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Antibacterial activity of the produced and purified L-glutamate oxidase from *Streptomyces* sp.

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ABSTRACT

From *Streptomyces* species isolated from agricultural soil, an extracellular L-glutamate oxidase was recovered. The *Streptomyces* bacterial isolates were grown in a selective medium that had L-glutamate as a substrate. The filtrate, which is representative of the crude enzyme, was subsequently obtained by extracting the extracellular enzyme using a cooling centrifugation procedure. L-glutamate oxidase purification operations were then performed, starting with fractionation with 40% ammonium salts and continuing with ion-exchange chromatography and gel filtration with a final 8.25 U mg^{-1} and 61.8% yield. Both positive and negative bacteria were sensitive to the pure L-glutamate oxidase effects. Purified L-glutamate oxidase outperformed other examined bacterial isolates in terms of activity, with MIC values of 32 µg mL^{-1} for *Klebsiella pneumonia* and 64 µg mL^{-1} for *Staphylococcus aureus* and *Escherichia coli*. Thus, pure L-glutamate oxidase may be viewed as a promising possibility in the rational design of new antibiotics.

Keywords: L-glutamate oxidase, *Streptomyces*, Antibacterial activity
Article type: Research Article.

INTRODUCTION

Gram-positive terrestrial bacteria called "actinomycetes" are heterotrophic, aerobic, stationary, spore-forming, filamentous, rod-shaped, and asexual (Barakate *et al.* 2018). The majority of soil actinomycetes belong to the genus *Streptomyces*, which is also the most prevalent, more than any other actinomycetes genus. *Streptomyces* spp. also produces a large range of new antibiotics. The largest taxonomic category of the bacterial kingdom, *Streptomyces*, are widely dispersed in both aquatic and terrestrial habitats (Chaudhary *et al.* 2017; Quinn *et al.* 2020). They can be found in a variety of settings, however, the soil is where they are most prevalent. *Streptomycetes* are known for their filamentous growth and hard, leathery colonies in terms of chemo hetero organography. They have a significant ecological function when organic stuff in the soil breaks down, and they can use complex organic resources like sources of energy and carbon (Chater 2016). L-glutamate oxidase (EC 1.4.3.11) with the presence of oxygen and water, promotes L-glutamate oxidative deamination, which results in the synthesis of α-ketoglutarate, NH_3, and H_2O_2 (Mohammed *et al.* 2022). L-glutamate oxidase is extensively utilized in the food, industrial fermentation, and pharmaceutical industries. It possesses excellent specificity and high affinity for the reaction substrate, gentle reaction conditions, and high catalytic efficiency. Although L-glutamic acid oxidase is a relatively recent practical tool enzyme, little is known about its mode of action and three-dimensional protein structure (Wachiratianchai *et al.* 2014). Antibiotic resistance is now a significant obstacle to treating infectious infections. Consequently, it is crucial to discover alternative antibiotics (Almeida & Marana 2019; Ainur *et al.* 2023; Hatif *et al.* 2023). Hundreds of centres worldwide are currently working in the field of isolating new potent strains of actinomycetes and extracting antibiotics substances as a result of compounds having been proven to be effective, among which the metabolites of actinomycetes like antibiotics

are of considerable value (Almashhadani 2021). So that the purpose of this research focuses on the isolation of *Streptomyces* from agricultural soils, screening L-glutamate oxidase productions besides to purification the enzyme and detection its ability to use as antibacterial against multi-resistant bacteria.

MATERIALS AND METHODS

Samples collection

At varying depths below the surface, 15 samples of agricultural soil were taken from various locations in the Army Channel, Iraq. Using a trowel, the sterile little plastic containers were used for the collection of samples and then taken to the lab for investigation.

Isolation of *Streptomyces* from soil

To eliminate pebbles and other debris, soil samples were thoroughly mixed before being run through a sieve filter. The samples were heated for 5 min at 55 °C as primary treatment. The dilution plate method was applied by suspending 1 g soil in 9 mL sterile water followed by serial dilutions up to 10^{-4}. An aliquot of 0.5 mL from the last dilution was spread on yeast-malt extract agar medium and then incubated at 28 °C for 3 days. The growing colonies were checked for the presence of *Streptomyces* after the incubation period. The characteristic spherical, small, opaque, compact, and frequently colourful colonies were examined under a light microscope (Maleki & Mashinchianm 2020). Using API biochemical kits, it was possible to determine an isolate's biochemical characteristics utilizing tests for oxidase, urease, carbohydrate fermentation, and nitrate reduction.

Screening L-glutamate oxidase production

All bacterial isolates were added to a medium containing glucose (3%), KCl (0.12%), $(NH_4)_2SO_4$ (0.6%), and monosodium glutamate (0.5%) (Chau *et al.* 2018). The culture was cultivated for three days at 28 °C. Using centrifugation, the supernatant was obtained after the incubation period and the activity and protein content were assessed.

L-glutamate oxidase assay

The following mixture was used to measure L-glutamate oxidase: 0.5 mL crude extract, 0.5 mL deionized water, 1.5 mL L-glutamate (16 Mm), and 0.8 mL potassium phosphate buffer (50 mM, pH 6). At 40 °C, the enzyme reaction took place. Nessler's solution was made by combining 0.5 mL of the mixture, 0.2 mL NaOH(0.2 N), 1 mL NaOH(1N), and 3.3 mL of deionized water to measure the ammonia that was created, then incubated for 15 min at 30 °C, with the ammonia detected at 420 nm (Kämpfer 2012). One unit of the enzyme was defined as the quantity of enzyme needed to produce 1 mol NH_3 per min under ideal circumstances.

Estimation of protein concentration

The Bradford method was used to calculate the protein concentration (Qingshan *et al.* 2009). At 595 nm, the total protein concentration was assessed using the standard of bovine serum albumin.

Steps of L-glutamate oxidase purification

By ammonium salts at a 60% saturation ratio, the crude enzyme was precipitated. The sample was placed into a DEAE-cellulose column following dialysis. The elution process was then carried out using a range of NaCl concentrations from 0.1 to 0.8 M, then each eluted fraction's absorbance was measured at 280 nm and the enzyme activity in the collected fractions was estimated. The sample was concentrated using sucrose, then using a phosphate buffer solution (0.2 M, pH 7.5). It was then processed over a Sephadex G-200 column. Protein peaks with high absorbency were examined for enzymatic activity, and the active fractions were combined.

Antibacterial activity of L-glutamate oxidase

A standardized dilution procedure was applied to calculate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The tested bacteria were suspended overnight to a turbidity of 1.5×10^8 CFU mL^{-1}: *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterobacter faecalis Bacillus subtilis,* and *Staphylococcus aureus*. Using the purified L-glutamate oxidase in a stock solution, successive quantities (10^2-10^4 µg mL^{-1}) of the enzyme were diluted and prepared in Meuller Hinton broth. Incubation at 37

°C after adding 25 µL of the bacterial suspension. The MBC was defined as the lowest dose at which no live cells were found after 24 h of incubation at 37 °C when streaked on an MH agar plate. The MIC was described as the lowest quantity that inhibited the organism from growing in an apparent manner (Oikawa *et al.* 2009).

RESULTS AND DISCUSSION

Isolation of *Streptomyces* from soil

The recovery and characterization of many suspected *Streptomyces* phenotypes. After incubation at 28 °C for 72 h, different bacterial colonies were developed on the plates of Yeast extract-Malt extract medium. By examining biochemical traits with API kits, the Actinomycete bacteria membership in the *Streptomyces* family was established. The results showed that out of 15 agricultural soil samples, only 6 isolates were *Streptomyces* spp.

Streptomyces are abundant in nature, particularly in soils with various chemical and structural makeups. *Streptomyces* species have developed symbiotic relationships with several types of animals, fungi, and plants, and as such, they serve an important ecological purpose in the soil (Bradford 1976). Many secondary metabolites produced by *Streptomyces* have been shown to reduce the development of infections, including those that affect humans and plants (Chowdhury *et al.* 2019; Seipke *et al.* 2020). *Streptomyces* was successfully isolated and recognized several from Sudan's two distinct ecozones (the desert and savanna ecozones).

Screening of L-glutamate oxidase production

The productivity ranged between 2.5-7.7 U mL-1 according to the qualitative screening for L-glutamate oxidase synthesis, with *Streptomyces* sp. S4 exhibiting the highest productivity (Fig. 1), to choose this isolate as the top L-glutamate oxidase producer.

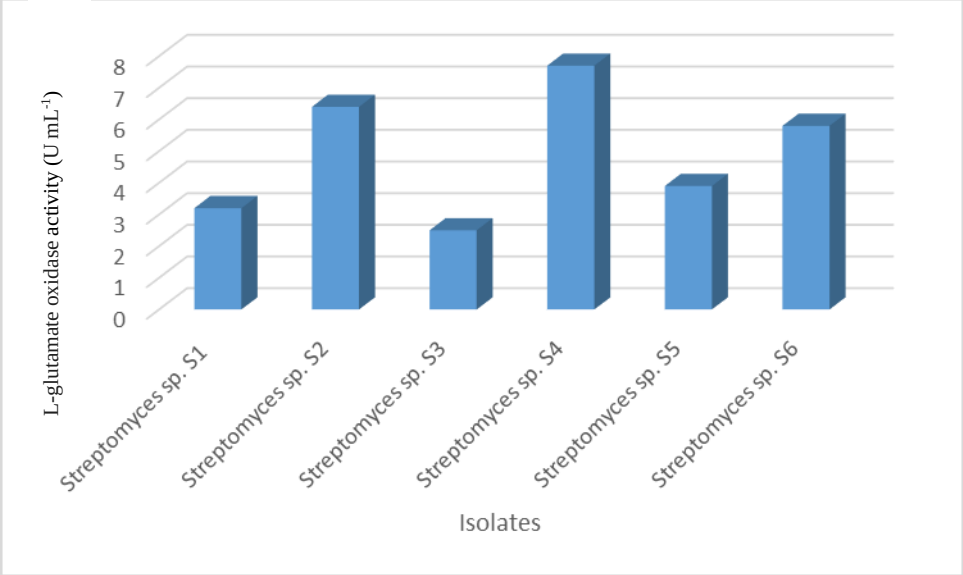

Fig. 1. Quantitative screening of L-glutamate oxidase production in *Streptomyces* spp.

After 33 h of fermentation and mycelium inoculation, the highest L-glutamate oxidase output was 2.7 U mL^{-1} (Newitt *et al.* 2019). When calcium was first added to the medium, the synthesis of L-glutamate oxidase could not or only little increased (Rashad *et al.* 2016).

Purification of L-glutamate oxidase

Streptomyces sp. S4 was grown on the screening medium enriched with monosodium glutamate, and after centrifugation, a fractionation process using ammonium sulphate at varying saturation rates (%) was used to separate the clear supernatant that was produced. At 40% salt saturation, the maximum L-glutamate oxidase activity was 11.5 U mL^{-1}. Following dialysis, the pooled fractions were run over a DEAE-cellulose column and a gradient of NaCl solutions was used for the elution, yielding 66.3 and 14.2 U mL^{-1} activities (Fig. 2). The pooled fractions were concentrated and placed into a Sephadex G-200 column then a phosphate buffer solution (0.2 M,

pH 7.5) was passed for the elution. The yield of the active components was 61.8, with an L-glutamate oxidase activity of 23.8 U mL^{-1} (Table 1 and Fig. 3).

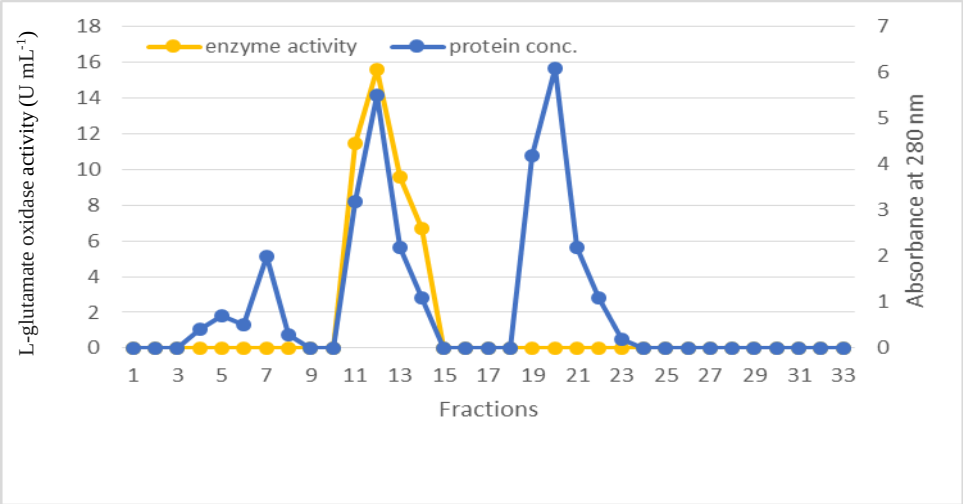

Fig. 2. An ion exchange chromatography with DEAE-Cellulose column for purification of L-glutamate oxidase.

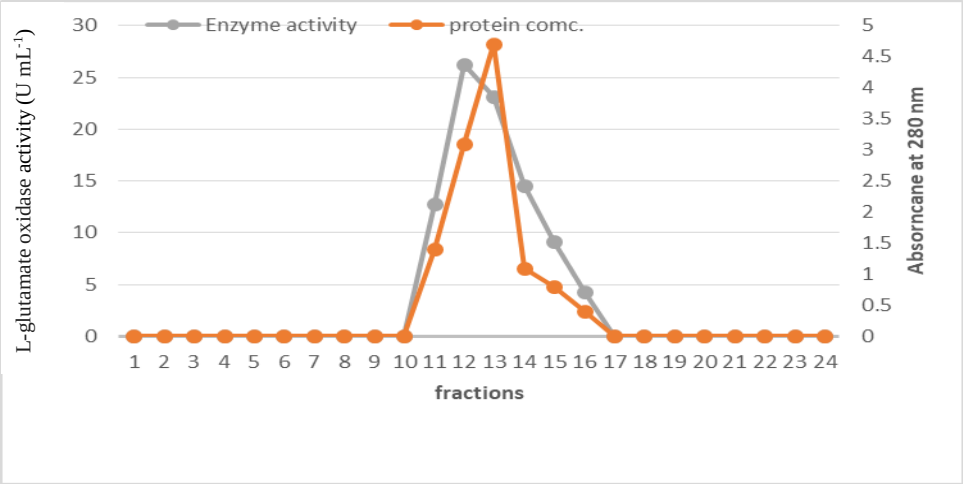

Fig. 3. Sephadex Gel filtration chromatography with Sephadex G-200 column for purification of L-glutamate oxidase.

Table 1. L-glutamate oxidase purification from *Streptomyces* sp. S4

Purification step	Size (mL)	L-glutamate oxidase activity (U mL^{-1})	Protein conc. (mg mL^{-1})	Specific activity (U mg^{-1})	Total activity	Purification fold	Yield (%)
Crude extract	50	7.7	2.3	3.34	385	1	100
$(NH_4)_2SO_4$ precipitation	23	11.5	1.7	6.76	264.5	2	68.7
DEAE-Cellulose	18	14.2	1.1	12.90	255.6	4	66.3
Sephadex G-200	12	23.8	0.7	34	238	10	61.8

The procedures used to purify L-glutamate oxidase included precipitation with ammonium sulphate as the first stage in the purification process, followed by dialysis to remove the salts. The purification procedure was then completed using gel filtration and ion exchange chromatography (Li & Zhong 2011). A 50% saturation ratio was employed in a study by (Li *et al.* 2014) then ion exchange and size exclusion chromatographies were next employed. These steps revealed the distinction between the earlier purification processes. This was a result of the enzyme high purity and greatest specific activity values being attained in these two phases.

Detection of L-glutamate oxidase antibacterial activity

Purified L-glutamate oxidase antibacterial activity was tested by measuring MIC and MBC values toward a variety of pathogenic bacteria (Fig. 4). Purified L-glutamate oxidase was effective against both G+ve and G-ve bacteria. The values of MIC were 32 µg mL^{-1} for *K. pneumonia* while 64 µg mL^{-1} for *S. aureus* and *E. coli*. The purified L-glutamate oxidase demonstrated greater activity. The pure L-glutamate oxidase, on the other hand, demonstrated reduced antibacterial activity with MICs of 256 µg mL^{-1} when tested against *P. aeruginosa*, *Enterobacter faecalis*, and other Gram-positive bacteria. The MBC values, in contrast, ranged from 64 to 1024 µg mL^{-1}. Hence, the rational design of novel antibiotics may be considered as a true L-glutamate oxidase for promising possibility.

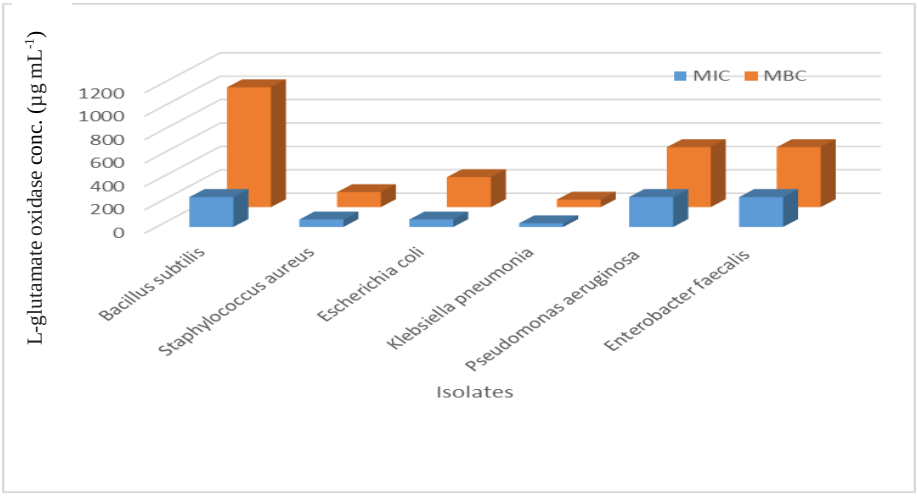

Fig. 4. Detection of MIC and MBC for L-glutamate oxidase against some pathogenic bacteria.

REFERENCES

Abdul Hameed, H & Ali, H 2021, Extraction and purification of extracellular l-glutamate oxidase from *Streptomyces*. *Archives of Razi Institute*, 76: 769-779.

Ainur, S, Indira, R, Abaibek, Z, Krykbaevna, ZM, Shoshanovna, BA & Gulzhas, T 2023, Bacterial Mediation of Environmental Impacts: Unveiling novel pathways for mitigation and stewardship. *Caspian Journal of Environmental Sciences*, 21: 1171-1183.

Almashhadani, HA 2021, Synthesis of a CoO–ZnO nanocomposite and its study as a corrosion protection coating for stainless steel in saline solution. *International Journal of Corrosion and Scale Inhibition*, 10: 1294-306.

Almeida, VM & Marana, SR 2019, The optimum temperature may be a misleading parameter in enzyme characterization and application. *PLOS ONE*, 14: e0212977.

Barakate, M, Ouhdouch, Y, Oufdou, KH & Beaulieu, C 2018, Characterization of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. *World Journal of Microbiology and Biotechnology*, 18: 49-54.

Bradford, M 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Annal. *Biochemistry*, 72: 248-254.

Chater, KF 2016, Recent advances in understanding *Streptomyces*. *F1000Research*, 5: 16-21.

Chau, R, Kalaitzis, JA, Wood, SA & Neilan, BA 2018, Diversity and biosynthetic potential of culturable microbes associated with toxic marine animals. *Marine Drugs*, 11: 2695-2712, https://doi.org/10.3390/md11082695

Chaudhary, HS, Yadav, J, Shrivastava, AR, Singh, S, Singh, AK & Gopalan, N 2017, Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city in central India). *Journal of Advanced Pharmaceutical Technology & Research*, 4: 118-123.

Chowdhury, AP, Singh, RR, Bharadwaj, B, Bhuyana, MP & Jyoti, S 2019, Qualitative assessment on isolated tartar forming bacteria from dental caries by pattern of screening, assembling and antibiotic sensitivity. *Journal of Research in Medical and Dental Science*, 7: 140-157.

Hatif, ST, Al Taie, SF, Zeadan, AM, Awad Al-Nuaimi, ZJ & Rasheed, ZS 2023, Effect of Sidr honey on production of bacteriocin like inhibitory substances in some pathogenic bacteria. *Caspian Journal of Environmental Sciences*, 21: 1037-1041.

Kämpfer, P 2012, Family Streptomycetaceae. Volume 5: The Actinobacteria, In: MGW, Whitman, P, Kämpfer, H, Busse, M, Trujillo, W, Ludwig, K, Suzuki, A, Parte, (Eds.), Bergey's Manual of Systematic Bacteriology Springer, New York, NY, USA.

Kusakabe, H, Midorikawa, Y, Kuninaka, A & Yoshino, H 2017, Occurrence of a new enzyme, l-glutamate oxidase in a wheat bran culture extract of *Streptomyces* sp. X-119-6. *Agricultural and Biological Chemistry*, 47: 179-182.

Li, QS & Zhong, JJ 2011, Short communication: Production of L-glutamate oxidase by *Streptomyces* sp. N1 in submerged fermentation. *World Journal of Microbiology and Biotechnology*, 12: 651-658.

Li, QS, Fang, QH, Zhu, JY & Zhong, JJ 2014, Hyperproduction of L-glutamate oxidase in submerged fermentation of *Streptomyces* sp. N1 with culture pH control and calcium addition. *Applied Biochemistry and Biotechnology*, 80: 97-106.

Maleki, H & Mashinchianm, O 2020, Characterization of *Streptomyces* Isolates with UV, FTIR Spectroscopy, and HPLC Analyses. *BioImpacts*, 1: 47-52.

Mohammed, AK, Al Shaheeb, S, Fawzi, OF, Almashhadani, HA & Kadhim, MM 2022, Evaluation of Interlukein-6 and Vitamin D in Patients with COVID-19. *Research Journal of Biotechnology*, 17.

Newitt, JT, Prudence, SMM, Hutchings, MI & Worsley, SF 2019, Biocontrol of cereal crop diseases using streptomycetes. *Pathogens*, 8: 78-82.

Oikawa, T, Watanabe, M, Makiura, H, Kusakabe, H, Yamade, K & Soda, K 2009, Production of d-glutamate from l-glutamate with glutamate racemase and l-glutamate oxidase. *Bioscience, Biotechnology, and Biochemistry*m, 63: 2168-2173.

Qingshan, L, Lijun, W & Yourong, L 2009, Colour development with rational screening method for improved L-glutamate oxidase-producing strains. *Enzyme and Microbial Technology*, 18: 7-9.

Quinn, GA, Banat, AM, Abdelhameed, AM & Banat, IM 2020, *Streptomyces* from traditional medicine: Sources of innovation in antibiotic discovery. *Journal of Medical Microbiology*, 69: 1040, 2020.

Rashad, FM, Fathy, HM, El Zayat, AS & Elghonaimy, AM 2016, Isolation and characterization of multifunctional *Streptomyces* species with antimicrobial, nematocidal, and phytohormone activities from marine environments in Egypt. *Microbiological Research*, 175: 34-47.

Seipke, RF, Kaltenpoth, M & Hutchings, MI 2020, *Streptomyces* as symbionts: An emerging and widespread theme? Fems. *Microbiological Reviews*, 36: 862-876.

Wachiratianchai, S, Bhumiratana, A & Udomsopagit, S 2014, Isolation, purification, and characterization of L-glutamate oxidase from *Streptomyces* sp. 18G. *Electronic Journal of Biotechnology*, 7: 09-10.