

MICROBIAL URICASE AND ITS UNIQUE POTENTIAL APPLICATIONS

Khaled Elbanna^{1, 2, 3*}, Atheer Alshareef^{1, 2}, Leena A. Neyaz^{1, 2},
Mahmoud Z. El-Readi^{4, 5}, Hussein H. Abulreesh^{1, 2}

¹ Department of Biology, Faculty of Science, Umm Al-Qura University, Makkah 21955, Saudi Arabia

² Research Laboratories Unit, Faculty of Science, Umm Al-Qura University, Makkah 21955, Saudi Arabia

³ Department of Agricultural Microbiology, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt

⁴ Department of Biochemistry, Faculty of Medicine, Umm Al-Qura University, Makkah 21955, Saudi Arabia

⁵ Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt

Submitted in January 2024, accepted in May 2024

Abstract: The uricase enzyme yields allantoin, hydrogen peroxide, and carbon dioxide by catalyzing the oxidative opening of the purine ring in the urate pathway. This enzyme is important for biochemical diagnosis and reduces toxic urate accumulation during various diseases (hyperuricemia, gout, and bedwetting). Direct urate oxidase injection is recommended in renal complications-associated gout and to prevent chemotherapy-linked hyperuricemia disorders. Thus, uricase is a promising enzyme with diverse applications in medicine. Microbial production of uricase is featured by high growth rates, cost-effective bioprocessing, and easy optimization of the medium. Microbes produce the enzyme extracellular or intracellular. Extracellular uricase is preferred for biotechnological applications as it minimizes time, effort, and purification processes. This review provides insights into uricase-producing microbes, bacterial uric acid degradation pathways, degrading enzymes, and uricase-encoding genes.

Furthermore, aspects influencing the microorganisms' production of the uricase enzyme, its activity, and its purification procedure are also emphasized. Cell disruption is mandatory for intercellular uricase production, which elevates production costs. Therefore, extracellular uricase-producing microbial strains should be investigated, and production factors should be optimized. Future techniques for obtaining extracellular enzymes should feature reduced time and effort, as well as a simple purification methodology. Furthermore, uricase gene-carrying recombinant probiotic microorganisms could become an effective tool for gout treatment.

1. Introduction. 1.1. Uric acid: chemical structure and biosynthesis. 1.2. Benefits and hazards of uric acid. 2. Uric acid degrading microorganisms. 3. Uric acid degradation pathway in bacteria. 4. Uric acid degrading enzymes. 5. Genetics and uricase encoding genes. 6. Uricase activity affecting factors. 7. Uricase production and purification. 7.1. Extracellular uricase. 7.2. Intercellular uricase. 7.3. Uricase purification from different microorganisms. 8. Applications of uricase. 8.1. Diagnostic role of uricase in clinical analysis. 8.2. Biosensor for bimolecular applications. 8.3. Agricultural applications of uricase. 8.4. Uricase-based detection of heavy metal water contamination. 8.5. Uricase application in nanomaterial manufacturing. Conclusion and future perspectives.

Keywords: gout, hyperuricemia disorders, microbial uricase, urate oxidase, uricase, uricase applications, uricase purification

1. Introduction

Uricase, a peroxisomal (oxidoreductase) enzyme, catalyzes the oxidative opening of the purine ring in the urate pathway to yield allantoin, hydrogen peroxide, and carbon dioxide (Roman 2023). Uricase is important for the biochemical diagnosis of uric acid in the serum and biological fluids (urine). Biosensors can readily detect uric acid more precisely and accurately than other methods (Aafaria *et al.* 2022). It also alleviates the accumulation of toxic urate during various diseases

(hyperuricemia, bedwetting, and gout). Uricase absence in some individuals could be attributed to metagenes, which prematurely terminate the translation process (Roman 2023). Direct urate oxidase injection is also suggested to treat renal complications-associated gout and prevent chemotherapy-linked hyperuricemia disorders (Cho *et al.* 2023). Microbes, animals, and plants can produce uricase. However, microbial production offers higher growth rates, cost-effective bioprocessing, and convenient optimization of the medium (Wan *et al.* 2023). The reported microbial uricase enzymes

* Corresponding Author: K. Elbanna, Department of Biology, Faculty of Science, Umm Al-Qura University, Makkah 21955, Saudi Arabia, kab00@fayoum.edu.eg

© 2024 Elbanna Khaled et al.

This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Cite as:

Microbial Uricase And Its Unique Potential Applications. Elbanna K. *et al.*, ADV MICROBIOL-NY, 2024, 63, 2, 81–91, <https://doi.org/10.2478/am-2024-0007>

mainly include intracellular enzymes released via cell disruption. Therefore, extracellular enzyme production is crucial to reduce yielding time and purification. This review elaborates on the uricase-producing microorganisms, bacteria-based uric acid degradation pathway, uricase activity affecting factors, microbial production and purification, and its applications. Cell disruption is mandatory for intracellular uricase synthesis and purification, which enhances the production cost. Identifying extracellular uricase-synthesizing microbial strains and optimizing conditions is highly advisable. Moreover, uricase gene-carrying recombinant probiotic microbes could emerge as an efficient gout treatment strategy.

1.1. Uric acid: chemical structure and biosynthesis

Uric acid ($C_5H_4N_4O_3$) is a heterocyclic compound of carbon, hydrogen, nitrogen, and oxygen (Fig. 1). It forms different salts and ions such as acid urates, urates, and ammonium acid urate (El Ridi *et al.* 2017).

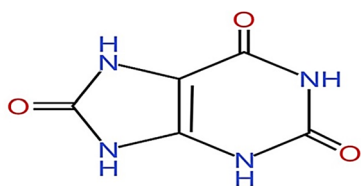


Fig. 1. Chemical structure of uric acid.

The liver and small intestine produce heterocyclic uric acid (7,9-dihydro-1H-purine-2,6,8(3H)-trione) that has a molecular weight of 168 Da. The diet contains a low urate concentration, whereas food purines are the main source of uric acid synthesis by producing new purine bases or internal breakdown of purine bases (Fauci *et al.* 2012). Hepatic uric acid generation and intestinal and renal excretion depend on multiple variables involving complicated metabolic mechanisms. Different enzymes can convert purine nucleic acids (guanine and adenine) into uric acid (Chaudhary *et al.* 2013). Two types of mechanisms initially convert Adenosine monophosphate (AMP) into inosine such as (a) deaminase-based removal of amino group to form inosine monophosphate (IMP) followed by nucleotidase-based dephosphorylation to generate inosine nucleoside, or (b) nucleotidase-based removal of phosphate group to form adenosine followed by deamination to produce inosine nucleoside. Nucleotidase also transforms guanine monophosphate (GMP) into guanosine nucleoside. Then, xanthine-oxidase (XO) oxidizes hypoxanthine to form xanthine, whereas guanine deaminase deaminates guanine to form xanthine. Xanthine oxidase further oxidizes xanthine into the final product known as uric acid. Fig. 2 demonstrates

the enzymatic pathway of purine degradation. Uric acid is a weak acid at physiologic pH with a pK_a value of 5.8. Uric acid mostly exists in the form of urate salt (Jin *et al.* 2012), and its crystal formation is enhanced with the high blood concentration of urate.

1.2. Benefits and hazards of uric acid

Uric acid, a urine component, is a metabolic breakdown product of purine nucleotides (guanine and adenine) and a derivative of proteins. Uric acid is a dominant natural antioxidant plasma factor that activates immunity responses in certain illnesses; however, gout and joint infections (chronic and acute) are associated with uric acid. High concentrations of blood uric acid can cause gout and other medical complications such as diabetes and kidney stone (ammonium acid urate) formation. The liver is the main uric acid-producing organ, along with the intestinal wall, endothelium of kidneys, and blood vessels (Yeum *et al.* 2004). Uric acid plays important physiological functions, and the body re-absorbs almost 90% of uric acid (Maiuolo *et al.* 2016; Roman 2023). Antioxidant activity is an essential feature of uric acid that could eliminate half of the blood plasma's free oxygen radicals (ROS) (Sautin and Johnson 2008; Roman 2023). Recent reports have highlighted uric acid-based initiation of inflammatory processes to facilitate tissue repair in addition to ROS removal (Nery *et al.* 2015). Lower blood uric acid levels might lead to mutations in renal carriers and blood cells (Sugihara *et al.* 2015). Contrarily, some studies have linked excess uric acid with kidney and cardiovascular diseases (Oberbach *et al.* 2014; Hammad *et al.* 2015; Roman 2023).

Uric acid is also known to protect the nervous system against various diseases. Autoimmune diseases such as lichen planus, Parkinson's disease, and pemphigus vulgaris are linked to lower uric acid levels (Bakhtiari *et al.* 2017; Kuwabara *et al.* 2023). Excessive protein intake or alleviated kidney-based excretion of uric acid results in increased uric acid levels in the blood (Bobulescu and Moe 2012; Xu *et al.* 2017). Higher uric acid levels damage the kidney's surface cells to cause their weak physiological activity, leading to chronic kidney diseases, particularly in Type 2 diabetes patients and individuals suffering from chemical intensification diseases (Xiao *et al.* 2015; Kim *et al.* 2015; Kuwabara *et al.* 2023). The uric acid accumulation in kidneys commonly results in kidney stone formation (Fathallah-Shaykh and Cramer 2014; Jalal 2016). Higher uric acid levels could also stimulate some autacoids and hormones to cause high blood pressure, whereas infiltration into smooth heart muscles leads to various cardiovascular illnesses (Kanbay *et al.* 2013; Kuwabara *et al.* 2023).

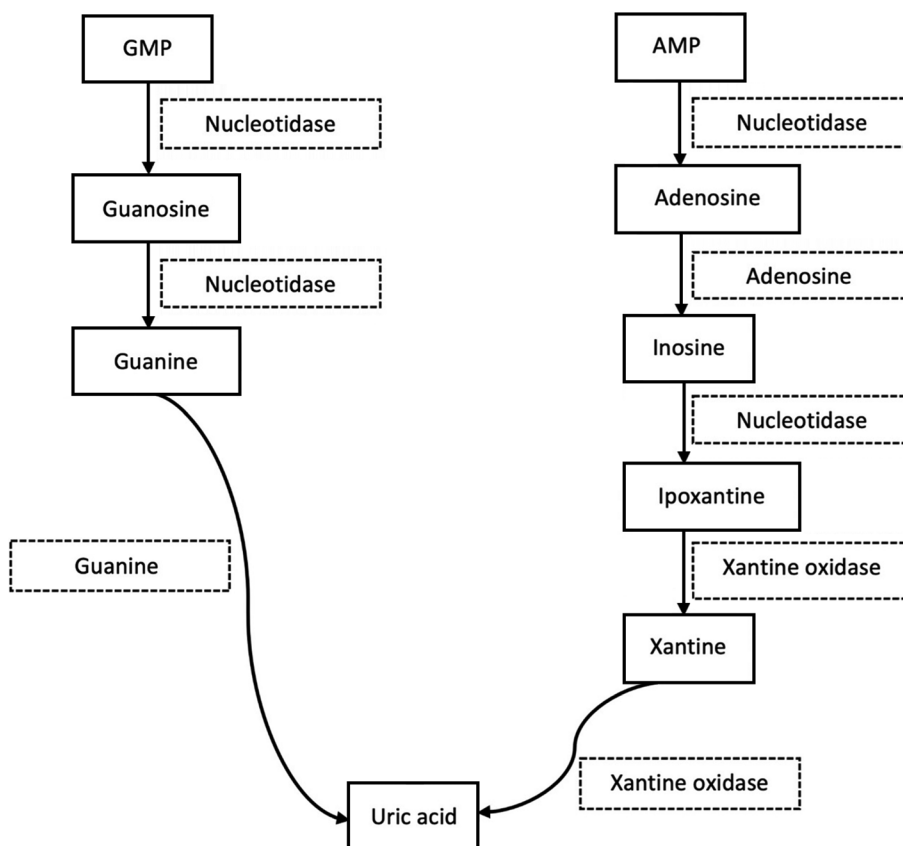


Fig. 2. Enzymatic degradation of purines to uric acid (Chaudhary *et al.* 2013).

2. Uric acid degrading microorganisms

The first microbial uricase was isolated from a fungus (*Neurospora crassa*) in 1957 (Perez-Ruiz *et al.* 2014). Later, it was discovered that various bacteria could degrade uric acid as well including *Pseudomonas aeruginosa*, *Bacillus thermocatenulatus*, *Microbacterium* sp., *Arthrobacter globiformis*, *Nocardia farcinica*, *Escherichia coli*, *Bacillus subtilis*, and *Bacillus fastidious* (Saeed *et al.* 2004; Suzuki *et al.* 2004; Zhou *et al.* 2005; Lotfy 2008; Xu *et al.* 2022; Chen and Li 2023). The uricase enzyme production in *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Alcaligenes*, and *Bacillus thermocatenulatus* is extracellular whereas its production is intracellular in *Microbacterium*, *Proteus vulgaris*, *Streptomyces albidoflavus*, *Streptomyces graminofaciens*, and *Streptomyces exofolitus* (Zhou *et al.* 2005; Ali *et al.* 2013; Chen and Li 2023). Lactic acid bacteria (*Pediococcus* species, *Bifidobacterium*, *Leuconostoc*, *Lactobacillus*, and *Enterococcus*) can degrade uric acid as their intake in rats/mice suppressed serum uric acid level (Ogawa 2006; Li *et al.* 2023; Negm El-Dein *et al.* 2023). Different soil fungi (*Fusarium*, *Helminthosporium*, *Spondilocladium*, *Curvularia*, *Stemphylium*, *Aspergillus*, *Geotrichum*, *Penicillium*, *Mucor*, *Rhizopus*, *Alternaria*, and *Chaetomium*) are capable of producing intracellular uricase at high rates, particularly in the presence of

urea or uric acid as the sole nitrogen source (Geweely and Nawar 2011; Ali *et al.* 2013; Rajagopalan *et al.* 2017; Moradpour *et al.* 2022), Table I, lists some of the significant uricase-producing microorganisms.

3. Uric acid degradation pathway in bacteria

The process of purine breakdown to uric acid is often conserved among organisms. However, uric acid degradation products could vary between species depending on the active catabolic enzymes, which can degrade or excrete uric acid in the peroxisomes (Lee *et al.* 2013). As a result of the degradation process, xanthine is the first intermediate product of all purine bases. Xanthine dehydrogenase degrades xanthine in the cytosol to generate urate. It is imported into the peroxisome and undergoes uricase-based oxidation to form 5-hydroxyisourate, which is converted to S-allantoin via 2-oxy-4-hydroxy-4-carboxy-5-ureidoimidazole by a functional allantoin synthase (Gabison *et al.* 2010). Urate oxidase evolution (allantoicase, uricase, and allantoinase) might be the reason behind varying degradation end-products of uric acid in different microorganisms. Most microorganisms can completely break down uric acid to ammonia through nitrogen catabolic enzymes (Marzluf *et al.* 1997). Allantoate

Table I
Uricase-producing microorganisms.

Microorganisms	Extracellular uricase	Intracellular uricase
Bacteria	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>
	<i>Bacillus pasteurii</i>	<i>Streptomyces albidoflavus</i>
	<i>Proteus mirabilis</i>	<i>Streptomyces graminofaciens</i>
	<i>Pseudomonas aeruginosa</i>	<i>Saccharopolyspora</i> sp.
	<i>Microbacterium</i> spp.	
	<i>Lactobacillus</i> sp.	
Filamentous fungi		<i>Fusarium</i> sp.
		<i>Geotrichum</i> sp.
		<i>Mucor</i> sp.
		<i>Alternaria</i> sp.
		<i>Penicillium</i> sp.
		<i>Aspergillus</i> sp.
		<i>Rhizopus</i> sp.
Yeast	<i>Candida tropicalis</i>	<i>Candida utilis</i>

Sources: Abdel-Fattah *et al.* (2005); Ali *et al.* (2012); Azab *et al.* (2005); Chen *et al.* (2008); Chen and Li (2023); Moradpour *et al.* (2022); Negm El-Dein *et al.* (2023); Khucharoenphaisan and Sinma (2011); Rando *et al.* (1990); Rajagopalan *et al.* (2017).

amidinohydrolase (allantoicase) in certain bacteria and fungi hydrolyze allantoate to generate s-ureidoglycolate and urea (Marzluf *et al.* 1997) (Fig. 3).

4. Uric acid degrading enzymes

Uricase enzymes (protein) possess the features of cofactors, they catalyze various biochemical reactions, and their deficiency could lead to different diseases. Uricase is often used in biochemical diagnosis, blood uric acid detection, and industrial processes (El Ridi *et al.* 2017; Tandon *et al.* 2021). Therapeutic enzymes differentiate from other drugs due to their relationship with pathogens, such as activating or inhibiting a specific reaction and direct association with the disease-causing substrate via its deposition in the body (Meletis and Barker 2005). Therapeutic enzymes could be biologically extracted from fungi, plants, and bacteria or synthesized in the laboratory (Babashamsi *et al.* 2009). Therapeutic uricase (urate oxidase) is generally not detected in humans. However, an RNA study has revealed uricase production in human liver cells (Kratzer *et al.* 2014). Uricase (urate oxidase, EC 1.7.3.3, oxidoreductase) carries out purine metabolism and activates uric acid oxidation into soluble allantoin. It is present in most vertebrates except higher apes and humans, where it became non-functional due to point mutation during evolution and formed a redundant protein (Wu *et al.* 1989). Uricase is localized in various microorganisms, including *Proteus mirabilis*, *Escherichia coli*, and *Bacillus pasteurii* (Cheristians

et al. 1986; Rando *et al.* 1990; Nakagawa *et al.* 1996). *Microbacterium*, *Candida tropicalis*, *Pseudomonas aeruginosa*, *Streptomyces albosriseolus*, and *Bacillus thermocatenulatus* can produce extracellular uricase after the optimization of culture media (Zhou *et al.* 2005; Abdel-Fattah *et al.* 2005; Loftly 2008). Uricase enzyme, containing four subunits, attracts and converts uric acid to hydrogen peroxide and allantoin through four identical type 2 copper binding sites (Fig. 4) (Wu *et al.* 1989). Several other enzymes, including xanthine oxidase, can also degrade uric acid by inhibiting a uric acid pathway reaction (hydrolysis). Xanthine oxidase is combined with different drugs for an efficient uric acid analysis (Li *et al.* 2005). *Pseudomonas* and other probiotics lactic acid bacteria produce other uric acid degrading proteins (Kanmani *et al.* 2013). Uricase catalyzes the *in-vivo* uric acid oxidation to generate CO₂ and allantoin in the presence of oxygen. The reduction of oxygen could also produce hydrogen peroxide (Fig. 5). Different types of thermostable microbial uricase enzymes are used in uric acid detection, which can sustain a wide range of pH (5, 6, 8, and 9) (Li *et al.* 2005; Ravichandran *et al.* 2015).

5. Genetics and uricase encoding genes

Fourteen functional genes have been discovered, which encode enzymes/proteins of the purine catabolic pathway. Xanthine dehydrogenase functioning requires the expression of five genes (*pucE*, *pucD*, *pucC*, *pucB*, and *pucA*), whereas two genes (*pucM* and *pucL*) encode

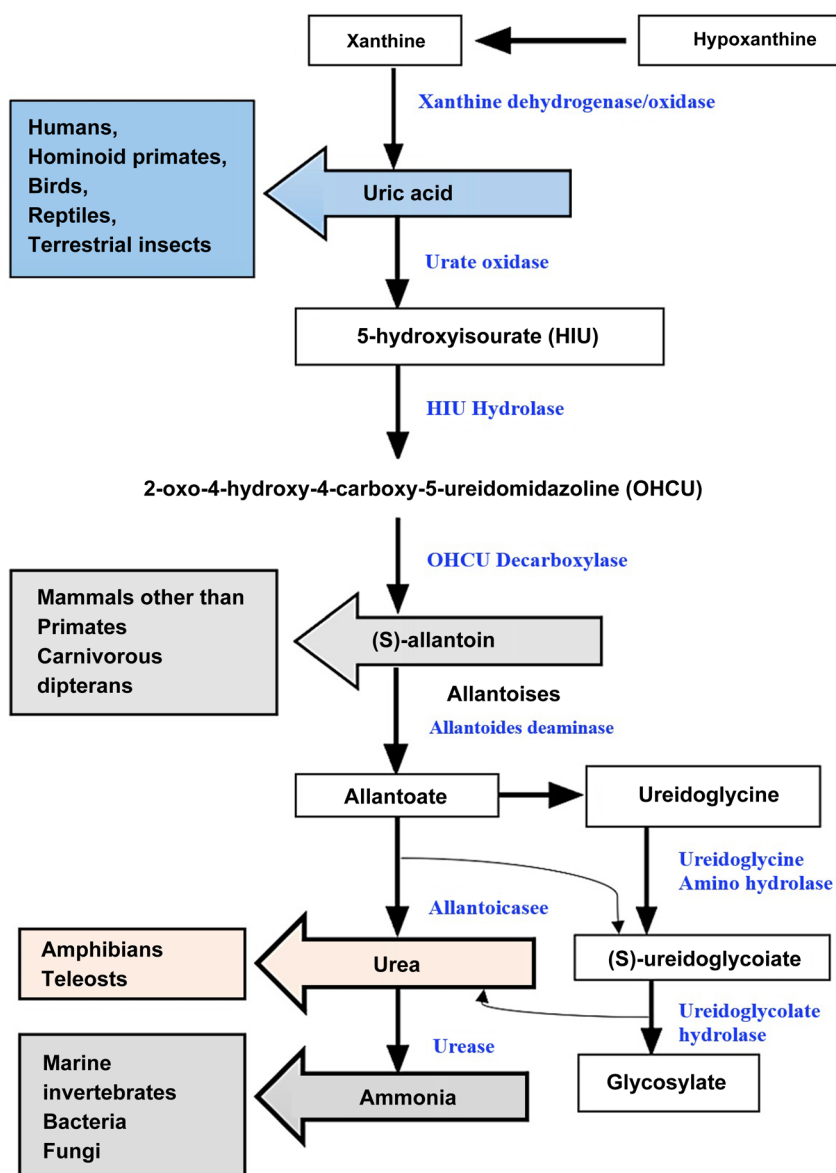


Fig. 3. The varying end products of purine metabolism in different species due to differential catabolic enzymes in the pathway (Lee *et al.* 2013).

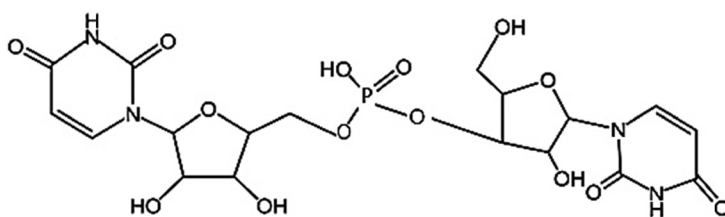


Fig. 4. Bacterial uricase (Wu *et al.* 1989).

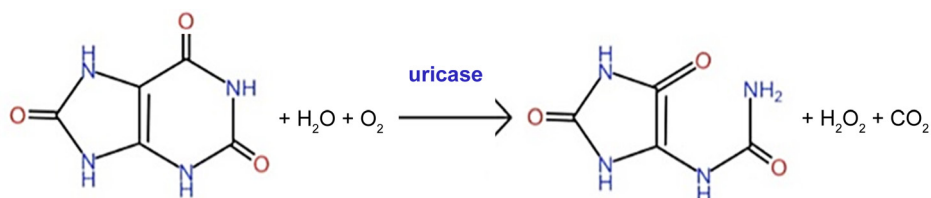


Fig. 5. Uricase catalyzes the reduction of dissolved oxygen to peroxide in the presence of uric acid (Li *et al.* 2005; Ravichandran *et al.* 2015).

uricase, and two genes (*pucK* and *pucJ*) encode uric acid transport system. The *pucI*, *pucH*, and *pucF* genes encode allantoin permease, allantoinase, and allantoate amidohydrolase. During a study, the *pucR*-mutant *Bacillus subtilis* expressed the lowest activity among all tested genes, indicating that PucR regulates *puc* gene expressions (Hafez *et al.* 2017). All 14 genes except *pucI* are located in a chromosomal gene cluster at 284–285° and participate in six transcription units. Uric acid, allantoic acid, and allantoin compounds regulate PucR for *puc* genes' expression (Argyrou *et al.* 2001). The utilization of uric acid initiates virulence factors (urease and capsule) synthesis in fatal meningitis-associated *Cryptococcus neoformans* that potentially regulate the host's immune response during the infection. Uricases (aquatic vertebrate and microbial) are mostly soluble and are found in bacterial cytoplasm or yeasts' peroxisome (Kratzer *et al.* 2014).

6. Uricase activity affecting factors

Multiple factors affect uricase activity, mainly pH and temperature. Ravichandran *et al.* (2015) reported an optimum pH (8) and temperature range (25–45°C) for uricase activity. They further noted an almost 50% decrease in uricase activity at 60°C after exposure for one hour (Chohan and Becker 2009). Contrarily, Geweely and Nawar (2011) have reported an optimum temperature of 35°C for *Aspergillus niger*-based uricase. Heavy metals serve as cofactors for some enzymes, but their large quantities could also inhibit enzyme activities (Suzuki *et al.* 2004; Witkowska *et al.* 2021). Nelson (2005) reported copper-based uricase inhibition, but Ravichandran *et al.* (2015) noted enhanced (140%) enzyme activity in response to copper stimulation. Chohan and Becker (2009) revealed enzyme stimulation by ethylenediaminetetraacetic acid (EDTA) (1 molar), whereas the same concentration inhibited the activity of ERW. Thus, unknown elements could contribute to enzyme activation and inhibition, varying in different bacteria (Ravichandran *et al.* 2015).

7. Uricase production and purification

Different carbon and nitrogen sources in the culturing media of uricase-producing microorganisms can influence uricase production. Uricase production with different carbon sources can be arranged as sucrose > glucose > cellulose > starch > maltose. Peptone is known for its higher uricase yield than other nitrogen sources (yeast extract, beef extract, and ammonium nitrate) (Pfrimer *et al.* 2010).

7.1. Extracellular uricase

Microorganisms and higher plants produce more uricase, whereas humans cannot produce this enzyme, which leads to purine breakdown-associated uric acid accumulation in the body (Hafez *et al.* 2017). Uricase alleviates hyperuricemia, whereas plant or human uricase stimulates immune responses (Roman 2023). Therefore, bacterial uricase is a therapeutic agent that removes excessive uric acid from the body (Abdel-Fattah *et al.* 2005). *Proteus mirabilis*, *Bacillus pasteurii*, and *Escherichia coli* are known to secrete uricase enzymes (Rando *et al.* 1990; Nakagawa *et al.* 1996; Hafez *et al.* 2017). *Pseudomonas aeruginosa*, *Tropical Candida*, *Thermobacilli*, *Alboseriolus*, and *Microbacterium* have been reported to yield extracellular uricase in the optimized media (Zhou *et al.* 2005; Abdel-Fattah *et al.* 2005; Anderson and Vijayakumar 2011). Extracellular uricase activity was assessed on a solidified medium by following the agar plate assay method, in which uric acid served as an inciting agent. The uric acid screening medium was comprised of sucrose (20 g/L), magnesium sulfate heptahydrate (0.5 g/L) sodium chloride (0.5 g/L), uric acid (3 g/L), di-potassium hydrogen phosphate (1 g/L), agar (15 g/L), and ferrous sulphate (0.01 g/L) (El-Naggar *et al.* 2019). The pH was adjusted to 6.8, and plates were incubated for 5–7 days at 30°C. A clear zone around the colony confirmed uricase production (Fig. 6).

7.2. Intracellular uricase

Fungal or bacterial growth is not directly associated with uric acid production. However, different fungi and bacteria can utilize uric acid as the only source of nitrogen (Baumgardner 2016). During a study, a starter culture of lactic acid bacteria (1%) was grown in uric acid (0.2%)-supplemented PGY broth to obtain intracellular uricase. The culture was incubated at 37°C for 24 hours. After fermentation, centrifugation (3000 rpm, 20 minutes) was carried out at 4°C to separate the supernatant, followed by bacterial cell-based stability testing of intracellular uricase (Carevic *et al.* 2015). Intracellular uricase production was noted in *Microbacterium* spp., *S. albidoflavus*, *P. vulgaris*, and *S. graminofaciens* (Zhou *et al.* 2005; Azab *et al.* 2005). The addition of uric acid into the growth media induced the uricase production. However, various influencing factors (heavy metals, temperature, and pH) are needed to disturb the cell to obtain intracellular uricase (Bongaerts *et al.* 1978). Generally, intracellular uricase production in the gastrointestinal system is considered more stable (O'Connell and Walsh 2007; Pugin *et al.* 2022).

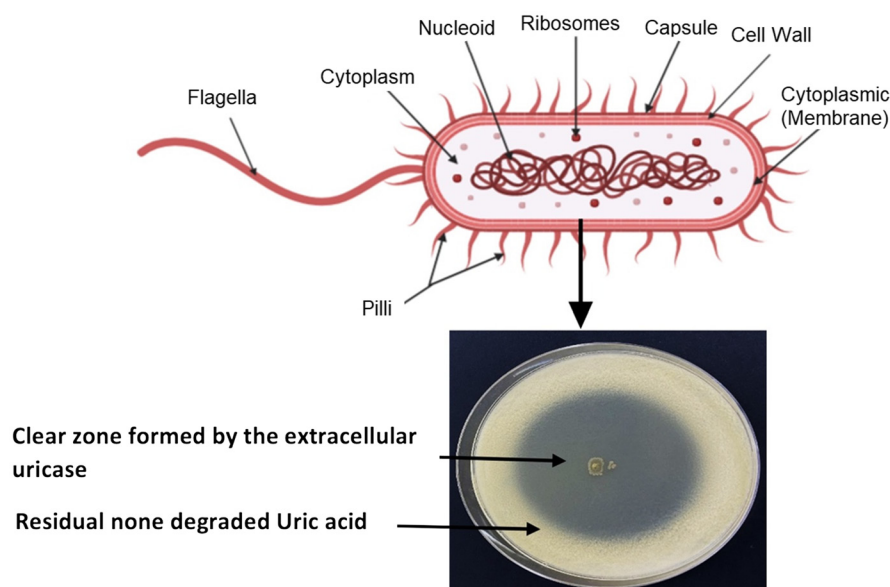


Fig. 6. A clear zone indicating *Alcaligenes faecalis*-secreted uricase on uric acid (0.3%) – supplemented BT medium.

7.3. Uricase purification from different microorganisms

Uricase purification is necessary to achieve its higher yield, which has been carried out through various approaches.

7.3.1. Precipitation by ammonium sulfate

Different concentrations of ammonium sulphate (20, 40, 60, and 70% w/v) can be used to separate uricase from secreted proteins-containing supernatant. Briefly, solid ammonium sulphate is slowly added to the culture filtrate on an ice bath with gentle stirring until the required ammonium sulphate saturation is reached. Then, the mixture is left overnight at 4°C followed by centrifugation at low temperature (8000 rpm, 30 minutes, 4°C) (Saeed *et al.* 2004; Ram *et al.* 2015).

7.3.2. Removal of ammonium sulfate salts

The Removal of ammonium sulphate is performed by dissolving the precipitate in Tris-HCl (0.01 M, 10 ml) buffer (pH 8.5). The solution is dialyzed overnight in ultra-pure distilled water/buffer (1 L) using a dialysis tube. Then, the concentrated dialyzed cell-free supernatant is subjected to the column chromatography technique (Saeed *et al.* 2004; Ram *et al.* 2015).

7.3.3. Uricase purification through ion exchange chromatography on DEAE-cellulose

A DEAE-cellulose-containing column is equilibrated with Tris-HCl (10 mM) buffer (pH 8.5). Dialyzed and concentrated cell-free supernatant is applied to it for uricase purification. The column is washed thrice with the Tris-HCl (10 mM) buffer (pH 8.5). The bound proteins are eluted in the same buffer with a linear NaCl

gradient (0–0.3 M), and collected fractions are analyzed at 280 nm using a UV spectrophotometer, whereas enzyme activity and protein concentration are detected at 293 nm (Saeed *et al.* 2004; Ram *et al.* 2015).

7.3.4. Uricase purification with gel filtration column

Potassium phosphate (50 mM) buffer (pH 8.2) is used to equilibrate the Superdex 200 HR-containing gel filtration column (Amersham Pharmacia Biotech, Germany). Ammonium sulfate-based partially purified uricase is dialyzed and applied to this column. The same buffer is used to elute uricase, and all fractions (0.5 ml) with high uricase activity are concentrated using an ultrafiltration membrane (YM 10) or following the lyophilized method. The concentrated fractions are stored at –20°C (Jianguo *et al.* 1994; Saeed *et al.* 2004; Ram *et al.* 2015). According to Laemmli *et al.* (1970), the molecular weight of the purified enzyme should be determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturation conditions to verify the purification stages of uricase. The determination of uricase's molecular weight involves comparing its electrophoretic mobility with that of marker proteins.

8. Applications of uricase

Uricase (Urate Oxidase, EC 1.7.3.3) is a diagnostic enzyme to measure uric acid (urate) levels in the body. It also synthesizes medicines (pegloticase and rasburicase) for hyperuricemia treatment (Yang *et al.* 2012). Uricase is crucial for the human body as it disintegrates

uric acid into allantoin, carbon dioxide, and hydrogen peroxide through oxidation. Generally, uric acid is excreted through the kidneys, but its blood solubility is extremely low (6.8 mg/L) (Ravichandran *et al.* 2015; Mei *et al.* 2022). Higher blood uric acid levels are associated with uric acid nephrolithiasis, gout, cardiovascular disease, hyperuricemia, diabetes, renal failure, and tumor lysis syndrome (Ganson *et al.* 2005; Roman 2023). The uricase enzyme remains inactive in humans because of frameshift mutation during evolution. Therefore, uricase synthesis from other sources is vital to counter associated disorders (Pawar *et al.* 2018).

8.1. Diagnostic role of uricase in clinical analysis

There are diverse applications of uricase enzyme, but its most important role is treating uric acid accumulation-related illnesses (nervous system, heart) (Hafez *et al.* 2017). It is commonly applied to assess blood uric acid levels in the blood. Moreover, it is also combined with a 4-amino-antipyrine-peroxidase system to determine uric acid levels in other biological fluids (Cheung *et al.* 2020). Rasburicase is frequently used to treat organ transplants and tumor lysis-associated hyperuricemia. Uricase is also a common additive of commercial hair coloring agents (Cheung *et al.* 2020).

8.2. Biosensor for bimolecular applications

Monitoring uric acid levels in urine and blood is necessary for the disease diagnosis. There are different methods of uric acid estimation, such as mass fragmentation, enzyme electrode, colorimetry, radiochemical-HPLC, fluorescent sol-gel, commercial uric acid kits, and chemiluminescence (Domagk and Schlicke 1968; Martinez-Pérez *et al.* 2003; Zhang *et al.* 2004; Bio-Assay Systems 2007; Chu *et al.* 2012). The colorimetric method is a simple, specific, and sensitive approach that employs peroxidase and uricase (Zhou *et al.* 2005). However, these enzymes are expensive, which makes this assay more costly than other methods. Different biosensing procedures have also been devised, which involve uricase immobilization on electrode surface using ZnO nanorods, polyaniline-polypyrrole film, polyaniline, ZnO nano-flakes, and polypyrrole nanoelectrode (Uchiyama and Sakamoto 1997; Zhang *et al.* 2004; Arora *et al.* 2007; Arslan 2008; Yang *et al.* 2012).

A transducer converts the energy alterations during the interaction of biological elements (protein, antibody, and enzyme) and an analyte into a quantifiable signal (Ravichandran *et al.* 2015). Modern miniature microelectronics are featured with lower cost, better processing power, and enhanced analytical efficiency, which broaden their potential applications. Cellular interactions, enzymatic contacts, antibody-antigen

interactions, nucleic acid connections, and artificial bioreceptor-based interactions are common biological recognition elements. Mass-sensitive, optical, and electrochemical transducers are frequently utilized for signal quantification (Javadi *et al.* 2018).

8.3. Agricultural applications of uricase

Biological compounds are known to enhance the quality of agricultural commodities. Soil microorganisms release vital secretions for better soil fertility, however, these microbial secretions could be hindered by different environmental factors (Javadi *et al.* 2018; Imran *et al.* 2021). Therefore, producing fungal and bacterial enzymes has been investigated to improve soil conditions and agricultural production at a reduced cost. The free uricase enzyme is utilized as calcium carbonate precipitate in the soil to promote soil mechanics by initiating urea breakdown (Hamdan *et al.* 2013).

8.4. Uricase-based detection of heavy metal water contamination

Heavy metals-containing toxic compounds are known to reduce enzyme activity, which is often used as a parameter to detect heavy metal contamination in water. The activity of uricase also decreases at varying levels in the presence of $\text{Hg}^{2+} > \text{Ag}^{+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Fe}^{2+} > \text{Pb}^{2+} > \text{Mn}^{2+}$. Therefore, it is a toxic compound detector in water samples (Zhylyak *et al.* 1995).

8.5. Uricase application in nanomaterial manufacturing

The importance of nanomaterials has significantly increased with diverse medical, agricultural, and industrial applications. Therefore, producing environment-friendly, low-cost, and stable industrial nanomaterials is being widely investigated worldwide. In this regard, enzyme applications in nanomaterial synthesis have become quite popular during the last decade (Durán *et al.* 2014; Adelere and Lateef 2016). *Canavalia ensiformis*-isolated uricase has produced Pt, Au, and Ag nanoparticles as a stabilizing and reducing agent. Similarly, the catalytic urease has been employed to synthesize core-shell ZnO nanomaterials at an ambient temperature. Exposed enzyme residue (Cys592) facilitates the synthesis of metal alloys and metallic nanoparticles (Sharma *et al.* 2013). During the process, the Zn^{2+} binds with negatively charged surface urease at pH 9 through a weak bond reaction to form intermediate zinc hydroxide. Further, zinc hydroxide dehydration under basic conditions yields ZnO on the precipitating enzyme's surface through the 'salting out' effect (Makarov *et al.* 2002).

Conclusion and future perspectives

This review elaborates on uricase-producing microorganisms, bacterial uric acid degradation pathways, degrading enzymes, and uricase-encoding genes. Moreover, the uricase activity affecting factors, microbial uricase production, and uricase purification and applications are also discussed. Cell disruption is mandatory for intercellular uricase production, elevating production costs. Therefore, extracellular uricase-producing microbial strains should be investigated, and production factors should be optimized. Future techniques for obtaining extracellular enzymes should feature reduced time and effort and a simple purification methodology. Furthermore, uricase gene-carrying recombinant probiotic microorganisms could become an effective tool for gout treatment.

ORCID

Khaled Elbanna <https://orcid.org/0000-0002-2011-9119>

Leena A. Neyaz <https://orcid.org/0009-0007-6664-007>

Mahmoud Z. El-Readi <https://orcid.org/0009-0007-0252-2370>

Hussein H. Abulreesh <https://orcid.org/0000-0002-3289-696X>

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.

Data availability

All data generated and gathered during this research have been used in the manuscript.

Funding

No funding was received for this research.

References

1. Aafaria S., Kumari P., Sharma S., Yadav S., Batra B., Rana J.S., Sharma M.: Electrochemical biosensing of uric acid: a review. *Microchem. J.* **182**, 107945 (2022)
2. Abdel-Fattah Y.R., Saeed H.M., Gohar Y.M., El-Baz M.A.: Improved production of *Pseudomonas aeruginosa* uricase by optimization of process parameters through statistical experimental designs. *Proc. Biochem.* **40**, 1707–1714 (2005)
3. Adelere I.A., Lateef A.: A novel approach to the green synthesis of metallic nanoparticles: the use of agro-wastes, enzymes, and pigments. *Nanotechnol. Rev.* **5**, 567–587 (2016)
4. Ali M., Tork S., Al-Garni S., Allam R.: Production and characterization of uricase from *Streptomyces exfoliatus* UR10 isolated from farm wastes. *Turk. J. Biol.* **37**, 520–529 (2013)
5. Anderson A. and Vijayakumar S.: Purification and optimization of uricase enzyme produced by *Pseudomonas aeruginosa*. *J. Exp. Sci.* **2**, 5–8 (2011)
6. Argyrou E., Sophianopoulou V., Schultes N., Dialis G.: Functional characterization of a maize purine transporter by expression in *Aspergillus nidulans*. *Plant Cell.* **13**, 953–964 (2001)
7. Arora K., Sumana G., Saxena V., Gupta R.K., Gupta S.K., Yakhmi J.V., Malhotra B.D.: Improved performance of polyaniline-uricase biosensor. *Anal. Chim. Acta.* **594**, 17–23 (2007)
8. Arslan F.: An amperometric biosensor for uric acid determination prepared from uricase immobilized in polyaniline-polypyrrole film. *Sensors.* **8**, 5492–5500 (2008)
9. Azab E.A., Ali M.M., Fareed M.F.: Studies on uricase induction in certain bacteria. *Egypt. J. Biol.* **7**, 44–54. (2005)
10. Babashamsi M., Razavian M.H., Nejadmoghaddam M.R.: Production and purification of streptokinase by protected affinity chromatography. *Avicenna J. Med. Biotechnol.* **1**, 47 (2009)
11. Bakhtiari S., Toosi P., Samadi S., Bakhshi M.: Assessment of uric acid level in the saliva of patients with oral lichen planus. *Med. Princ. Pract.* **26**, 57–60 (2017)
12. Baumgardner D.J.: Disease-causing fungi in homes and yards in the Midwestern United States. *J. Patient-Centered Res. Rev.* **3**, 99–110 (2016)
13. BioAssay Systems: QuantiChrom™ Uric Acid Assay Kit, DIUA250. (2007) [Internet: <http://www.medibena.at/media/BAS/product-sheets/diua>]
14. Bobulescu I.A., Moe O.W.: Renal transport of uric acid: evolving concepts and uncertainties. *Adv. Chronic Kidney Dis.* **19**, 358–371 (2012)
15. Bongaerts G.P., Uitzetter J., Brouns R., Vogels G.D.: Uricase of *Bacillus fastidiosus*. Properties and regulation of synthesis. *Biochim. Biophys. Acta.* **527**, 348–358 (1978)
16. Carevic M., Vukašinović-Sekulić M., Grbavčić S., Stojanović M., Mihailović M., Dimitrijević A., Bezbradica D.: Optimization of (-galactosidase production from lactic acid bacteria. *Hem. Ind.* **69**, 305–312 (2015)
17. Chaudhary K., Malhotra K., Sowers J., Aroor A.: Uric acid-key ingredient in the recipe for cardiorenal metabolic syndrome. *Cardiorenal Med.* **3**, 208–220 (2013)
18. Chen M.H. and Li S.Y.: Extra-cellular production of uricase through the sec-type secretion system in *Escherichia coli*. *Biochem. Eng. J.* **194**, 108894 (2023)
19. Chen Z., Wang Z., He X., Guo X., Li W., Zhang B.: Uricase production by a recombinant *Hansenula polymorpha* strain harboring *Candida utilis* uricase gene. *Appl. Microbiol. Biotechnol.* **79**, 545–554 (2008)
20. Cheristans S., Kaltwasser H.: Nickel-content of urease from *Bacillus pasteurii*. *Arch. Microbiol.* **145**, 51–55 (1986)
21. Cheung W.L., Hon K.L., Fung C.M., Leung A. K.: Tumor lysis syndrome in childhood malignancies. *Drugs Context*, **9**, (2020)
22. Cho J., Yang B., Lee J.H., Kim H., Kim H., Go E.B., Bak D.H., Park S.J., Kwon I., Choi J., Lee K.: In vivo study of newly developed albumin-conjugated urate oxidase for gout treatment. *Arthritis Res. Ther.* **25**, 247 (2023)
23. Chohan S. and Becker M.A.: Update on emerging urate-lowering therapies. *Curr. Opin. Rheumatol.* **21**, 143–149 (2009)
24. Chu H., Wei X., Wu M., Yan J., Tu Y.: An electrochemiluminescent biosensor based on polypyrrole immobilized uricase for ultrasensitive uric acid detection. *Sens. Actuators B Chem.* **163**, 247–252 (2012)
25. Domagk G.F., Schlicke H.H.: A colorimetric method using uricase and peroxidase for the determination of uric acid. *Anal. Biochem.* **22**, 219–224 (1968)
26. Durán N., Cuevas R., Cordi L., Rubilar O., Diez M.C.: Biogenic silver nanoparticles associated with silver chloride nanoparticles (Ag@AgCl) produced by laccase from *Trametes versicolor*. *SpringerPlus.* **3**, 1–7 (2014)
27. El Ridi R., Tallima H., Migliardo F.: Biochemical and biophysical methodologies open the road for effective schistosomiasis therapy and vaccination. *Biochim. Biophys. Acta.* **1861**, 3613–3620 (2017)

28. El-Naggar N.E.A., Haroun S.A., El-Wesly E.M., Metwally E.A., Sherief A.A.: Mathematical modeling for bioprocess optimization of a protein drug, uricase, production by *Aspergillus welwitschiae* strain 1–4. *Scientific Reports*, **9**(1), 12971 (2019)
29. Fathallah-Shaykh S.A. and Cramer M.T.: Uric acid and the kidney. *Pediatr. Nephrol.* **29**, 999–1008 (2014)
30. Fauci A.S., Kasper D.L., Braunwald E., Hauser S.L., Longo D.L., Jameson J.L.: Neoplasias malignas hematológicas menos frecuentes. Longo DL, Harrison TR. *Harrison's principles of internal medicine. 18th ed. New York: McGraw Hill*, 2150–2160 (2012)
31. Gabison L., Chiadmi M., El Hajji M., Castro B., Colloc'h N., Prangé T.: Near-atomic resolution structures of urate oxidase complexed with its substrate and analogues: the protonation state of the ligand. *Acta Crystallogr. D*. **66**, 714–724 (2010)
32. Ganson N.J., Kelly S.J., Scarlett E., Sundry J.S., Hershfield M.S.: Control of hyperuricemia in subjects with refractory gout, and induction of antibody against poly (ethylene glycol) (PEG), in a phase I trial of subcutaneous PEGylated urate oxidase. *Arthritis Res. Ther.* **8**, 1–10. (2005)
33. Geweely N.S., Nawar L.S.: Production, optimization, purification and properties of uricase isolated from some fungal flora in Saudi Arabian soil. *Aust. J Basic Appl. Sci.* **5**, 220–230 (2011)
34. Hafez R.M., Abdel-Rahman T.M., Naguib R.M.: Uric acid in plants and microorganisms: Biological applications and genetics-A review. *J. Adv. Res.* **8**, 475–486 (2017)
35. Hamdan N., Kavazanjian Jr. E., O'donnell S.: Carbonate cementation via plant derived urease. In *Proceedings of the 18th International Conference on Soil Mechanics and Geotechnical Engineering* September (pp. 2–6) (2013)
36. Hammad H., Lambrecht B.N.: Barrier epithelial cells and the control of type 2 immunity. *Immunity*. **43**, 29–40 (2015)
37. Imran M., Abulreesh H.H., Monjed M.K., Elbanna K., Samreen, Ahmad I.: Multifarious functional traits of free-living rhizospheric fungi, with special reference to *Aspergillus* spp. isolated from North Indian soil, and their inoculation effect on plant growth. *Ann. Microbiol.* **71**, 31 (2021)
38. Jalal D.I.: Hyperuricemia, the kidneys, and the spectrum of associated diseases: a narrative review. *Curr. Med. Res. Opin.* **32**, 1863–1869 (2016)
39. Javadi N., Khodadadi H., Hamdan N., Kavazanjian Jr. E.: EICP treatment of soil by using urease enzyme extracted from watermelon seeds. In: *IFCEE*. 115–124 (2018)
40. Jianguo L., Gaoxiang L., Hong L., Xiukai Z. Purification and properties of uricase from *Candida* sp. and its application in uric acid analysis in serum. *Appl Biochem. Biotechnol.* **47**, 57–63 (1994)
41. Jin M., Yang F., Yang I., Yin Y., Luo J.J., Wang H., Yang X.F.: Uric acid, hyperuricemia and vascular diseases. *Front. Biosci.* **17**, 656 (2012)
42. Kanbay M., Segal M., Afsar B., Kang D.H., Rodriguez-Iturbe B., Johnson R.J.: The role of uric acid in the pathogenesis of human cardiovascular disease. *Heart*. **99**, 759–766 (2013)
43. Kanmani P., Satish Kumar R., Yuvaraj N., Paari K.A., Pattukumar V., Arul V.: Probiotics and its functionally valuable products – a review. *Crit. Rev. Food Sci. Nutr.* **53**, 641–658 (2013)
44. Khucharoenphaisan K., Sinma K.: Production and partial characterization of uric acid degrading enzyme from new source *Saccharopolyspora* sp. PNR11. *Pak. J. Biol. Sci.* **14**, 226–231(2011)
45. Kim S.M., Lee S.H., Kim Y.G., Kim S.Y., Seo J.W., Choi Y.W., Moon J.Y.: Hyperuricemia-induced NLRP3 activation of macrophages contributes to the progression of diabetic nephropathy. *Am. J. Physiol.* **308**, F993–F1003 (2015)
46. Kratzer J.T., Lanaspa M.A., Murphy M.N., Cicerchi C., Graves C.L., Tipton P.A., Gaucher E.A.: Evolutionary history and metabolic insights of ancient mammalian uricases. *Proc. Nat. Acad. Sci.* **111**, 3763–3768 (2014)
47. Kuwabara M., Fukuchi T., Aoki Y., Mizufa E., Ouchi M., Kurajoh M., Maruhashi T., Tanaka A., Morikawa N., Nishimiya K., Akashi N., Tanaka Y., Oani N., Morita M., Miyata H., Takada T., Tsutani H., Ogino K., Ichida K., Hisatome I. et al.: Exploring the multifaceted nexus of uric acid and health: a review of recent studies on diverse diseases. *Biomolecules*. **13**, 1519 (2023)
48. Laemmli U.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685 (1970)
49. Lee I.R., Yang L., Sebetso G., Allen R., Doan T.H., Blundell R., Fraser J.A.: Characterization of the complete uric acid degradation pathway in the fungal pathogen *Cryptococcus neoformans*. *PLoS One*. **8**, e64292 (2013)
50. Li D., Zhang M., La A. L. T. Z., Lyu Z., Li X., Feng Y., Liu D., Guo Y., Hu Y.: Quercetin-enriched *Lactobacillus aviaries* alleviated hyperuricemia by hydrolase-mediated degradation of purine nucleosides. *Pharmacol. Res.* **196**, 106928. (2023)
51. Li J., Laursen T.M., Precht D.H., Olsen J., Mortensen P.B.: Hospitalization for mental illness among parents after the death. *N. Engl. J. Med.* **352**, 1190–1196 (2005).
52. Lotfy W.A.: Production of a thermostable uricase by a novel *Bacillus thermocatenuatus* strain. *Bioresour. Technol.* **99**, 699–702 (2008)
53. Maiuolo J., Oppedisano F., Gratteri S., Muscoli C., Mollace V.: Regulation of uric acid metabolism and excretion. *Int. J. Cardiol.* **213**, 8–14 (2016)
54. Makarov V., Pettitt B.M., Feig M.: Solvation and hydration of proteins and nucleic acids: a theoretical view of simulation and experiment. *Acc. Chem. Res.* **35**, 376–384 (2002)
55. Martinez-Pérez D., Ferer M.L., Mateo C.R.: A reagent less fluorescent sol-gel biosensor for uric acid detection in biological fluids. *Anal. Biochem.* **322**, 238–242 (2003)
56. Marzluf G.A.: Genetic regulation of nitrogen metabolism in the fungi. *Microbiol. Mol. Biol. Rev.* **61**, 17–32 (1997)
57. Mei Y. Dong B., Geng Z., Xu L.: Excess uric acid induces gouty nephropathy through crystal formation: a review of recent insights. *Front. Endocrinol.* **13**, 911968 (2022)
58. Meletis C.D. and Barker J.E.: Therapeutic enzymes: using the body's helpers as healers. *Altern. Complement. Ther.* **11**, 74–77 (2005)
59. Moradpour Z., Daryani M. E., Diba K., Ghasemian A.: Medium optimization for extracellular urate oxidase production by a newly isolated *Aspergillus niger*. *Int. J. Health Sci.* **6**, 5964–5977 (2022)
60. Nakagawa S., Ishino S., Teshiba S.: Construction of catalase deficient *Escherichia coli* strains for the production of uricase. *Biosci. Biotechnol. Biochem.* **60**, 415–420 (1996)
61. Negm El-Dien A., Ezzat A., Aly H.F., Younis E.A., Awad G.A., Farid M.A.M.: Hypouricemic, anti-inflammatory, and antioxidant activities of *Lactobacillus*-based functional yogurt in induced-arthritic male Wistar rats: therapeutic and protective potentials. *Biocatal. Agric. Biotechnol.* **47**, 102597 (2023)
62. Nelson D.L.: *Lehninger Principles of Biochemistry 4th edition*. WH Freeman, USA (2005)
63. Nery R.A., Kahlow B.S., Skare T.L., Tabushi F. I.: Uric acid and tissue repair. *Arq. Bras. Cir. Dig.* **28**, 290–292 (2015)
64. O'Connel S., Walsh G.: (2007). Purification and properties of a β -galactosidase with potential. *Appl. Biochem. Biotechnol.* **141**, 1–13 (2007).
65. Oberbach A., Neuhaus J., Jehmlich N., Schlichting N., Heinrich M., Kullnick Y., Adams V.: A global proteome approach in uric acid stimulated human aortic endothelial cells revealed regulation of multiple major cellular pathways. *Int. Journal Cardiol.* **176**, 746–752 (2014)

66. Ogawa J.: Analysis of microbial purine metabolism and its application for hyperuricemia prevention. Institute for Scientific Research Grant, Noda, Japan (2006)
67. Pawar S.V., Rathod V.K.: Ultrasound assisted process intensification of uricase and alkaline protease enzyme co-production in *Bacillus licheniformis*. *Ultrason. Sonochem.* **45**, 173–179 (2018)
68. Perez-Ruiz F., Martínez-Indart L., Carmona L., Herrero-Beites A.M., Pijoan J.I., Krishnan E.: Tophaceous gout and high level of hyperuricaemia are both associated with increased risk of mortality in patients with gout. *Ann. Rheum. Dis.* **73**, 177–182 (2014)
69. Pugin B., Pluss S., Mujezinovic D., Nielsen R.C., Lacroix C.: Optimized UV-spectrophotometric assay to screen bacterial uricase activity using whole cell suspension. *Front. Microbiol.* **13**, 853735 (2022)
70. Rajagopalan P., Chandramoorthy H.C., Elbessoumy A.A.: Pilot scale production and characterization of uricase from *Penicillium purpurescens*. *Ind. J. Biotechnol.* **16**, 570–577 (2017)
71. Ram S.K., Raval K., Jagadeesh Babu P.E.: (2015). Enhancement of a novel extracellular uricase production by media optimization and partial purification by aqueous three-phase system. *Prep. Biochem. Biotechnol.* **45**, 810–824 (2015)
72. Rando D., Steglitz U., Mörsdorf G., Kaltwasser H.: Nickel availability and urease expression in *Proteus mirabilis*. *Arch. Microbiol.* **154**, 428–432 (1990)
73. Ravichandran R., Hemaasri S., Cameotra S.S., Jayaprakash N.S.: Purification and characterization of an extracellular uricase from a new isolate of *Sphingobacterium thalpophilum* (VITPCB5). *Protein Expr. Purif.* **114**, 136–142 (2015)
74. Roman Y.M.: The role of uric acid in human health. *J. Pers. Med.* **13**, 1409 (2023)
75. Saeed H.M., Yousry Y.R.A.F., Gohar M., Elbaz M.A.: Purification and characterization of extracellular *Pseudomonas aeruginosa* urate oxidase enzyme. *Polish J. Microbiol.* **53**, 045–052 (2004)
76. Sautin Y.Y. and Johnson R.J.: Uric acid: the oxidant-antioxidant paradox. *Nucleos. Nucleot. Nucl.* **27**, 608–619 (2008)
77. Sharma B., Mandani S., Sarma T.: Biogenic growth of alloy and core-shell nanostructures using urease as a nanoreactore at ambient conditions. *Sci. Rep.* **3**, 2601 (2013)
78. Sugihara S., Hisatome I., Kuwabara M., Niwa K., Maharani N., Kato M., Yamamoto K.: Depletion of uric acid due to SLC22A12 (URAT1) loss-of-function mutation causes endothelial dysfunction in hypouricemia. *Circ. J.* **79**, 1125–1132 (2015)
79. Suzuki K., Sakasegawa S.I., 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.* **98**, 153–158 (2004).
80. Tandon S., Sharma A., Singh S., Sharma S., Sarma S.J.: Therapeutic enzymes: Discoveries, production and applications. *J. Drug Deliv. Sci. Technol.* **63**, 102455 (2021).
81. Uchiyama S., Sakamoto H.: Immobilization of uricase to gas diffusion carbon felt by electropolymerization of aniline and its application as an enzyme reactor for uric acid sensor. *Talanta*, **44**, 1435–1439 (1997).
82. Usman Ali S.M., Ibupoto Z.H., Kashif M., Hashim U., Willander M.: A potentiometric indirect uric acid sensor based on ZnO nanoflakes and immobilized uricase. *Sensors*. **12**, 2787–2797 (2012).
83. Wan S., Liu X., Sun W., Lv B., Li C.: Current advances for omics-guided process optimization of microbial manufacturing. *Bio-resour. Bioprocess.* **10**, 30. (2023)
84. Witkowska D., Slowik J., Chilicka K.: Heavy metals and human health: possible exposure pathways and the competition for protein binding sites. *Molecules*. **26**, 6060. (2021)
85. Wu X.W., Lee C.C., Muzny D.M., Caskey C.T.: Urate oxidase: primary structure and evolutionary implications. *Proc. Nat. Acad. Sci.* **86**, 9412–9416 (1989).
86. Xiao J., Zhang X.L., Fu C., Han R., Chen W., Lu Y., Ye Z.: Soluble uric acid increases NALP3 inflammasome and interleukin-1 β expression in human primary renal proximal tubule epithelial cells through the Toll-like receptor 4-mediated pathway. *Int. J. Molec. Med.* **35**, 1347–1354 (2015).
87. 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.* **18**, 1–14 (2017).
88. Xu X., Yan Y., Huang J., Zhang Z., Wang Z., Wu M., Liang H.: Regulation of uric acid glyoxylate metabolism by UgmR protein in *Pseudomonas aeruginosa*. *Environ. Microbiol.* **24**, 3242–3255. (2022)
89. Yang X., Yuan Y., Zhan C.G., Liao F.: Uricases as therapeutic agents to treat refractory gout: Current states and future directions. *Drug Develop. Res.* **73**, 66–72 (2012).
90. Yeum K.J., Russell R.M., Krinsky N.I., Aldini G.: Biomarkers of antioxidant capacity in the hydrophilic and lipophilic compartments of human plasma. *Arch. Biochem. Biophys.* **430**, 97–103 (2004).
91. Zhang F., Wang X., Ai S., Sun Z., Wan Q., Zhu Z., Yamamoto K.: Immobilization of uricase on ZnO nanorods for a reagentless uric acid biosensor. *Anal. Chim. Acta.* **519**, 155–160 (2004).
92. Zhou X.L., Ma X.H., Sun G.Q., Li X., Guo K.P.: Isolation of a thermostable uricase-producing bacterium and study on its enzyme production conditions. *Process Biochem.* **40**, 3749–3753 (2005).
93. Zhylyak G.A., Dzyadevich S.V., Korpan Y.I., Soldatkin A.P., El'Skaya A. V.: Application of urease conductometric biosensor for heavy-metal ion determination. *Sens. Actuators B.* **24**, 145–148 (1995).