Iran. J. Biotechnol. January 2024;22(1): e3602 DOI: 10.30498/ijb.2024.379614.3602

Research Article

Recombinant Expression and Functional Assessment of Uricase from a Pertinent Origin of the Enzyme, *Streptomyces* sp. Strain 17-1

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Received: 2023/01/04 ; **Accepted:** 2023/10/30

Background: Uricase or urate oxidase, as a therapeutic enzyme, is extensively applied to oxidize accumulated uric acid in the body to soluble form to treat related illnesses.

Objectives: This study was conducted with the aim of searching for potential sources of uricase-producing *Streptomyces* from Eshtehard salt desert in Alborz province, Iran and heterologous expression, purification and functional assay of the enzyme.

Materials and Methods: Main screening was conducted by cultivation of the strains on a medium enriched with 0.3 percent (w/v) uric acid. The uricase gene from the most potent strain was then recombinantly expressed in *E. coli* BL21 (DL3)

Results: Out of the tested strains, only seven showed uricase activity. The highest level of native uricase activity (11.5735 $U.mL^{-1}$) belonged to strain 17-1, which had the closest similarity to *Streptomyces nigra*. A recombinant uricase with a molecular mass of approximately 38 kDa was produced. The purified uricase exhibited a specific activity of about 28.29±0.59 $U.mg^{-1}$, which is among the highest level of uricase activity reported by other studies.

Conclusions: This enzyme is a promising candidate for further applicable investigations and large-scale production in terms of its large volume of soluble expression and selective competitive activity.

Keywords: Actinobacteria, pET28a^{+}, *Streptomyces*, Uricase

1. Background

As a characteristic product of digestion resulting from the disintegration of DNA molecules, uric acid is regularly excreted from the body (1). On the other hand, the pathogenesis of human ailments, for example, gout and hyperuricemia, are solidly connected to the high-lev-

el gradual addition of uric acid in the body or its low elimination rate (2). Due to its severe implications, the treatment countermeasures for hyperuricemia have received a lot of attention in the medical world. This disease afflicts more than 10 million patients worldwide, and its prevalence is increasing. Allopurinol has been considered a first-choice drug for the past three decades, which manages the biogenesis of uric acid via hindering xanthine uricase (XO) enzyme. Likewise, Febuxostat is applied to lessen uric acid production by restraining the XO enzyme in addition to Allopurinol, and the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA or USFDA) are affirmed it in 2008 and 2009, respectively. Using enzyme medicines is considered a better alternative to these treatments since they have fewer side effects while still controlling the accumulation of uric acid in soft tissues and serum. Uricase (EC 1.7.3.3), as a homotetramer drug enzyme, oxidatively changes uric acid to allantoin, CO_2 (Carbon dioxide), and H_2O by unlocking its purine ring (3,4). As a soluble molecule in plasma, Allantoin can be effortlessly discarded through the kidneys, as compared to uric acid, it is 5-10 times more soluble (5) the key molecule leading to gout in humans, into allantoin, but it is absent in humans. It has been produced as a PEGylated pharmaceutical where the purification is performed through three sequential chromatographic columns. More recently an aqueous two-phase system (ATPS. However, humans lack uricase due to developmental changes in its gene (6) high activity, high thermostability, high solubility and low immunogenicity. The latter property is believed to depend largely on sequence identity to the deduced h uman UOX (dH-UOXhigh activity, high thermostability, high solubility and low immunogenicity. The latter property is believed to depend largely on sequence identity to the deduced human UOX (dH-UOX, but the gene is highly-preserved in mammals (7) or uricase (EC 1.7.3.3 or uricase (EC 1.7.3.3, plants (8), fungi, bacteria and yeasts (4). In this way, uricase from different microorganisms was intravenously administered for nearly 40 years to treat hyperuricemia and gout (9). Moreover, uricase is valuable for clinical investigations for the enzymatic measurement of uric acid in organic liquids. Uricase may be used as a protein treatment to reduce the toxic rate of uric acid buildup. As a recombinant form of Uox (Urate oxidase), Rasburicase (Fasturtec®), with *Aspergillus flavus* origin, is the primary marketed Uox developed as preventive and for the treatment of intense hyperuricemia resulting from acute lymphoblastic leukemia in children (10) which catalyzes uric acid to allantoin, is highly effective in treating tumor lysis syndrome (TLS. Notwithstanding, the immunogenicity and short half-life of Rasburicase have constrained its clinical utilization (11). The FDA has endorsed Peglotticase, a PEGylated (PEG: Polyethylene glycol) form of chimeric porcine-baboon Uox (Krystexxa), in 2010 to treat adult patients with chronic gout which resists traditional treatments (12) allopurinol, febuxostat. Pegloticase has significantly reduced immune response stimulation and an extended half-life due to its higher homology to human uricase and the use of PEGylation. The examinations on a few other fanciful uricases have likewise been accounted for, such as chimeric uricases of porcine–human and canine–human origins, besides the FDA affirmed porcine–baboon chimera (PBC) (13). Functionality, consistency, and immunoreactivity of the enzyme should all be considered because developing therapeutic uricase for human use is a challenging task. In general, the medical community is enthusiastic about developing a recombinant enzyme that is homologous to human uricase to treat gout and hyperuricemia (14). While other microorganisms could deliver enzymes extracellularly by changing certain components of the culture media, as in *Bacillus thermocatenulatus* (15), and *Pseudomonas aeruginosa* (16) based on statistical experimental designs, was employed to enhance the production of uricase by Pseudomonas aeruginosa local isolate. Glucose supplementation to the basal production medium inhibits uricase production, perhaps by catabolic repression. For screening of bioprocess parameters significantly influencing uricase activity, the two-level Plackett-Burman design was used. Among fifteen variables tested; pH, $CuSO_4$ and $FeSO_4$ were selected based on their high significant effect on uricase activity. A near optimum medium formulation was obtained using this method with increased uricase yield by 15-folds. Response surface methodology (RSM, uricase is limited inside microorganisms, particularly in *Bacillus pasteurii* (17), and *Escherichia coli* (18). As one of the first attempts, Magda *et al*. separated *Streptomyces exofolitus* from soil, which was found to be a high producer of uricase (19).

Due to the increasing importance of uricase in clinical treatments and laboratory analysis, researchers are searching for novel sources of the enzyme that can

provide higher output and activity. *Streptomyces* is the biggest and most famous genus of the order *Actinomycetales*. As the industrious manufacturers of secondary metabolites, such as antibiotics and biological compounds like enzymes which have imperative utilizations either in medicine or agriculture, it accounts for up to 90% of actinomycetes detached from the soil samples (20,21)a zygomycete fungus with well-developed columellae was recognized to produce high levels of the enzyme in a short time. Classification of the isolated fungus was carried out according to the morphological and culture characteristics of the organism, and it was identified as *Mucor hiemalis*. The fungus was able to produce an intracellular urate oxidase in a fermentation medium mainly containing uric acid. Optimized composition of the medium consisted of (l-1 of distilled water.

2. Objectives

Access to microbial enzyme resources (in this case uricase) with high production ability and activity, especially from industrially important strains such as actinobacteria, can help to provide successful industrial-pharmaceutical cases. This study was conducted with the aim of searching for potential sources of uricase-producing *Streptomyces*, which are collected and identified by Iranian Biological Resource Center (IBRC) from a wide area of Eshtehard salt desert in Alborz province of Iran, and gene isolation and cloning, heterologous expression, purification and functional assay of the enzyme.

3. Materials and Methods

3.1.Microbial Strains and Growth Conditions

Seventy-six Actinobacteria strains were selected from Microorganism bank at IBRC. All strains were cultured on a specific malt-yeast agar medium (ISP2) consisting of ($g.L^{-1}$): Malt extract 10g, Yeast extract 4g, Glucose monohydrate 4g; distilled water up to 1 L; pH 7. Agar was added (20.0 g. L^{-1}) to prepare plates, and incubation was carried out at 28 °C for seven days.

3.2. Primary and Secondary Screening of Uricase-Producing Strains

Modified Bennett's agar medium for preliminary screening consisted of ($g.L^{-1}$): Yeast extract 1.0 g; Malt extract 1.0 g; Peptone, 2.0 g; Uric acid, 3.0 g; glycerol, 2.0 g; distilled water up to 1 L; pH 7. Agar plates were prepared by adding 20.0 g. L^{-1} agar. The first selection for uricase-producing strains was done by inoculating pure actinobacteria strains as spots on agar medium and growing them for seven days at 30 °C. The presence of microorganisms-derived uricase in the medium is specified by forming a transparent zone surrounding the colonies. The strains that produced more zones in a shorter period of time were chosen for further testing. In a 250 mL shaking flask, 50 mL of culture media was dispensed, followed by sterilization and inoculation. The media were incubated at 28 °C in a rotatory shaker (180 rpm). The amount of the enzyme which was produced in the medium was measured after seven days. The strain with the most voluminous amount of enzyme production was affirmed for additional experiments.

3.3. Uricase Assay

Uricase produces allantoin, carbon dioxide, and hydrogen peroxide by catalyzing the oxidation of uric acid. The products of this reaction are then measured by the oxidative coupling reaction with chromogens like 4-aminoantipyrine, phenol, and peroxidase. The activity of the enzyme was measured by incubating 300 µL cell lysate (containing enzyme) with a mixture of 400 µL sodium borate buffer (pH 8.5, 0.1 M) comprising 2 mM uric acid, 30 mM 4-aminoantipyrine, 100 µL of 1.5% phenol, and 50 µL peroxidase at 37 °C for 20 min (22). The reaction was terminated by adding 0.2 reaction volume of 0.1 M potassium cyanide solution. The samples were analyzed in a spectrophotometer at 540 nm against the blank. Under standard assay conditions, the amount of enzyme that produces 1 µmoL of H_2O_2 (Hydrogen peroxide) per minute is equal to one unit of uricase (21,23)

3.4. Identification of the Designated Actinobacteria Isolate

The actinobacteria strain with the highest uricase activity (strain 17-1) underwent detailed identification. Morphological studies were followed-up by growing on ISP media 2-7 as defined by Shirling and Gottlieb (1966) (24); Followed by incubation at 28 °C for 14 days. 2-dimensional thin-layer chromatography was used to determine phospholipid types.

3.5. Phylogenetic Investigation of 16S rRNA Sequence

The genomic DNA extraction from the strain was

performed according to the method described by Wilson (25). A total reaction volume of 50 µL was used for polymerase chain reaction, PCR, containing 10 ng of DNA, 0.4 mM dNTPs mix: 5%(v/v) DMSO (Dimethylsulfoxide); 1X PCR buffer; 2.5 mM $MgCl_2$; 2 units of Taq DNA polymerase; 0.4 µM of each forward 16S rRNA primer 27F (5'-AGAGTT TGATCMTGGCTCAG-3') and reverse 16S rRNA primer 1492R (5' GGTTA CCTTGTTACGACTT-3') and filled-up with distilled water up to 50 µL. The PCR profile was as follows: 5 min initial denaturation at 95 °C, followed by 35 cycles of fragment amplification, including denaturing step at 95 °C for 1 min, 1 min of primer annealing at 55 °C, and 90 sec of extension at 72 °C, with a subsequent final extension at 72 °C for 10 min. PCR result was separated on the agarose gel (21) and then sequenced. The sequence was then compared with EzBioCloud database, and its similarity was recognized, followed by drawing a phylogenetic tree by the Molecular Evolutionary Genetics Analysis (MEGA7) software.

3.6. Cloning Uricase Gene

The uricase coding sequence (903pb) was attained by PCR amplification using primers Uricase-F (5′-*TATACACATATG*CTSGGMCAGAACCAGTAC-3′) and Uricase-R (5′-TTGTTACTCGAGGAGGTTGG TSAKGTC-3′) which were synthesized based on the uricase gene sequence. Primer Uricase-F contained *Nde*I site (underlined), whereas *Xho*I site was added to Uricase-R primer. Histidine-tag sequences were implemented at both ends of uricase gene to help with more efficient purification. For PCR amplification, the reaction conditions were 95 °C for 30 s, 53 °C for 20 s, and 72 °C for 60 s with 35 amplification cycles. PCR product was ligated into the $pET28a^+$ expression vector to form the recombinant plasmid Uricase-$pET28a^+$, which was transformed into the expression host *E. coli* BL21(DL3). The positive clones were selected, sequenced, and screened for expression studies.

3.7. Expression of Uricase

A transformed colony with Uricase-$pET28a^+$ was inoculated in 6ml Luria- Bertani (LB) medium with 50 μg. mL^{-1} kanamycin incubated at 37 °C with overnight shaking (180-200 rpm). 100mL of LB medium in a 500mL Erlenmeyer was inoculated with 5 mL of the preculture. The culture was grown under the same conditions until the OD600 (Optical Density 600) reached 0.6, at which point induction was commenced with a final concentration of 0.1mM IPTG (Isopropyl ß-D-1-thiogalactopyranoside). Every hour, 1 mL of samples were pelleted, and the collected cells were resuspended in 50 µL of SDS-PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis) sample buffer, lyzed for 5 min by boiling, and then electrophoresed on SDS polyacrylamide gel (26). Western blot validation of the expressed protein was achieved using diluted (1:4,000) monoclonal anti-6X histidine-tag antibody (SAB2702218, Sigma Aldrich, USA). Subsequently, the membrane was incubated with tetramethylbenzidine (TMB) substrate solution at ambient temperature and then visualized (27)

3.8. Purification of Recombinant Enzyme

To purify and examine the recombinant protein, 100 mL of fresh LB culture (OD600=0.6) was induced with 0.1mM IPTG at 17 °C with shaking (180-200 rpm) overnight. The cells were collected by centrifugation at 7000 g for 8 min in cold conditions. The pellet was resuspended in 6 mL lysis buffer (5mM Imidazole, 50mM Tris-HCL pH 8.8, 0.3M NaCl) and disrupted by sonication (2 cycles of pulse sonication, each time 2 min at 0.5% amplitude -60W). The suspension was separated by 20 min of centrifugation at 10000 g, and the soluble phase was transferred into a new vial. The insoluble fraction was then suspended and solved in lysis buffer containing 8M Urea followed by centrifugation at 10000 g for 20 min. Both soluble and insoluble forms were analyzed on 12% SDS-PAGE, and the result was visualized by Coomassie brilliant blue staining. The soluble fraction was loaded into a Ni-NTA (Nickel-Nitrilotriacetic Acid) column, including 1mL of pre-equilibrated resin. Washing step was done three times with 2mL of wash buffer (50mM sodium phosphate pH 8.0, 20mM imidazole, 0.3M NaCl); afterwards, 1mL elution buffer containing 0.5M imidazole, 50mM sodium phosphate pH 8.0, 0.3M NaCl was added to the column twice. As previously disclosed, the enzymatic functionality of the His-tag purified uricase was assessed by spectrophotometric monitoring of the rise in hydrogen peroxide absorbance at 540 nm.

3.9. Activity Assay of the Recombinant Enzyme

Bradford protein assay procedure was used to

measure the concentration of purified uricase. The standard curve was drawn to calculate the protein concentration (27). The enzymatic activity of crude and pure uricase was next evaluated by spectrophotometry using the same methodology as previously reported for the assessment of native uricase activity, with the exception that 0.1 mL and 0.05 mL of crude and purified enzymes were utilized, respectively.

4. Results

4.1. Primary Screening and Secondary Examination of Uricase Production

Among the tested strains, only seven strains of bacteria received from IBRC were found to be capable of utilizing uric acid (0.3% uric acid) in a growth medium (Modified Bennett's agar). These strains were named strains 1-7, 17-1, MN-198, yellow, M77, M75, and 4-43. The utilization of uric acid by these strains was observed by the formation of a clear zone around the colonies. Of these strains, strain 17-1 showed the biggest zone formation within a shorter time period and was subsequently transferred to the broth media (**Fig. 1A, 1B**). After 96 hours of incubation at 28 °C, the uricase activity of the culture was measured to be 11.5735 $U.mL^{-1}.min^{-1}$ using spectrophotometry analysis.

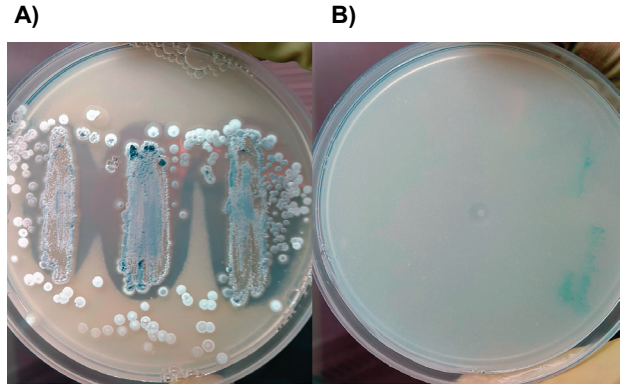

Figure1. Primary screening of the strains: **A**) Screening of strain 17-1 for uricase activity using plate method (formation of clear zones around the colonies indicated the presence of uricase activity); **B**) Negative Control

Table 1. Culture properties of the 17-1 strain

Growth medium	Growth	Aerial mycelium	Soluble pigment	Reverse color
ISP2 (Yeast extract -malt extract agar)	Good	bluish gray	strong yellow	bluish black
ISP3 (Oatmeal agar)	Good	Deep olive green	Yellowish green	Dark grayish olive green
ISP4 (Inorganic salt-starch agar)	Good	Pale blue	Yellowish white	Grayish blue
ISP5 (Glycerol asparagine agar)	Good	Yellowish white	Pinkish white	Yellowish gray
ISP6 (Peptone-yeast extract iron agar)	Good	Yellowish white	Moderate yellow	Yellowish white
ISP7 (Tyrosine agar)	Good	Olive gray	Dark yellow	Olive black

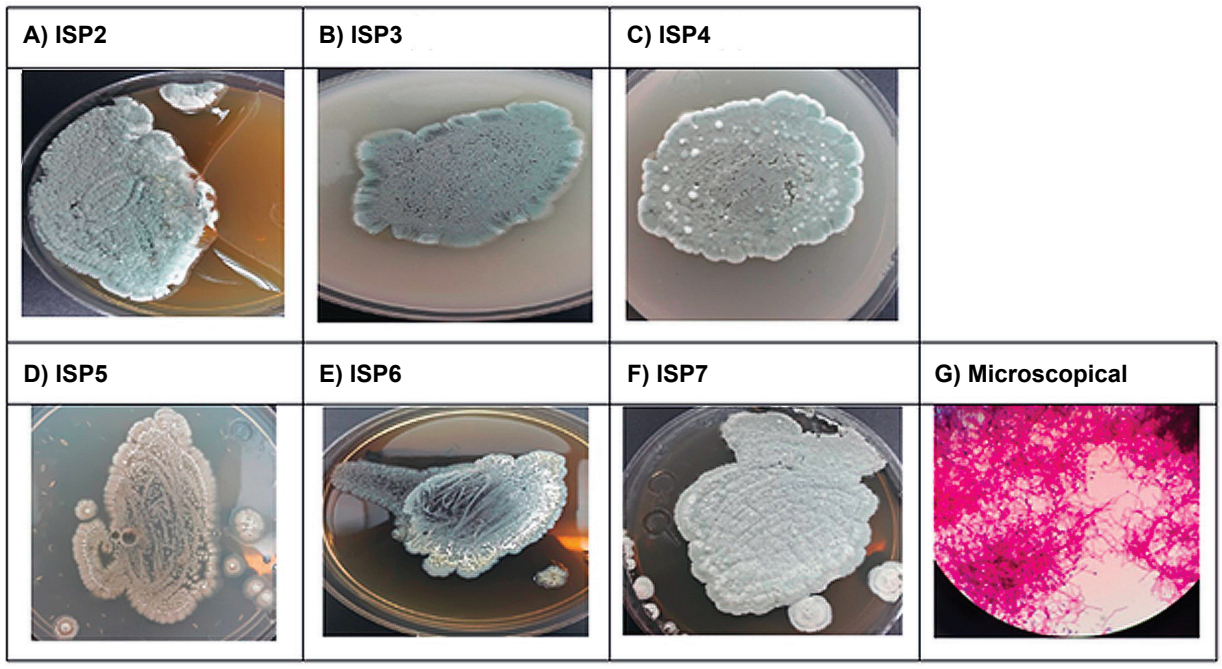

Figure 2. Characteristics of the selected strain: Morphological A-F) and microscopical **G**) characteristics of strain 17-1. Morphological features are studied in six culture media, which indicates the good growth of strain in all culture environments, but the colors of mycelium and pigments are different. complete explanations are given in the table1.

4.2. Polyphasic Identification and Molecular Phylogeny of the Uricase Producer Strain

Under a light microscope, the chosen strain, 17-1, was found to be a gram-positive bacterium capable of generating spore-chain with a filamentous structure that included aerial mycelium. Characterization of this strain is achieved by morphological and physiological properties (**Table 1** and **Fig. 2**). Further analysis of the bacterial cell wall and whole cell hydrolysates demonstrated diaminopimelic acid in the form of L-isomer. 16S rRNA sequencing results shows 99.71% identity with the *Streptomyces nigra* (accession number OK560047) (**Fig. 3**).

4.3. Recombinant Expression of Uricase and Purification of the Enzyme

The sequencing result of the recombinant plasmid containing uricase gene (Uricase-pET28a^{+}) confirmed the accuracy of gene sequence, and the sequence is now accessible from GenBank, NCBI with the accession number MW892815 (**Fig. S1**). The highest level of protein expression was reported 24 hours post-induction. SDS-PAGE results support the expression of a protein with a size of approximately 38 kDa and show the capability of *E. coli* expression system for overexpression of the enzyme. Despite the fact that both soluble and insoluble forms of expressed protein were visible on the gel, the amount of soluble protein was significantly higher, according to the gel data. An acceptable degree of purity of recombinant uricase is attained by using of Ni^{+2} affinity chromatography (**Fig. 4A**). To verify the identity of protein, western blot analysis was performed using an anti-histidine tag monoclonal antibody (Sigma Aldrich, USA), and a 38kDa protein band corresponding to the molecular weight of the expressed recombinant uricase is perceived (**Fig. 4B**).

4.4. Uricase Specific Activity

To assay the enzyme activity of purified fraction, spectrophotometric analysis was conducted as described in Methods. The activity of crude enzyme was calculated to be 13.63±0.69 $U.mg^{-1}$. Furthermore, the purified enzyme showed specific activity of

approximately 28.29±0.59 U.mg^{-1} affirming high-level functionality of pure uricase with 2- fold increase compared with the crude enzyme (**Table 2**).

5. Discussion

The conserved uricase enzyme is found in a wide range of taxa, from microbes to mammals, and more recently was isolated from a variety of bacteria and fungi (3,10,19). However, genetic changes during the evolution of primates have led to its inactivation in mammals (29). This enzyme catalyzes the oxidative reaction of converting urate to allantoin. Regarding its more solubility, allantoin can be easily excreted. Uricase can be used for therapeutic and diagnostic applications thanks to this property. The major part of the enzyme available in *Microbacterium* ZZJ4-1*, B. subtilis, Candida utilis, Pseudomonas vulgaris* B317-C*, Aspergillus flavus Proteus vulgaris* 1753*, Streptomyces albidoflavus, Streptomyces cyanogenus* and *Streptomyces graminofaciens* is intracellular; therefore, its extraction requires cellular destruction (19). However, in some microbial sources, such as *Pseudomonas aeruginosa* and *Bacillus fastidiosus*, uricase is available extracellularly, and there is no need for cellular destruction. Actinobacteria are among the main sources of producing bioactive metabolites and important medical enzymes. Among the strains generating uricase, *Streptomyces sclerotialus, Streptomyces alboniger*, *Streptomyces citreus*, *Streptomyces orientalis*, and *Streptomyces corchorusii* can be mentioned (30). Despite the numerous resources available for producing this enzyme, there are some challenges as low expression level; low stability and low efficiency in its mass production. Additionally, the steps involved in protein purification have some complications for clinical applications. Another chal-

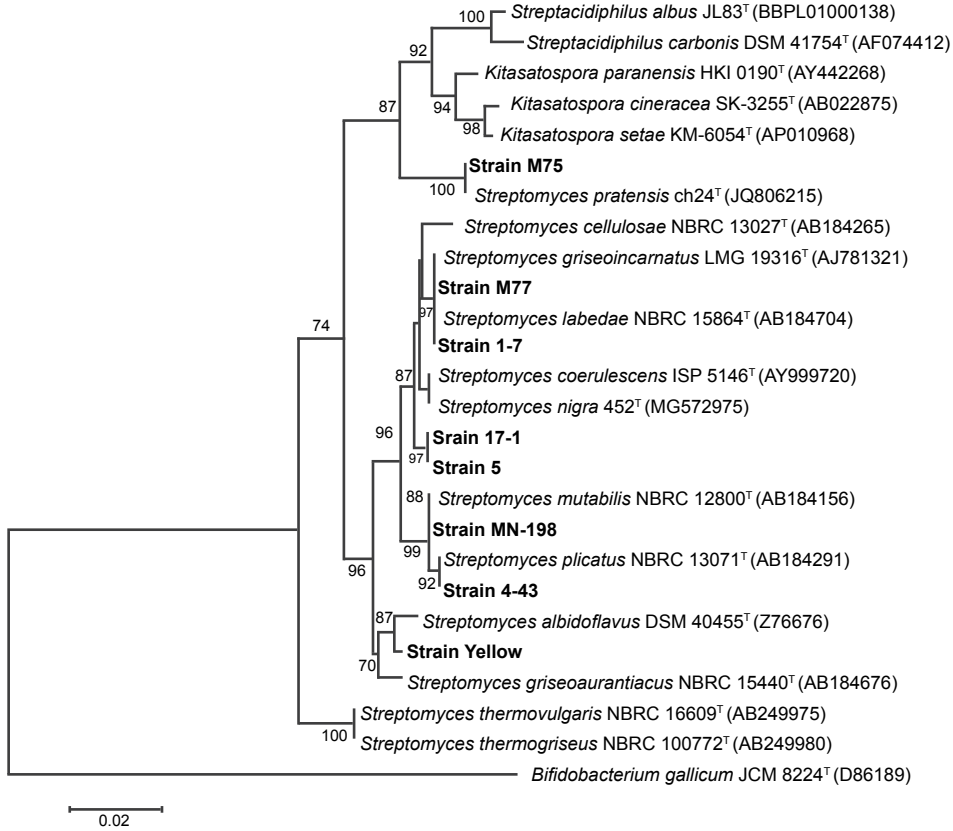

Figure3. Relationships between strain 17-1 and related species of the genus *Streptomyces*; Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences was conducted in MEGA7 software with bootstrap degree of 10000. GenBank sequence accession numbers are indicated in parentheses after the strain names.

Table 2. The enzymatic activity of the crude and purified uricase

Protein				Enzyme			
Purification step	Fraction volume (ml)	Concentration ($mg.ml^{-1}$)	Total protein (mg)	Total activity (units)	Specific activity ($units.mg^{-1}$)	Purification yield (%)	Purification factor (fold)
Crude extract	10	10	100	1362.7	13.63±0.69	100	1
Nickel gel filtration	2	5	10	282.9	28.29±0.59	21.3	2.08

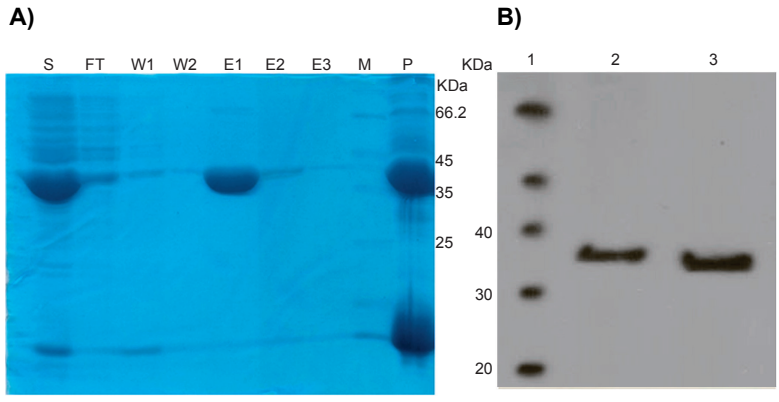

Figure 4. Recombinant uricase purification steps: **A**) Fractions collected after $nickel^{2+}$-nitrilotriacetic acid (Ni-NTA) chromatography were visualized on 12% SDS-PAGE. Lane S, soluble purified protein; Lane FT, flow-through; Lane M, protein Marker(SM0431); Lane W1-W3, washing step (20mM imidazole); Lane E1-E3, purified uricase after elution with 800mM imidazole; Lane P, precipitate. **B**) Western blot detection of recombinant uricase using anti-histidine tag monoclonal antibody. lane 1: protein ladder; lane 2 and 3: purified uricase, 1 and 2 µL, respectively.

lenge is the high cost of treatment with recombinant pharmaceutical enzymes, including uricase. Therefore, it is necessary to develop efficient expression systems to cheaply produce large volumes of recombinant enzymes. Currently, recombinant uricase from *S. cerevisiae* or *E.coli* was commercially marketed (31) It was shown that, at sufficient concentrations of uric acid in the culture medium, *P. aeruginosa* Ps43*, A. niger, Proteus vulgaris* 1753 and B-317-C are induced to uricase production (28).

Seventy-six 76 bacterial strains with the capability of growing in the media containing uric acid, as the major source of carbon and energy, were screened. Based on the uricase test findings, the 17-1 strain was chosen for future investigation as the most gifted in uricase synthesis. The activity is assessed by clear zones that coincided with the bacteria growth areas using Bennett's modified agar medium containing uric acid at a concentration of 0.3 percent. Hence, the maximum activity of uricase production by 17-1 as 11.5735 $U.mL^{-1}.min^{-1}$ was achieved using uric acid at the concentration of 0.3%. This result is in accordance with the report of Lotfy *et al*. about *Bacillus thermocatenulatus* increased uricase production in the presence of 0.3% uric acid. However, the efficiency of proposed process is greater than that of their reported value. Moreover, a similar

result was reported about *Microbacterium sp.* ZZJ4-1 indicating the best concentration of uric acid as 0.3% for uricase generation (0.6 $U.mL^{-1}$) (15,32). Based on its biochemical analysis, as well as the morphological and physiological features, isolate 17-1 belongs to the genus *Streptomyces*. The identified characteristics were confirmed utilizing 16S rRNA gene sequencing, which is a helpful route for deducing phylogenetic and evolutionary correlations among different bacterial species. The 16S rRNA gene sequence of strain 17-1 was compared with the EzBiocloud database and it was found that this strain is similar to *Streptomyces nigra* (strain 452) with a similarity rate of 99.71%. The specific activity of the purified uricase (28.29 $U. mL^{-1}$) was two times higher than that of the crude enzyme (13.63 $U.mL^{-1}$). Some studies revealed that pure enzyme generated by *Gliomastix gueg* (NRC 1A) has a high uricolytic activity of 146.84 $U.mL^{-1}$ and the specific activity of 157.13 $U.mg^{-1}$ protein (33), while others have lower activity e.g. *Streptomyces sp*. (2.25 $U.g^{-1}$ wet cells), *Streptomyces graminofaciens* (8 $U.mg^{-1}$), and *Streptomyces cyanogenus* (12.3 $U.mg^{-1}$) (34). In another investigation, researchers cloned and expressed the uricase gene of *B. subtilis* and *Pseudomonas aeruginosa* Ps43 in *E. coli*. Purification of uricase generated by *P. aeruginosa* Ps43 results in a two-fold increase in the specific activity compared to the crude enzyme (15 $IU.mg^{-1}$ vs. 7.2 $IU.mg^{-1}$) (28). Based on SDS-PAGE result, the molecular weight of purified recombinant uricase was estimated around 38 kDa. The amino acid sequences and molecular weights of the uricase molecules synthesized in various projects from various sources varied. According to published reports, the molecular weights monomer form of uricase produced by *P. aeruginosa, Candida utilis,* and *Nocardia farcinica* are 33, 34 and 54 kDa, respectively (35). Thus, we indicated, using the native PAGE that the functional form of our uricase is a homo-tetramer molecule with approximately 140 kDa in size (gel data not shown). According to Montalbini *et al*. (1997), the molecular weight of native uricase originating from plant leaves was 120-130 kDa as a tetramer molecule (36); However, Saeed *et al*.'s study (2004) showed that uricase originating from *Pseudomonas aeruginosa* has a molecular weight of 68.0 kDa for one subunit while *Bacillus fastidiosus* produces an enzyme with a molecular weight of about 145-150 kDa in tetramer form (37,38). Likewise, the uricase produced by *N. crassa* was purified up to a 1000 times to be homogenous (39) and showed a tetramer molecule made up of four identical subunits.

In conclusion, this work presents the efficient production and purification of recombinant uricase from an enzyme-producing *Streptomyces* strain in *E. coli*. The product was purified and contained a sufficient quantity of the enzyme's active form and may be used to enzymatically determine the urate levels in blood and urine during clinical trials. It is also useful to detect gout in which urate accumulation is a causative factor.

6. Conclusion

This study presents an efficient method for producing and purifying recombinant uricase from a *Streptomyces* strain in *E. coli*, and the resulting enzyme, Due to its high potential for soluble expression and competitive specific activity, may be useful for clinical purposes. For example, it can be employed in detecting gout by measuring urate levels in blood and urine. It is important to note that further research is required to address the challenges in uricase production and purification. Nevertheless, this work has provided a promising avenue for future studies and applications, considering the potential implications of molecular docking studies and PEG attachment.

Acknowledgment

The authors gratefully thank to the Iranian Biological Resource Center, where our research was conducted, and Research Council of the University of Guilan for technical support of this study.

Conflict of Interest

There is no conflict of interest.

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