH 8.5, 0.1M) containing 2mM uric acid,

Received: Aug 26, 2011; Revised: Oct 24, 2011, Accepted: Nov 24, 2011

*Corresponding Author

A. Anderson
Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College, Poondi,
Thanjavur-613503, Tamilnadu, India.

Tel: +91 9944557848
Email: ansanmicro@gmail.com

0.15ml 4-aminoantipyrine (30mM), 0.1ml phenol (1.5%), 0.05ml peroxidase (15U/ml) at 37 °C for 20 min [25]. The reaction was stopped by addition of 1.0ml ethanol, and the absorbance at 540 nm was read against the blank by a spectrophotometer. One unit of enzyme was defined as the amount of enzyme that produces 1.0mmol of H₂O₂ per minute under the standard assay conditions.

Purification of Uricase

The enzyme purification processes were performed with 0.1 M phosphate buffer at pH 7.0 (buffer A) at 4 °C. Cells from 10.0 l of media were harvested by centrifugation and washed twice with 50 mM phosphate buffer (pH 7.5), re-suspended in buffer A and disrupted by ultrasonic oscillation (120 W oscillating for 3 s with 6 s intervals, repeated 100 times). After the cell debris had been separated by centrifugation, solid ammonium sulfate was added to the enzyme solution and the precipitate of the fractions from 30% to 80% saturation was collected by centrifugation (8000 rpm, 40 min, and 4 °C). The enzyme was then dissolved in a small amount of buffer (2:1, volume of the buffer/weight of the precipitate), dialyzed against 0.01 M phosphate buffer until the ammonium sulfate was removed. The purified enzyme was collected for further studies.

SDS-PAGE protein Electrophoresis

The proteins were separated by SDS-PAGE electrophoresis and the size of polypeptide chains of given protein can be determined by comparing its electrophoretic mobility in SDS-PAGE gel with mobility marker proteins of known molecular weight [26].

Characterization of purified uricase

pH value

To investigate the effect of pH on the activity of uricase, the enzyme was assayed at different pH; 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0 with the intervals of 0.5 and 100Mm buffers were used. For 6.0–8.0 pH, phosphate buffer was used, and borate buffer was used for 8.0–10.0 pH.

Temperature

To study the effect of temperature on uricase activity, the standard enzyme reaction solution was pre-incubated at different temperatures, 20, 25, 30, 35, 40, 45, 50, 55 and 60 °C for 5 min and the enzyme solution was then added and incubated for 20 min at the same temperature to measure its activity.

Metal ions

To study the effect of metal ions on uricase activity, the different metal ions such as Ca²⁺, Co²⁺, Mn²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Cu²⁺ were applied into the reaction mixture at 10-3M concentration.

RESULTS AND DISCUSSION

The present study was conducted to obtain the purified form of uricase and to determine its optimum conditions for maximum activity. In the first step, the uricase was obtained as a crude extract from *Pseudomonas aeruginosa* and then the uricase was purified by different concentrations of ammonium sulphate (30%, 40%, 50%,

60%, 70% and 80%). The results presented in Table 1 indicates that, 70% ammonium sulphate concentration with dialysis shown to be high uricase activity (40.0 U/ml) than 50% and 60% concentration and cell free supernatant. There is no enzyme activity was noticed with 30%, 40% and 80%. Similar result was observed from the recent study that the ammonium sulphate 70% saturation proved to be high uricolytic activity (146.84 U/ml) comparing with crude uricase and other concentrations [27]. The previous study also reported that *P. aeruginosa* uricase was purified using ammonium sulphate selective precipitation (70% concentration) and dialysis [28]. A study reported that the fractions with ammonium sulfate concentrations from 65% to 80% had the highest uricase enzyme specific activity [29].

Table 1 . Purification of uricase enzyme produced by *Pseudomonas aeruginosa*

Ammonium sulphate Concentration (%)	Uricase activity U/ml
Crude	11.0
30	0
40	0
50	5.14
60	14.72
70	40.0
80	0

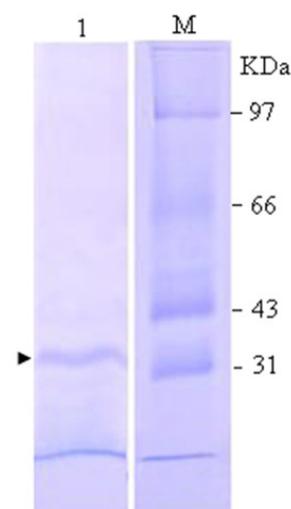


Fig 1. SDS-PAGE profile of purified uricase

Lane1: Ammonium sulphate precipitate and dialyzed sample: Arrow indicates the uricase (protein) band.

Lane M: Standard protein molecular weight marker.

The molecular weight of purified uricase was determined by SDS- poly acrylamide gel electrophoresis (SDS-PAGE) using standard marker proteins of known molecular weight. The results given in Figure 1 showed that the molecular weight of uricase was estimated to be 33 kDa. It was found and reported that the molecular weight of purified uricase from *Pseudomonas aeruginosa* was 68.0 kDa for one subunit [28]. A study found that the purified uricase enzyme by *Microbacterium* sp. strain ZZJ4-1 showed the molecular mass was estimated to be 34 kDa by SDS-PAGE [29]. The previous studies reported that molecular weight of uricase enzyme produced and purified from *Bacillus fastidiosus* was 145-150 kDa (30), 34 kDa uricase from *Candida utilis* [31] and 60 kDa uricase from *Glomastix*

gueg [27]. The present study was carried out to observe the effect of different pH (6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0) and temperatures (20 - 60°C) on purified uricase activity. The result recorded in Figure 2 & 3 shows that the highest uricase activity was attained at pH 8.5 and 35°C temperature.

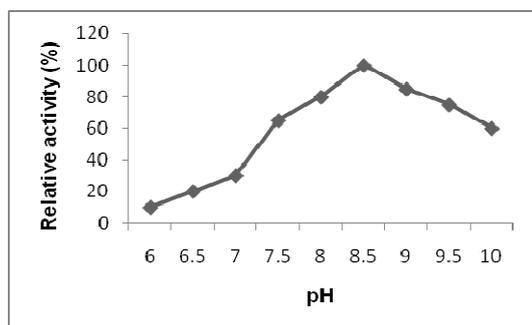


Fig 2. Effect of pH on uricase activity

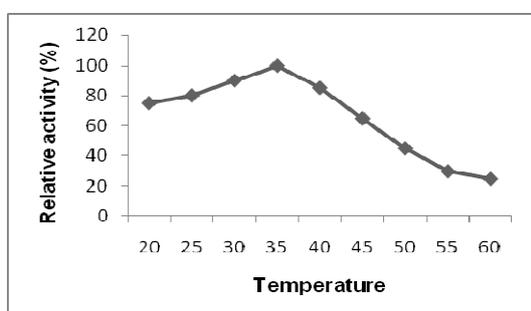


Fig 3. Effect of temperature on uricase activity

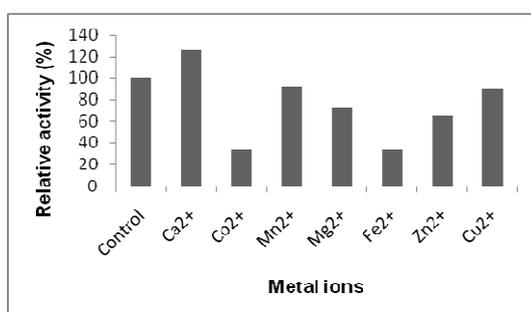


Fig 4. Effect of metal ions on uricase activity

The recent study also reported that the maximum activity of purified uricase produced by *Gliomastix gueg* was optimized at 35°C temperature and 9.0 pH [27]. The previous study was observed that the optimum temperature and pH for uricase activity of *Saccharopolyspora* sp. PNR11 was 37°C and 8.5 [32]. The early study documented that the optimum temperature and pH was 30°C and 8.5 for purified uricase produced by *Microbacterium* sp. strain ZZJ4-1 [29]. This experiment was carried out in order to study the effect of various metal ions such as Ca²⁺, Co²⁺, Mn²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Cu²⁺ on uricase activity. The results illustrated in Figure 4 that Ca²⁺ enhanced the uricase activity to 126%, whereas Co²⁺, Mn²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Cu²⁺ metal ions reduced the enzyme activity to 33.17%, 92.18%, 72.47%, 32.73%, 64.32% and 90.10% respectively. The earlier study reported that metal ions such as Co²⁺,

Mg²⁺ and Fe²⁺ reduce the enzyme activity and maximum increasing of uricase activity was indicated in the presence of Ca²⁺ ions [27]. The previous study also reported that the inhibition of uricase activity was noticed with Co²⁺, Fe²⁺ and Zn²⁺ and enhanced with Cu²⁺ and Ca²⁺ [28]. The earlier study conducted the effect of metal ions on uricase activity produced from *Microbacterium* sp. strain ZZJ4-1 that, Mn²⁺, Fe³⁺, Zn²⁺ and Ca²⁺ had no inhibitory activity on uricase enzyme [29].

CONCLUSION

We demonstrated that the uricase enzyme by *Pseudomonas aeruginosa* was purified using 70% ammonium sulphate precipitation and dialysis. The optimum temperature and pH of purified uricase was 35°C and 8.5 to get maximum enzyme activity. The metal ions like Co²⁺, Mn²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Cu²⁺ reduced the enzyme activity and the enhanced activity achieved with Ca²⁺. The purified uricase enzyme will be investigated for proteomics and animal studies in the future experiments.

ACKNOWLEDGEMENTS

The authors are thankful to the management, A.V.V.M. Sri Pushpam College for having provided the facilities to carry out this study.

REFERENCES

- [1] Fraisse L., Bonnet MC., Farcy JP., Agut C., Dersigny D. and 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.
- [2] Schiavon O., Caliceti P., Ferruti P. and Veronese F M. 2000. Therapeutic proteins: a comparison of chemical and biological properties of uricase conjugated to linear or branched poly (ethylene glycol) and poly (Narryloylmorpholine). *Il Farmaco* 55: 264-269.
- [3] Friedman TB., Polanco GE., Appold JC. and Mayle JE. 1985. On the loss of uricolytic activity during primate evolution. I. Silencing of urate oxidase in a hominoid ancestor. *Comp Biochem Physiol B* 81(3): 653- 659.
- [4] Yeldandi AV., Wang X., Alvares K., Kumar S., Rao MS. and Reddy JK. 1990. Human urate oxidase gene: cloning and partial sequence analysis reveal a stop codon within the fifth exon. *Bioch Biophys Res Comm* 171: 641- 645.
- [5] Nakagawa T., Mazzali M., Kang DH., Sanchez-Lozada LG., Herrera-Acosta J. and Johnson RJ. 2006. Uric acid - a uremic toxin? *Blood Purif* 24(1): 67-70.
- [6] S.J. Lee. and R.A. Terkeltaub. 2006. New developments in clinically relevant mechanisms and treatment of hyperuricemia, *Curr Rheumatol Rep* 8: 224-230.
- [7] Alderman MH., Cohen H., Madhavan S. and S Kivlighn. (1999) Serum uric acid and cardiovascular events in successfully treated hypertensive patients. *Hypertens* 34: 144-50.
- [8] Maseoud D., Rott K., Liu-Bryan R. and Agudelo C. 2005. Overview of hyperuricaemia and gout. *Curr Pharm Des* 11: 4117-4124.
- [9] Capasso G., Jaeger Ph., Robertson WG. and Unwin RJ. 2005. Uric acid and the kidney: urate transport, Stone

- disease and progressive renal failure. *Curr Pharm Des* 11: 4153–4159.
- [10] Larsen G. and Loghman-Adham M. 1996. Acute renal failure with hyperuricemia as initial presentation of leukemia in children. *J Pediatr Hematol Oncol* 18: 191–194.
- [11] Gochman N. and Schmitz M.J. 1971. Automated determination of uric acid, with use of a uricase – peroxidase system. *Clin. Chem* 17: 1154–1159.
- [12] Colloch., Hajji ME., Bachet B., L Hermite G., Schiltz M. and Parange T. 1997. Crystal structure of the protein drug urate oxidase-inhibitor complex at 2.05 Å resolution. *Nat. Struct. Biol* 4: 947–952.
- [13] . Montalbini., J. Redondo., J L. Caballero., J Cardenas. and M. Pineda. 1997. Uricase from leaves: its purification and characterization from three different higher plants. *Planta* 202 (3): 277-283.
- [14] A Azab., Magda M Ali. and Mervat F. Fareed. 2005. Studies on uricase induction in certain bacteria. *Egyptian Journal of Biology* 7: 44-54.
- [15] Kida J. and Kuniyoshi 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.
- [16] Cheristians S. and Kaltwasser H. 1986. Nickel-content of urease from *Bacillus pasteurii*. *Archives of Microbiology* 145: 51-55.
- [17] Rando D, Steglitz U, Morsdorf G and Kaltwasser H. 1990. Nickel availability and urease expression in *Proteus mirabilis*. *Archives of Microbiology* 154: 428-432.
- [18] Nakagawa S., Ishino S. and Teshiba S. 1996. Construction of catalase deficient *Escherichia coli* strains of the production of uricase. *Bioscience Biotechnology and Biochemistry* 60: 415-420.
- [19] Xue-lai Zhou., Xiao-hang Ma., Gui-qin Sun., Xia Li. and Kang-ping Guo. 2005. Isolation of a thermostable uricase-producing bacterium and study on its enzyme production conditions. *Process Biochemistry* 40: 3749–3753.
- [20] Franke W., Taha E E M. and L Krieg. 1952. Purinoxydierende fermente aus Schimmelpilzen. I. Uricase der Schimmelpilze. *Arch. Mikrobiol* 17: 255-259.
- [21] Atsuo Tanaka., Midori Yamamura., Susumu Kawamoto. and Saburo Fukui. 1977. Production of Uricase by *Candida tropicalis* Using n-Alkane as a Substrate. *Applied and Environmental Microbiology* 34: 342-346.
- [22] Mahmoud M., Nour El Dein. and A El- Fallal. 1996. Screening of some fungi for uricolytic activity. *Qatar Univ. Sci.J* 16 (1): 71-76.
- [23] Adamek V., Kralova B., Suchova M., Valentova O. and Demnerova k. 1989.. Purification of microbial uricase. *Journal of Chromatography* 497: 268- 275.
- [24] Anderson A and Vijayakumar S. 2012. Isolation and Optimization of *Pseudomonas aeruginosa* for uricase production.3 (1): 143-150
- [25] Masaru S. 1981. Purification and some properties of sarcosine oxidase from *Corynebacterium* sp. U-96. *J. Biochem* 89: 599–607.
- [26] Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685.
- [27] Atalla., M. Mabrouk., Eman R., Hamed., Farag M. M. and Nehad E. Ahmed. 2010. Purification and Characterization of Uricase Enzyme Produced by *Gliomastix gueg*. www.Gate2Biotech.com 2 (11): 1-13
- [28] Hesham M Saeed., Yasser R., Abdel-Fattah Yousry., Gohar M. and Mohammed A Elbaz. 2004. Purification and Characterization of extracellular *Pseudomonas aeruginosa* urate oxidase enzyme. *Polish Journal of Microbiology* 53 (1): 45-52.
- [29] Lei Kai., Xiao-Hang Ma., Xue-Lai Zhou., Xiao-Ming Jia., Xia Li. and Kang Ping Guo. 2007. Purification and characterization of a thermostable uricase from *Microbacterium* sp. strain ZZJ4-1. *World Journal of Microbiology and Biotechnology* 24 (3): 401-406.
- [30] Bongaerts G.P.A. and G.D. Vogels. 1976. Uric acid degradation by *Bacillus fastidiosus* strains. *J. Bacteriol* 125: 689-697.
- [31] Koyama Y, Ichikawa T and Nakano E. 1996. Cloning, sequence analysis, and expression in *Escherichia coli* of the gene encoding the *Candida utilis* urate oxidase (uricase). *J Biochem* 120: 969–973.
- [32] K. Khucharoenphaisan. and K. Sinma. 2011. Production and Partial Characterization of Uric Acid Degrading Enzyme from New Source *Saccharopolyspora* sp. PNR11. *Pakistan Journal of Biological Sciences* 14: 226-231.