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Screening, production and optimization of Uricase from *P. aeruginosa*

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Abstract
Uricase is very important for the determination of uric acid in biological fluids. Uricase has effective role in prophylaxis and treatment of tumor lysis hyperuricemia. In the present study, the uricase enzyme production was evaluated using *P. aeruginosa.* Enzyme production was studied for different pH, temperature, carbon, nitrogen source.In this current study the amount of enzyme produced was 73U/ml. The optimum temperature and pH was detected at 45°C and 5 for maximum uricase activity. The objective of the work was to develop a new method for 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 higher production of uricase was obtained in this study. The proposed method is a novel, inexpensive, simple and sensitive technique for screening and estimation of uricase.

Keywords: uricase, *p. aeruginosa*, enzyme, optimization

Introduction
Urate oxidase or uricase (urate: oxygen oxidoreductase, EC (1.7.3.3) is an enzyme that catalyse the oxidation of uric acid to allantoin and plays an important role in purine metabolism (Wu *et al*., 1994) [17]. This enzyme is widely present in most vertebrates but is absent in humans (Schiavon *et al*., 2000) [18]. It was first found in bovine kidney. Various natural sources such as bacteria (Mansour *et al*., 1996) [19], fungi (Farley and Santosa, 2002) [10] and eukaryotic cells (Montalbini *et al*., 1997) [2] have also been found to be uricase producers.
The first important application discovered for uricase was in clinical biochemistry as a diagnostic reagent for measurement of uric acid in blood and other biological fluids (Adamek *et al*., 1989) [20]. Higher primates (apes and humans) lack functional uricase and excrete uric acid as the end product of purine degradation (Friedman *et al*., 1985; Yeldandi *et al*., 1990) [21, 22]. In some individuals, uric acid precipitates, leading to gout symptoms.
Basically, many drugs are the xanthine oxidase inhibitors that block the formation of uric acid, while, urate oxidase degraded uric acid (purine catabolic product or pre-existing) and formed water soluble metabolites that readily excrete out of the body through kidney. It shows better results by reducing 50 % hyperuricemia in less than 24 hours (Cheng *et al.*, 2000. Goth *et al.*, 2008; Juan *et al.*, 2008; Altarsha *et al.*, 2009; Terzuoli *et al.*, 2009; Gibison *et al.*, 2010; Dong *et al.*, 2011; Meotti *et al.*, 2011) [25, 27, 11].
Uricase is mainly localized in liver of animals and in microorganisms especially bacteria such as. *Pseudomonas aeruginosa, Arthrobacter globiformis, Bacillus subtillus fastidious, Nocardia farcinica and Microbacterium sp.* (Poovizh *et al.*, 2014) [32]. Uricase enzyme also used in biosensor in immobilized condition. This enzyme used to analyze the uric acid in aqueous solution. (Ahuja *et al.*, 2015) [34].

Materials and Methods
Sample collection
P. aeruginosa were obtained from Department of Biotechnology. Srimad andavan arts and science college, Trichy. The obtained culture was maintained on nutrient broth.

Screening of bacterial strains for uricase production
P. aeruginosa were screened for Uricase production in medium containing 0.3% uric acid. Plate were incubated at 37^0C for 24 hours. After incubation zone formation was observed. (Buchanam *et al.*, 1974) [29].

Optimization of uricase production medium
Effect of pH
The production media prepared with all the nutritional components with the alteration in the pH alone varying from (4, 5, 6, 7, 8 and 9), was inoculated with the cultures (*P. aeruginosa*a) and incubated. After incubation the uricase activity was assayed. (Srivastava *et al.*, 1997).

Effect of Temperature
The production media was prepared with all the nutritional components with the alternative temperatures (30˚C, 35˚C, 40˚C, 45˚C, 50˚C) was inoculated with the cultures and incubated. After incubation the uricase activity was estimated. (Lamed and Zeikus, 1980).

Effect of Incubation time
The production media was prepared with all the nutritional components. The culture were inoculated and kept at variuos time duration (24, 48, 72 and 96 hours). After incubation the uricase activity was estimated.

Effect of Substrate concentration
To find the suitable substrate concentration of (uric acid),

production was carried out using different concentration & such as 0.1g, 0.2g, 0.3g, 0.4g, 0.5g, and 0.6g % /100 ml of the production medium. The enzyme activity was determined.

Effect of Carbon source
Different carbon sources like dextrose, maltose, sucrose, glucose and lactose were tested in order to determine their influences in the uricase production. (Palanivelu, 2004) [30].

Effect of Nitrogen source
Different nitrogen sources like peptone, ammonium nitrate and yeast extract were tested in order to determine their influences in the uricase production.Uricase activity was estimated. (Palanivelu, 2004) [30].

Uricase assay method
The enzymatic assay was carried out by the method described by Mahler *et al* (1970) [31]. 3 ml of 20mM boric acid buffer solution having pH of 9.0 was added with 75 µl of 3.57 mM uric acid solution and 20 µl of cell free supernatant (used as crude enzyme), at 25^0C. Blank solution was separately prepared by adding 20 µl of buffer solution instead of the cell free supernatant. The blank and the test solutions were incubated at 25^0C for 10 mins. The reduction in the uric acid concentration in the test sample was measured using UV-visible spectrophotometer at 293nm.

Result
Plate assay method
P. aeruginosa were screened for uricase production in medium containing 0.3% uric acid. Plate was incubated for 24 hrs. After incubation zone formation was observed. (Fig. 1).

Optimization of bacterial growth
Optimum condition like pH, temperature, substrate concentration, incubation time, carbon and nitrogen sources were found to influence the growth of *P. aeruginosa* uricase production. The identified optimized parameters are as follows:

Effect of pH
The optimum pH for maximum uricase production by *P. aeruginosa* was identified. The results showed that optimum pH for the growth of *P. aeruginosa* was attained at pH 5 and pH 6 for maximum uricase production of 73U/ml and 56U/ml. (Fig. 2).

Effect of Temperature
An experiment was designed to establish the optimum temperature for bacterial growth to produce high quantity of uricase enzyme. The results showed that (Fig.3) was optimum temperature for maximum uricase production (53U/ml) by *P. aeruginosa.*

Effect of Incubation time
The optimum incubation time for maximum uricase production by *P. aeruginosa* was identified. The results showed that optimum time for the growth of *P.aeruginosa* was attained at 72 hours and 48 hours for maximum uricase production of 58U/ml (Fig. 4).

Effect of Substrate concentration
The influence of different uric acid concentration (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6%) was studied and the results presented in Fig.6,indicated that 0.2% and 0.3% uric acid were shown to increase the uricase production by *P. aeruginosa.*(Fig.5).

Effect of Carbon source
Different carbon sources (dextrose, maltose, lactose, sucrose and glucose) were tested in order to determine their influences in *P. aeruginosa* growth for uricase production. The results showed that, the large amount of uricase (48 U/ml) was produced in the medium containing glucose from *P. aeruginosa* and the amount of uricase. (Fig.6).

Effect of Nitrogen source
The present experiment was conducted to test the effect of different nitrogen sources (yeast extract, ammonium nitrate and peptone) for bacterial growth. The result showed that peptone proved to be the best nitrogen source for maximum uricase production (73U/ml) by *P. aeruginosa.* (Fig.7).

Production of uricase enzyme
The uricase enzyme was produced by optimized condition. *P. aeruginosa* were produce 123U/ml uricase in 48hours and 72 hours respectively.

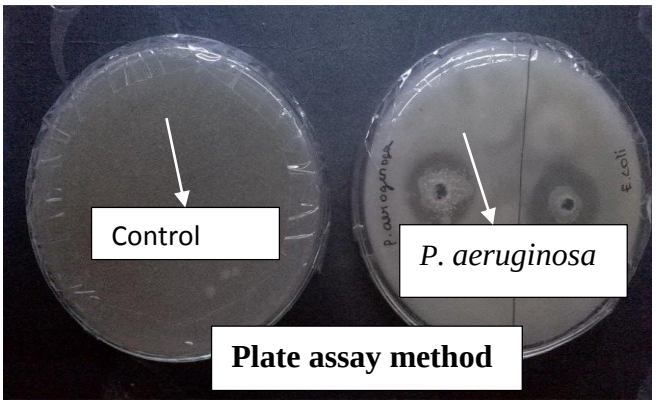

Fig 1: Zone of uric acid utilization by uricase producing *Pseudomonas aeruginosa* nutrient agar supplemented with 0.3% uric acid

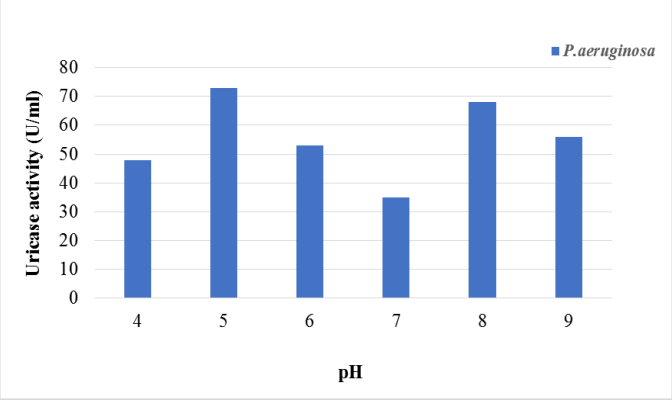

Fig 2: Effect of pH on uricase production

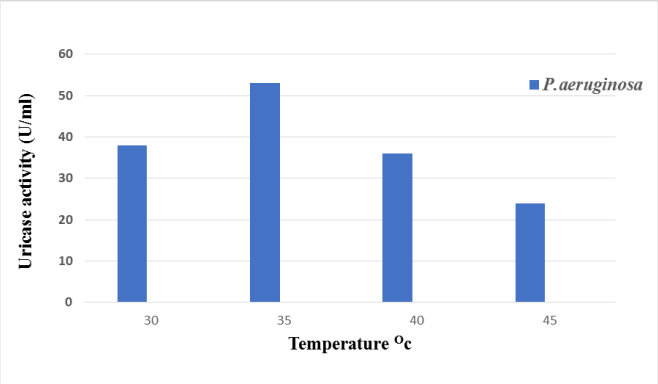

Fig 3: Effect of Temperature on uricase production

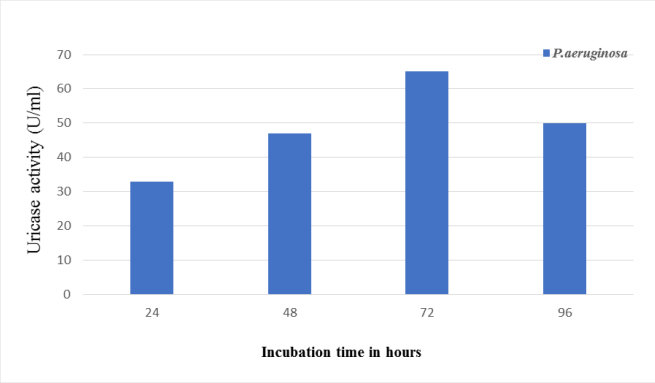

Fig 4: Effect of Incubation time on uricase production

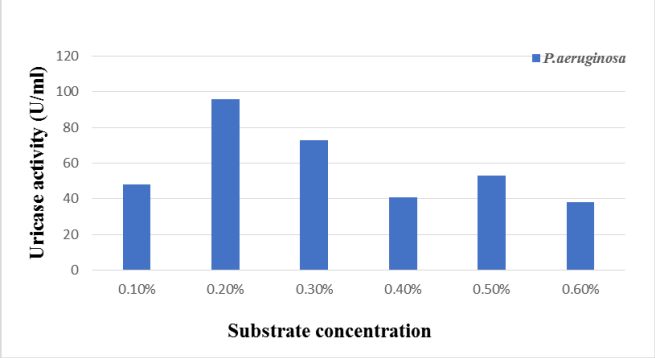

Fig 5: Effect of Substrate concentration on uricase production

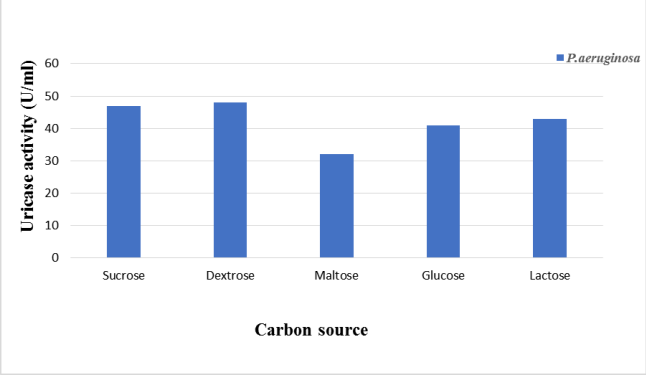

Fig 6: Effect of Carbon source on uricaseproduction

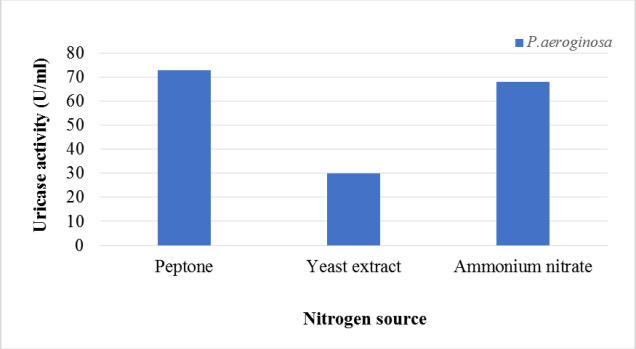

Fig 7: Effect of Nitrogen source on production media

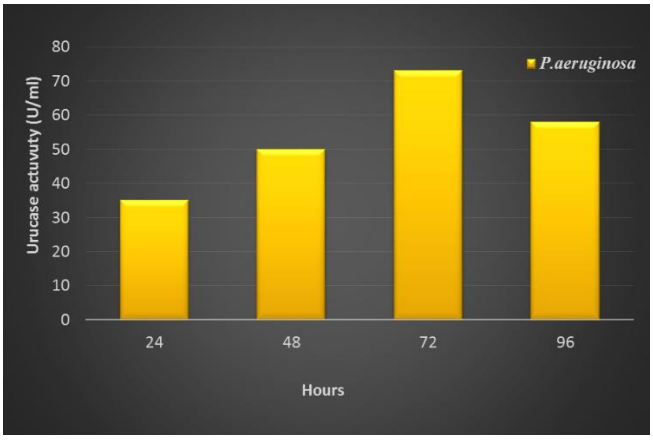

Fig 8: Uricase production under Optimization condition

Discussion

Uricase catalyze the oxidative reaction that converts urate to allantoin, a more soluble and easily excreted compound. Due to this property urate oxidase may be used for both therapeutically and diagnostic purposes (Chohan and Becker, 2009) [28].

In the present study, *P.aeruginosa* were screened by plate assay method for uricase production. *P.aeruginosa* produces uricase enzyme was confirmed by zone formation observed. Prior study also documented that the *P.aeruginosa* produce extracellular inducible active uricase enzyme on nutrient agar media contained uric acid and was identified by several biochemical tests. (Saeed *et al.*, 2004) [13].

The initial medium pH ranging from and 4, 5, 6, 7, 8 and 9 was studied on detect the effect on uricase production by *P. aeruginosa*. In the present study, the optimum pH was observed at 5 pH for *P. aeruginosa*and growth for maximum enzyme production. But the other studies, uricase production was better in 7 pH for *Pseudomonas aeruginosa.* (Anderson and Vijayakumar, 2012) [15].

In the present study, the effect of uric acid was examined in the range of 0.1% - 0.6% for bacterial growth. The uric acid 0.2% of uric acid concentration optimum for *P. aeruginosa* to obtain maximum uricase. The 96U/ml/min level of uricase produced by *P. aeruginosa* in 0.2%. In other studies, 0.3% uric acid concentration was used to produce maximum uricase (0.45U/ml) by *Bacillus cereus,* (Amirthanathan and Subramaniyan, 2012) [33], *Bacillus thermocatenulatus.* (Lotfy

et al., 2008) [24] and *Microbacterium sp.* ZZJ_{4-1} that 0.3% uric acid was the best concentration for uricase production (0.6 U/ml) (Zhou *et al.*, 2005) [23].

The present experiment was conducted to test the suitability of different carbon sources such as sucrose, dextrose, maltose, glucose, and lactose for uricase production and found the dextrose was a best for *P. aeruginosa.* In *P. aeruginosa* produce maximum level of uricase utilizing dextrose than maltose, sucrose, starch, and cellulose. (Anderson and Vijayakumar, 2012) [15].

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Reference

1. Alvarez-Lario B, Macarron-Vicente J. Is there anything good in uric acid? QJM, 2011; 104:1015-1024.
2. Montalbini P, Redondo J, Caballero J, Cardenas J, Pineda M. Uricase from leaves: its purification and characterization from three different higher plants. Planta, 1997; 202:277-283.
3. Koyama Y, Ichikawa T, Nakano E, Cloning. Sequence analysis, and expression in Escherichia coli of the gene encoding the Candida utilisurate oxidase (uricase). J. Biochem. 1996; 120:969-973.
4. Khucharoenphaisan K, Sinma K. Production and partial characterization of uric acid degrading enzyme from new source Saccharopolysporasp.Pak. J. Biol. Sci, 2011; 14:226-231.
5. Chen Z, Wang Z, He X, Guo X, Li W, Zhang B. Uricase production by a recombinant Hansenula polymorpha strain harboring Candida utilis uricase gene. Appl. Microbiol. Biotechnol. 2008; 79:545-54.
6. Chen Z, Wang Z, Zhang B. Uricase production by a recombinant Hansenula polymorpha strain harboring Candida utilis uricase gene. Appl. Microbiol. Biotechnol. 2008; 79:545-554.
7. Abdel-Fattah YR, Saeed HM, Gohar YM, El- Baz M. A.Improved production of Pseudomonas aeruginosa uricase by optimization of process parameters through statistical experimental designs.Proc Biochemistry, 2005; 40:1707-1714.
8. Kai L, Xiao HM, Xue LZ, Xiao MJ, Xia L, Guo KP. Purification and characterization of a thermostable uricase from Microbacterium sp. strain ZZJ4-1. J. Microbiol. Biotechnol. 2008; 24:401-406.
9. Geweely NS, Nawar LS. Production, optimization, purification and properties of uricase isolated from some fungal flora in Saudi Arabian soil. Aust J Basic Appl Sci. 2011; 5:220-230.
10. Farley PC, Santosa S. Regulation of expression of the Rhizopusoryzaeuricase and urecase enzymes. Can. J. Microbiol. 2002; 48:1104-1108.
11. Dong Y, Chi Y, Lin X, Zheng L, Chen G. Nano-sized platinum as a mimic of uricase catalyzing the oxidative degradation of uric acid. Phys Chem Chem Phys. 2011; 13:6319-6324.
12. Kraalentova VB, Suchova M, Valentova O, Demnerova K. Purification of microbial uricase. J. Chromatogr. B Biomed. Sci. Appl. 1989; 497:268-275.
13. Saeed MH, Abdel-Fatah Y, Gohar M, Elbaz AM. Purification and characterization of extracellular Pseudomonas aeruginosa urate oxidase enzyme. Pol. J. Microbiol. 2004; 53(1):45-52.
14. Hesham Saeed M, Yasser R, Abdel-Fattah Yousry, Gohar M, Mohammed Elbaz A. Purification and Characterization of extracellular Pseudomonas aeruginosa urate oxidase enzyme. Pol. J. Microbiol. 2004; 53:45-52.
15. Anderson A, Vijayakumar S. Isolation and optimization of Pseudomonas aeruginosa for uricase production. Int. J. Pharma Biol. Sci. 2012; 3:143-150.
16. Wang LC, Marzluf GA. Purification and characterization of uricase a nitrogen regulated enzyme from Neurospora crassa, Biochem. Biophys, 1980; 201:185-193.
17. Wu X, Wakamiya M, Vaishnav S, Geske R, Montgomery C, Jones P, et al. Hyperuricemia and urate nephropathy in urate oxidase deficient mice. Proceedings in Natural Academy Science of USA, 1994; 91:742-746.
18. Schiavon O, Caliceti P, Ferruti P, Veronese FM. Therapeutic proteins: a comparison of chemical and biological properties of uricase conjugated to linear or branched poly (ethylene glycol) and poly (Narryloylmorpholine). II Farmaco. 2000; 55:264-269.
19. Mansour FA, Nour-El-Dein MM, El-Fallel AA, Abou-Do-Bara MIM. Purification and general properties of uricase from Streptomyces aureomonopodiales. Acta Microbiologica Polonica. 1996; 45:45-53.
20. Adamek V, Kralova B, Suchova M, Valentova O, Demnerova K. Purification of microbial uricase. Journal of Chromatography. 1989; 497:268-275.
21. Friedman TB, Polanco GE, Appold JC, Mayle JE. On the loss of uricolytic activity during primate evalution. I. Silencing of urate oxidase in a hominoid ancestor. Comparative Biochemistry and Physiology. 1985; 81(B):653-659.
22. Yeldandi AV, Wang X, Alvares K, Kumar S, Rao MS, Reddy JK. Human urate oxidase gene: colning and partial sequence analysis reveal a stop codon within the fifth exon. Biochemical and Biophysical Research Communications. 1990; 171:641-645.
23. Zhou X, X, Ma G, Sun Xli, Guo K. Isolation of a thermostable uricase producing bacterium and study on its enzyme production conditions. Proc. Biochem. 2005; 40:3749-3753.
24. Lotfy WA. Production of a thermostableuricase by a novel Bacillus thermo catenulatus strain. Bioresource technology. 2008; 99(4):699-702.
25. Goth L. (Rasburicase therapy may cause hydrogen peroxide shock). Orvosihetilap. 2008; 149(34):1587-1590.
26. Chang K, Farnden K. Purification and properties of Asparaginase EC-3.5.1.1 from Lupinus arboreus and Lupinus angustifolius. Archives of Biochemistry and Biophysics. 1981; 241(2):571-576.
27. Juan EC, Hoque MM, Shimizu S, Hossain MT, Yamamoto T, Imamura S, et al. Structures of Arthrobacter globiformis urate oxidase-ligand complexes. Act. Cryst. 2008; 64(8):815-822.

28. Chohan S, Becker MA. Update on emerging urate-lowering therapies. Curr. Opin. Rheumatol. 2009; 21:143-149.
29. Buchanan RE, Gibbons NE, Cowan ST, Holt JG, Liston J, Murray RGE, et al. Bergey's manual of determinative bacteriology. 8th ed. William and Wilkins, Baltimore, 1974; 1268p.
30. Palanivelu P. Analytical Biochemistry and Separation Techniques. 2004; 1:255-267.
31. Mahler JL. A new bacterial uricase for uric acid determination. Anal Biochem. 1970; 38:65-84.
32. Poovizh T, Gajalakshmi P, Jayalakshmi S. Production of uricase a therapeutic enzyme from Pseudomonas putida isolated from poultry waste. Inter J Adv Res. 2014; 2(1):34-40.
33. Amirthanathan A, Subramaniyan A. Isolation and optimization of Pseudomonas aeruginosa for uricase production. Int J Pharm Bio Sci. 2012; 3:143-150.
34. Ahuja SK, Ferrerira GM, Morrerira AR. Application of placket and burman design and respons methodology to achieve exponential growth of aggregated shipworm bacterium, Biotechnol. Bioenging, 2015.