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Identification, Cloning and Expression of *Pseudomonas aeruginosa* Ps-x Putative Urate Oxidase Gene in *Escherichia coli*

HESHAM M. SAEED[1], YASSER R. ABDEL-FATTAH[2], MAHMOUD M. BEREKAA[3],
YOUSRY, M. GOHAR[3] and MOHAMED A. ELBAZ[5]

[1, 5] Institute for Graduate Studies and Research, University of Alexandria, Chatby 21526, Alexandria, Egypt
[2] Mubarak City for Scientific Research and Technological Applications
[3] Faculty of Sciences, University of Alexandria

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Abstract

In a previous study we reported for the first time the isolation and characterization of urate oxidase enzyme from *Pseudomonas aeruginosa.* In this work we isolated and cloned a 1.350 kilobase DNA fragment that encode a putative urate oxidase gene from the genomic library of *P. aeruginosa* Ps-x. The nucleotide sequence of the cloned DNA insert revealed an open reading frame that encodes a protein of a molecular weight of 54.0 kDa. The cloned DNA fragment showed an uricolytic activity when expressed in *E. coli* DH5α. Surprisingly, the nucleotide sequence of the cloned gene showed more than 99% identity to the gene encoding hypothetical protein of *P. aeruginosa* PAO1. Moreover, the sequence of the cloned gene was closely similar to the corresponding uricase gene of *Cellulomonas flavigena* (44% similarity), but showed lower similarity values to that of *Bacillus* sp. BT-90 (24% similarity), *Candida utilis* (24% similarity). Interestingly, the isolated uricase gene showed closer similarity to uricase from yeast-like symbiotic fungi *Beauveria bassiana* (35%), *Tolypocladium inflatum* (29%), *Paecilomyces tenuipes* (27%) and *Cerataphis fransseni* (24%).

Key words: Urate oxidase (uricase), *P. aeruginosa*, *C. flavigena*, partial coding sequence

Introduction

Urate oxidase or uricase (urate oxygen oxidoreductase, EC 1.7.3.3) is an enzyme that catalyzes the oxidation of uric acid to unidentified product then to a more soluble compound, allantoin. Uricase occupies a pivotal position in the chain of enzymes responsible for the metabolism of purines (Wu *et al.*, 1994). In human and other higher primates, uricase gene is absent as a result of two mutations in the gene coding sequence. As a result, uric acid is the end product of purines metabolism, which is present largely as insoluble monosodium salt and in some individuals, uric acid can precipitate, leading to gout symptoms (Lee *et al.*, 1988). Gout is a painful disorder, characterized by uricemia, recurrent attack of acute arthritis, deposition of sodium urate in and around joints and in many cases, formation of uric acid calculi (Lee *et al.*, 1988; Richard *et al.*, 1992). Allopurinol is considered as one of the most potent xanthine oxidase competitive inhibitor and thus an efficient drug for gouty patients (Massey *et al.*, 1970). In case of gout associated with renal complications, direct injection of urate oxidase allows a much more rapid resorption of urate nephrolithiases (Richard *et al.*, 1992). The presence of uricase in many microorganisms during purines catabolism has been well studies and characterized (Xi *et al.*, 2000; Nygaard *et al.*, 2000 and Schultz *et al.*, 2001). In spite of the importance of the purines degradation pathway, there are only a few reports about the genetics of such a pathway in bacteria (Schultz *et al.*, 2001).

In this study, we reported the isolation and cloning of *P. aeruginosa* urate oxidase gene. Furthermore; characterization of the uricase gene in terms of similarity with other known uricases was also studied. The possible uricolytic activity of the previously characterized *P. aeruginosa* PAO1 hypothetical protein was also examined.

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[1] Correspondence: tel: 002034297005, fax: 002034285792, e-mail: hesham25166@yahoo.com

Experimental

Materials and Methods

Bacterial strains and plasmids. The bacterial strain used in this study was a gift from Dr. Yossry Gohar, department of Microbiology, Faculty of Science, Alexandria University. This strain was previously identified as *P. aeruginosa* by Saeed *et al.*, 2004 and was used for uricase gene isolation and characterization. *Escherichia coli* DH5α host strain was obtained from Stratagene Co., and this strain was used as a host strain for recombinant plasmids and in pilot protein expression study. The pTrcHis (A, B, C) plasmid cloning vector is a 4.4 Kb in size and was used for construction of *P. aeruginosa* genomic library and for the expression of the recombinant protein. This plasmid was obtained from Invitrogen Corporation, USA.

Culture media and growth of microorganisms. *P. aeruginosa* Ps-x cultivated and tested for uricase enzyme production using nutrient agar medium pH 7.5 and contained per liter: peptone, 5.0 g; beef extract, 3.0 g; NaCl, 2.0 g; agar-agar, 23.0 g and uric acid 3.0 g. *E. coli* DH5α strain was cultivated in Luria Bertani (LB) medium containing per liter: Trypton, 10.0 g; Yeast extract, 5.0 g and sodium chloride, 10.0 g. The pH was adjusted to 7.5 and autoclaved. Recombinant *E. coli* DH5α strain was cultivated in LB medium supplemented with ampicillin to 100 μg/ml and IPTG to 1 mM final concentration. Recombinant *E. coli* DH5α strain was tested for their ability to produce recombinant uricase by cultivation on LB-agar medium supplemented with ampicillin to 100 μg/ml, IPTG to 1 mM and uric acid substrate to 0.2% final concentration.

Chemicals and enzymes. All chemicals used were of analytical reagent and molecular biology, chromatographic grade as appropriate. GeneClean II kit was obtained from Anachem. Co. Restriction endonucleases and DNA modifying enzymes were obtained from Promega (Southampton, UK), Boehringer Mannheim (Germany) and Stratagene, UK.

Uricase assay. Uricase was assayed by following the disappearance of uric acid, detected by decrease in absorbance at 292 nm in the presence of cell free extract or purified uricase. The assay mixture contained about 50–100 μl of the enzyme preparation in 0.1 M borate buffer pH 9.0 and 0.12 mM of uric acid in a final volume of 3.0 ml. One unit is defined as the amount of enzyme necessary to transform 1 μmole of uric acid into allantoin in 1 minute at 30°C and pH 9.0 (Mahler, 1970).

Protein analysis. Total protein concentrations of cell free supernatant and purified samples were assayed by the method of Bradford (Bradford, 1976) using a calibration curve established with bovine serum albumin as a standard. Proteins in these preparations were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels according to the method of (Laemmli, 1970). Protein molecular weight markers for SDS-PAGE was SeeBlue® Plus2 prestained standard and obtained from Invitrogen life technologies.

Pilot expression study in *E.coli* cells. Recombinant protein was expressed in *E. coli* DH5α cells. 100 ml of LB medium supplemented with ampicillin at a concentration 100 μg/ml were inoculated with 1 ml of an overnight culture of *E. coli* DH5α pTrcHis B8. The flasks were shaken at 200 rpm and at 37°C until the absorbance at 600 nm was 0.6 and then point IPTG (1 mM) and uric acid (0.15%) were added to the induced cultures separately followed by continued incubation at 200 rpm and at 37°C. Samples were taken from each flasks at zero induction time and after 12 and 24 hours. Then the induced and uninduced cells were pelleted by centrifugation at 8 000 rpm and 4°C. The cells were then washed with 100 mM Tris-HCl, pH 7.4 and pelleted a second time. The cells were resuspended in 4 ml of 100 mM Tris-Hcl, pH 7.4 and sonicated using 4 × 15 second pulses. Cells debris were removed by centrifugation at 10 000 rpm, 4°C for 10 minutes after which the supernatant was taken for protein analysis and for uricase assay.

Nucleic acid techniques. Genomic DNA and plasmid DNA purification, competent cells preparation, transformation and all other standard DNA methodologies were performed according to Sambrook *et al.* (1989).

Construction and screening of *P. aeruginosa* genomic DNA library. *P. aeruginosa* DNA was isolated and purified according to Sambrook *et al.*, 1989. The prepared DNA was partially cut with EcoRI according to the following reaction conditions: purified DNA, 20 μg (25 μl); 10x EcoRI buffer (H), 5 μl; EcoRI enzyme 120 units (10 μl); d-H_2O up to 50 μl. The reaction was carried out at 37°C for 3 hours after which the reaction was terminated at 70°C for 10 minutes and the product was analysed and resolved on agarose gel (1%) along with phage λDNA Hind III cut molecular weight markers. DNA fragments of 1.5–3.0 kbp average size were separated from the agarose gel and was purified using GeneClean II kit and used for ligation reactions. The plasmid pTrcHis (A, B, C) vector used for ligation reaction and library construction was obtained from Invitrogen corporation, UK. This plasmid (4.4 kbp) contains a unique EcoRI site in the multiple cloning site that was used in ligation reaction. Ligation reactions utilizing all the three possible reading frames of the plasmid (A, B, C) and transformation was carried out according to Sambrook *et al.*, 1989. Recombinant *E. coli* DH5α cells harbouring recombinant plasmids were screened separately utilizing LB-agar plates supplemented with ampicillin, IPTG and uric acid substrate at previously indicated concentration.

DNA sequencing. DNA was sequenced by the dideoxy chain termination method according to Sanger *et al.* (1977) using ABI Prism Ready Reaction Dye Terminator Sequencing Kit and analyzed on an ABI 377 automated sequencer.

DNA sequencing data analysis. Analysis of the nucleotide and amino acid sequence data was performed using Clone Manager 5 and BioEdit computer based program. Alignments and comparison of sequences were carried out using Blast program. The nucleotide and amino acid sequencing data reported in this work have been submitted to the EMBL nucleotide sequence database and is listed under the accession number AY484467.

Results and Discussion

Construction and screening of *P. aeruginosa* genomic library. In an attempt to produce recombinant urate oxidase enzyme from *P. aeruginosa* Ps-x, genomic library was constructed by ligating the EcoRI partially digested and purified DNA with average insert size of 1.5–3.0 kb, onto EcoRI digested, Calf intestinal alkaline phosphatase (CIAP) treated pTrcHis A, B, C plasmid expression vector. The pTrcHis A,

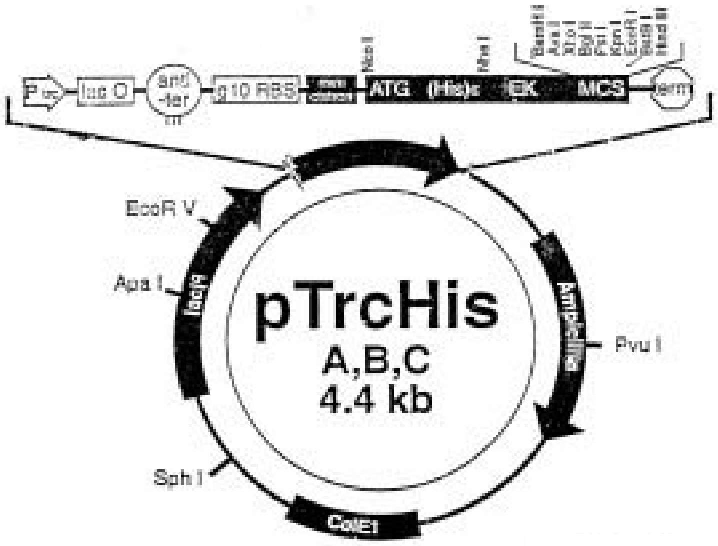


Fig. 1. Schematic representation of pTrc His A, B, C plasmid

B, C plasmid (Figure 1) is a 4.4 kb that carries a unique EcoRI site and a 6 histidine tag that facilitates the purification of the expressed protein using metal affinity chromatography. The ligation products were transformed into competent *E. coli* DH5α and the recombinant cells were plated onto LB agar plates supplemented with ampicillin as selectable marker and incubated overnight at 37°C.

Screening, identification and characterization of urate oxidase recombinant clones. *E. coli* DH5α harbouring pTrcHis A, B, C recombinant plasmids were subjected to more than one round of screening for the identification of potential positives urate oxidase producing clones. The screening process was carried out as mentioned earlier on LB agar plates supplemented with ampicillin, uric acid and IPTG. Results of the screening process showed that 8 potential positives clones were obtained and most of them were found to be on the pTrcHis B frame rather than the other two open reading frames, A and C, and thus they were given individual clones number B1-B8. Figure 2 shows the results of screening process when these clones were tested on LB agar ampicillin, uric acid and IPTG plates. Urate oxidase recombinant clones characterized by the presence of clear zone around the bacterial growth colony indicated for the hydrolysis of uric acid substrate due to enzyme secretion by the recombinant clones. It is worthwhile noting that although *E. coli* cells does not produce any endogenous or exogenous uricase enzyme as mentioned by Richard *et al.*, 1992, it is capable of producing a recombinant enzyme at quite different level of expression since some of the recombinant clones produce more uricase enzyme than the other clones as shown in Figure 2. Moreover, since *E. coli* cells normally does not produce extracellular recombinant enzyme in the surrounding medium, it was suggested that the produced recombinant enzyme found its way through the *E. coli* periplasmic space and membrane to the outer medium and this explain the clear zones around the recombinant colonies that resulted from uricase action on its uric acid substrate. These results indicated that background uricase activities from *E. coli* host strains was extremely unlikely to account for the results produced upon using recombinant clones. Indeed, the expression level of uricase enzyme utilizing the agar-uric acid plate method was fluctuating and it was found that the most potent uricase producing recombinant clone was pTrcHis-B8. Therefore, this clone was subjected to further characterization.

Plasmid DNA preparation and insert size determination. Plasmid DNA was prepared from recombinant *E. coli* DH5α pTrcHis-B8 and analyzed for DNA insert size by digestion of the prepared plasmid using EcoRI enzyme. It was found that the cloned DNA fragment has a size of 1.4 kb (Figure 3) which is encode a protein of molecular weight around 54.0 kDa that was close to the molecular weight of the purified native protein from *P. aeruginosa* under investigation.

Sequencing analysis and characterization of the gene encoding putative urate oxidase enzyme. Sequencing analysis of the cloned DNA insert on pTrcHis B8 showed 1350 bp fragment that have a unique EcoRI site (Figure 4 A and B) with an open reading frame of 450 amino acid residues and of a molecular weight of 54 kDa. Surprisingly, by comparing the molecular weight of the *P. aeruginosa* purified native urate oxidase with the recombinant one it was found that, the molecular weight of the native protein is higher than the recombinant protein (64 kDa *versus* 54 kDa). Sequencing data analysis using Blast search

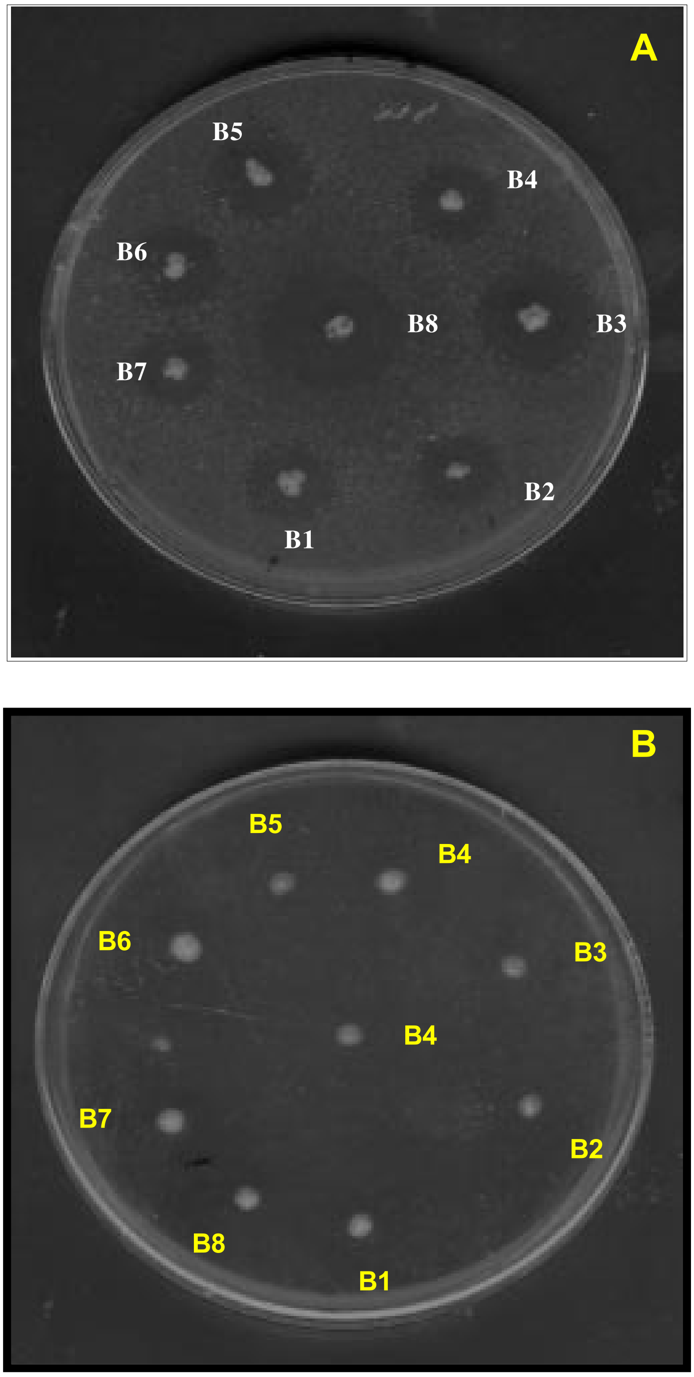


Fig. 2. Recombinant *E. coli* DH5α pTrc His B (clones B1-B8)
on LB/agar/ampicillin/uric acid plates IPTG induced (A) and uninduced (B)

program revealed that, the sequence of the cloned fragment was approximately 99.5% similar to a corresponding sequence of *P. aeruginosa* PAO1 isolate (accession number AF 540933) that encode a hypoothetical protein of 495 amino acids and with a molecular weight 59.4 kDa but still of unknown function. Of more interest however, is to study and to record the functional uricase activity of the previously characterized hypothetical protein from *P. aeruginosa* PAO1 along with the new recombinant urate oxidase clone. Moreover, alignment of sequencing data of putative uricase gene isolated from *P. aeruginosa* with other available uricases on GeneBank (from different organisms) indicated that, the highest similarity was to the gene encoding uricase enzyme from *Cellulomonas flavigena* (accession no. E06699) (Yagasaki *et al.*, 1994) that have approximately 49% amino acids similarity and 35% for nucleotides (Figure 5, 6). This was followed by lower similarity to the uricase gene of yeast-like symbiotic fungal ascomycetes species (Hongoh,

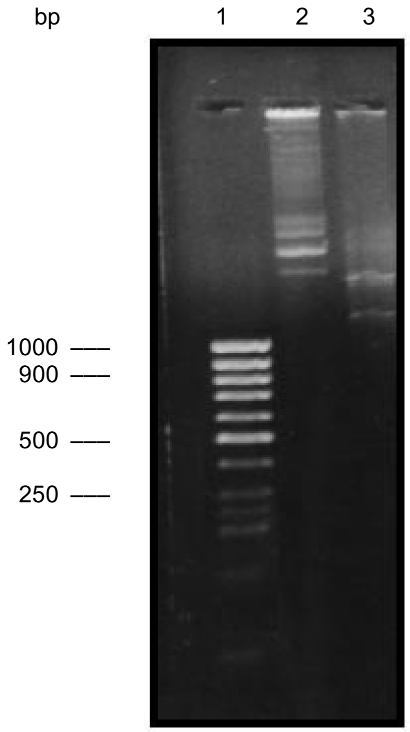


Fig. 3. Agarose gel (1%) electrophoresis for pTrcHis B (B8 clone) plasmid; uncut (Lane 2) and EcoRI cut (Lane 3). Lanes 1 represents 50 bp molecular weight DNA markers

2000): *Beauveria bassiana*, *Tolypocladium inflatum*, *Paecilomyces tenuipes* and *Cerataphis fransseni* and this was 35%, 29%, 27% and 24%, respectively. Lower similarity value still excise in comparison the uricase sequence of the *P. aeruginosa* with the corresponding sequence from *Candida utilis* (Koyama *et al*., 1996) and *Bacillus* sp. BT-90 (Yamamoto, 1996) that was 24% for both sequences. Sequences used for similarity search are shown in Table I.

Pilot expression study on *E.coli* DH5α pTrcHis-B8. A preliminary pilot expression study using *E. coli* DH5α pTrcHis-B8, the clone harboring the 1.4 kb DNA insert showed that, clear and discrete protein bands of molecular weight 52 and 59.4 kDa were obtained in IPTG induced cells (Figure 7, lane 1). On the other hand, these two uniques protein bands were very faint in uninduced *E. coli* DH5α pTrcHis-B8 (Figure 7, lane 2). The result of this expression study indicated that, the protein expressed in *E. coli* was of higher molecular weight (by approximately 5.4 kDa) than the expected size, 54 kDa. The difference in size could be due to a small leader sequence of amino acids added to the N-terminal region of the recombinant protein. This leader sequence represents 45 amino acids of molecular weight 5.4 kDa and encodes an ATG start codon, a histidine tag, enterokinase cleavage site and some other plasmid specific features. To complement what has been investigated before, uricolytic activity of the recombinant *E. coli* DH5α pTrcHis-B8 induced

Table I
Sequences used for similarity studies

Organism	Sequence description	Accession number	Reference
Cellulomonas flavigena	Uricase gene (CDS)	E06699	Yagasaki *et al*., 1994
Beauveria bassiana (Ascomycete)	Uricase gene, partial CDS	AB038707	Hongoh *et al*., 2000
Paecilomyces tenuipes (Ascomycete)	Uricase gene (CDS)	AB038709	Hongoh and Ishikawa, 2000
Tolypocladium inflatum	Uricase gene (CDS)	AB038708	Hongoh and Ishikawa, 2000
Cerataphis fransseni (yeast-like symbiont)	Uricase gene (CDS)	AB038706	Hongoh and Ishikawa, 2000
Candida utilis	Uricase gene	E05948	Koyama *et al*., 1996
Bacillus sp.	*Bacillus* sp. DNA for uricase gene	D49974	Yamamoto *et al*., 1996

A

1 TGGGGGGGTC TC**ATCATCAT CATCATCATG** GTATGGCTAG CATGACTGGT GGACAGCAAA
TGGGTCGGGA TCTGTACGAC GATGACGATA AGGATCGATG GATCCGACCT CGAGATCTGC
AGATGGTACC ATATGG**GAAT TC**GCCACCCA GGTCCTCGAC GAGGGCATGG CCGTCGACAA
GGACACCGTG GCGATGATCA ACGACCGCAT CAGCCAGATC GATGCGCTGA TCAGCGACCA
GCTCAACCAG ATCATCCACC ACCCCGAGTT GCAGAAGCTG GAAGCCTCCT GGCGCGGCCT
GCACCAACTG GTGAGCAACA CCGAGACCAG CGCGCGGCTC AAGCTGCGCC TGCTGAACGT
CGGCAAGAAC GAACTGCAGA ACGACCTGGA GAAGGCGGTC GAGTTCGACC AGAGCGCACT
GTTCAAGAAG ATCTACGAAG AGGAATACGG CACCTTCGGC GGACACCCCT TCAGCCTGCT
GATCGGCGAC TTCACCTTCG GTCGCCATCC GCAGGACATC GGCCTGCTGG AGAAGCTGTC
GAACGTCGCC GCGGCTGCCC ACGCGCCGTT CATCGCCGCC GCCAGCCCAC GCCTGTTCGA
CATGAACAGC TTCACCGAAC TGGCCGTGCC ACGCGACCTG ACCAAGATCT TCGAGAGCCT
GGAGCTGATC AAGTGGCGCG CCTTCCGCGA GAGCGAGGAC TCGCGCTACG TGTCGCTGGT
GCTGCCGAAC TTCCTCTTGC GCCTGCCCTA CGGCCCGGAG ACGCGACCGG TGGAAGGCAT
GAACTATGTC GAGGACGTCA ACGGCACCGA CCACTCCAAG TACCTCTGGG GCAACGCCGC
CTGGGTCCTG GCGCAGCGCA TCACCGAGGC CTTCGCCAAG TACGGCTGGT GCGCGGCGAT
CCGCGGCGCG GAAGGCGGCG GCGCGGTCGA GGGCCTACCG GCGCACACGT TCCGCACCAG
CTCCGGCGAC CTGTCGCTGA AGTGCCCGAC CGAGGTGGCG ATCACCGACC GCCGCGAGAA
GGAACTCAAC GATCTCGGCT TCATTTCCCT GTGCCACAAG AAGAACAGCG ACGTGGCGGT
GTTCTTCGGC GGCCAGACCA CCAACAAGGC CAGGCTCTAC AACACCAACG AGGCCAACGC
CAACGCGCGC CTGTCGGCGA TGCTGCCGTA CGTGCTGGCG GCATCGCGCT TCGCCCACTA
CCTGAAGGTG ATCATGCGCG ACAAGGTCGG CAGCTTCATG ACCCGCGACA ACGTGCAGAC
CTACCTGAAC AACTGGATCG CCGACTACGT GCTGATCAAC GACAACGCAC CGCAGGAAAT
CAAGGCGCAG TACCCGCTGC GCGAGGCGCG GGTGGATGTC AGCGAGGTGG CCGGCAAACC
GGGAGCCTAC CGCGCCACGG TGTTCCTCCG GCCGCACTTC CAGCTCGAGG AACTCAGCGC
1441 GTCGATCCGC CTGGTCGCCA ACCTGCCGCC GCCGGTAGCG GCGTGA 1486

B

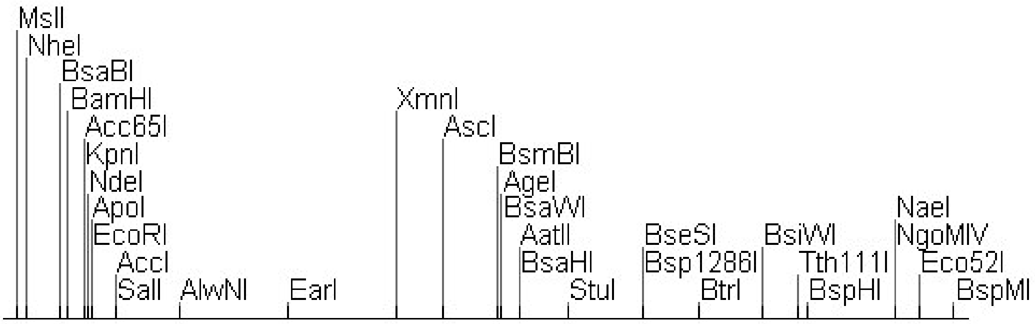


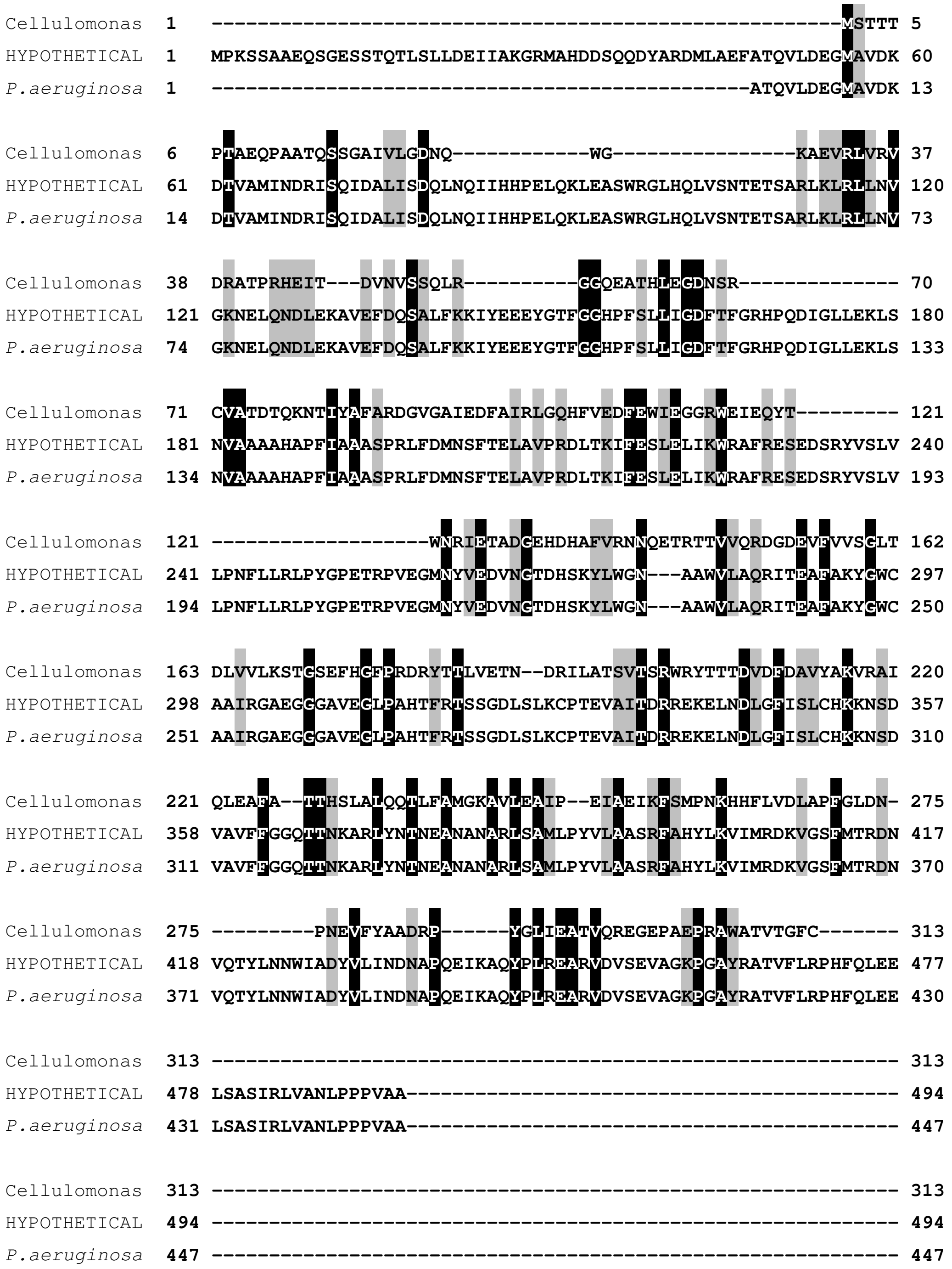
Fig. 4. (A) DNA sequence of *P. aeruginosa* DNA insert onto pTrcHis B (B8 clone) vector. The unique EcoRI site that was used for cloning is shown in bold and double underlined. The codon for the 6 histidine tag is shown in bold and underlined.
(B) Restriction map for the cloned *P. aeruginosa* DNA insert that represent partial coding sequence of putative uricase gene

with IPTG (1 mM), uric acid (0.15%) and uninduced cells were determined. Table II showed that, uninduced culture had no uricase activity utilizing uric acid as a substrate compared to either IPTG or uric acid induced cultures. Uricase activity after 12 hours of induction was almost 10 times higher in case of IPTG than in of uric acid induced culture under the same experimental conditions and under identical assay conditions.

Table II
Uricase activity of recombinant *E.coli* DH5α pTrcHis-B8 upon induction with IPTG and uric acid

Time (Hours)	Activity in uninduced culture (Units/ml)*	Activity in IPTG (1 mM) induced culture (Units/ml)*	Activity in uric acid (0.15%) induced culture (Units/ml)*
Zero time	0.1	0.45	1.0
12 hrs	0.026	190.0	19.0
24 hrs	0.03	196.0	31.0

* One unit of uricase is the amount of enzyme that consume one micromole of uric acid substrate per minute at 37°C



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Cellulomonas   1   -------------------------------------------------------MSTTT 5
HYPOTHETICAL   1   MPKSSAAEQSGESSTQTLSLLDEIIAKGRMAHDDSQQDYARDMLAEFATQVLDEGMAVDK 60
P.aeruginosa   1   -----------------------------------------------ATQVLDEGMAVDK 13

Cellulomonas   6   PTAEQPAATQSSGAIVLGDNQ------------WG----------------KAEVRLVRV 37
HYPOTHETICAL   61  DTVAMINDRISQIDALISDQLNQIIHHPELQKLEASWRGLHQLVSNTETSARLKLRLLNV 120
P.aeruginosa   14  DTVAMINDRISQIDALISDQLNQIIHHPELQKLEASWRGLHQLVSNTETSARLKLRLLNV 73

Cellulomonas   38  DRATPRHEIT---DVNVSSQLR----------GGQEATHLEGDNSR-------------- 70
HYPOTHETICAL   121 GKNELQNDLEKAVEFDQSALFKKIYEEEYGTFGGHPFSLLIGDFTFGRHPQDIGLLEKLS 180
P.aeruginosa   74  GKNELQNDLEKAVEFDQSALFKKIYEEEYGTFGGHPFSLLIGDFTFGRHPQDIGLLEKLS 133

Cellulomonas   71  CVATDTQKNTIYAFARDGVGAIEDFAIRLGQHFVEDFEWIEGGRWEIEQYT--------- 121
HYPOTHETICAL   181 NVAAAAHAPFIAAASPRLFDMNSFTELAVPRDLTKIFESLELIKWRAFRESEDSRYVSLV 240
P.aeruginosa   134 NVAAAAHAPFIAAASPRLFDMNSFTELAVPRDLTKIFESLELIKWRAFRESEDSRYVSLV 193

Cellulomonas   121 -------------------WNRIETADGEHDHAFVRNNQETRTTVVQRDGDEVFVVSGLT 162
HYPOTHETICAL   241 LPNFLLRLPYGPETRPVEGMNYVEDVNGTDHSKYLWGN---AAWVLAQRITEAFAKYGWC 297
P.aeruginosa   194 LPNFLLRLPYGPETRPVEGMNYVEDVNGTDHSKYLWGN---AAWVLAQRITEAFAKYGWC 250

Cellulomonas   163 DLVVLKSTGSEFHGFPRDRYTTLVETN--DRILATSVTSRWRYTTTDVDFDAVYAKVRAI 220
HYPOTHETICAL   298 AAIRGAEGGGAVEGLPAHTFRTSSGDLSLKCPTEVAITDRREKELNDLGFISLCHKKNSD 357
P.aeruginosa   251 AAIRGAEGGGAVEGLPAHTFRTSSGDLSLKCPTEVAITDRREKELNDLGFISLCHKKNSD 310

Cellulomonas   221 QLEAFA--TTHSLALQQTLFAMGKAVLEAIP--EIAEIKFSMPNKHHFLVDLAPFGLDN- 275
HYPOTHETICAL   358 VAVFFGGQTTNKARLYNTNEANANARLSAMLPYVLAASRFAHYLKVIMRDKVGSFMTRDN 417
P.aeruginosa   311 VAVFFGGQTTNKARLYNTNEANANARLSAMLPYVLAASRFAHYLKVIMRDKVGSFMTRDN 370

Cellulomonas   275 ---------PNEVFYAADRP------YGLIEATVQREGEPAEPRAWATVTGFC------- 313
HYPOTHETICAL   418 VQTYLNNWIADYVLINDNAPQEIKAQYPLREARVDVSEVAGKPGAYRATVFLRPHFQLEE 477
P.aeruginosa   371 VQTYLNNWIADYVLINDNAPQEIKAQYPLREARVDVSEVAGKPGAYRATVFLRPHFQLEE 430

Cellulomonas   313 ------------------------------------------------------------ 313
HYPOTHETICAL   478 LSASIRLVANLPPPVAA------------------------------------------- 494
P.aeruginosa   431 LSASIRLVANLPPPVAA------------------------------------------- 447

Cellulomonas   313 ------------------------------------------------------------ 313
HYPOTHETICAL   494 ------------------------------------------------------------ 494
P.aeruginosa   447 ------------------------------------------------------------ 447
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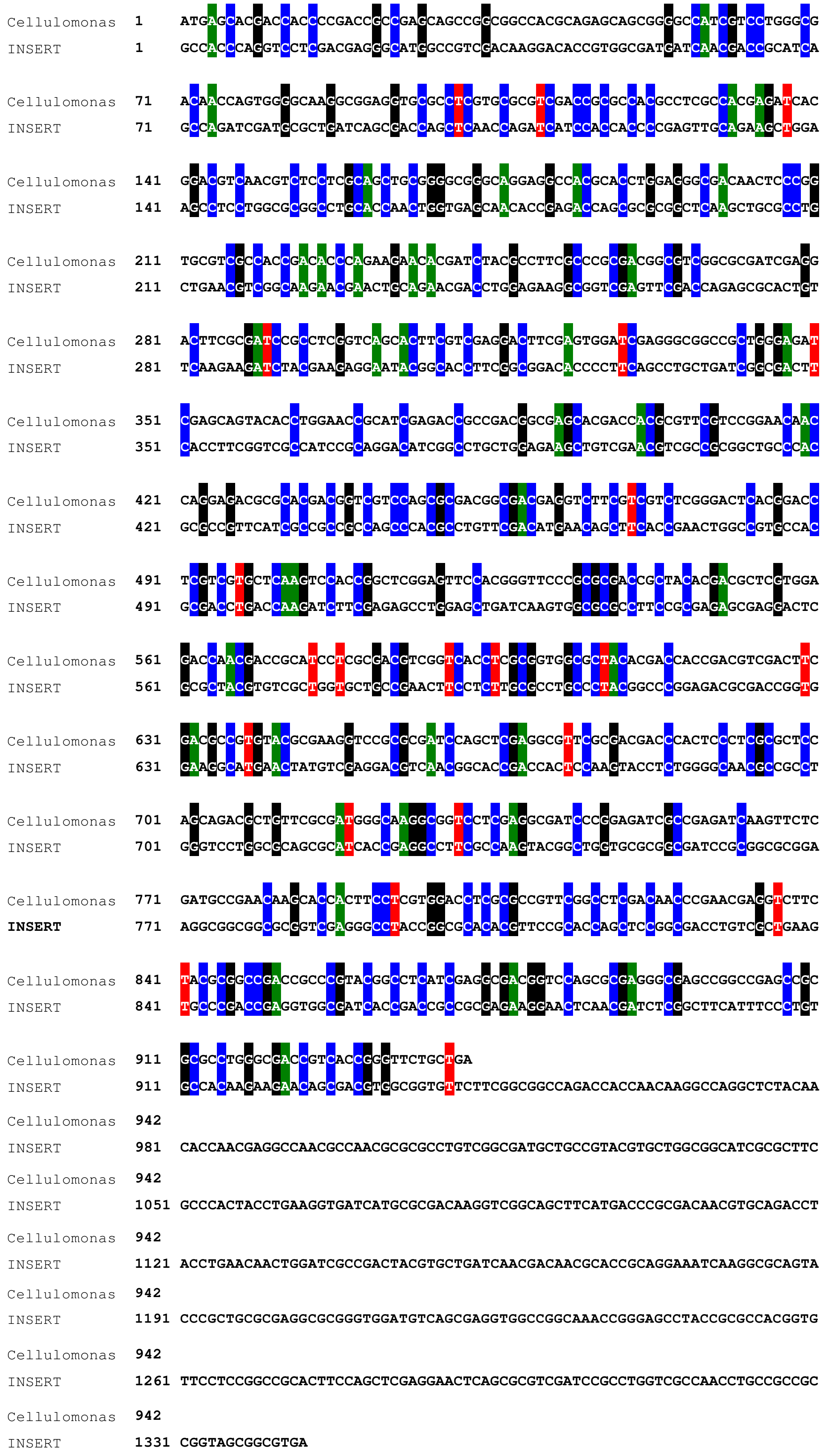
Fig. 5. Amino acid sequence alignment of uricase gene. The uricase came from *Cellulomonas flavigena*, *P. aeruginosa* PAO1 and *P. aeruginosa*. Conserved amino acids are dark shaded and residues that may be conserved are grey shaded.

Moreover, after 24 hours of induction no significant change in uricase activity was noted for both induced cultures as shown in Table II. Comparing the uricolytic activity of recombinant *E. coli* DH5α pTrcHis-B8 induced with IPTG with that of native uricase enzyme produced from *P. aeruginosa* it was found that, recombinant cells produced 16.21 times more (196 U/ml *versus* 12.09 U/ml) uricase enzyme than did the native strain after 24 hours of induction. The work undertaken in this study raises a number of interesting points with respect to *P. aeruginosa* Ps-x putative urate oxidase gene. First, sequencing data analysis of the cloned gene and the unidentified hypothetical gene of *P. aeruginosa* PAO1 revealed about 99% similarity but still some part of the cloned gene is missing probably near the N-terminal part of this gene. Second, pilot protein expression and activity studies clearly demonstrated that uric acid act as an inducer for the recombinant gene when expressed in *E. coli* cells but the exact mechanism for induction is



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Cellulomonas  1     ATGAGCACGACCACCCCGACCGCCGAGCAGCCGGCGGCCACGCAGAGCAGCGGGGCCATCGTCCTGGGCG
INSERT        1     GCCACCCAGGTCCTCGACGAGGGCATGGCCGTCGACAAGGACACCGTGGCGATGATCAACGACCGCATCA

Cellulomonas  71    ACAACCAGTGGGGCAAGGCGGAGGTGCGCCTCGTGCGCGTCGACCGCGCCACGCCTCGCCACGAGATCAC
INSERT        71    GCCAGATCGATGCGCTGATCAGCGACCAGCTCAACCAGATCATCCACCACCCCGAGTTGCAGAAGCTGGA

Cellulomonas  141   GGACGTCAACGTCTCCTCGCAGCTGCGGGGCGGGCAGGAGGCCACGCACCTGGAGGGCGACAACTCCCGG
INSERT        141   AGCCTCCTGGCGCGGCCTGCACCAACTGGTGAGCAACACCGAGACCAGCGCGCGGCTCAAGCTGCGCCTG

Cellulomonas  211   TGCGTCGCCACCGACACCCAGAAGAACACGATCTACGCCTTCGCCCGCGACGGCGTCGGCGCGATCGAGG
INSERT        211   CTGAACGTCGGCAAGAACGAACTGCAGAACGACCTGGAGAAGGCGGTCGAGTTCGACCAGAGCGCACTGT

Cellulomonas  281   ACTTCGCGATCCGCCTCGGTCAGCACTTCGTCGAGGACTTCGAGTGGATCGAGGGCGGCCGCTGGGAGAT
INSERT        281   TCAAGAAGATCTACGAAGAGGAATACGGCACCTTCGGCGGACACCCCTTCAGCCTGCTGATCGGCGACTT

Cellulomonas  351   CGAGCAGTACACCTGGAACCGCATCGAGACCGCCGACGGCGAGCACGACCACGCGTTCGTCCGGAACAAC
INSERT        351   CACCTTCGGTCGCCATCCGCAGGACATCGGCCTGCTGGAGAAGCTGTCGAACGTCGCCGCGGCTGCCCAC

Cellulomonas  421   CAGGAGACGCGCACGACGGTCGTCCAGCGCGACGGCGACGAGGTCTTCGTCGTCTCGGGACTCACGGACC
INSERT        421   GCGCCGTTCATCGCCGCCGCCAGCCCACGCCTGTTCGACATGAACAGCTTCACCGAACTGGCCGTGCCAC

Cellulomonas  491   TCGTCGTGCTCAAGTCCACCGGCTCGGAGTTCCACGGGTTCCCGCGCGACCGCTACACGACGCTCGTGGA
INSERT        491   GCGACCTGACCAAGATCTTCGAGAGCCTGGAGCTGATCAAGTGGCGCGCCTTCCGCGAGAGCGAGGACTC

Cellulomonas  561   GACCAACGACCGCATCCTCGCGACGTCGGTCACCTCGCGGTGGCGCTACACGACCACCGACGTCGACTTC
INSERT        561   GCGCTACGTGTCGCTGGTGCTGCCGAACTTCCTCTTGCGCCTGCCCTACGGCCCGGAGACGCGACCGGTG

Cellulomonas  631   GACGCCGTGTACGCGAAGGTCCGCGCGATCCAGCTCGAGGCGTTCGCGACGACCCACTCCCTCGCGCTCC
INSERT        631   GAAGGCATGAACTATGTCGAGGACGTCAACGGCACCGACCACTCCAAGTACCTCTGGGGCAACGCCGCCT

Cellulomonas  701   AGCAGACGCTGTTCGCGATGGGCAAGGCGGTCCTCGAGGCGATCCCGGAGATCGCCGAGATCAAGTTCTC
INSERT        701   GGGTCCTGGCGCAGCGCATCACCGAGGCCTTCGCCAAGTACGGCTGGTGCGCGGCGATCCGCGGCGCGGA

Cellulomonas  771   GATGCCGAACAAGCACCACTTCCTCGTGGACCTCGCGCCGTTCGGCCTCGACAACCCGAACGAGGTCTTC
INSERT        771   AGGCGGCGGCGCGGTCGAGGGCCTACCGGCGCACACGTTCCGCACCAGCTCCGGCGACCTGTCGCTGAAG

Cellulomonas  841   TACGCGGCCGACCGCCCGTACGGCCTCATCGAGGCGACGGTCCAGCGCGAGGGCGAGCCGGCCGAGCCGC
INSERT        841   TGCCCGACCGAGGTGGCGATCACCGACCGCCGCGAGAAGGAACTCAACGATCTCGGCTTCATTTCCCTGT

Cellulomonas  911   GCGCCTGGGCGACCGTCACCGGGTTCTGCTGA
INSERT        911   GCCACAAGAAGAACAGCGACGTGGCGGTGTTCTTCGGCGGCCAGACCACCAACAAGGCCAGGCTCTACAA

Cellulomonas  942
INSERT        981   CACCAACGAGGCCAACGCCAACGCGCGCCTGTCGGCGATGCTGCCGTACGTGCTGGCGGCATCGCGCTTC

Cellulomonas  942
INSERT        1051  GCCCACTACCTGAAGGTGATCATGCGCGACAAGGTCGGCAGCTTCATGACCCGCGACAACGTGCAGACCT

Cellulomonas  942
INSERT        1121  ACCTGAACAACTGGATCGCCGACTACGTGCTGATCAACGACAACGCACCGCAGGAAATCAAGGCGCAGTA

Cellulomonas  942
INSERT        1191  CCCGCTGCGCGAGGCGCGGGTGGATGTCAGCGAGGTGGCCGGCAAACCGGGAGCCTACCGCGCCACGGTG

Cellulomonas  942
INSERT        1261  TTCCTCCGGCCGCACTTCCAGCTCGAGGAACTCAGCGCGTCGATCCGCCTGGTCGCCAACCTGCCGCCGC

Cellulomonas  942
INSERT        1331  CGGTAGCGGCGTGA
```

Fig. 6. Nucleotides sequence similarity of *P. aeruginosa* DNA insert that encode uricase gene and *Cellulomonas flavigena* uricase gene. Conserved nucleotides are shaded

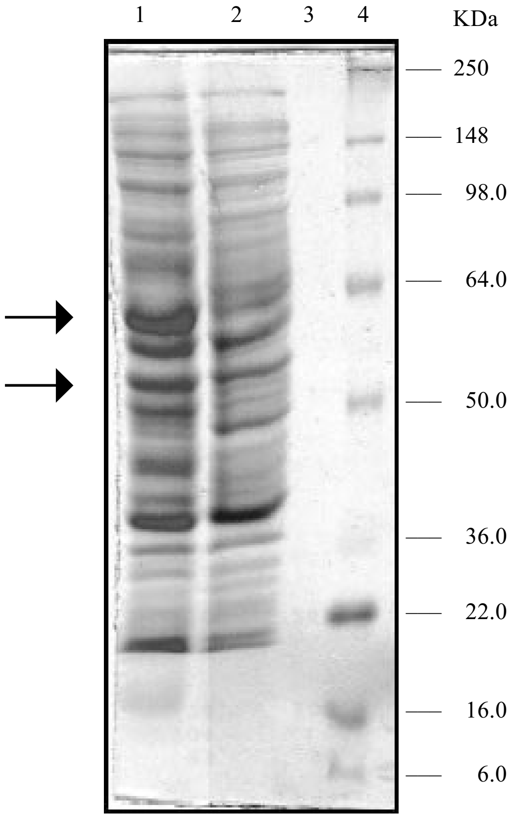

Fig. 7. Sodium dodecyl sulphate polyacrylamide gel (10%) (SDS-PAGE) electrophoresis of uninduced (Lane 2) and IPTG induced (Lane 1) *E. coli* DH5α pTrc His B8 cells. Lanes 3 and 4 represent an empty lane and prestained molecular weight markers proteins, respectively. Arrows indicate the location of the expressed proteins

not clear and need more investigation. From this and previous study can be concluded that *P. aeruginosa* possess urate oxidase gene and express it in active form.

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