

## Isolation and Characterization of Highly Active Uricase from *Alcaligenes* spp. Strain UR1

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### Abstract

For the first time, this study reports extracellular uricase enzyme isolation and characterization from strain UR1 of *Alcaligenes* spp. from Western Saudi Arabia. The strain efficiently produced highly active extracellular uricase for therapeutic applications. It offers a simplified enzyme purification approach rather than complicated intracellular enzyme purification from other microbes. Strain UR1 exhibited significantly higher uricase synthesis potential [916 U/mg (specific activities) and 275 U/ml (volume)]. The study optimized the conditions (37°C and pH 7.4) for 10% enhanced uricase production in the BT medium where sucrose served as the carbon source. Uricase enzyme remained stable at various pH levels (5–9) up to 50°C, however, the optimal activity was noted at 40°C and pH 7.5. The strain was sensitive to EDTA-like inhibitors. Ca<sup>2+</sup> improved the strain activity, which could yield potent formulations for clinical and industrial applications. This novel aspect presents *Alcaligenes* spp. strain UR1 as a promising candidate for the treatment of hyperuricemia and gout. It offers an efficient and inexpensive alternative for uricase synthesis at the industrial scale. These findings encourage further investigations regarding genetic aspects of uricase for improved bioprocessing and therapeutic applications.

**Key words:** Uricase, uricase-producing-bacteria, therapeutic enzyme, *Alcaligenes* spp., uricase purification, uricase characterization

### Introduction

Uric acid (UA) is well known to prevent nervous system diseases and its lower level contributes to various autoimmune diseases such as lichen planus, Parkinson's disease, and pemphigus vulgaris (Bakhtiari et al. 2017). Humans' metabolism of purine derivatives yields uric acid as the end product. Metagenes in the gene coding sequence prematurely terminate the translation process, leading to the absence of uricase in humans. Excessive uric acid production could cause gout, kidney disorder, and hyperuricemia. Direct injection of urate oxidase is suggested to counter gout-linked renal complications and prevent chemotherapy-associated hyperuricemia

disorders. Thus, uricase is a promising enzyme with clinical applications. High protein intake or reduced uric acid excretion via the kidneys increases blood uric acid levels (Xu et al. 2017; Bobulescu and Moe 2012). High uric acid levels damage kidneys' surface cells and weaken their physiological activity, leading to chronic kidney diseases, particularly in Type-II diabetic patients (Kim et al. 2015; Xiao et al. 2015). Uric acid accumulation in the kidneys is the most common cause of kidney stones (Fathallah-Shaykh and Cramer 2014; Jalal 2016). A high level of uric acid also stimulates autacoids and hormones' growth leading to high blood pressure. The infiltration of uric acid into smooth muscles weakens the heart muscle causing various cardiovascular

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diseases (Kanbay et al. 2013). High uric acid levels also complicate the refractory gout treatment. The peroxisomal (oxidoreductase) uricase enzyme catalyzes the purine ring's oxidative opening in the urate pathway to generate allantoin, hydrogen peroxide, and carbon dioxide (Chaudhary et al. 2013; El Ridi et al. 2017). Uricase is used as a protein drug to reduce the accumulation of toxic urate and treat hyperuricemia, gout, and bedwetting. Recent studies have used uricase as a diagnostic reagent in biochemical diagnosis and as a sensitive, accurate biosensor to monitor uric acid levels in the urine and blood (Hafez et al. 2017; Cheung et al. 2020; Tandon et al. 2021). Uricase also serves as an additive in hair coloring agents' commercial formulations, and in various medical, industrial, and agricultural applications (Elbanna et al. 2024). Diverse applicability has significantly enhanced the demand for this therapeutic enzyme.

Several organisms (microbes, animals, and plants) are known for uricase production (Elbanna et al. 2024). However, microbes offer cost-effective bioprocessing, convenient optimization of the medium, and higher growth rates. Green and Mitchell isolated the first microbial uricase in 1957 from the fungus *Neurospora crassa* (Perez-Ruiz et al. 2014). Then, multiple researchers isolated it from different bacteria such as *Nocardia farcinica*, *Pseudomonas aeruginosa*, *Microbacterium* spp., *Bacillus thermocatenulatus*, *Bacillus fastidious*, *Arthrobacter globiformis*, and *Bacillus subtilis* (Saeed et al. 2004; Suzuki et al. 2004; Zhou et al. 2005; Lotfy 2008;). Uricase production is extracellular in *B. subtilis*, *P. aeruginosa*, and *B. thermocatenulatus* whereas it is intercellular in *Microbacterium* spp., *Proteus vulgaris*, *Streptomyces exfolitus*, *Streptomyces graminofaciens*, and *Streptomyces albidoflavus* (Zhou et al. 2005; Aly et al. 2013). Lactic acid bacteria (*Pediococcus* spp., *Leuconostoc* spp., *Bifidobacterium* spp., *Lactobacillus* spp., and *Enterococcus* spp.) are known to degrade uric acid and their administration in rats effectively suppressed serum uric acid levels (Ogawa 2006). *Alcaligenes faecalis* could synthesize high uricase levels. This bacteria flourishes in birds and poultry intestines, soil, water, hospitals, and moist environments. Despite its presence in diabetic ulcers, it is non-pathogenic to humans (Simmons et al. 1979; Versalovic 2011; Abo-Amer et al. 2015).

Multiple factors could affect uricase enzyme activity. Previous studies have reported an optimum pH of 8 for uricase activity within a thermal range of 25–45°C. Uricase exposure to 60°C for an hour could reduce its activity by 50% (Chohan and Becker 2009; Ravichandran et al. 2015). Heavy metals are established cofactors for specific enzymes, but their higher concentration could inhibit enzyme activity (Suzuki et al. 2004). Copper inhibited uricase activity, which contradicts the

findings of Ravichandran et al. (2015), who reported a copper-based enzyme stimulation by 140%. Similarly, EDTA (1M) based enzyme stimulation has also been reported in various studies (Chohan and Becker 2009; Ravichandran et al. 2015). Nitrogen and carbon sources in the fermentation media are crucial for the uricase-producing microorganisms' growth and uricase productivity. Uricase production with different carbon sources has been reported whereas peptone is known to facilitate the highest uricase production among various other nitrogen sources (Pfrimer et al. 2010). Most published microbial uricase enzymes are intracellular, requiring cellular disruption to yield uricase. The production of extracellular enzymes is crucial for reducing the time and effort during the purification process (Elbanna et al. 2024).

Most uricase production-related studies focus on labor-intensive and expensive intracellular purification methods. *Alcaligenes* spp. It inhabits diverse types of environments; however, its extracellular uricase-producing potential remains unexplored. This study highlights the significantly higher uricase synthesizing potential of *Alcaligenes* spp. Strain UR1 as compared to previous reports from other microbes. The findings of this study could help devise large-scale economic uricase production systems. The study further elaborates on optimized uricase production parameters for its improved applications. Thus, the study collectively involves isolating, identifying, and characterizing a novel uricase-producing bacterial strain and analyzing its industrial and therapeutic potential.

## Experimental

### Materials and Methods

**Sample collection and preparation.** Different types of environmental samples from the cities of Makkah and Taif, Saudi Arabia, were used to isolate uricase-producing bacteria. Samples comprised 20 pigeon and 20 chicken fecal droppings, which were collected from the pigeon and poultry farmers. Samples also included 10 agricultural soils, which were collected at a depth of 10–15 cm after removing the surface layer. An ice box was used to transfer the collected samples to the laboratory. High urea levels in chicken fecal droppings and urea-added agricultural soils make them a reliable source for isolating uricase-producing bacteria.

**Isolation and screening of uricase-producing bacteria.** A uric acid enrichment broth medium [uric acid (8 g/l),  $\text{KH}_2\text{PO}_4$  (0.5 g/l),  $\text{K}_2\text{HPO}_4$  (2 g/l), NaCl (0.1 g/l),  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  (0.1 g/l), and  $\text{CaCl}_2$  (0.1 g/l)] was prepared with an initial pH of 7.4 followed by sterilization for 15 min at 121°C to enrich uric acid-degrading bacterial

cultures (Ghosh and Sarkar 2014). Diluted samples (5 ml) were inoculated in the uric acid enrichment broth medium followed by incubation (48 h and 37°C) under shaking conditions. To isolate uricase-degrading bacteria, serial dilutions of enrichment cultures were transferred into the Petri dishes followed by the pouring of basal trace agar medium [uric acid (3 g/l),  $\text{KH}_2\text{PO}_4$  (0.5 g/l),  $\text{K}_2\text{HPO}_4$  (2 g/l), NaCl (0.1 g/l),  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  (0.1 g/l),  $\text{CaCl}_2$  (0.1 g/l),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (2.5 g/l),  $\text{CuSO}_4$  (2.5 g/l), and agar (15 g/l) at pH 7.2] (Ghosh and Sarkar 2014). The inoculated plates were incubated for 48 h at 37°C. Uric acid-degrading bacterial colonies appearing with clear zones were inoculated in new uric acid agar plates [uric acid (8 g/l),  $\text{KH}_2\text{PO}_4$  (0.5 g/l),  $\text{K}_2\text{HPO}_4$  (2 g/l), NaCl (0.1 g/l),  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  (0.1 g/l), and  $\text{CaCl}_2$  (0.1 g/l), and agar (15 g/l)]. The clear zone-forming isolates were sub-cultured multiple times in nutrient agar (NA) (HiMedia Laboratories Pvt. Ltd., India) and microscopic examinations were performed to confirm their purity. These isolates were coded with a “UR” and kept at 4°C as slant cultures.

**Preservation and purity control.** Uricase-producing strains were streaked onto nutrient agar (NA) plates and incubated for 24 h at 37°C. After the purity check, the strains were inoculated in Nutrient broth (NB) (HiMedia Laboratories Pvt. Ltd., India) for 24 h at 37°C. Then, 500 µl of sterile glycerol was added to the 500 µl of each culture to make a final concentration of 20% and 7.5% (v/v), respectively. The tubes were stored at –80°C until further use. Culture purity was regularly confirmed through microscopic examinations, and single colonies were obtained by streaking on NA.

**Phenotypic characterization of uricase-producing isolates.** Uric acid agar was used to test the uricase activity of all isolates, which were then partially characterized based on the Gram staining, cell morphology, motility, and sporulation. The most promising uricase-producing isolates were selected based on the uricase activity and subjected to phenotypic and genotypic characterizations. The method of Gerhardt et al. (1994) was adopted for the phenotypic characterization. API 20E test kit (bioMérieux, France) was utilized for further biochemical and sugar fermentation tests, whereas more biochemical tests were performed using the Vitek® 2 compact system (bioMérieux, France) (Yang et al. 2012). Nutrient broth medium was used to study the differential growth at varying pH (pH 4–9), temperatures (20–45°C), and salt concentrations (0–9% NaCl), and a UV-VIS spectrophotometer (JASCO V-530; JASCO International Co., LTD., Japan) was employed to monitor bacterial growth at 650 nm.

**Hemolytic activity and antimicrobial susceptibility testing.** The procedure of Abdalrahim et al. (2019), was followed to perform the hemolytic activity test. Briefly, the bacterial cultures of selected uricase-pro-

ducing strains were spotted on sheep blood agar plates (Saudi Prepared Laboratory Media – SPLM-Riyadh, Saudi Arabia) that were incubated for 24 h at 37°C. The antibiotic susceptibility profiling of the selected uricase-producing strains was conducted as well employing the Kirby-Bauer disk diffusion method (Bauer et al. 1966) according to the guidelines of Clinical and Laboratory Standards Institute (CLSI 2024), briefly, a 0.5 McFarland standard suspension was prepared from each isolate and spread onto Mueller-Hinton agar (HiMedia Laboratories Pvt. Ltd., India) plates and incubated at 37°C for 18–24 h after placing the antibiotic disks. Antimicrobial susceptibility testing was performed against 12 antibiotics (Oxoid™, Thermo Fisher Scientific Inc., USA) as follows: Piperacillin (100 µg), Ceftazidime (30 µg), Cefepime (30 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Gentamicin (10 µg), Amikacin (30 µg), Tetracycline (10 µg), Aztreonam (30 µg), Chloramphenicol (30 µg), and Trimethoprim-Sulfamethoxazole (1.25/23.75 µg).

**Genomic DNA isolation, PCR amplification, and 16S rRNA gene sequencing.** Nutrient broth (HiMedia Laboratories Pvt. Ltd., India) medium (50 ml) was used to grow (24 h at 37°C) uricase-producing strains for genomic DNA isolation. The cultures were centrifuged (4,000 rpm, 20 min) and pellets were washed once with a TE-buffer. Genomic DNA isolation was performed by following the method of Elbanna et al. (2010). PCR amplification of the 16S rRNA gene was carried out using the universal eubacteria primers. Electrophoresis was performed to confirm the PCR products (~1,540 bp), which were further purified by using a PCR extraction kit (Macherey-Nagel GmbH & Co. KG, Germany). The purified PCR samples were sent to Macrogen, South Korea to sequence 16S rRNA fragments with an Applied Biosystems™ 3730x1 automated DNA sequencer (Thermo Fisher Scientific Inc., USA). Then, the Clustal-X tool was employed to align obtained sequences of selected and reference strains (retrieved from the NCBI database) (Thompson et al. 1997). Tree View was used to construct and display the phylogenetic tree (Page 1996). The neighbor-joining method with a 100 bootstrap value was adopted to prepare a phylogenetic tree of uricase-producing other closely related strains of genus *Alcaligenes* spp. (Saitou and Nei 1987). The *E. coli* strain ATCC® 35218™ (J01859.1) and *B. subtilis* strain ATCC® 21331™ (AB018487.1) served as the out-group.

**Factors affecting the growth and uricase secretion of strain UR1.** The growth and uricase secretion of strain UR1 were assessed under various parameters, including pH (pH 5–9), temperature (20–50°C), carbon sources (maltose, glucose, dextrose, sucrose, cellulose, and fructose), and nitrogen sources (urea, beef extract, peptone, yeast extract, casein,  $(\text{NH}_4)_2\text{SO}_4$ , and  $\text{NaNO}_3$ ).

Uric acid medium (uric acid, 0.3% w/v) was added into an Erlenmeyer flask (250 ml) and inoculated with a pre-culture (10 ml) that was grown on the nutrient broth for 15 h (Ghosh and Sarkar 2014). Nutrient agar plates were used to count the viable cells of strain UR1 and to monitor its growth. In contrast, cell-free supernatant was used to measure the uricase-secretion activity at 293 nm spectrophotometrically.

**Production and purification of uricase from strain UR1.** The uricase enzyme was electrophonically purified from one liter of cell-free strain UR1 culture supernatant with diethylaminoethyl-cellulose (DEAE cellulose) followed by Sephadex G100 column chromatography. Briefly, strain UR1 was cultivated in Erlenmeyer flasks (250 ml) with 50 ml BT, and 0.3% uric acid as a sole carbon source. The flasks were incubated (48 h, 37°C) on a rotary shaker (180 rpm) followed by centrifugation (8,000 rpm). A rotary evaporator was used to concentrate one litter of cell-free culture supernatant to 100 ml, and uricase was precipitated by cooling with ammonium sulfate (60%). The resultant precipitate was dissolved in 5 ml Tris-HCl buffer (0.02 M, pH 7.5) and dialyzed overnight in the same buffer (2 l) in a dialysis tube to remove salts and ammonium sulphate traces. This concentrated, partially purified, and dialyzed cell-free supernatant was applied onto a Superdex-200 HR gel filtration column (Amersham Pharmacia Biotech, Germany) and equilibrated at pH 7.5 with 50 mM Tris-HCl buffer. The same buffer was used to elute

uricase at a 2 ml/min flow rate and a fraction size of 1 ml. A UV spectrophotometer was used to analyze the purified enzyme's activity at 293 nm. Fractions with higher uricase activity were further concentrated under vacuum and subjected to uricase enzyme assays (Saeed et al. 2004; Ram et al. 2015).

**Uricase enzyme assays.** The clear zone method was followed to perform uricase enzyme assays. Briefly, purified uricase enzyme (50 µl) was transferred onto uric acid (0.3% w/v)-containing opaque agar plates and incubated for 24 h at 37°C. The presence of active uricase was depicted by the clear zone formation (mm).

**Characterization of purified uricase. Uricase enzyme activity assay.** The reaction mixture was comprised of uric acid solution (3.57 mM, 100 µl)-containing sodium borate buffer (20 mM, 2 ml) at pH 7.5. The crude enzyme (100 µl) was added to the mixture to initiate the reaction followed by incubation for 30 min at 37°C. A UV visible spectrophotometer was used to measure the absorbance at 293 nm whereas the reduction in uric acid concentration was measured from the difference between blank and test absorbance. One unit (U) of enzyme activity was defined as the amount of uricase required to convert 1 µmol of uric acid into allantoin/minute at pH 7.5 and 37°C, whereas the millimolar extinction coefficient of uric acid (ε) was considered as 12.6 mM<sup>-1</sup> cm<sup>-1</sup> at 293 nm (Adámek et al. 1989; Pustake et al. 2019) The following equation was used to calculate the enzyme activity:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_{293 \text{ nm/min}} \text{Test} - \Delta A_{293 \text{ nm/mm}} \text{Blank}) \times (B) \times (DF)}{(\text{Extinction coefficient of uric acid at 293 nm}) \times (C)}$$

where:

ΔA – absorbance; B – total volume of reaction mixture; C – volume of enzyme; DF – dilution factor; extinction coefficient of uric acid at 293 nm = 12.5.

Total activity = Volume activity × Total volume

$$\text{Specific activity (U/mg)} = \frac{\text{Volume activity (U/ml)}}{\text{Total protein (mg/ml)}}$$

$$\text{Purification fold} = \frac{\text{Specific activity step 2 or 3}}{\text{Specific activity step 1}}$$

$$\text{Yield recovery(\%)} = \frac{\text{Total activity step 2 or 3}}{\text{Total activity step 1}} \times 100\%$$

**Uricase molecular weight estimation by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).** SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions was performed to assess the uricase enzyme's molecular weight. Discontinuous SDS-PAGE consisted of a stacking gel (4% (w/v), pH 6.8) and a separation gel (12% (w/v), pH 8.9) (Laemmli 1970)

a mini gel chamber (8.5 × 7 cm; Biometra GmbH, Germany) was used for electrophoresis, and brilliant blue R 250 stained the protein.

**Determination of protein concentration.** Laemmli's technique (Laemmli 1970) was adopted to determine protein concentration. The protein-dye complex of the Bradford reagent shifted the maximum dye absorption from 465 to 595 nm. A protein sample (cell-free supernatant) of 50 µl was added to the Bradford reagent (950 µl) and incubated at room temperature for 5 min. Then, the absorbance was spectrophotometrically (Ultraspec IIIR, Sweden) measured at 595 nm. A standard curve was generated using different BSA concentrations (6 µg to 200 µg).

**Effect of different temperatures on uricase activity.** The purified enzyme reaction mixture was incubated at different temperatures (10–70°C) to find the optimum temperature for uricase. Then, the tubes were cooled and uricase activity was spectrophotometrically measured at 293 nm. Uricase stability at different temperatures (4–100°C) was assessed by incubating purified uricase (10 µg) in Tris-HCl buffer (50 mM, pH 7.4) in



a thermos-block for 24 h. Uricase residual activity was spectrophotometrically determined at 293 nm.

**Effect of different pH levels on uricase stability.** Different buffers (phosphate and Tris-HCl buffer) were used to incubate the purified enzyme mixture at various pH levels (4 to 9.0) and 40°C. Then, tubes were assayed for uricase activity.

**Effect of substrate concentration on uricase activity.** Different concentrations (100–4,000 µg/ml) of substrate (uric acid) were applied to the reaction mixture at pH 7.4 and 40°C. Then, the mixture was assayed for uricase activity (Sarni et al. 2016).

**Effect of enzyme concentration on uricase activity.** Different concentrations (25, 50, 75, 100, 120, and 130 µl) of purified enzyme (0.2–1 U/ml) were added to the reaction mixture to find the optimum enzyme concentration. The reaction mixture was incubated (pH 7.4, 37°C), and assayed for uricase activity.

**Effect of Inhibitors on uricase activity.** The effect of inhibitors on uricase activity was investigated by preparing a 500 µl mixture of the purified strain UR1 solution (pH 7.5) and 500 µl of inhibitors [EDTA (10 mM), mercaptoethanol (0.1%), and 2 mM of Ca<sup>2+</sup>, K<sup>+</sup>, Cu<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, and Zn<sup>2+</sup>]. The mixture (1 ml) was initially incubated for 30 min at 40°C and then kept at room temperature. The residual uricase activity was spectrophotometrically measured at 293 nm and compared with untreated enzyme activity (Ravichandran et al. 2015).

Results

**Isolation and screening of uricase-producing isolates.** A total of fifty-two uricase-producing bacteria were isolated from the collected samples. Poultry waste samples yielded forty-two uricase-producing isolates, whereas the remaining isolates came from the soil samples (Table I). Clear zone forming isolates were purified and characterized (Fig. 1). All these isolates appeared as Gram-negative motile, and non-spore-forming short rods, and catalase, and oxidase positive. They exhibited uricase activity through clear zone formations around their colonies (Table I, II, and Fig. 1). The most promising of these isolates (UR1, UR2, UR5 UR6, and UR10) were selected for further genotypic and phenotypic characterizations.

**Phenotypic and biochemical characterizations of uricase-producing strains.** The selected strains' morphological, physiological, and biochemical activities were monitored under similar conditions to obtain comparable results (Table I–III). The colonies of strains UR1, UR2, UR5 UR6, and UR10 appeared whitish opaque, non-pigmented, and irregular on nutrient agar. The cell dimensions of strains UR1, UR2, UR5, UR6, and UR10 were observed as 2.02 × 0.63 µm, 2.51 × 0.70 µm, 1.86 × 0.67 µm, 2.99 × 0.53 µm, and 1.83 × 0.53 µm, respectively. These isolates were negative for protease, amylase, and lipase and positive for

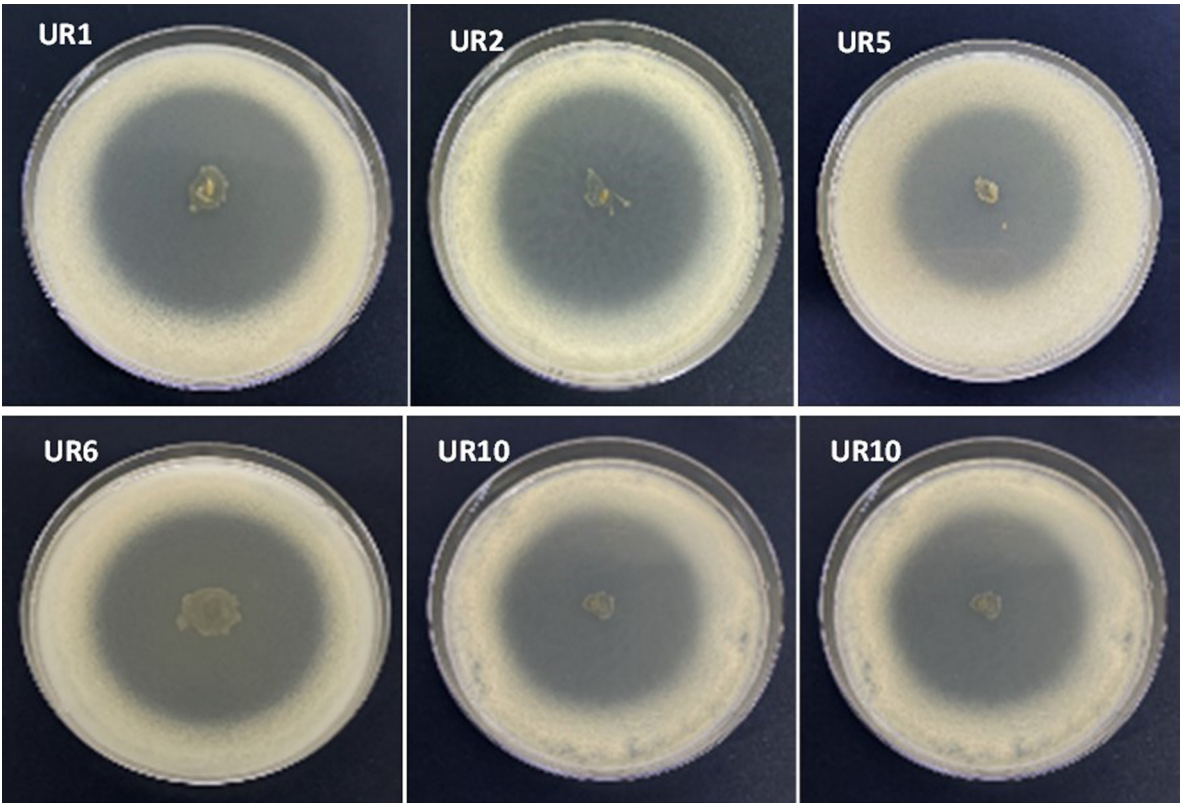


Fig. 1. Uricase activity of the selected strains based on the clear zone formation around colonies on the uric acid-supplemented (0.3%) BT agar.

Table I  
Screening and partial characterization of uricase-producing isolates.

Isolate code	Source of strains	Motility		Sporulation	Gram test	Uricase activity (mm)
		Gram stain	KOH test			
UR1	Soil	–	+	+	–	75
UR2	Soil	–	+	+	–	74
UR3	Poultry wastes	–	+	+	–	73
UR4	Poultry wastes	–	+	+	–	70
UR5	Poultry wastes	–	+	+	–	70
UR6	Soil	–	+	+	–	70
UR7	Soil	–	+	+	–	70
UR8	Poultry wastes	–	+	+	–	70
UR9	Poultry wastes	–	+	+	–	70
UR10	Poultry wastes	–	+	+	–	70
UR11	Poultry wastes	–	+	+	–	70
UR12	Poultry wastes	–	+	+	–	70
UR13	Poultry wastes	–	+	+	–	68
UR14	Poultry wastes	–	+	+	–	68
UR15	Soil	–	+	+	–	67
UR16	Poultry wastes	–	+	+	–	66
UR17	Poultry wastes	–	+	+	–	65
UR18	Poultry wastes	–	+	+	–	65
UR19	Poultry wastes	–	+	+	–	65
UR20	Poultry wastes	–	+	+	–	65
UR21	Poultry wastes	–	+	+	–	65
UR22	Poultry wastes	–	+	+	–	65
UR23	Poultry wastes	–	+	+	–	65
UR24	Soil	–	+	+	–	62
UR25	Poultry wastes	–	+	+	–	60
UR26	Poultry wastes	–	+	+	–	60
UR27	Poultry wastes	–	+	+	–	60
UR28	Soil	–	+	+	–	60
UR29	Poultry wastes	–	+	+	–	60
UR30	Poultry wastes	–	+	+	–	60
UR31	Poultry wastes	–	+	+	–	60
UR32	Poultry wastes	–	+	+	–	60
UR33	Poultry wastes	–	+	+	–	60
UR34	Poultry wastes	–	+	+	–	60
UR35	Poultry wastes	–	+	+	–	60
UR36	Poultry wastes	–	+	+	–	60
UR37	Poultry wastes	–	+	+	–	60
UR38	Poultry wastes	–	+	+	–	55
UR39	Poultry wastes	–	+	+	–	55
UR40	Poultry wastes	–	+	+	–	55
UR41	Poultry wastes	–	+	+	–	55
UR42	Poultry wastes	–	+	+	–	55
UR43	Poultry wastes	–	+	+	–	50
UR44	Poultry wastes	–	+	+	–	50
UR45	Poultry wastes	–	+	+	–	50
UR46	Poultry wastes	–	+	+	–	50

Table I  
Continued.

Isolate code	Source of strains	Motility		Sporulation	Gram test	Uricase activity (mm)
		Gram stain	KOH test			
UR47	Poultry wastes	–	+	+	–	50
UR48	Poultry wastes	–	+	+	–	45
UR49	Soil	–	+	+	–	45
UR50	Poultry wastes	–	+	+	–	45
UR51	Soil	–	+	+	–	40
UR52	Soil	–	+	+	–	40

Uric acid degrading bacteria were enriched in uric acid-supplemented (0.8%) enrichment broth followed by incubation for 48 h at 37°C under shaking conditions. Uric acid turbidity gradually disappeared during fermentation. Then, dilutions of enrichment cultures were inoculated on uric acid-supplemented (0.3%) BT agar plates where all the isolates formed clear zones.

Table II  
Morphological and partial characterization of uricase-producing isolates.

Characteristics	Most promising uricase-producing isolates				
	UR1	UR2	UR5	UR6	UR10
Colony morphology:					
Form	Irregular	Irregular	Irregular	Irregular	Irregular
Color on NA	whitish	Whitish	Whitish	Whitish	Whitish
Pigment	–	–	–	–	–
Elevation	Flat	Flat	Flat	Flat	Flat
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Consistency	Dry	Dry	Dry	Dry	Dry
Cell morphology:					
Shape	Rods shaped	Rods shaped	Rods shaped	Rods shaped	Rods shaped
Gram stain	–	–	–	–	–
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Sporulation	+	+	+	+	+
Motility	+	+	+	+	+
Carbon source utilization:					
Maltose	+/-	+	+	+	+
Dextrose	+	+	+	+	+
Fructose	+	+	+	+	+
D-Glucose	+	+	+	+	+
Sucrose	+	+	+	+	+
Nitrogen source utilization:					
Casein	+	+	+	+	+
Yeast extract	+	+	+	+	+
Urea	+	+	+	+	+
Growth on NaCl (%):					
0	+	+	+	+	+
1%	+	+	+	+	+
2%	+/-	+/-	+/-	+/-	+/-
5%	–	+/-	+/-	+/-	+/-
10%	–	–	–	–	–

Table II  
Continued.

Characteristics	Most promising uricase-producing isolates				
	UR1	UR2	UR5	UR6	UR10
pH optimum:					
5	+	+/-	+/-	+/-	+/-
6	+	+/-	+/-	+/-	+/-
7	+	+	+	+	+
8	+	+	+	+	+
9	+/-	+/-	+/-	+/-	+/-
Temperature optimum:					
20°C	+/-	+/-	–	+/-	+/-
30°C	+	+	+	+	+
37°C	+	+	+	+	+
40°C	+	+	+	+	+
50°C	–	–	–	–	–
MacConkey agar:	–	–	–	–	–
Blood agar hemolysis	α-hemolytic	α-hemolytic	α-hemolytic	α-hemolytic	α-hemolytic
Enzyme secretion:					
Uricase	+	+	+	+	+
Urease	+	+	+	+	+
Protease	–	–	–	–	–
Amylase	–	–	–	–	–
Lipase	–	–	–	–	–
Cellulose	+	+	+	+	+

+ – good growth; – – no growth; +/- – weak growth

cellulase and uricase (Table II). They could grow at a wide range of temperatures (20–45°C) and pH (5–9), whereas optimum pH and temperature were noted as 7 and 37°C, respectively. These isolates tolerated a range of NaCl (0–2%), but weak growth was observed with more than 3% NaCl. Moreover, these non-lactose fermenters exhibited α hemolysis on blood agar (Table II).

Biochemical features and analytical profile index 20E (API 20E kit) revealed that the selected uricase-producing strains were negative to *O*-nitrophenyl-β-D-galactopyranoside (ONPG), tryptophan deaminase (TDA), arginine dihydrolase (ADH), hydrogen sulfide, lysine decarboxylase (LDC), gelatinase (GEL), indole (IND), and ornithine decarboxylase (ODC), but remained positive to Voges-Proskauer test (VP), citrate (CIT), and urease (URA). Uricase-producing strains demonstrated a narrow growth range on sugar fermentation. They utilized glucose (GLU), fructose, sucrose (SAC), dextrose, and maltose. However, they were unable to ferment rhamnose (RHA), mannose (MAN), sorbitol (SOR), inositol (INO), arabinose (ARA), melibiose (MEL), and amygdalin (AMY) (Table III).

The pattern of biochemical reactions in the bioMérieux Vitek® 2 compact system database depicted

that uricase-producing isolates were positive to biochemical reactions such as L-lactate alkalization (ILATk), D-glucose (dGLU), urease (URE), L-proline arylamidase (ProA), and tyrosine arylamidase (TyrA). Contrarily, they were negative to a wide range of biochemical reactions such as L-pyrrolydonyl-arylamidase (PyrA), Ala-Phe-Pro-arylamidase (APPA), D-cellobiose (dCEL), adonitol (ADO), L-arabitol (IARL), β-galactosidase (BGAL), Glutamyl arylamidase pNA (AGLTp), and other biochemical traits as shown in Table IV. Moreover, most of the selected strains showed resistance to Cefepime, Amikacin, and Aztreonam, however, they were sensitive to Tetracycline, Gentamicin, Chloramphenicol, and Imipenem. Strain UR1 was also resistant to Ceftazidime, Amikacin, and Aztreonam, but it was found to be sensitive to Trimethoprim, Gentamicin, Piperacillin, Tetracycline, Imipenem, Ciprofloxacin, Chloramphenicol, Cefotaxime, and Cefepime (Table V).

bioMérieux Vitek® 2 compact system identification revealed the identity of strains UR1 and UR2 as *Pseudomonas oleovorans* with a 95% similarity. Strain UR5 exhibited 97% similarity with *A. faecalis* whereas strains UR6 and UR10 were identified as



Table III  
API-20E profile of sugar fermentation and enzyme activity of uricase-producing isolates.

Biochemical test		Uricase-producing isolates					Reference strain
		UR1	UR2	UR5	UR6	UR10	<i>Alcaligenes faecalis</i> DSM13644
1	O-nitrophenyl-b D-galactopyranoside (ONPG)	–	–	–	–	–	–
2	Arginine dihydrolase (ADH)	–	–	–	–	–	–
3	Lysine decarboxylase (LDC)	–	–	–	–	–	–
4	Ornithine decarboxylase (ODC)	–	–	–	–	–	–
5	Citrate (CIT)	+	+	+	+	+	+
6	Hydrogen sulfide (H <sub>2</sub> S)	–	–	–	–	–	+
7	Urease (URA)	+	+	+	+	+	–
8	Tryptophan deaminase (TDA)	–	–	–	–	–	+
9	Indole (IND)	–	–	–	–	–	+
10	Voges-Proskauer test (VP)	+	–	+	+	+	+
11	Gelatinase (GEL)	–	–	–	–	–	–
Sugar fermentation:							
12	Glucose (GLU)	+	+	+	+	+	+
13	Mannose (MAN)	–	–	–	–	–	–
14	Inositol (INO)	–	–	–	–	–	–
15	Sorbitol (SOR)	–	–	–	–	–	–
16	Rhamnose (RHA)	–	–	–	–	–	–
17	Sucrose (SAC)	+	+	+	+	+	+
18	Melibiose (MEL)	–	–	–	–	–	–
19	Amygdalin (AMY)	–	–	–	–	–	–
20	Arabinose (ARA)	–	–	–	–	–	–

+ – good growth, – – no growth, +/- – weak growth  
+ – positive enzyme reaction, – – negative enzyme reaction

*Acinetobacter lwoffii* with a respective similarity of 92% and 96%. Due to the lower identity ratio of the Vitek 2 compact system, the most promising uricase-producing isolates were further subjected to 16S rRNA-based molecular identification.

**Genotypic characterizations of uricase-producing strains.** PCR amplification and sequencing of 16S rRNA gene (~1,540 bp) was carried out by using a genomic DNA template to confirm the identification of selected uricase-producing strains. 16S rRNA gene sequences of selected strains were deposited in the National Center for Biotechnology Information (NCBI) nucleotide sequence database under the accession numbers NR113993.1 (UR1), NR137421.1 (UR2), NR157609.1 (UR5), NR113993.1 (UR6), and NR181952.1 (UR10). These sequences were compared with similar ones on the NCBI database using the BLAST tool (<http://www.ncbi.nlm.nih.gov>). ClustalX program was employed to construct a phylogenetic tree of these sequences by adopting a neighbor-joining and maximum composite likelihood model with a bootstrap value of 100. *B. subtilis* strain ATCC® 21331™ (AB018487.1) and *E. coli* strain ATCC® 35218™ (J01859.1) served as the

outgroup (Fig. 2, Table VI). 16S rRNA gene sequences indicated a close relationship of strain UR10 with *Alcaligenes ammonioxydans* strain HO-1 (NR\_180910.1) and *A. faecalis* strain NBRC 13111 (NR\_113606.1) with similarities of 98.84% and 98.02%, respectively. Strains UR1, UR2, UR5, and UR6 showed higher similarity of 96.96%, 95.40%, 97.28%, and 98.77% to *A. faecalis* (NR\_113606.1) and *A. ammonioxydans* (NR\_180910.1) respectively. Based on the phenotypic and genotypic features, the selected uricase-producing strains were characterized as the members of the genus *Alcaligenes* spp.

**Growth and uricase secretion affecting factors in strain UR1.** The growth conditions of the uricase-secreting strain UR1 were optimized by incubating for different time intervals (24, 48, 72, and 96 h) at different temperatures (20, 25, 30, 37, 40, 45, and 50°C), and initial pH levels (pH 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, and 9). The results revealed maximum UR1 growth (2.5 OD) and uricase activity (65 U/ml) after 50 h of incubation (Fig. 3). The data depicted a drastic decrease in the growth and uricase activity of strain UR1 at temperatures over 40°C whereas the maximum growth

Table IV  
bioMérieux VITEK®-based biochemical tests of uricase-producing isolates.

Carbon source utilization, enzymatic activities and resistance		Conc. (mg)	Uricase isolates/identification results				
			UR1	UR2	UR5	UR6	UR10
1	Ala-Phe-Pro-arylamidase (APPA)	0.0384	–	–	–	–	–
2	Adonitol (ADO)	0.1875	–	–	–	–	–
3	L-Pyrrolydonyl-arylamidase (PyrA)	0.018	–	–	–	–	–
4	L-Arabitol (IARL)	0.3	–	–	–	–	–
5	D-Cellobiose (dCEL)	0.3	–	–	–	–	–
6	β-Galactosidase (BGAL)	0.036	–	–	–	–	–
7	Production (H2S)	0.0024	–	–	–	–	–
8	β-N-acetyl-glucosaminidase (BNAG)	0.0408	–	–	–	–	–
9	Glutamyl arylamidase pNA (AGLTp)	0.0324	–	–	–	–	–
10	D-Glucose (dGLU)	0.3	+	+	+	+	+
11	γ-Glutamyl-transferase (GGT)	0.0228	–	–	–	–	–
12	Fermentation/glucose (OFF)	0.45	+	+	+	+	+
13	β-Glucosidase (BGLU)	0.036	–	–	–	–	–
14	D-Maltose (dMAL)	0.3	–	–	–	–	–
15	D-Mannitol (dMAN)	0.1875	–	–	–	–	–
16	D-Mannose (dMNE)	0.3	–	–	–	–	–
17	β-Xylosidase (BXYL)	0.0324	–	–	–	–	–
18	β-Alanine arylamidase pNA (BALap)	0.0174	–	–	–	–	–
19	L-Proline arylamidase (ProA)	0.0234	+	+	+	+	+
20	Lipase (LIP)	0.0192	–	–	–	–	–
21	Palationse (PLE)	0.3	–	–	–	–	–
22	Tyrosine arylamidase (TyrA)	0.0276	+	+	+	+	+
23	Urease (URE)	0.15	+	+	–	+	+
24	D-Sorbitol (dSOR)	0.1875	–	–	–	–	–
25	Saccharose/sucrose (SAC)	0.3	–	–	–	–	–
26	D-Tagatose (dTAG)	0.3	–	–	–	–	–
27	D-Trehalose (dTRE)	0.3	–	–	–	–	–
28	Citrate (sodium) (CIT)	0.054	–	–	+	–	–
29	Malonate (MNT)	0.15	–	–	+	–	–
30	5-Keto-D-gluconate (5KG)	0.3	–	–	–	–	–
31	L-Lactate alkalisation (ILATk)	0.15	+	+	+	+	+
32	α-Glucosidase (AGLU)	0.036	–	–	–	–	–
33	Succinate alkalisation (SUCT)	0.15	–	–	+	+	–
34	β-N-acetyl-galactosaminidase (NAGA)	0.0306	–	–	–	–	–
35	α-Galactosidase (AGAL)	0.036	–	–	–	–	–
36	Phosphatase (PHOS)	0.0504	–	–	–	–	–
37	Glycine arylamidase (GlyA)	0.012	–	–	–	–	–
38	Ornithine decarboxylase (ODC)	0.3	–	–	–	–	–
39	Lysine decarboxylase (LDC)	0.15	–	–	–	–	–
40	L-Histidine assimilation (IHISa)	0.087	–\+	–	–	+	+\–
41	Coumarate (CMT)	0.126	+	–	–	–	–
42	β-Glucuronidase (BGUR)	0.0378	–	–	–	–	–
43	O/129 Resistance (comp.vibrio.) (O129R)	0.0105	–	–	–	–	–
44	Glu-Gly-Arg-arylamidase (GGAA)	0.0576	–	–	–	–	–
45	L-malate assimilation (IMLTa)	0.042	–\+	+\–	+	–	–
46	Ellman (ELLM)	0.03	–\+	–\+	–	+	–
Identification and similarity according to VITEK® data base			<i>Pseudomonas oleovorans</i> (95%)	<i>Pseudomonas oleovorans</i> (95%)	<i>Alcaligenes faecalis</i> (97%)	<i>Acinetobacter lwoffii</i> (92%)	<i>Acinetobacter lwoffii</i> (96%)

+ – good growth, – – no growth, +/- – weak growth; + – positive enzyme reaction, – – negative enzyme reaction

Table V  
Antibiotic susceptibility of uricase-producing promising isolates.

Antibiotics	Antibiotics/ Disc potency	Uricase-producing isolates				
		UR1	UR2	UR5	UR6	UR10
Amikacin	30 µg (AK)	R	R	S	R	S
Gentamicin	10 µg (GM)	S	S	S	S	R
Tetracycline	30 µg (T)	S	S	S	S	S
Aztreonam	30 µg (ATM)	R	R	R	R	R
Ciprofloxacin	5 µg (CIP)	S	S	R	S	S
Cefotaxime	30 µg (Cf)	S	S	R	R	R
Ceftazidime	30 µg (CAZ)	S	R	S	R	S
Cefepime	30 µg (CPM)	R	R	S	R	R
Imipenem	10 µg (IMI)	S	S	S	S	S
Piperacillin	100 µg (PRL)	S	S	R	R	S
Trimethoprim/sulfamethoxazole	25 µg (TS)	S	S	R	S	R
Chloramphenicol	30 µg (C)	S	S	S	S	S

R – resistant; S – sensitive

Table VI  
16S rRNA gene sequence-based molecular identification of uricase-producing promising strains.

Isolate code	Blast match	Gene Bank Accession No.	16S rRNA Identity (%)
Strain UR1 (PP563675)	– <i>Alcaligenes faecalis</i> strain NBRC 13111	– NR_113606.1	96.96
	– <i>Alcaligenes ammonioxydans</i> strain HO-1	– NR_180910.1	96.86
	– <i>Alcaligenes aquatilis</i> strain LMG 22996	– NR_104977.1	96.54
	– <i>Alcaligenes endophyticus</i> strain AER10	– NR_156855.1	95.91
	– <i>Alcaligenes pakistanensis</i> strain NCCP-650	– NR_145932.1	95.58
Strain UR2 (PP563676)	– <i>Alcaligenes faecalis</i> strain NBRC 13111	– NR_113606.1	95.40
	– <i>Alcaligenes ammonioxydans</i> strain HO-1	– NR_180910.1	95.19
	– <i>Alcaligenes aquatilis</i> strain LMG 22996	– NR_156855.1	94.97
	– <i>Alcaligenes endophyticus</i> strain AER10	– NR_156855.1	95.15
	– <i>Alcaligenes pakistanensis</i> strain NCCP-650	– NR_145932.1	93.79
Strain UR5 (PP563677)	– <i>Alcaligenes ammonioxydans</i> strain HO-1	– NR_180910.1	97.28
	– <i>Alcaligenes faecalis</i> strain NBRC 13111	– NR_113606.1	97.20
	– <i>Alcaligenes aquatilis</i> strain LMG 22996	– NR_104977.1	96.89
	– <i>Alcaligenes pakistanensis</i> strain NCCP-650	– NR_145932.1	96.11
	– <i>Alcaligenes endophyticus</i> strain AER10	– NR_156855.	95.96
Strain UR6 (PP563678)	– <i>Alcaligenes ammonioxydans</i> strain HO-1 16S	– NR_180910.1	98.77
	– <i>Alcaligenes faecalis</i> strain NBRC 13111 16S	– NR_113606.1	98.56
	– <i>Alcaligenes aquatilis</i> strain LMG 22996	– NR_104977.1	98.15
	– <i>Alcaligenes endophyticus</i> strain AER10	– NR_156855.1	98.15
	– <i>Alcaligenes pakistanensis</i> strain NCCP-650	– NR_145932.1	98.15
Strain UR10 (PP563679)	– <i>Alcaligenes ammonioxydans</i> strain HO-1	– NR_180910.1	98.84
	– <i>Alcaligenes faecalis</i> strain NBRC 13111	– NR_113606.1	98.02
	– <i>Alcaligenes aquatilis</i> strain LMG 22996	– NR_104977.1	97.73
	– <i>Alcaligenes pakistanensis</i> strain NCCP-650	– NR_145932.1	96.98
	– <i>Alcaligenes faecalis</i> subsp. <i>phenolicus</i>	– NR_042830.1	95.87

Similarity of uricase-producing isolates and closely related strains of genus *Alcaligenes* spp.

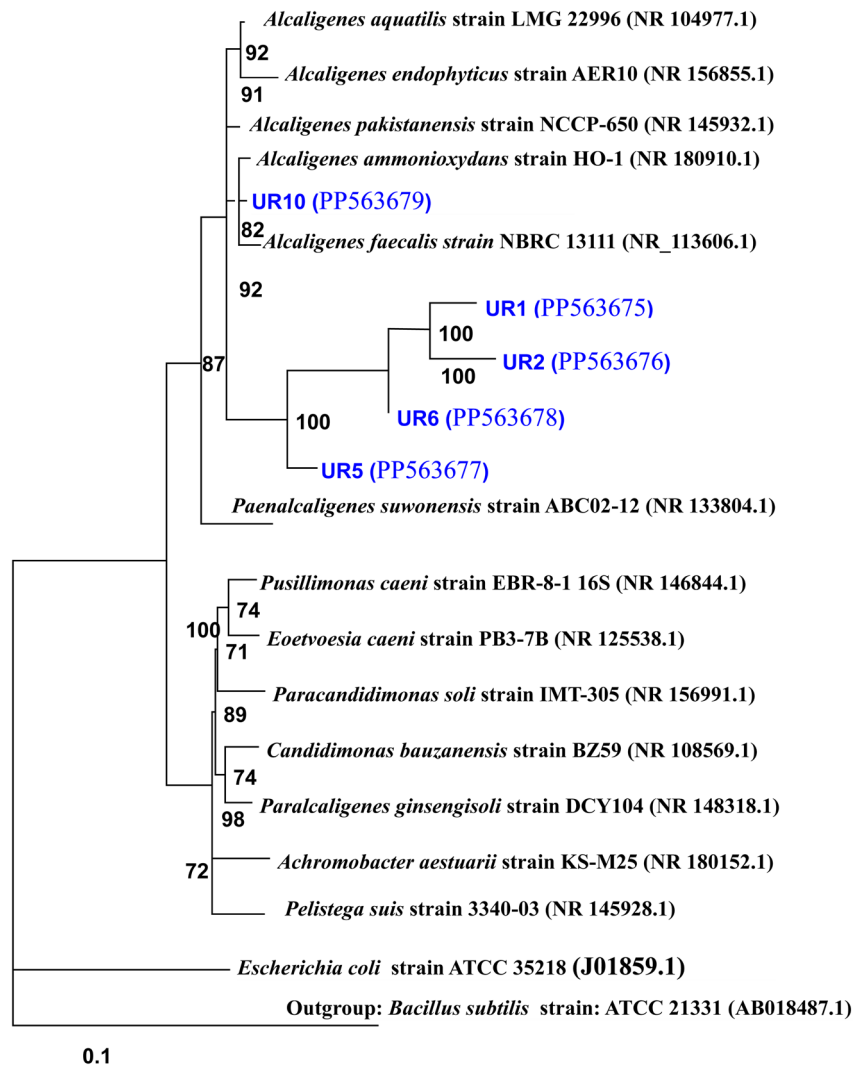


Fig. 2. Phylogenetic tree of uricase-producing isolates (shown in blue) and closely related strains of genus *Alcaligenes*. Bootstrap values out of 100 are mentioned at the nodes. *Escherichia coli* strain ATCC® 35218™ (J01859.1) and *Bacillus subtilis* strain: ATCC® 21331™ (AB018487.1) served as the out-group.

(2.5 OD) and uricase activity (67 U/ml) was observed at 37°C (Fig. 4). Similarly, an initial pH 7 yielded the maximum UR1 growth (3 OD) and uricase activity (80 U/ml) (Fig. 5).

**Impact of carbon and nitrogen sources on strain UR1 growth and uricase secretion.** Different carbon sources (fructose, glucose, sucrose, cellulose, maltose, and dextrose) were examined for uricase secretion.

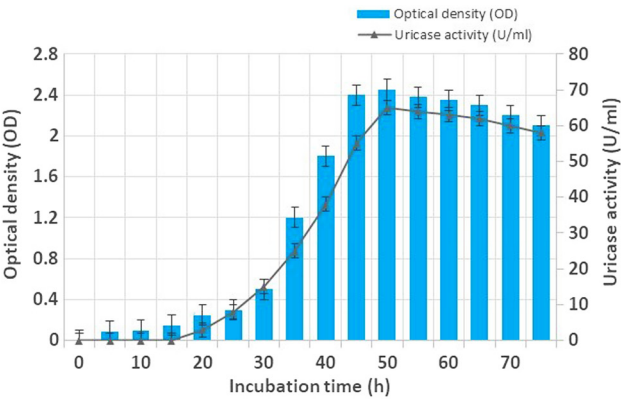


Fig. 3. Effect of incubation period on the growth (OD) and uricase secretion of strain UR1

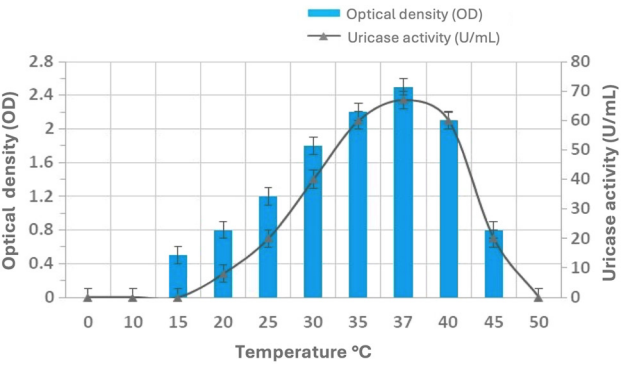


Fig. 4. Effect of temperature on the growth (OD) and uricase activity (U/ml) of strain UR1.



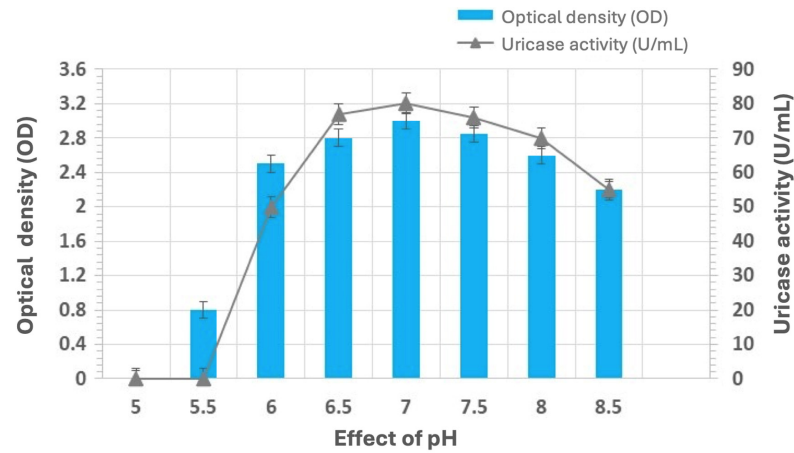


Fig. 5. Effect of pH on the growth (OD) and uricase activity (U/ml) of strain UR1.

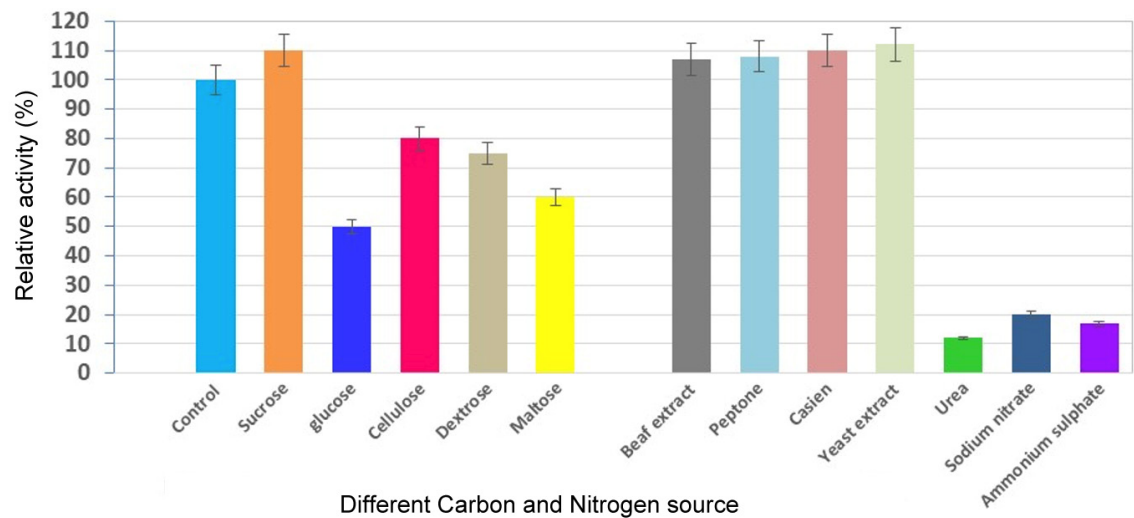


Fig. 6. Impact of carbon and nitrogen sources on strain UR1 growth and uricase activity (U/ml).

Optical density was spectrophotometrically measured at 600 nm and 293 nm to monitor growth and uricase activity, respectively. The maximum activity of the control treatment (only containing 0.3% uric acid in BT) was considered 100% to calculate the relative activity of various nitrogen and carbon sources. Data revealed that only sucrose enhanced the uricase secretion from strain UR1 with a 10% activity. In contrast, other carbon sources such as glucose, maltose, dextrose, and cellulose caused a reduction in uricase secretion by 50, 40, 25, and 20%, respectively compared to the control (Fig. 6).

Similarly, uric acid-supplemented (0.3% w/v) BT medium was individually supplemented with different nitrogen sources [yeast extract, beef extract, peptone, casein, urea (organic), (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub> (inorganic), and NaNO<sub>3</sub>] at an initial pH 7. Optical density was spectrophotometrically measured at 600 nm and 293 nm to monitor growth and uricase activity, respectively. The results revealed that all organic nitrogen sources (e.g., yeast extract, beef extract, peptone, casein, and urea)

enhanced the uricase secretion of strain UR1 by 12, 10, 8, and 7% as compared to the control treatment (100%). Inorganic nitrogen sources such as sodium nitrate, ammonium sulfate, and urea significantly inhibited the uricase secretion of strain UR1 by 80, 83, and 88% compared to control (Fig. 6).

**Impact of salt on strain UR1 growth and uricase secretion.** The impact of salt was assessed by adding different NaCl concentrations (0.1, 0.2, 0.5, 1, 1.5, and 2%, w/v) in uric acid-containing BT medium at an initial pH 7. The results indicated a significant reduction in strain UR1 growth and uricase activity with the rise in salt concentration whereas the maximum growth (3 OD) and uricase secretion (80 U/ml) were observed at a NaCl concentration of 0.1% (Fig. 7).

**Uricase purification from strain UR1.** DEAE sepharose-based uricase purification from strain UR1 remained unsuccessful. Therefore, the Gel filtration column (Sephadex G100 Column chromatography) was used for the protein purification, which was confirmed

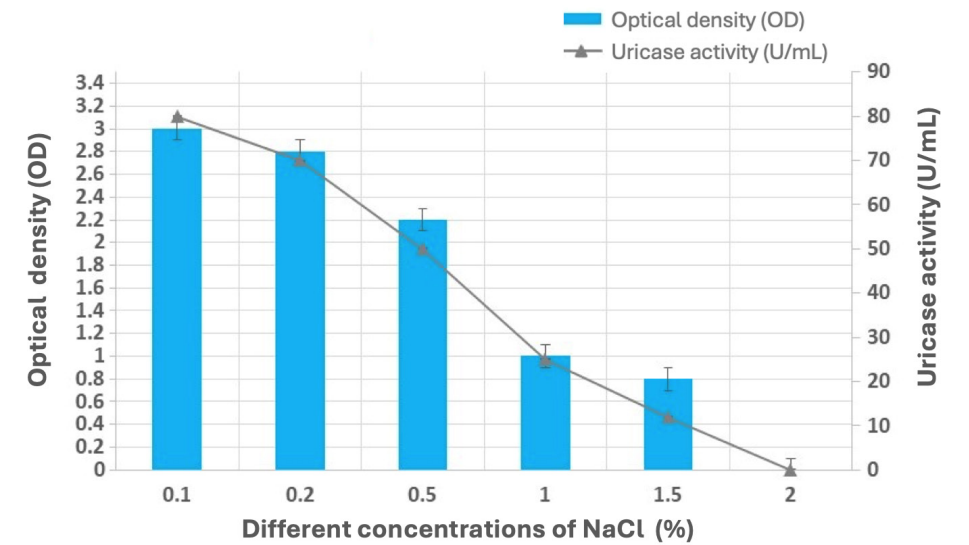


Fig. 7. Impact of different NaCl (%) concentrations on strain UR1 growth (OD) and uricase activity (U/ml).

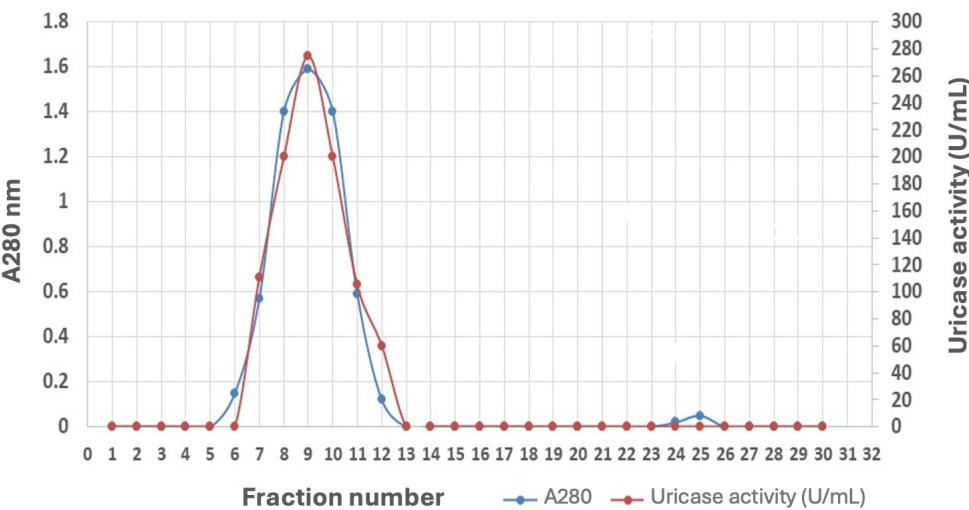


Fig. 8. Uricase purification from strain UR1 by gel filtration. Uricase activity exhibiting fractions (F7-F12).

by measuring the absorption at 293 nm. Fractions of 5 ml to 12 ml were used to detect uricase activity (Fig. 8). SDS-PAGE was performed to check the purity of the fractions with high uricase activity (Fig. 9). Uricase purification by gel filtration chromatography yielded the fractions with purities of 66.37% (275 U/ml)

and 19% (916 U/mg) (Table VII). Notably, the enzyme presented a very high uricase activity (275 U/ml) compared to the previous reports.

**Characterization of the purified uricase from strain UR1. Molecular mass of purified uricase.** SDS-PAGE revealed the homogeneity of purified uricase.

Table VII  
Gel-filtration-based uricase purification steps from strain UR1.

Purification steps	Volume (ml)	Uricase activity (U/ml)	Total uricase activity (U)	Protein concentration (mg/ml)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Crude enzyme (10 fold concentration)	100	75	7,500	5.4	13.8	1	100
Dialysis after ammonium sulfate precipitation	5	112	560	3.8	29.74	2.13	7.40
Gel filtration (Sephadex)	5	275	1,375	0.3	916	66.73	18.33

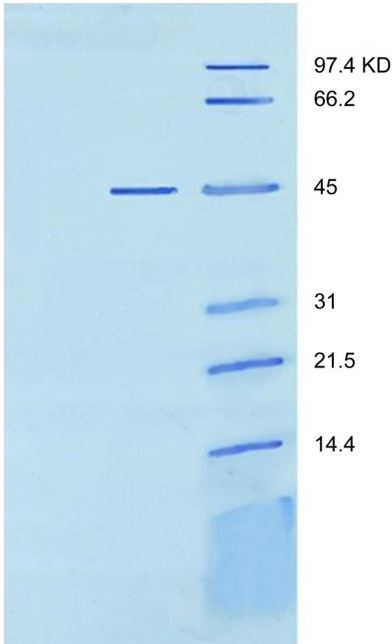


Fig. 9. SDS-PAGE of the purified uricase from strain UR1. SDS-denatured samples (20–40 µl) were separated in an SDS-polyacrylamide gel (12%) and stained with Serva blue R. Lane 1: molecular mass standard, Lane 2: protein (10 µg) of purified enzyme.

The brilliant blue R-250 staining method was followed to visualize protein bands, which revealed a molecular mass of 45 kDa in a single polypeptide form (Fig. 9).

**Effect of temperature on purified uricase activity.** The data revealed maximum uricase activity at 40°C whereas uricase activity was not observed below 20°C and above 70°C (Fig. 10a). Uricase stability at different temperatures (0–100°C) was assessed through overnight incubation followed by the measurement of residual activity. The maximum activity was referred to as 100% relative activity. The enzyme was found to maintain its activity up to 50°C. The results depicted that enzyme activity was significantly reduced by 20, 60, and 95% at 60, 70, and 80°C, respectively, whereas a complete loss of activity was observed at 90°C (Fig. 10b).

**Effect of pH on purified uricase activity.** The activity of purified uricase was noted at a broad pH range (5–9) whereas the maximum activity (275 U/ml) was recorded at pH 7.5. It also retained 94, 80, and 20% activity at pH 8, 8.5, and 9, respectively. Alkaline pH above 9 resulted in a complete loss of enzyme activity. Similarly, only 6% activity was noted at pH 5.0 whereas enzyme activity was completely lost at pH 4 (Fig. 11).

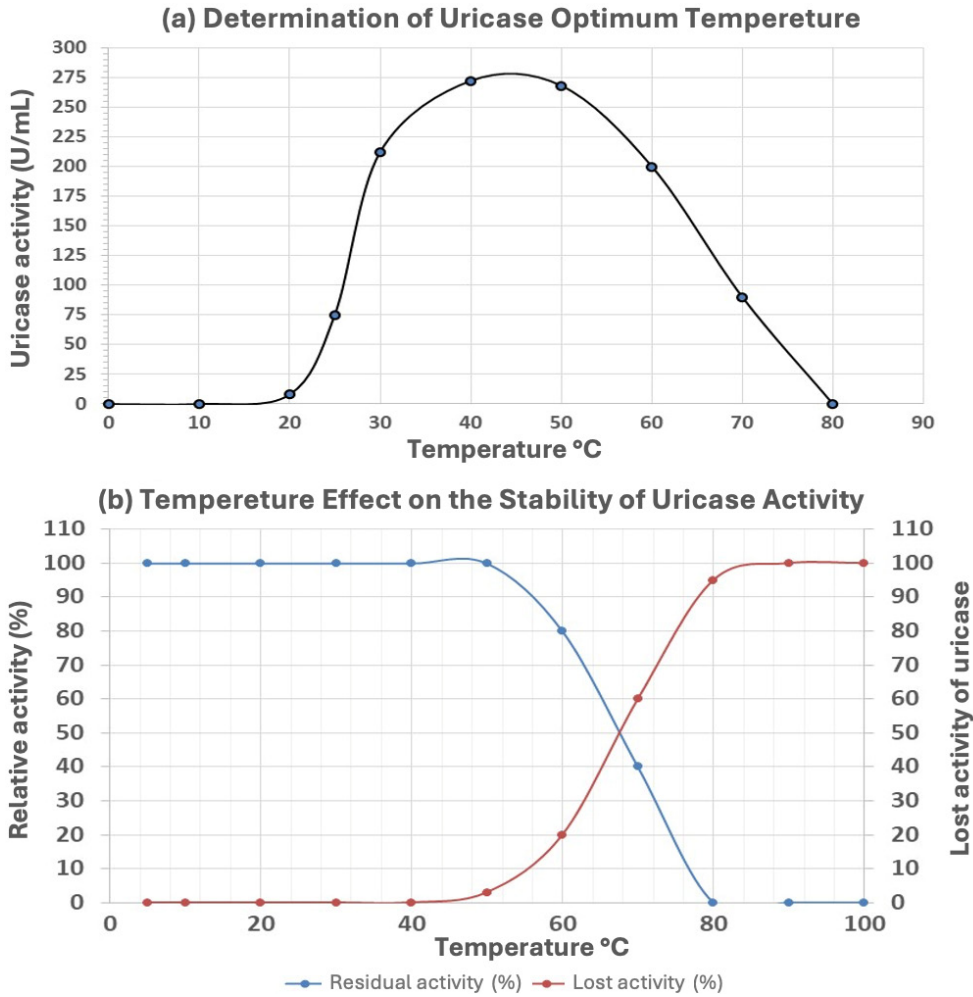


Fig. 10. Effect of temperature on a) uricase activity, and b) stability.

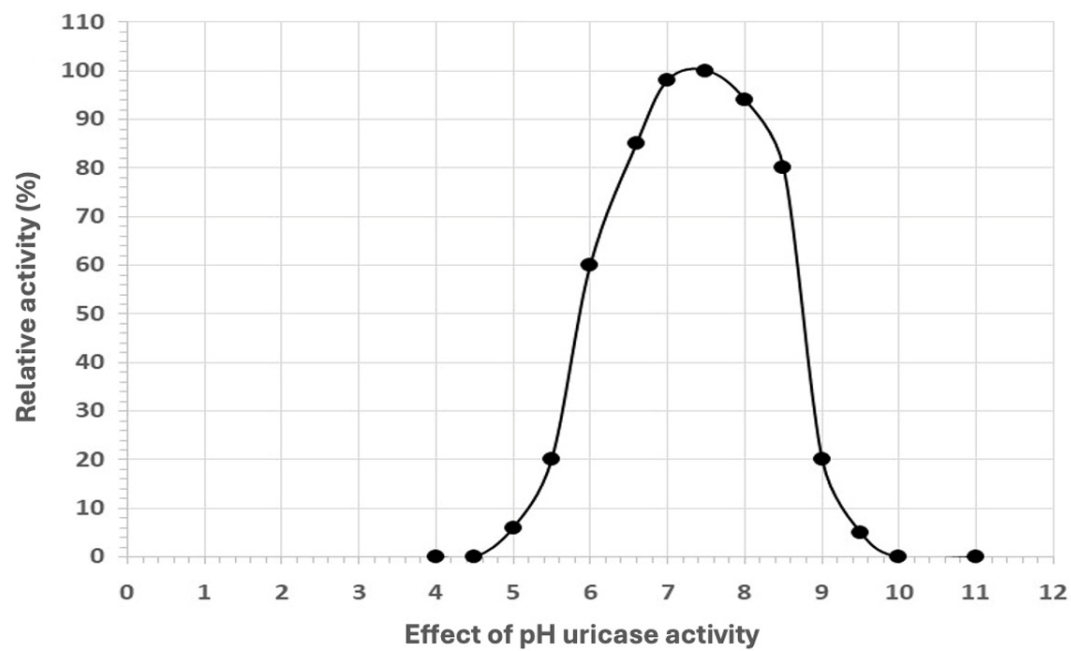


Fig. 11. Effect of pH on the uricase activity of strain UR1.

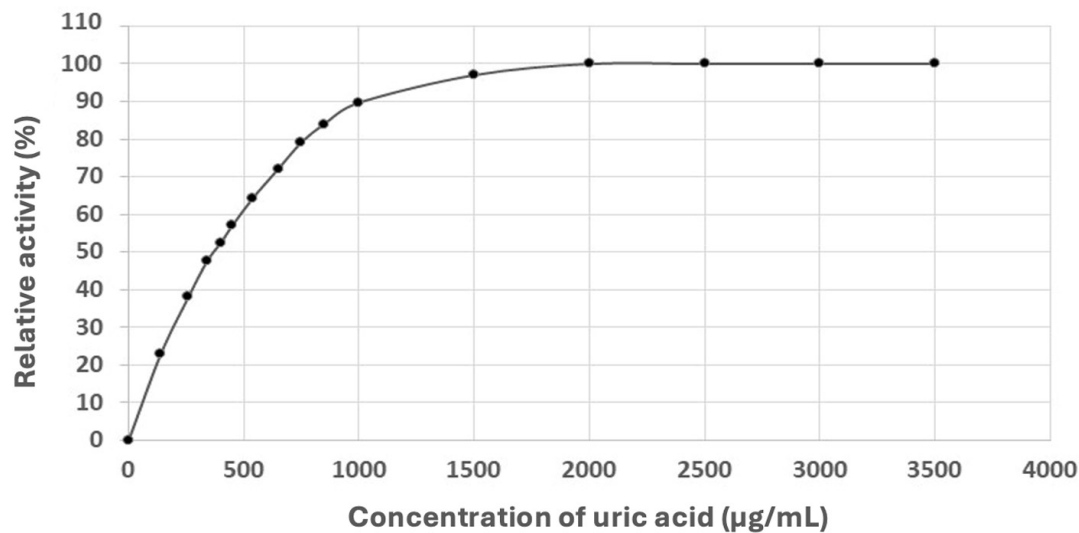


Fig. 12. Effect of substrate concentration on uricase activity of strain UR1.

**Effect of substrate concentration on uricase activity.** The enzyme activity gradually increased with the rising uric acid concentration, and maximum activity was observed at 2,000 µg. Then, it remained constant, indicating that the enzyme had already been filled/saturated with the substrate and achieved its optimal condition (Fig. 12).

**Effect of metal ions and inhibitors on uricase activity.** The results indicated drastic inhibition in uricase activity of strain UR1 by β-mercaptoethanol (94.6%) followed by EDTA (66.8%), Co<sup>2+</sup> (61.81%), and Zn<sup>2+</sup> (31.20%). Contrarily, Ca<sup>2+</sup> strongly enhanced the uricase activity (20%), whereas other metal ions such as Cu<sup>2+</sup>, Mg<sup>2+</sup>, and Mn<sup>2+</sup> caused a slight increase of 6, 5, 3, and 2%, respectively (Fig. 13).

Discussion

This study isolated fifty-two uricase-producing bacteria from environmental samples (poultry fecal droppings and soil). Traditional laboratory protocols and the bioMérieux Vitek® 2 compact system were employed for these isolates' biochemical and phenotypic characterization (Gerhardt et al. 1994). bioMérieux Vitek® 2 compact data identified strains UR1 and UR2 as *P. oleovorans* with a 95% similarity. Strain UR5, with a similarity of 97%, was identified as *A. faecalis* whereas strains UR6 and UR10 exhibited a 92% and 96% similarity with *A. lwoffii*. Due to the lower identity of the selected isolates by the Vitek® 2 compact system, these uricase-producing isolates were further subjected



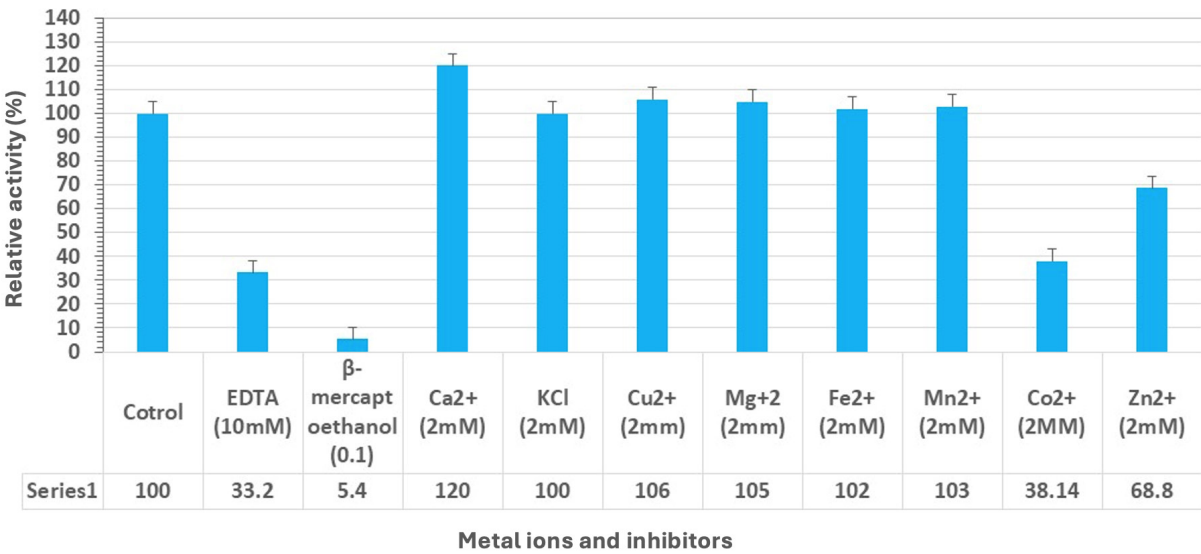


Fig. 13. Effect of the metal ions and inhibitors on uricase activity of strain UR1.

to 16S rRNA gene sequence-based molecular identification. Kim et al. (2017) also reported misidentification of four *Alcaligenes* spp. strains as *A. faecalis* by the Vitek® 2 compact system. Biochemical patterns showed a wide range of negative results, possibly due to the misidentification of four slow-growing *A. baumannii* clinical isolates by the Vitek® 2 compact system. The system could not differentiate *A. baumannii* from *A. faecalis* (Kim et al. 2018). Guo et al. (2014) also revealed two generic errors in Vitek® 2 compact system-based *A. baumannii* identification. Therefore, the results of the Vitek® 2 compact system should be carefully interpreted followed by the confirmation through 16S rRNA sequencing or MALDI-TOF methods, which provide rapid and precise bacterial identification in clinical laboratories.

Uricase-producing strains were further confirmed through PCR amplification and sequencing of 16S rRNA gene (~1,540 bp). The 16S rRNA gene sequences of selected isolates were compared with identical sequences on NCBI databases. Phylogenetic tree of the 16S rRNA gene sequences categorized uricase-producing strains in the genus *Alcaligenes* spp. (Castellani and Chalmers 1919) belonging to the *Alcaligenaceae* family under the Proteobacteria β subgroup. These results confirmed uricase production in various bacteria including genus *Alcaligenes* in addition to previously reported bacteria such as *S. graminofaciens*, *P. aeruginosa*, *S. albidoflavus*, *Microbacterium*, *Bacillus cereus*, *P. vulgaris*, *B. thermocatenulatus*, and *Lactobacillus plantarum* (Saeed et al. 2004; Azab et al. 2005; Zhou et al. 2005; Lotfy, 2008; Amirthanathan and Subramaniyan 2012).

The analysis of 16S rRNA gene sequences indicated a close relationship of strain UR10 with *A. faecalis* strain NBRC 13111 (NR\_113606.1) and *A. ammonioxydans* strain HO-1 (NR\_180910.1) with a similar-

ity of 98.02% and 98.84%, respectively. Other strains (UR1, UR2, UR5, and UR6) clustered together in a new clade and exhibited similarity (96.96, 95.40, 97.28, and 98.77%) to *A. ammonioxydans* (NR\_180910.1) and *A. faecalis* (NR\_113606.1). *Alcaligenes* spp. is common in water and soil environments, and intestinal tracts of vertebrates (Ray and Pattnaik 2024). Conventional phenotypic bacterial identification is generally not as accurate as genotypic methods.

Therefore, comparing the 16S rRNA gene sequence has an established genetic identification method, which can efficiently identify phenotypically aberrant, poorly described, and rarely isolated strains (Clarridge 2004). Generally, this technique can differentiate organisms at the genus, species, and subspecies levels across major bacterial phyla. The rare limitation of this method could occur with highly similar sequences (Clarridge 2004). Prokaryotic species exhibit a minimum of 3% difference in the 16S rRNA sequence. Therefore, a bacterial species is referred to as a collection of strains with a higher overall similarity, which differs from related strain groups in multiple independent features (Madigan et al. 2000). The 16S rRNA gene is as a base for modern bacterial classification with a limited variation for closely related taxa (Fox et al. 1992). The members of the genus *Alcaligenes* are considered opportunistic human pathogens (Cruz et al. 2021). The Gram-negative genus *Alcaligenes* belongs to the *Alcaligenaceae* family under the order Burkholderiales. The type species *A. faecalis* further contains three subspecies (*A. faecalis* subsp. *phenolicus*, *A. faecalis* subsp. *para faecalis*, *A. faecalis* subsp. *faecalis*), whereas the genus has three other species such as *A. endophyticus*, *A. pakistanensis*, and *A. aquatilis* (Mehanni and Abd El-Aziz 2019). The limitation of 16S rRNA sequence in differentiating

closely related species could be overcome by DNA-DNA hybridization method by whole genome sequencing or housekeeping gene sequence (Murray et al. 1990). Therefore, further studies involving more advanced genetic and molecular techniques are needed to validate novel uricase-producing strains.

Uricase is found in almost all organisms (mammals, bacteria, yeasts, fungi, and plants except humans (Honarbakhsh et al. 2021)). The uricase enzyme, predominantly found in the liver, forms a tetramer with each subunit weighing approximately 32–34 kDa, and this enzyme is associated with peroxisomes (Wu et al. 1989). Uricase synthesizes crystalloid core in hepatocytes' peroxisomes and is well-known to treat gout arthritis. Higher uric acid levels in biological fluids (3.6 mg dl-L in children, 6 mg dl-L in females, and > 7 mg dl-L in males) cause hyperuricemia, which refers to monosodium urate crystal formation in the blood leading to inflammation and discomfort in and around joints (Nelapati et al. 2021). The lack of uricase also contributes to gout in humans and thus its administration is suggested to decrease uric acid levels in the plasma to treat gout and hyperuricemia (Nanda and JagadeeshBabu 2016). Initially, *Aspergillus flavus*-based uricase was used to treat gout, and tumor lysis-associated hyperuricemia. European and the United States Food and Drug Administration (US FDA) have approved a recombinant *A. flavus* uricase (Rasburicase, Elitek®, Sanofi-Aventis) and a PEGylated chimeric porcine-baboon uricase (Pegloticase, Krystexxa®, Savient Pharmaceuticals) to safely and rapidly reduce uric acid levels in plasma (Gabison et al. 2008). Rasburicase, a *Saccharomyces cerevisiae* polypeptide chain (301 amino acids), acts as an allopurinol alternative, and it controls uric acid levels more effectively than allopurinol (Zhu et al. 2022).

Higher uric acid levels in urine and blood raise the risk of neurological diseases, kidney illness, stroke, cardiovascular diseases, and hypertension. Childhood leukemia is also linked to higher urate content (Roopa et al. 2022). Metabolic diseases generating and forcing diuresis and urine alkalinization are treated through medication. Xanthine oxidase inhibitors (febuxostat and allopurinol) are often used to alleviate uric acid production. Frequent uricase administration induces anaphylaxis and allergic responses as it is primarily composed of foreign antigenic proteins. Allopurinol is commonly recommended for hyperuricemia treatment (Beedkar et al. 2012). Moreover, uricosuric medicines and recombinant uricase preparations are used to reduce uric acid concentrations (Pierzynowska et al. 2020).

An appropriate medium with optimized contents and parameters is crucial for cost-effective industrial-scale enzyme production. Generally, the one factor at a time (OFAT) method is considered an effective

approach for devising optimal medium components and parameters. OFAT has been adopted to optimize and increase uricase production in various strains, such as *Halobacillus* spp. strain GCFx14, *Bacillus licheniformis*, and *B. subtilis* strain SP6 (Pawar and Rathod, 2018; Honarbakhsh et al. 2021; Pustake et al. 2019; 2022). Thus, the OFAT approach can reveal effective nutrient sources to enhance extracellular enzyme production in the target bacterium. Several factors contribute to uricase production in bacteria. Thus, important factors such as production strain, temperature, incubation time, pH, salinity, fermentation media, and different carbon and nitrogen sources were investigated during this study. Out of the fifty-two uricase-producing strains, the most promising strain, UR1, producing the largest clear zone diameter in minimum time, was selected for further studies based on the uric acid plate assay. The OFAT approach was adopted to optimize the growth and uricase secretion of strain UR1.

During this study, the growth and uricase secretion of strain UR1 were optimized by evaluating different incubation times (24, 48, 72, and 96 h), temperatures (20, 25, 30, 37, 40, 45, and 50°C), and initial pH (pH 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, and 9). The results revealed that strain UR1 achieved maximum growth (2.5 OD) and uricase activity (65 U/ml) after 50 h incubation period. Initial growth phases were characterized by a high uric acid consumption rate, leading to reduced uric acid concentration and increased biomass. Ghosh and Sarkar (2014) have also reported 99% uric acid consumption by *Comamonas* spp. in 33 h at 37°C. Atty and Joseph (2016) reported a higher uric acid consumption rate by *Ochrobactrum anthropic* strain during initial growth phases, which reduced the uric acid concentration with an increase in biomass. Most strains consume more than 25% uric acid within 20 h of bacterial growth.

The data demonstrated a drastic reduction in strain UR1 growth and uricase activity at a temperature of more than 40°C whereas the maximum growth (2.5 OD) and uricase activity (67 U/ml) were observed at 37°C. The maximum strain UR1 growth (3 OD) and uricase activity (80 U/ml) were noted at an initial pH of 7.5. Jagathy et al. (2016) have also reported an optimum initial pH of 7 and an optimum temperature range of 30–37°C for growth and uricase secretion from *B. subtilis*. However, other studies have stated a lower optimum pH of 5.5 for uricase activity (Abdel-Fattah et al. 2005; Li et al. 2006).

Different nitrogen and carbon sources in the media play a pivotal role in the growth of uricase-producing microorganisms. The results revealed a sequence of various carbon sources' efficiency as sucrose > glucose > cellulose > starch > maltose. On the other hand, peptone appeared to be the most efficient nitrogen source for uricase production among different sources

(Pfrimer et al. 2010). It seems that only sucrose was able to enhance uricase secretion of strain UR1 by 10%, whereas other carbon sources such as glucose, maltose, dextrose, and cellulose reduced uricase secretion by 50, 40, 25, and 20% as compared to control, which could be caused by glucose effect. These results align with the findings of Thillainayagi et al. (2021), who found that sucrose facilitated the maximum growth as a carbon source whereas uric acid favored the highest growth and uricase activity as the sole nitrogen source. Liu et al. (2023), also suggested the possibility of glucose-based suppression of uric acid metabolism. However, some glucose-containing media (BHI and GAM) support uric acid consumption. Watanabe and Morita (1973), also reported a glucose-linked inhibition of uricase formation in the absence of potassium ions. Recent reports have suggested the role of potassium ions in protein synthesis. It highlights an increased need for potassium ions in the presence of glucose in the medium. The impact of glucose on uricase formation is associated with glucose metabolism, which might depend on the presence of potassium ions. Glucose metabolism might produce an inhibitory metabolite without potassium ions to restrict enzyme formation. Jagathy et al. (2016), reported a decrease in enzyme productivity as sucrose > starch > glucose > citric acid > lactose. Handayani et al. (2018), also reported an inhibition in *L. plantarum*-based uricase production at high glucose concentrations. However, uricase synthesis in most microorganisms is regulated by growth medium components and their uric acid degrading capability. Moreover, metabolite (nitrogen or carbon origin)-based repression could also control uricase production in microorganisms (Van Der Drift et al. 1975; Bongaerts et al. 1977).

Yeast extract, casein, peptone, and beef promoted the uricase secretion of strain UR1 by 12, 10, 8, and 7% compared to control (100%). Contrarily, inorganic nitrogen sources such as urea, ammonium sulfate, and sodium nitrate significantly inhibited the uricase production of strain UR1 by 88, 83, and 80% as compared to control. Lotfy (2008) also noticed enhanced uricase production with various nitrogen sources such as corn steep liquor, maize milk, peptone, yeast extract, soybean, beef extract, and sodium glutamate. Amirthanathan and Vijayakumar (2011) found yeast extract and sucrose as the best nitrogen sources for uricase production whereas inorganic nitrogen sources (potassium nitrate and ammonium sulfate) yielded the lowest values. Organic nitrogen sources might contain growth-promoting amino acids, which cells could metabolize directly to enhance uricase production (El-Naggar 2015; Lotfy, 2008; Pustake et al. 2019). Pustake et al. (2019) reported a significant impact of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , lactose, and soya peptone on uricase production,

whereas the effects of  $\text{NaCl}$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{K}_2\text{HPO}_4$ , and  $\text{NH}_4\text{NO}_3$  were non-significant.

Thillainayagi et al. (2021) stated that sucrose induced the maximum growth (2.1 OD) and uricase activity (36 U/ml/min) whereas fructose remained the most ineffective carbon source. Similarly, uric acid facilitated the highest uricase production (31 U/ml/min) and growth (1.83 OD), whereas potassium dihydrogen phosphate remained the least effective nitrogen source. In line with the current study, Atalla et al. (2010) noted uric acid and sucrose as the best nitrogen and carbon sources for *Gliomastix guei* isolate-based uricase production. Zhou et al. (2005) demonstrated maize milk as the best nitrogen and carbon source for a *Microbacterium* spp. isolate. The findings of Nanda and Babu (2014) contradicted the current study as they reported sucrose as the less effective carbon source. Furthermore, they found asparagine as the best nitrogen source whereas ammonium nitrate and yeast extract supported a low uricase productivity in a *B. cereus* strain. Lekshmy and Raval (2016) isolated a *Comamonas testosteroni* strain from poultry waste that exhibited maximum uricase activity (0.15 U/ml) at pH 7 and 30°C with peptone and cellulose as the ideal nitrogen and carbon sources. Selvaraj and Thirumalai (2017) reported 35°C, pH 5, glucose (carbon source), and peptone (nitrogen source) as the optimum parameters for *P. aeruginosa* isolate growth and uricase production. Higher salt concentrations significantly impacted the UR1 growth whereas the lower  $\text{NaCl}$  (0.1%) concentration favored the maximum growth (3 OD) and uricase secretion (80 U/ml). These findings contradict the results of Atty and Joseph (2016) who achieved optimum *O. anthropic* growth at 0.5%  $\text{NaCl}$  concentration. Amirthanathan and Vijayakumar (2011) also noticed a salinity concentration of 1.5% optimum for *P. aeruginosa* growth and uricase secretion.

This study purified an extracellular uricase produced by strain UR1 from the culture supernatant without cell disruption, indicating uric acid's key role in uricase production. Contrarily, most published microbial uricase enzymes are intracellular, requiring cell disruption for uricase secretion (Yokoyama et al. 1988; Zhou et al. 2005; Azab et al. 2005; Kai et al. 2008). DEAE-Sepharose-based uricase purification was not successful during this study. However, the addition of the gel filtration chromatography step yielded 66.37% and 18.33% pure uricase with 275 U/ml and 916 U/mg of volume and specific activity, respectively. Interestingly, this purified uricase from strain UR1 exhibited a very high uricase activity (275 U/ml) as compared to published uricase enzymes from *S. graminofaciens* (8 U/ml), *A. globiformis* (22 U/ml), *Microbacterium* spp. (1.0 U/ml), *P. vulgaris* (8.7 U/ml), *P. aeruginosa* (7.1 U/ml) and (40 U/ml), *Comamonas* spp. (80 U/ml),



and *B. thermocatenuatus* (1.25 U/ml) (Kiba et al. 2000; Abdel-Fattah et al. 2005; Azab et al. 2005; Zhou et al. 2005; Khucharoenphaisan and Sinma 2011; Ghosh and Sarkar 2014; Amirthanathan and Vijayakumar 2011). Multiple studies purified uricase from *P. aeruginosa* strain's culture filtrate in two steps involving ammonium sulfate (70%)-based precipitation saturation and ion exchange chromatography on DEAE-cellulose. The process purified 7.2 fold uricase with a yield of 43.79%. This method has been followed in various studies for the precipitation and partial purification of uricase enzymes from *Gliomastix gueg*, *P. aeruginosa*, *Candida utilis*, *Streptomyces* spp., *Proteus* spp., *B. cereus* using ammonium sulphate (60–80% w/v) (Adámek et al. 1989; Saeed et al. 2004; Azab et al. 2005; Atalla et al. 2010; Nanda et al. 2012).

This study used SDS-PAGE to evaluate purified uricase from strain UR1, which appeared as a single polypeptide of 45 kDa molecular mass. However, the molecular mass of purified uricase from different sources could be different. SDS-PAGE of purified enzymes from different microorganisms revealed varying molecular masses enzyme of 35 kDa from *Comamonas* spp. BT UA strain with a single protein band (Ghosh and Sarkar 2014) 33 kDa from *P. aeruginosa* (Amirthanathan and Vijayakumar 2011) 48 kDa from *B. subtilis* (Jagathy et al. 2016), 34 kDa from *C. utilis* (Koyama et al. 1996), and 60 kDa from *B. subtilis* (Pfrimer et al. 2010). Several factors, including pH and temperature, could affect uricase enzyme activity and stability. Different studies have reported an optimum pH of 8 and a temperature range of 25–45°C for better enzyme activity. The enzyme activity is often reported to decrease by 50% after exposure to 60°C for one hour (Chohan and Becker 2009; Ravichandran et al. 2015). This study demonstrated a maximum activity of purified uricase enzyme at 40°C that was sustained up to 50°C. However, the enzyme activity was completely lost at 90°C. Moreover, the purified uricase remained active within a broad range of pH (5–9) and exhibited the maximum activity (275 U/ml) at pH 7.5, which gradually decreased to 94, 80, and 20% at pH 8, 8.5, and 9, respectively. The activity was completely lost above pH 9. The enzyme activity was also not detected at pH 4.0 whereas only 6% of the relative activity was observed at pH 5. Thillainayagi et al. (2021) have also reported greater stability of crude enzyme from *A. faecalis* at a wide range of temperatures and pH as it retained more than 70% and 60% of residual activity, respectively. Zhou et al. (2005) reported enzyme stability at 37°C and pH 8.5 for more than 40 days whereas uricase remained stable with 100% activity even after heat treatment at 70°C for 30 min. Multiple studies reported stable uricase activity at pH 8–9 with peak enzyme activity and stability at pH 8.5 and 30°C

(Kai et al. 2008; Nanda and Babu 2014). Jagathy et al. (2016) noticed a maximum activity of *B. subtilis*-yielded uricase at pH 7 whereas other studies reported a relatively lower pH of 5.5 as an optimum pH for uricase activity (Abdel-Fattah et al. 2005; Li et al. 2006). An optimum temperature range of 30°C to 35°C has been revealed in some studies for uricase production (Abdel-Fattah et al. 2005).

Uricase activity was significantly reduced against various inhibitors such as  $\beta$ -mercaptoethanol (94.6%), EDTA (66.8%),  $\text{Co}^{2+}$  (61.81%), and Zn (31.20%).  $\text{Ca}^{2+}$  strongly promoted the uricase activity (20%) whereas the increase in uricase activity with other metal ions [ $\text{Cu}^{2+}$  (6%),  $\text{Mg}^{2+}$  (5%), and  $\text{Mn}^{2+}$  (2%)] was comparatively low. It suggests that metal ions could bind to the uricase enzyme to change the enzymatic activity through destabilization or stabilization of the enzyme's conformation. EDTA-based uricase activity inhibition might be overcome by adding metal ions as cofactors. In this regard, several studies have reported varying effects of cations on enzyme stability and activity (Bakhtiar et al. 2003; Patel et al. 2006; Kai et al. 2008; Atalla et al. 2010; de Oliveira et al. 2010; Ravichandran et al. 2015).

The novelty of this study the enzyme volume and activity of uricase-producing strain of *Alcaligenes* spp. (UR1). Uricase enzyme exhibited significant specific activity of 916 U/mg and volume activity of 275 U/ml. These values are significantly higher than *Microbacterium* spp. (1 U/ml) and *S. graminofaciens* (8 U/ml). Moreover, extracellular uricase synthesis by strain UR1 distinguishes it from other bacteria (*P. aeruginosa* and *B. subtilis*), which are only capable of intracellular uricase production (Azab et al. 2005; Zhou et al. 2005). The extracellular uricase synthesis alleviates the processing cost and presents strain UR1 as an efficient tool for industrial applications.

**Relevance to Current Knowledge.** This study aligns with previous investigations, highlighting the uricase enzyme's industrial and therapeutic roles. Uricase efficacy has been established for the clinical treatments of hyperuricemia and gout. Similarly, Pegloticase and Rasburicase recombinant enzymes have already been approved for clinical applications (Gabison et al. 2008). However, the high cost of production and immunogenicity are major restrictions of these enzymes. Strain UR1-associated extracellular uricase enzyme's higher stability and activity offer an economical alternative to avoid these hurdles. During this study, sucrose served as the carbon source, considerably improving uricase synthesis and activity. Contrarily, glucose restricted the uricase synthesis, which aligns with previous reports (Watanabe and Morita 1973). Organic nitrogen sources (yeast extract and peptone) also improved enzyme growth and yield whereas inorganic nitrogen sources restricted enzyme synthesis. These findings agree with



previous studies and provide valuable information regarding fermentation media optimization during uricase synthesis (Lotfy 2008; Thillainayagi et al. 2021).

**Implications of This Research.** The findings of this study offer effective therapeutic and industrial applications.

**Therapeutic Applications.** Highly stable and active uricase enzyme from strain UR1 can be used as an effective tool for treating gout and hyperuricemia. Extracellular synthesis of this enzyme further promotes its scalability at an affordable cost. Moreover, lesser regulatory limitations for the non-genetically modified strain UR1 enhance its accessibility for clinical use.

**Industrial Scale-Up.** This study's findings could facilitate large-scale uricase production with strain UR1. The optimization of medium composition and growth conditions provides guidelines for inexpensive uricase production at the industrial level. Uricase stability at various temperatures and pH levels further improves its suitability for a range of industrial applications.

**Future Directions.** Though this study confirms the uricase synthesis efficacy of strain UR1, further investigations are necessary for its enhanced applicability. Whole-genome sequencing (WGS) could elaborate on the genetic basis of uricase synthesis to enable specific genetic alterations for improved stability and yield. Moreover, advanced bioprocessing approaches (fed-batch fermentation) could also improve industrial-scale enzyme yield. The therapeutic potential of uricase should also be clinically analyzed through efficacy and immunogenicity assessment along with the exploration of influential cofactors for enhanced activity in diverse formulations and improved shelf-life.

## Conclusion

The study highlights the uricase synthesis potential of *Alcaligenes* spp. strain UR1. It also addresses key gaps in previous uricase production techniques and optimizes and analyzes the enzyme's properties. Moreover, the results provide a foundation for industrial and therapeutic applications of strain UR1. Future investigations should focus on enhancing uricase production and clinical applications through advanced molecular and genetic analyses.

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## Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

## Literature

- Abdalahim S, Zohri ANA, Khider M, Kamal El-Dean AM, Abulreesh HH, Ahmad I, Elbanna K. Phenotypic and genotypic characterization of exopolysaccharide producing bacteria isolated from fermented fruits, vegetables and dairy products. *J Pure Appl Microbiol.* 2019 Sep;13(3):1349–1362. <https://doi.org/10.22207/jpam.13.3.06>
- Abdel-Fattah YR, Saeed HM, Gohar YM, El-Baz MA. Improved production of *Pseudomonas aeruginosa* uricase by optimization of process parameters through statistical experimental designs. *Process Biochem.* 2005 Apr;40(5):1707–1714. <https://doi.org/10.1016/j.procbio.2004.06.048>
- Abo-Amer AE, El-Shanshoury AERR, Alzahrani OM. Isolation and molecular characterization of heavy metal-resistant *Alcaligenes faecalis* from sewage wastewater and synthesis of silver nanoparticles. *Geomicrobiol J.* 2015 Aug;32(9):836–845. <https://doi.org/10.1080/01490451.2015.1010754>
- Adámek V, Králová B, Süchová M, Valentová O, Demnerová K. Purification of microbial uricase. *J Chromatogr.* 1989 Dec;497:268–275. [https://doi.org/10.1016/0378-4347\(89\)80028-3](https://doi.org/10.1016/0378-4347(89)80028-3)
- Aly M, Tork S, Al-Garni S, Allam R. Production and characterization of uricase from *Streptomyces exfoliatus* UR10 isolated from farm wastes. *Turk J Biol.* 2013;37(5):520–529. <https://doi.org/10.3906/biy-1206-3>
- Amirthanathan A, Subramaniyan V. Studies on uricase production by marine *Bacillus cereus* and its optimum conditions. *Int J Med. Biosci.* 2012;1:5–12.
- Amirthanathan A, Vijayakumar S. Purification and optimization of uricase enzyme produced by *Pseudomonas aeruginosa*. *J Exp Sci.* 2011;2(11):5–8.
- Atalla MM, Hamed ER, Ahmed NE, Farag MM. Purification and characterization of uricase enzyme produced by *Gliomastix gueg*. *Gate2Biotech.* 2010;2(11):1–13.
- Atty FK, Joseph J. Isolation and identification of uric acid degrading bacteria, optimization of uricase production and purification of uricase enzyme. *Int J Ad. Res.* 2016 Dec;4(12):2732–2742. <https://doi.org/10.21474/ijar01/2702>
- Azab EA, Ali MM, Fareed MF. Studies on uricase induction in certain bacteria. *Egypt J Biol.* 2005;7:44–54.
- Bakhtiar S, Andersson MM, Gessesse A, Mattiasson B, Hatti-Kaul R. Stability characteristics of a calcium-independent alkaline protease from *Nesterenkonia* sp. *Enzyme Microb Technol.* 2003 Apr;32(5):525–531. [https://doi.org/10.1016/S0141-0229\(02\)00336-8](https://doi.org/10.1016/S0141-0229(02)00336-8)
- Bakhtiar S, Toosi P, Samadi S, Bakhshi M. Assessment of uric acid level in the saliva of patients with oral lichen planus. *Med Princ Pract.* 2017;26(1):57–60. <https://doi.org/10.1159/000452133>
- Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 1966 Apr;45(4\_ts):493–496. [https://doi.org/10.1093/ajcp/45.4\\_ts.493](https://doi.org/10.1093/ajcp/45.4_ts.493)

- Beedkar SD, Khobragade CN, Bodade RG, Vinchurkar AS. Comparative structural modeling and docking studies of uricase: Possible implication in enzyme supplementation therapy for hyperuricemic disorders. *Comput Biol Med.* 2012 Jun;42(6):657–666. <https://doi.org/10.1016/j.combiomed.2012.03.001>
- Bobulescu IA, Moe OW. Renal transport of uric acid: Evolving concepts and uncertainties. *Adv Chronic Kidney Dis.* 2012 Nov; 19(6):358–371. <https://doi.org/10.1053/j.ackd.2012.07.009>
- Bongaerts GP, Sin IL, Peters AL, Vogels GD. Purine degradation in *Pseudomonas aeruginosa* and *Pseudomonas testosteroni*. *Biochim Biophys Acta.* 1977 Aug 25;499(1):111–118. [https://doi.org/10.1016/0304-4165\(77\)90233-1](https://doi.org/10.1016/0304-4165(77)90233-1)
- Castellani A, Chalmers AJ. Manual of tropical medicine. London (UK): Baillière, Tindall and Cox; 1919. <https://doi.org/10.5962/bhl.title.84653>
- Chaudhary K, Malhotra K, Sowers J, Aroor A. Uric acid – key ingredient in the recipe for cardiorenal metabolic syndrome. *Cardiorenal Med.* 2013 Oct;3(3):208–220. <https://doi.org/10.1159/000355405>
- Cheung WL, Hon KL, Fung CM, Leung AK. Tumor lysis syndrome in childhood malignancies. *Drugs Context.* 2020 Feb;9:2019-8-2. <https://doi.org/10.7573/dic.2019-8-2>
- Chohan S, Becker MA. Update on emerging urate-lowering therapies. *Curr Opin Rheumatol.* 2009 Mar;21(2):143–149. <https://doi.org/10.1097/bor.0b013e328325bd94>
- Clarridge JE. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev.* 2004 Oct;17(4):840–862. <https://doi.org/10.1128/cmr.17.4.840-862.2004>
- CLSI. Performance standards for antimicrobial susceptibility testing. 34<sup>th</sup> ed. CLSI supplement M100. Wayne (USA): Clinical and Laboratory Standards Institute; 2024.
- Cruz D, Cisneros R, Benítez Á, Zúñiga-Sarango W, Peña J, Fernández H, Jaramillo A. Gram-negative bacteria from organic and conventional agriculture in the hydrographic basin of Loja: Quality or pathogen reservoir? *Agronomy.* 2021 Now;11(11):2362. <https://doi.org/10.3390/agronomy11112362>
- de Oliveira AN, de Oliveira LA, Andrade JS. Production and some properties of crude alkaline proteases of indigenous Central Amazonian rhizobia strains. *Braz Arch Biol Technol.* 2010 Oct;53(5):1185–1195. <https://doi.org/10.1590/s1516-89132010000500024>
- El Ridi R, Tallima H, Migliardo F. Biochemical and biophysical methodologies open the road for effective schistosomiasis therapy and vaccination. *Biochim Biophys Acta Gen Subj.* 2017 Jan; 1861(1): 3613–3620. <https://doi.org/10.1016/j.bbagen.2016.03.036>
- Elbanna K, Alshareef A, Neyaz LA, El-Readi MZ, Abulreesh HH. Microbial uricase and its unique potential applications. *Adv Microbiol.* 2024;63(2):81–91. <https://doi.org/10.2478/am-2024-0007>
- Elbanna K, Hassan G, Khider M, Mandour R. Safe biodegradation of textile azo dyes by newly isolated lactic acid bacteria and detection of plasmids associated with degradation. *J. Bioremed Biodegrad.* 2010;1:110. <https://doi.org/10.4172/2155-6199.1000110>
- El-Naggar NEA. Isolation, screening and identification of actinobacteria with uricase activity: Statistical optimization of fermentation conditions for improved production of uricase by *Streptomyces rochei* NEAE-25. *Int J Pharmacol.* 2015;11(7):644–658. <https://doi.org/10.3923/ijp.2015.644.658>
- Fathallah-Shaykh SA, Cramer MT. Uric acid and the kidney. *Pediatr Nephrol.* 2014 Jun;29(6):999–008. <https://doi.org/10.1007/s00467-013-2549-x>
- Fox GE, Wisotzkey JD, Jurtshuk P Jr. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int J Syst Bacteriol.* 1992 Jan;42(1):166–170. <https://doi.org/10.1099/00207713-42-1-166>
- Gabison L, Prangé T, Colloc'h N, El Hajji M, Castro B, Chiadmi M. Structural analysis of urate oxidase in complex with its natural substrate inhibited by cyanide: Mechanistic implications. *BMC Struct Biol.* 2008 Jul;8:32. <https://doi.org/10.1186/1472-6807-8-32>
- Gerhardt P, Murray RGE, Wood WA, Krieg NR. Methods for general and molecular bacteriology. Washington (USA): American Society for Microbiology; 1994.
- Ghosh T, Sarkar P. Isolation of a novel uric-acid-degrading microbe *Comamonas* sp. BT UA and rapid biosensing of uric acid from extracted uricase enzyme. *J Biosci.* 2014 Dec;39(5):805–819. <https://doi.org/10.1007/s12038-014-9476-2>
- Guo L, Ye L, Zhao Q, Ma Y, Yang J, Luo Y. Comparative study of MALDI-TOF MS and VITEK 2 in bacteria identification. *J Thorac Dis.* 2014 May;6(5):534–538.
- Hafez RM, Abdel-Rahman TM, Naguib RM. Uric acid in plants and microorganisms: Biological applications and genetics – A review. *J Adv Res.* 2017 Sep;8(5):475–486. <https://doi.org/10.1016/j.jare.2017.05.003>
- Handayani I, Utami T, Hidayat C, Rahayu ES. Screening of lactic acid bacteria producing uricase and stability assessment in simulated gastrointestinal conditions. *Int Food Res J.* 2018 Aug;25(4): 1661–1667.
- Honarbaksh F, Abolmaali S, Amoozegar MA. Uricase activity of halophilic bacteria from Iranian salt lakes. *Iran J Sci Technol Trans Sci.* 2021 Oct;45:1597–1606. <https://doi.org/10.1007/s40995-021-01179-0>
- Jagathy K, Pushparaj A, Ronald J. Uricase production from *Bacillus subtilis* isolated from poultry waste. *Int. J Adv Res Biol Sci.* 2016; 3(6):255–262.
- Jalal DI. Hyperuricemia, the kidneys, and the spectrum of associated diseases: A narrative review. *Curr Med Res Opin.* 2016 Nov; 32(11):1863–1869. <https://doi.org/10.1080/03007995.2016.1218840>
- Kai L, Ma XH, Zhou XL, Jia XM, Li X, Guo KP. Purification and characterization of a thermostable uricase from *Microbacterium* sp. strain ZZJ4-1. *World J Microbiol Biotechnol.* 2008 Mar;24:401–406. <https://doi.org/10.1007/s11274-007-9489-1>
- Kanbay M, Segal M, Afsar B, Kang DH, Rodriguez-Iturbe B, Johnson RJ. The role of uric acid in the pathogenesis of human cardiovascular disease. *Heart.* 2013 Jun;99(11):759–766. <https://doi.org/10.1136/heartjnl-2012-302535>
- Khucharoenphaisan K, Sinma K. Production and partial characterization of uric acid degrading enzyme from new source *Saccharopolyspora* sp. PNR11. *Pak J Biol Sci.* 2011 Feb;14(3):226–231. <https://doi.org/10.3923/pjbs.2011.226.231>
- Kiba N, Suzuki K, Miwa T, Tachibana M, Koizumi H, Tani K. Chemiluminometric determination of uric acid in plasma by closed-loop FIA with a coimmobilized enzyme flow cell. *Anal Sci* 2000 Nov; 16:1203–1205. <https://doi.org/10.2116/analsci.16.1203>
- Kim S, Kim MH, Lee WI, Kang SY, Jeon YL. Misidentification of *Acinetobacter baumannii* as *Alcaligenes faecalis* by VITEK 2 System; Case report. *Lab Med.* 2017 Dec;49(1):e14–e17. <https://doi.org/10.1093/labmed/lmx062>
- Kim SM, Lee SH, Kim YG, Kim SY, Seo JW, Choi YW, Kim DJ, Jeong KH, Lee TW, Ihm CG, et al. Hyperuricemia-induced NLRP3 activation of macrophages contributes to the progression of diabetic nephropathy. *Am J Physiol Renal Physiol.* 2015 May;308(9):F993–F1003. <https://doi.org/10.1152/ajprenal.00637.2014>
- Koyama Y, Ichikawa T, Nakano E. Cloning, sequence analysis, and expression in *Escherichia coli* of the gene encoding the *Candida utilis* urate oxidase (uricase). *J Biochem.* 1996 Nov;120(5):969–973. <https://doi.org/10.1093/oxfordjournals.jbchem.a021514>
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970 Aug;227(5259):680–685. <https://doi.org/10.1038/227680a0>

- Lekshmy V, Raval K.** Isolation and production studies of uricase producing bacterial strains from poultry waste. *Int J Adv Res Trends Eng Technol.* 2016;3:152–157.
- Li J, Chen Z, Hou L, Fan H, Weng S, Xu C, Ren J, Li B, Chen W.** High-level expression, purification, and characterization of non-tagged *Aspergillus flavus* urate oxidase in *Escherichia coli*. *Protein Expr Purif.* 2006 Sep;49(1):55–59. <https://doi.org/10.1016/j.pep.2006.02.003>
- Liu Y, Jarman JB, Low YS, Augustijn HE, Huang S, Chen H, DeFeo ME, Sekiba K, Hou BH, Meng X, et al.** A widely distributed gene cluster compensates for uricase loss in hominids. *Cell.* 2023 Aug;186(16):3400–3413.e20. <https://doi.org/10.1016/j.cell.2023.06.010>
- Lotfy WA.** Production of a thermostable uricase by a novel *Bacillus thermocatenulatus* strain. *Bioresour Technol.* 2008 Mar;99(4):699–702. <https://doi.org/10.1016/j.biortech.2007.01.048>
- Madigan MT, Martinko JM, Parker J.** Brock biology of microorganisms. London (UK): Prentice Hall; 2000.
- Mehanni M, Abd El-Aziz S.** A new bacterial strain *Alcaligenes aquatilis* MAG 1 associated with the Mediterranean Sea *Lithophaga* (Bivalvia) precipitating in nutrient free medium shell-like calcium carbonate polymorphs. *Egypt Acad J Biol Sci.* 2019;11(1):111–124. <https://doi.org/10.21608/eajbsg.2019.153533>
- Murray RGE, Brenner DJ, Colwell RR, De Vos P, Goodfellow M, Grimont PAD, Pfennig N, Stackebrandt E, Zavarzin GA.** Report of the ad hoc committee on approaches to taxonomy within the Proteobacteria. *Int J Syst Bacteriol.* 1990 Apr;40(2):213–215. <https://doi.org/10.1099/00207713-40-2-213>
- Nanda P, Babu PE.** Isolation, screening and production studies of uricase producing bacteria from poultry sources. *Prep Biochem Biotechnol.* 2014;44(8):811–821. <https://doi.org/10.1080/10826068.2013.867875>
- Nanda P, Jagadeesh Babu PE.** Studies on the site-specific PEGylation induced interferences instigated in uricase quantification using the Bradford method. *Int J Pept Res Ther.* 2016 Sep;22:399–406. <https://doi.org/10.1007/s10989-016-9518-8>
- Nanda P, Babu PJ, Fernandes J, Hazarika P, Dhabre RR.** Studies on production, optimization and purification of uricase from *Gliocladium viride*. *Res. Biotechnol.* 2012;3(4):35–46.
- Sarni, Natsir H, Dali S.** Production and characterization chitosanase of sponge symbiont bacteria *Klebsiella* sp. to hydrolyze chitosan be chitoooligosaccharides. *Mar Chim Acta.* 2016 Apr; 17(1):1441–2132.
- Nelapati AK, Meena S, Singh AK, Bhakta N, Ponnann Ettiyappan J.** *In silico* structural and functional analysis of *Bacillus uricaes*. *Curr. Proteomics.* 2021;18(2):124–142. <https://doi.org/10.2174/1570164617999200512081127>
- Ogawa J.** Analysis of microbial purine metabolism and its application for hyperuricemia prevention. Noda City (Japan): Noda Institute for Scientific Research; 2006. [cited 2024 September 24]. Available from <https://www.nisr.or.jp/wp-content/uploads/NISR06ogawa.pdf>
- Page RD.** TreeView: An application to display phylogenetic trees on personal computers. *Comput Appl Biosci.* 1996 Aug;12(4):357–358. <https://doi.org/10.1093/bioinformatics/12.4.357>
- Patel RK, Dodia MS, Joshi RH, Singh SP.** Purification and characterization of alkaline protease from a newly isolated haloalkaliphilic *Bacillus* sp. *Process Biochem.* 2006 Sep;41(9):2002–2009. <https://doi.org/10.1016/j.procbio.2006.04.016>
- Pawar SV, Rathod VK.** Ultrasound assisted process intensification of uricase and alkaline protease enzyme co-production in *Bacillus licheniformis*. *Ultrason Sonochem.* 2018 Jul;45:173–179. <https://doi.org/10.1016/j.ultsonch.2018.03.004>
- Perez-Ruiz F, Martínez-Indart L, Carmona L, Herrero-Beites AM, Pijoan JI, Krishnan E.** Tophaceous gout and high level of hyperuricaemia are both associated with increased risk of mortality in patients with gout. *Ann Rheum Dis.* 2014 Jan;73(1):177–182. <https://doi.org/10.1136/annrheumdis-2012-202421>
- Pfrimer P, de Moraes LM, Galdino AS, Salles LP, Reis VC, De Marco JL, Prates MV, Bloch C Jr, Torres FA.** Cloning, purification, and partial characterization of *Bacillus subtilis* urate oxidase expressed in *Escherichia coli*. *J Biomed Biotechnol.* 2010;2010:674908. <https://doi.org/10.1155/2010/674908>
- Pierzynowska K, Deshpande A, Mosiichuk N, Terkeltaub R, Szczurek P, Salido E, Pierzynowski S, Grujic D.** Oral treatment with an engineered uricase, ALLN-346, reduces hyperuricemia, and uricosuria in urate oxidase-deficient mice. *Front Med.* 2020 Nov; 7:569215. <https://doi.org/10.3389/fmed.2020.569215>
- Pustake SO, Bhagwat PK, Dandge PB.** Statistical media optimization for the production of clinical uricase from *Bacillus subtilis* strain SP6. *Heliyon.* 2019 May;5(5):e01756. <https://doi.org/10.1016/j.heliyon.2019.e01756>
- Pustake SO, Bhagwat P, Pillai S, Dandge PB.** Purification and characterisation of uricase from *Bacillus subtilis* SP6. *Process Biochem.* 2022 Feb;113:55–61. <https://doi.org/10.1016/j.procbio.2021.12.010>
- Ram SK, Raval K, Jagadeesh Babu PE.** Enhancement of a novel extracellular uricase production by media optimization and partial purification by aqueous three-phase system. *Prep Biochem Biotechnol.* 2015;45(8):810–824. <https://doi.org/10.1080/10826068.2014.970690>
- Ravichandran R, Hemaasri S, Cameotra SS, Jayaprakash NS.** Purification and characterization of an extracellular uricase from a new isolate of *Sphingobacterium thalpophilum* (VITPCB5). *Protein Expr Purif.* 2015 Oct;114:136–142. <https://doi.org/10.1016/j.pep.2015.06.017>
- Ray RR, Pattnaik S.** *Alcaligenes faecalis*: a bacterium for sustainable management of environment. *Environ Qual Manag.* 2024; 34:e22189. <https://doi.org/10.1002/tqem.22189>
- Roopa RA, Mantelingu K, Guin M, Thimmaiah SB.** Bionzymatic spectrophotometric method for uric acid estimation in human serum and urine. *J Anal Chem.* 2022 Mar; 77:301–307. <https://doi.org/10.1134/s1061934822030091>
- Saeed HM, Abdel-Fattah YR, Gohar YM, Elbaz MA.** Purification and characterization of extracellular *Pseudomonas aeruginosa* urate oxidase enzyme. *Pol J Microbiol.* 2004;53(1):45–52.
- Saitou N, Nei M.** The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol.* 1987 Jul;4(4):406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Selvaraj C, Thirumalai VP.** Screening, production and optimization of uricase from *P. aeruginosa*. *Eur J Biotechnol Biosci* 2017 Jan; 5(1):57–61.
- Simmons DG, Gray JG, Rose LP, Dillman RC, Miller SE.** Isolation of an etiologic agent of acute respiratory disease (rhinotracheitis) of turkey poult. *Avian Dis.* 1979;23(1):194–203. <https://doi.org/10.2307/1589686>
- Suzuki K, Sakasegawa S, Misaki H, Sugiyama M.** Molecular cloning and expression of uricase gene from *Arthrobacter globiformis* in *Escherichia coli* and characterization of the gene product. *J Biosci Bioeng.* 2004;98(3):153–158. [https://doi.org/10.1016/s1389-1723\(04\)00259-2](https://doi.org/10.1016/s1389-1723(04)00259-2)
- Tandon S, Sharma A, Singh S, Sharma S, Sarma SJ.** Therapeutic enzymes: Discoveries, production and applications. *J Drug Delivery Sci Technol.* 2021 Jun;63:102455. <https://doi.org/10.1016/j.jddst.2021.102455>
- Thillainayagi S, Harikrishnan S, Jayalakshmi S.** Screening, optimization and production of uricase from *Alcaligenes faecalis*. *Int J Res Educ Sci Methods.* 2021 Apr;9(4):69–74.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG.** The CLUSTAL\_X windows interface: Flexible strategies for multiple

- sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 1997 Dec;25(24):4876–4882  
<https://doi.org/10.1093/nar/25.24.4876>
- Van der Drift L, Vogels GD, Van der Drift C.** Allantoin racemase: A new enzyme from *Pseudomonas* species. *Biochim Biophys Acta.* 1975 May;391(1):240–248. [https://doi.org/10.1016/0005-2744\(75\)90170-9](https://doi.org/10.1016/0005-2744(75)90170-9)
- Versalovic J.** *Manual of Clinical Microbiology* 10<sup>th</sup> ed. ASM Press, 2011. USA.
- Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW.** *Manual of clinical microbiology.* Washington (USA): American Society for Microbiology; 2011.
- Watanabe Y, Morita M.** Effect of glucose on the uricase formation by *Streptomyces* sp. *Agric Biol Chem.* 1973 Dec;37(12):2735–2741. <https://doi.org/10.1080/00021369.1973.10861086>
- Wu XW, Lee CC, Muzny DM, Caskey CT.** Urate oxidase: Primary structure and evolutionary implications. *Proc Natl Acad Sci USA.* 1989 Dec;86(23):9412–9416. <https://doi.org/10.1073/pnas.86.23.9412>
- Xiao J, Zhang XL, Fu C, Han R, Chen W, Lu Y, Ye Z.** Soluble uric acid increases NALP3 inflammasome and interleukin-1 $\beta$  expression in human primary renal proximal tubule epithelial cells through the Toll-like receptor 4-mediated pathway. *Int J Mol Med.* 2015 May; 35(5):1347–1354. <https://doi.org/10.3892/ijmm.2015.2148>
- Xu X, Hu J, Song N, Chen R, Zhang T, Ding X.** Hyperuricemia increases the risk of acute kidney injury: A systematic review and meta-analysis. *BMC Nephrol.* 2017 Jan;18(1):27. <https://doi.org/10.1186/s12882-016-0433-1>
- Yang X, Yuan Y, Zhan CG, Liao F.** Uricases as therapeutic agents to treat refractory gout: Current states and future directions. *Drug Dev Res.* 2012 Mar;73(2):66–72. <https://doi.org/10.1002/ddr.20493>
- Yokoyama S, Ogawa A, Obayashi A.** Rapid extraction of uricase from *Candida utilis* cells by use of reducing agent plus surfactant. *Enzyme Microb Technol.* 1988 Jan;10(1):52–55. [https://doi.org/10.1016/0141-0229\(88\)90099-3](https://doi.org/10.1016/0141-0229(88)90099-3)
- Zhou XL, Ma XH, Sun GQ, Li X, Guo KP.** Isolation of a thermostable uricase-producing bacterium and study on its enzyme production conditions. *Process Biochem.* 2005 Dec;40(12):3749–3753. <https://doi.org/10.1016/j.procbio.2005.05.002>
- Zhu T, Chen H, Yang L, Liu Y, Li W, Sun W.** Characterization and Cys-directed mutagenesis of urate oxidase from *Bacillus subtilis* BS04. *Biologia.* 2022 Jan;77:290–301. <https://doi.org/10.1007/s11756-021-00941-4>