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FULL PAPER

An antibacterial activity of the produced and purified L-glutamate oxidase from *Streptomyces* sp

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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 units/mg and 61.8% yield. Both positive and negative bacteria were sensitive to the pure L-glutamate oxidase's effects. Purified L-glutamate oxidase outperformed the other examined bacterial isolates in terms of activity, with MIC values of 32 µg/mL for *Klebsiella pneumonia* and 64 µg/mL 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.

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Introduction

Gram-positive terrestrial bacteria called "actinomycetes" are heterotrophic, aerobic, stationary, spore-forming, filamentous, rod-shaped, and asexual [1]. 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 [2,3].

They can be found in a variety of settings, but the soil is where they are most prevalent. *Streptomycetes* are chemo hetero organography known for their filamentous growth and hard, leathery colonies. 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 [4].

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 [5]. 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 [6].

Antibiotic resistance is now a significant obstacle to treating infectious infections. Consequently, it is crucial to discover alternative antibiotics [7].

Hundreds of centers 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 [8] so that the purpose of this research focuses on the isolation of *Streptomyces* from agricultural soils, screening of 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, fifteen samples of agricultural soil were taken from various locations in the army channel. 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 of 1 g of soil in 9 mL of sterile water followed by serial dilutions up to 10-4. 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 colorful colonies were examined under a light microscope [9]. With API biochemical kits, it was possible to determine an isolate's biochemical characteristics utilizing tests for oxidase, urease, carbohydrate fermentation, and nitrate reduction.

Screening of 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%) [10]. 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 of 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 of 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 minutes at 30 °C, with the ammonia detected at 420 nm [11].

One unit of the enzyme was defined as the quantity of enzyme needed to produce 1 mol of NH_3 per minute under ideal circumstances.

Estimation of Protein Concentration

The Bradford method has been used to calculate the protein concentration [12].

At 595 nm, the total protein concentration was assessed with the standard of bovine serum albumin.

Steps of L-glutamate oxidase purification

Ammonium salts at a 60% saturation ratio, the crude enzyme was precipitated. The sample was put 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, and 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, and then using a (0.2 M, pH 7.5) phosphate buffer solution, it was 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 the minimum bactericidal concentration (MBC). The tested bacteria were suspended overnight to a turbidity of 1.5 x 108 cfu/mL:

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 (1–1024 µg/ml) 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 hours 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 [13].

Results and discussion

Isolation of Streptomyces from soil

The recovery and characterization of many suspected *Streptomyces* phenotypes. After incubation at 28 °C for 72 hours, different bacterial colonies developed on the plates of Yeast extract-Malt extract medium. By examining biochemical traits with API kits, the actinomycete bacteria's membership in the Streptomyces family was established. The results showed that out of 15 agricultural soil samples, there were only 6 isolates of *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 [14].

Many secondary metabolites produced by *Streptomyces* have been shown to reduce the development of infections, including those that affect humans and plants [15,16].

Streptomyces has successfully isolated and recognized several s 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 according to the qualitative screening for L-glutamate oxidase synthesis, with *Streptomyces* spp. S4 has the highest productivity (Figure 1).

To choose this isolate as the top L-glutamate oxidase producer.

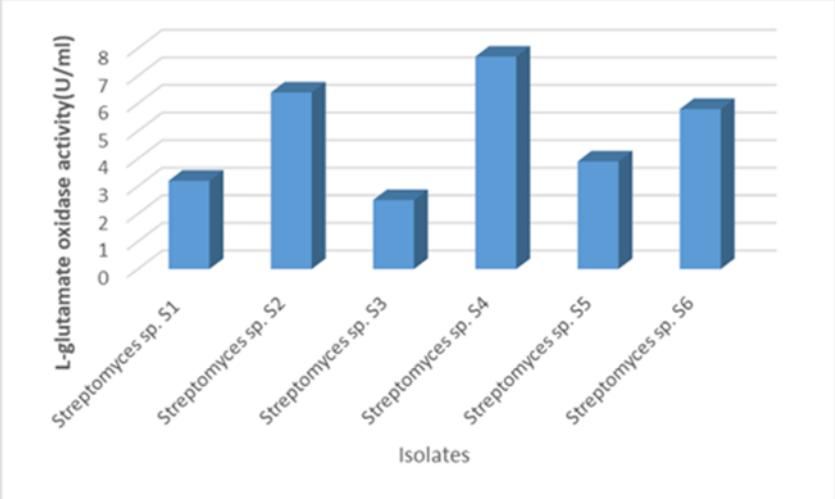

FIGURE 1 Quantitative screening of L-glutamate oxidase production in *Streptomyces* spp

After 33 hours of fermentation and mycelium inoculation, the highest L-glutamate oxidase output was 2.7 U/mL [17]. When calcium was first added to the medium, the synthesis of L-glutamate oxidase could not or only little increased [18].

Purification of L-glutamate oxidase

Streptomyces spp. S4 was grown on the screening medium enriched with monosodium glutamate, and after centrifugation, A fractionation process using ammonium sulfate at varying saturation percentages 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. 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 of activity (Figure 2).

The pooled fractions were concentrated and placed into a Sephadex G-200 column, and 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, as demonstrated in Table 1 and Figure 3.

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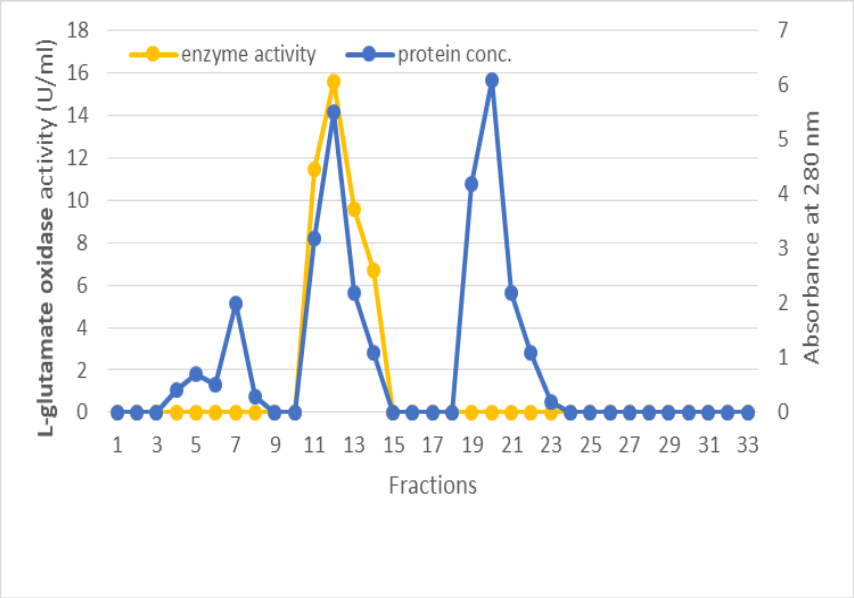

FIGURE 2 An ion exchange chromatography with DEAE-Cellulose column for purification of L-glutamate oxidase

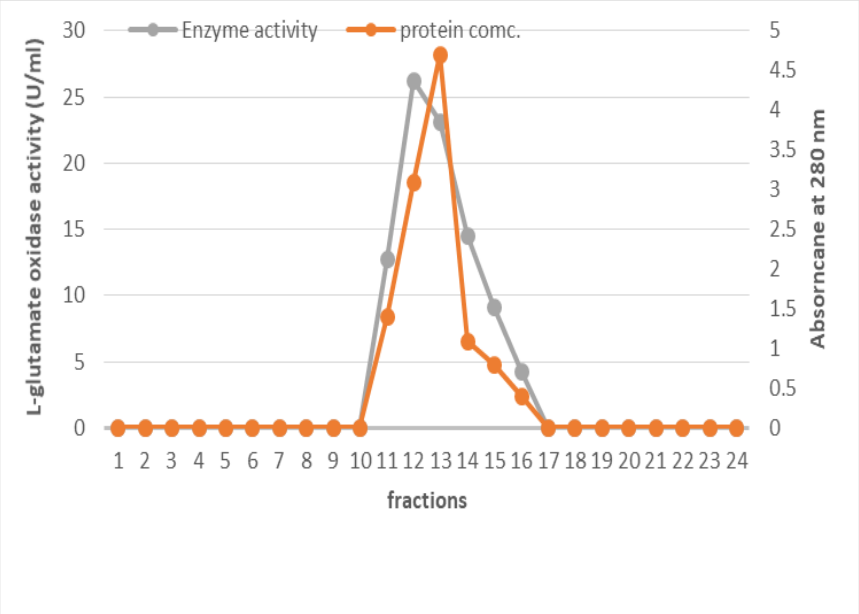

FIGURE 3 Sephadex Gel filtration chromatography with Sephadex G-200 column for purification of L-glutamate oxidase

The procedures used to purify L-glutamate oxidase included precipitation with ammonium sulfate as the initial 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 [19].

A 50% saturation ratio was employed in a study by [20-23] 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's high purity and the greatest specific activity values being attained in these two phases.

Detection of L-glutamate oxidase antibacterial activity

Purified L-glutamate oxidase's antibacterial activity was tested by measuring MIC and MBC values toward various pathogenic bacteria.

The results are demonstrated in Figure 4; purified L-glutamate oxidase was effective against both G+ve AND G-ve bacteria. The MIC values at 32 µg/mL for Klebsiella pneumonia and 64 µg/mL for *Staphylococcus aureus* and *Escherichia 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 when tested against *Pseudomonas aeruginosa*, Enterobacter faecalis, and other GGGram-positive bacteria The MBCS values, in contrast, range from 64 to 1024 µg/mL.

Hence, the rational design of novel antibiotics may consider ttrueL-glutamate oxidase as a promising possibility.

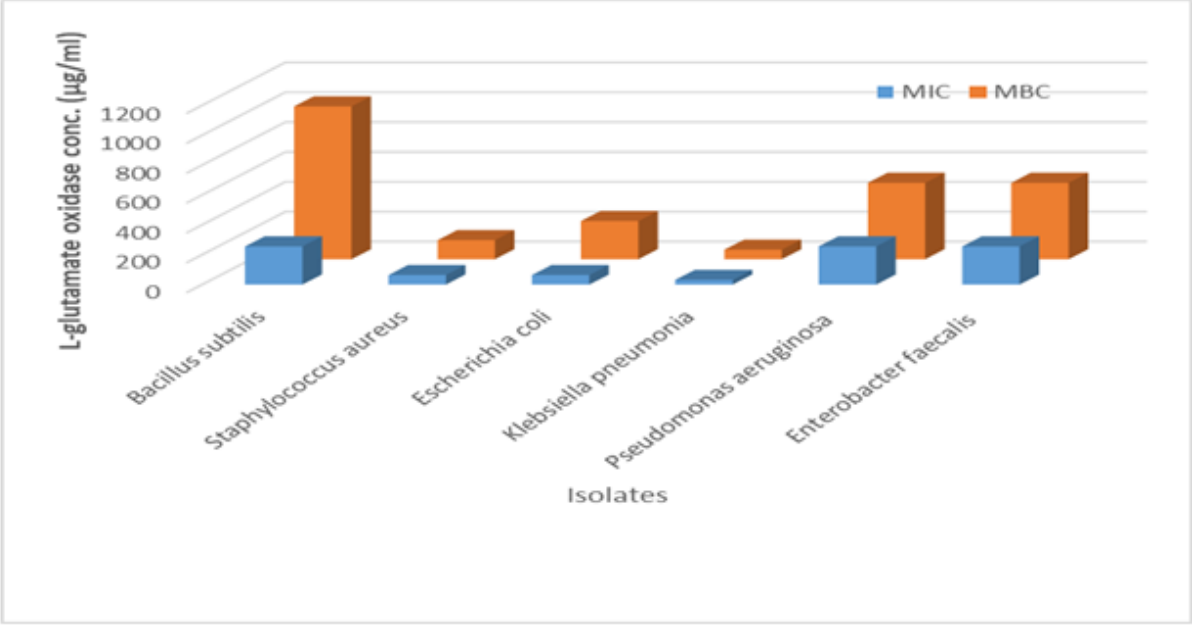

FIGURE 4 Detection of MIC and MBC for L-glutamate oxidase against some pathogenic bacteria

TABLE 1 L-glutamate oxidase purification from *Streptomyces* spp. S4

Purification step	Size (ml)	L-glutamate oxidase activity (U/mL)	Protein conc. (mg/ mL)	Specific activity (U/ mg)	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

Conclusion

Through this study, pure L-glutamate oxidase can be considered as a promising possibility in the rational design of new antibiotics by isolating Streptomyces from agricultural soil, examining the production of L-glutamate oxidase along with purifying the enzyme and revealing its ability to use it as an antibacterial against multi-resistant bacteria. Both positive and negative bacteria were sensitive to the effects of pure L-glutamate oxidase. Purified L-glutamate oxidase outperformed other bacterial isolates examined in terms of efficacy.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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