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Generation of fungal L-glutaminase enzyme as an antineoplastic agent from various Egyptian country soil environments

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ABSTRACT

Background: Acute lymphocytic leukaemia and auxtrophic hepatocellular carcinoma for L-glutaminase are two of the leading causes of mortality worldwide. The bacterial L-glutaminase enzyme, which is normally produced under harsh circumstances from Escherichia coli or Bacillus cereus, was used to treat acute lymphocytic leukaemia. Fungal L-glutaminase is preferred over bacterial L-glutaminase due to less hypersensitivity events, such as allergic responses and medicine neutralization; as well as higher efficiency against hepatic carcinoma. **Methodology:** In the current screening experiment, mineral glutamine agar (MGA), a selective medium, was employed for the production of fungal L-glutaminase (only fungi that used L-glutaminase as a single metabolic source for carbon and nitrogen could grow). The incubation conditions comprised PH 6.5, 25°C, and for 3 days. Malt agar media was further used to sub-cultivate fungal L-glutaminase producing strains. Thereafter, these strains were determined by means of biochemical procedures, microscopic analysis, and morphology. L-glutaminase-producing strains were molecularly identified by using the northern blotting method to detect molecular DNA hybridization. The fungal L-glutaminase gene was firstly isolated using specific PCR primers then the gene was subcloned and inserted into a DNA vector to be produced via recombinant DNA technology. In order to assess anticancer activity, the MTT test was performed. **Results:** Morphological, biochemical, and DNA blotting hybridization studies identified Aspergillus niger Strain ATCC 1015 as the major fungal isolate that produced this enzyme. The in vitro performance of fungal L-glutaminase as an antileukemic and anticancer mediator for hepatic auxotrophic tumours was very good. MTT test results showed that the IC50 values for anticancer activity against the cancer cell lines CCL-120 and JHH4 were 38.9 and 40.3 g/ml respectively. L-glutaminase had a molecular mass of 65 kDa, a specific activity of 15.3 U/mg protein, a yield of 57.6%, and a purity factor of 3.8. Extracellular L-glutaminase, 6.8U/ml, was generated. The conventional vitamin C and L-glutaminase have IC50s for their antioxidant activity of 89 g/ml and 189 g/ml, respectively. The inoculum contained 1*108 spores/ml.

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Conclusion: The current study was a promising study because it evolved a novel source of fungal L-glutaminase from various soil environments in Egypt with high efficacy as an anticancer agent against cancers that are auxotrophic for L-glutamine, such as hepatocellular carcinoma and acute lymphocytic leukaemia. It was also free of the enormous difficulties that bacterial enzymes present, such as hypersensitivity reactions, as shown by numerous previous studies. It is advised that more studies look at how to make fungal L-glutaminase more effective as an anticancer therapy.

Introduction

Acute lymphocytic leukemia (ALL) and auxotrophic hepatocellular carcinoma (AHC) for L-glutaminase are two of the leading causes of mortality worldwide. Globally, the 5-year relative survival rate for ALL patients 20 years and older is 43%. For those under 20, the close 5-year survival rate is 90%. On the other hand, the worldwide mortality rate for AHC is less than 50%, and the 5-year survival rate is below 10%[1].

L-glutamine is hydrolytically deaminated by L-glutaminase, an amidohydrolase, producing L-glutamic acid and ammonia in the process.[2]

Mold and yeast are the two different categories of fungus.[3] Molds generate a mat by growing as long filaments (hyphae) (mycelium).[4] Certain hyphae(septate hyphae) form transverse walls,[5] but other hyphae do not form these transverse walls (nonseptate hyphae).[6] The expansion of the hyphal tip instead of cell division is the mechanism by which hyphae develop.[7] The multicellular nonseptate hyphae are coenocytic.[8] None of the fungi are obligatory anaerobes,[9] although the majority of them are obligate aerobes.[10]. Several important thermal dimorphic fungi are used in medicine[11]. They live as molds in the environment[12] and as yeasts in human tissues.[13]

Acute lymphocytic leukaemia was treated with the bacterial L-glutaminase enzyme, which is typically generated by Bacillus cereus or Escherichia coli.[14]

L-glutaminase breaks down L-glutamine amino acid (which is necessary for the proliferation of cancer cells since they cannot synthesis it and must acquire it from extracellular fluids, blood, and lymph) into L-glutamate and ammonia.[15]

Nevertheless, since Escherichia coli and Bacillus cereus are both prokaryotes. A number of adverse events, including allergic reactions and medication neutralization, are brought on by bacterial L-glutaminase.[16]

L-glutaminase from fungal eukaryotic cells on the other hand, is comparable to human enzymes.[17]

Excellent scavenging and antioxidant properties were shown by L-glutaminase. For increasing synthesis of fungal L-glutaminase, starch and L-glutamine at 1% w/v have been shown to be the optimum sources of nitrogen and carbon.[18]

The current study's goal was to produce a fungal L-glutaminase from a novel yeast source in order to overcome the limitations of bacterial L-glutaminase.

Methodology

Ethical statement:

All appropriate national, international, and/or institutional standards for the care and use of people and animals were followed in the current study. The ethical committee for animal handling at Cairo University CU- IACUC (Institutional Animal Care & Use Committee), at the Faculty of Pharmacy, Cairo University, Egypt, approved all procedures used in the study, including the use of animals, in accordance with the recommendations of the weatherall report with permission number P-18-7-2021. Every attempt was made to reduce the number of animals used in the study and their suffering. All animal experiments were complied with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Type of study:

A screening experimental investigation was conducted in this one.

Place and date of the study:

In Egypt, Cairo University's pharmacy school, this study was conducted between November 2021 and December 2022.

Collection of the samples:

100 soil samples with a depth of 10 cm were taken (Menofia, Sharqia, Qalyobia, Giza and Cairo governorates) from various locations in Egypt.

Material source:

The chemical and biological components were bought from the Egyptian Algomhuria Pharmaceutical Chemical Company. Cell lines were bought from the Accegen biological firm.

Screening of positive fungal L-glutaminase producing isolates:

Mineral glutamine agar (MGA):

To screen isolates of fungi that produce L-glutaminase, selective medium was employed. KH2PO4 (1.2), MgSO4 (0.6), FeSO4 (0.005), KCL (0.4), D-glucose (7), Agar (13), L-glutaminase (15), and antibiotic Thiamphenicol made up the substance (0.15). Thiamphenicol was included to stop the development of bacteria. The incubation period was three days, with a PH of 6.5 for the medium. In this selective media, only the colonies that could use L-glutaminase as a source of nitrogen and carbon could grow. The positive isolates were kept at 4 0C for further research. List of instruments is shown in Table 5. Morover, subculturing was achieved via malt extract agar media for increasing the yield, productivity, and purity of bacterial isolate producing L-glutaminase.

Malt extract agar medium:

It is an all-purpose acidic growth medium for the isolation and growing of fungi. NaOH was used to alter the pH to 6.5. After three days of incubation, subcultures of the positive isolates (produced on MGA) were colonized and isolated on malt extract agar medium at 20 0C, PH 6.5.

After being dissolved in 10% KOH, positive fungal isolates may be examined under a standard microscope. Using DNA probe hybridization, the molecular identification of positive fungal isolates was completed.

Production of fungal L-glutaminase by recombinant DNA technology:

The following are the primary procedures in designing homologous primers (known sequences): Recovering or obtaining the target gene (nucleotide sequence) through national centre of biological science[NCBI] was performed. The target gene was then subjected to a restriction analysis using the software tool restriction Mapper. The Staden package program's Spin subprogram was then used to determine the reading frames. Also, the nucleotide sequences of the reverse primer on the complementary strand and the forward primer on the parent DNA strand were determined.

The whole L-glutaminase genome from NCBI was cloned using fasta. Moreover, the coding sequence[CDS] of the L-glutaminase enzyme from NCBI was copied. The PUC18 vector plasmid was selected.

Suitable restriction enzymes for forward and reverse primers were chosen according to the web-cutter website. Selected restriction enzyme has to be in the plasmid vector's multiple cloning sites (MCS), accessible, and in line with the orientation of the gene that will be copied and expressed.

Using the PDRAW32 Microsoft tool, the T annealing for forward and reverse primers was calculated after appropriate restriction enzymes, forward and reverse enzymes, and primers were identified. The difference in annealing temperature between forward and reverse primers shouldn't be greater than 3 degrees Celsius. This provided the best conditions for the protein of interest to be cloned and expressed.

Procedure:

The new primer for recombinant expression of L-glutaminase as anticancer agent included forward and reverse primers:

Forward primer for expression: Direction of sequence was from 5-to 3-

AGCTGCCCTTGTCATAGTTG

Annealing temperature=melting temperature- 5=57.88-5=52.88 0C.

Reverse primer for expression: Direction of sequence is from 5- to 3-

AAGGCAGTTCTCCTTTGCCT

Annealing temperature = melting temperature- 5=59.52- 5=54.52 0C.

The expression vector was the PUC 18 plasmid.

The two restriction enzymes utilized and selected were Bam HI and Sphl according to the web-cutter website. The L-glutaminase enzyme's complementary DNA nucleotide sequence(cDNA)

was successfully isolated from the yeast extract and then, amplified with PCR. Finally, this sequence was inserted into an appropriate expression vector, such as PUC18. Also, when the expression vector was transformed into Escherichia, inducible IPTG was added to the expression host coli BL21(DES) POLYSS for the activation of the transcription process. Expression was carried out throughout production in Escherichia coli. The expression vector which used was PUC18.

T7 lac served as the promotor, and the tag protein had a 6x histidine tag at the C terminus. The recombinant protein was partly purified from the supernatant by salting out using ammonium sulphate. Next, using nickel affinity chromatography on immobilized nickel columns, 85% of the L-glutaminase was purified. For the initial culture, the yield was close to 650 mg/L.

For a standard bacterial culture, LB agar and broth were used, and an incubation period of 24 hours at 37 0C was completed. In accordance with the manufacturer references' instructions, antibiotics were applied to the medium. The production of fusion proteins containing a 6× histidine tag, an L-glutamine recognition site, and a T7 tag at the N-terminal of the target protein was made possible using the PUC18 expression vector. This procedure was according to the protocol followed in recombinant DNA expression of the vital therapeutic proteins.

Clarification and purification of L-glutaminase:

Initially, a centrifuge for three minutes at a speed of 4,000 rpm was achieved. The process was completed via precipitation using ammonium sulphate and partial extraction of L-glutaminase precursor from the culture's supernatant, followed by purification with nickel affinity chromatography. After extraction by precipitation (salting out) of 100 ml of the supernatant with 53 ml of a 4.1 M[Molar concentration unit] ammonium sulphate saturated solution at 25 0C, recombinant L-glutaminase fused with polyhistidine-tagged proteins could be quickly purified from the supernatant using nickelcolumns using nickel-based immobilized metal affinity chromatography. The preparations were filtered using Whatman-1541-042 filter paper (0.22 micron), which was acquired in the USA, to sterilize them before the final formulation was completed.[19]

Determination of fungal L-glutaminase production and activity:

Direct Nesslerization test:

A positive fungal isolate cultured in MGA was utilised to characterise the enzyme synthesis and activity. The hydrolysis of L-glutamine amino acid into L-glutamic acid and ammonium is catalyzed by L-glutaminase. The released ammonium was recognized and measured using a spectrophotometer at 425 nm. The relationship between light intensity and ammonium concentration was linear. The enzyme activity was directly inversely correlated with the amount of released ammonium.[20]

In vitro cell viability assay:

The physiologic, pharmacologic, and toxicological effects of the enzyme on cancer cells were evaluated using the CCL-120 cancer cell line[obtained from Accegen company, USA]. The physiologic, pharmacologic, and toxicological effects of the enzyme on hepatic cancer cells were evaluated using the JHH4 hepatic carcinoma cell line. On mammalian cells, the Vero cell line was employed[21].

Fungal L-glutaminase was tested for in vitro cell viability using the MTT ((dimethylthiazol-2-yl)diphenyl tetrazolium) method[22].

Formulation of fungal L-glutamine degrading enzymes as anticancer agents:

The optimum dosage forms and mode of administration of fungal glutamine-degrading enzymes as an anticancer treatment against auxotrophic tumors were performed victimizing several pharmaceutical dosage forms and routes of administration: The preparation of injectable goods took place using physiologic isotonic aqueous solutions (PH 7.4). The antibacterial agent thiamphenicol was added to injections. The release of L-glutamine-degrading enzymes from aqueous solutions administered intramuscularly was slowed down by increasing vehicle viscosity with carboxymethylcellulose (CMC), in order to extend the duration of effect to once-daily dosage administration rather than multiple-dose injections, ethylene glycol was added afterwards. Wet granulation was used to create tablets containing10 mcg/g of L-glutamine-degrading enzymes. As an excipient, 3% magnesium aluminium silicate was added. It was a glidant, disintegrant, and binder. 17% w/w starch was included in the preparation as a diluent. On the other hand, 1% w/v magnesium stearate was applied as lubricating ingredient.

Estimation of antioxidant actions of L-glutaminase:

This was accomplished using an assay for the scavenging of nitric oxide.

Briefly, 6ml of the reaction mixture containing 6 mM sodium nitroprusside (SNP) in phosphate buffered saline pH 7.4 with L-glutaminase extract was incubated at 25oC for 3 hours in front of a viewable polychromatic light-colored source (tungsten lamp 25 Watt). As a result, the radical of NO produced interacted with oxygen to produce nitrite ion (NO2 -), which was measured at intervals of thirty minutes by mixing two millilitres of the incubation mixture with a balanced amount of the Griess reagent (sulphanilamide 2% in phosphoric acid 6% and N-naphthylethylenediamine dihydrochloride 0.2%). The chromophore optical density was measured at 540 nm. The amount of nitrite produced in the presence of L-glutaminase extract was calculated by using a standard curve built on noteworthy sodium nitrite solution values.

Assessment of secretion of antibodies to L-glutaminase:

With the use of an effective ELISA operator, the quantity of IgG anti-L-glutaminase antibodies in mouse serum was sealed.

The kinetic parameters Km and Vmax determination:

Purified L-kinetic glutaminase's parameters, Michaelis-Menten constant (Km), and maximum velocity (Vmax) were determined using the proper substrate concentrations of L-glutaminase (2–11 mM). The nonlinear exponential stage union regression curve was attached to the data. Software called GraphPad Prism 5 was used for this. By measuring the rate at which L-glutamine is hydrolyzed in a conventional laboratory setting, the Michaelis-Menten equation was used to get the L-glutaminase result.

The deactivation rate constant (KD) and half-life time (t1/2)Estimation:

KD and t1/2 of the purified L-glutaminase enzyme were determined by running software of Graph-pad Prism number 5.

Molecular weight of L-glutaminase determination:

Using the western blot technique, which combines a 10% detachable acrylamide gel (pH 8.8) with a 5% stacking gel and 0.2% SDS according to Laemmli perception, it was possible to observe the mass and purity of the purified L-glutaminase enzyme. pH was 6.8. Gel staining was carried out using R-250 blue brilliant Coomassie, followed by a staining stage using a 5:2:6 solution of methanol, acetic acid, and water. Using a protein marker with an observed molecular weight range of 20–130 kDa, the molecular weight of L-glutaminase was determined. As a result, L-molecular glutaminase's weight was determined, and then it was confirmed using a mass spectrometer.

Determination of selectivity index of L-glutaminase:

The following formula was used to calculate an antineoplastic agent's selectivity index: SI is the ratio of the IC50 values for the test enzyme in healthy vs cancerous cell lines. The test enzyme's IC50 value denotes the concentration needed to kill 50% of the cells. Software called Graph Pad Prism version 5 was used for the deliberation. Also, the impact of varying PH, temperature, incubation duration, and substrate concentrations on the enzyme activity was evaluated. By a direct Nesslerization test, the location of L-glutaminase within the cell was determined.

Statistical analysis

Every culture was carried out in triplicate. They used standard deviation and means to present their findings. One-way analysis of variance (p value≤ 0.05) and statistical analysis using Excel spreadsheet micro-soft office were both employed as techniques for doing statistical analysis. For this investigation, the F test was used.

Results

In present investigation, L-glutaminase was produced majorly via the yeast Aspergillus niger Strain ATCC 1015. L-glutaminase had a specific activity of 15.3 U/mg protein, a molecular mass of 65 KDa, a yield of 57.6%, and a 3.8 fold increase. L-glutaminase has an extracellular productivity of 6.8 U/ml. At an acidic pH, this enzyme exhibited no activity. It had more activity at alkaline PH, peaking at PH 9, but not reaching PH 10. L-glutaminase performed equally well in neutral PH 7.3 circumstances with Mg as a cofactor and alkaline conditions. L-glutaminase is administered intramuscularly or subcutaneously via injection. It needs to be administered numerous times daily and has a brief half-life. In our investigation, we were

able to get around this issue by adding polyethylene glycol to make the activity last longer.

With increasing incubation durations up to 50 minutes, the impact of Aspergillus niger L-glutaminase was steadily increased (L-glutaminase effect 70.099 U/ml). With the use of a mass spectrometer, the molecular mass was determined to be 65 KDa. L-glutaminase had a Vmax of 159.7 UML in 1 min and a Km value of 4.011 10 3 M. At 66°C (Kd 0.039 min-1), the heat inactivation half-life time (t1/2) was 69.24 min, but at 54°C, it was 72.05 min (Kd 0.061 min-1). The fungus L-glutaminase has a 3.97 selectivity index against the cancer cell line CCL-120 and a 3.42 selectivity index against the cancer cell line JHH4.

The ELISA test revealed no detectable antibodies against fungus L-glutaminase. The conventional vitamin C and L-glutaminase have IC50s for their antioxidant activity of 89 g/ml and 189 g/ml, respectively. MTT test results showed that the IC50 values for anticancer activity against the cancer cell lines CCL-120 and JHH4 were 38.9 and 40.3 g/ml, respectively.

The purity and yield of the produced test enzyme:

High yield and purity were attributes of the L-glutaminase synthesised in our investigation using the novel expression primer.

Determination of activity of L-glutaminase enzyme by direct Nesslerization test:

According to the results of this test, the test L-glutaminase catalyzed the transformation of L-glutamine amino acid into L-glutamic amino acid and ammonia. A spectrophotometer was used to calculate the concentration of ammonia that was released. The concentration of freed ammonia was directly correlated with the L-glutaminase concentration and activity.

In vitro cell viability assay:

This experiment demonstrated greater anticancer activity of L-glutaminase against auxotrophic cancer cell lines, including acute lymphocytic leukaemia cancer cells produced by tissue culture. Acidic PH has no effect on this enzyme's activity. The maximum activity was at PH 9, with increased activity at alkaline PH but not reaching PH 11. L- glutaminase was equally efficient in neutral PH 7.3 circumstances with Mg ions acting as cofactor. In this work, L-glutaminase showed thermal stability up to 61 0C with a Tm value of 57.5 0C. It was also stable in a wide range of PH between 5 and 10.

The determination of optimum and suitable dosage form and route of administration of L-glutaminase enzyme:

A subcutaneous or intramuscular injection of L-glutaminase was used to deliver it. It had a short half-life and was given several times each day. By adding polyethylene glycol to the experiment, this problem was solved and the activity lasted longer. The performance and yield of this new enzyme were superior to those of bacterial L-glutaminase without any of its unfavourable side effects.

Table 1. It displays a computation of fungal L-glutamine degrading action via the direct Nesslerization technique:.

Concentration Of soil bacterial culture containing L-glutamine degrading enzymes (serial dilutions from 10^{-1} to 10^{-6} mg/l)	The absorbance of liberated ammonia at 425 nm by UV spectrophotometer
0.00	0.00
1/10	0.410
1/100	0.382
1/1000	0.341
1/10000	0.283
1/100000	0.177
1/1000000	0.076

Table 2. It displays a computation of fungal L-glutamine degrading enzymes action via the salicylate technique:

Concentration Of soil fungal culture containing L-glutamine degrading enzymes (serial dilutions from 10-1 to 10-6 mg/l)	The absorbance of liberated ammonia at 425 nm by UV spectrophotometer
0.00	0.00
1/10	0.421
1/100	0.386
1/1000	0.372
1/10000	0.297
1/100000	0.188
1/1000000	0.082

Table 3. Ingredients of Metallic glutamine Agar.

Ingredient	**Unit of measurement**
Potassium chloride	0.5 mg
Magnesium sulfate	0.5 mg
KH2PO4	1.0 g
Ferrous sulfate	0.1 g
Zinc sulfate	0.1 g
L-glutamine	1.0 g
Agar	2%

Table 4. Distribution of cellular positions of L-glutaminase

Proportion	**Percent(%)**
Extracellular	69
Surface bound	21
Cytosolic	10

Table 5. It shows the formulation of L-glutaminase as a sterile solution at PH 7:

Ingredient	**concentration**
L-glutaminase	15 mg
PEG20	45 mg
Mono-basic sodium phosphate	USP, 1.5 mg +5%
Di-basic sodium phosphate	USP, 3 mg + 5%
Sodium chloride	USP, 7.5 mg +5%
Water for injection	Query size to 1 ml

Table 6. It exhibits the scavenging(antioxidant) activity of L-glutaminase via nitric oxide scavenging assay:

Concentration of sample(mcg/l)	Percentage of inhibition(%)
100	20
200	30
300	52
400	68
500	79

Figure 1. It shows recombinant fused L-glutaminase that has been purified using a gel electrophoresis process and a western blot. Recombinant L-glutaminase has an 85% purity level. The molecular weight of L-glutaminase was calculated using this method to be around 65 kDa.

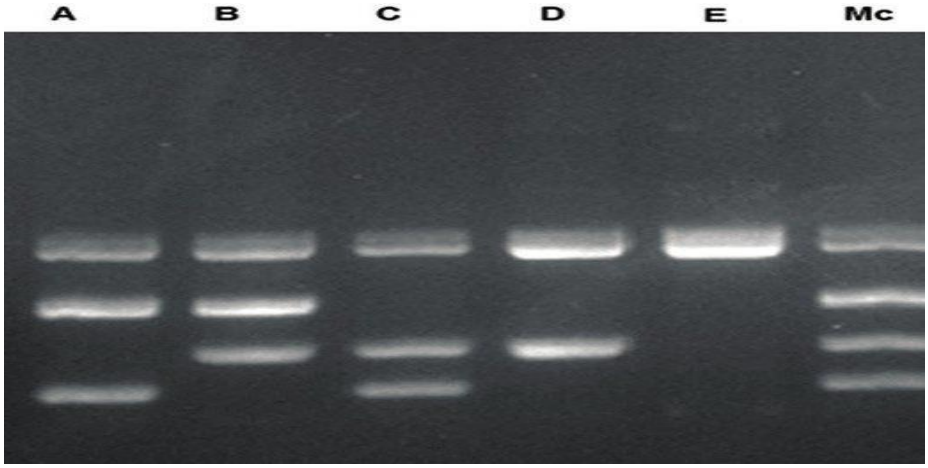


Figure 2. It acts as a Nesslerization screening test for various amounts of L-glutamine-degrading enzymes in soil fungus. L-glutaminase activity increases in proportion to ammonia liberation.

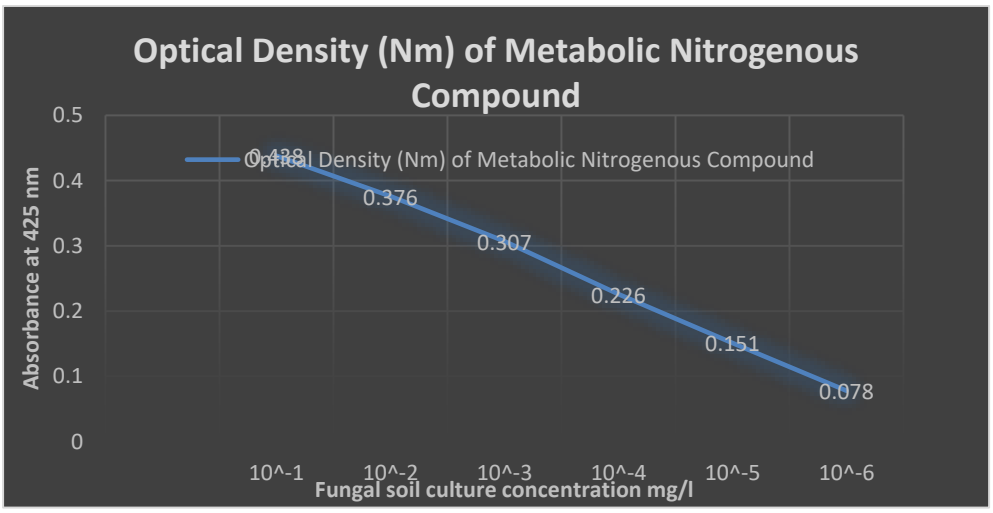
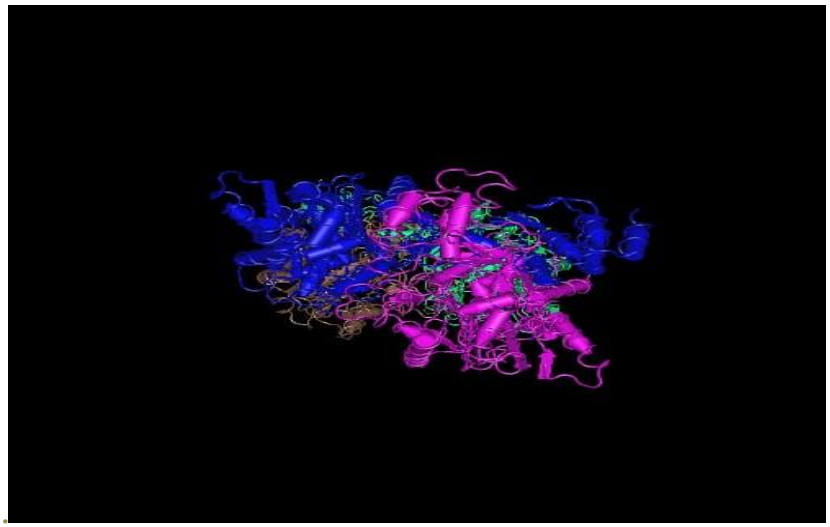


Figure 3. It shows the test L-glutaminase's three-dimensional structure. With 321 amino acids, L-glutaminase was an extracellular protein.



Discussion

The main findings of the present investigation were the discovery of a novel fungal source of L-glutaminase as an anticancer agent from various soil conditions in Egypt.

The major isolate that produced L-glutaminase when grown on MGA selective medium and malt extract agar was the fungus Aspergillus Niger Strain ATCC 1015, which was discovered through analysis of 74 soil samples from various locales in Egypt. It was distinguished by mold with septate hyphae that could be observed under a light microscope. Using MGA selective medium and malt extract agar, molds with green spores and conidia in radiating channels were developed. This was verified by using DNA probes in a molecular method hybridization. The hydrolysis of L-glutamine amino acid into L-glutamic acid and ammonia is catalysed by L-glutaminase. Direct nesslerization and salicylate assays were used to estimate the ammonia content. The enzyme activity was directly correlated with the amount of released ammonium.

PH 6.5 at a temperature of 250C were the ideal conditions for the synthesis and biological activity of the enzyme. The enzyme synthesis was triggered by KH2PO4 (1.2 g), MgSO4 (0.6 g), FeSO4 (0.005 g), and KCL (0.4 g). As compared to bacterial L-glutaminase made from Escherichia coli or Bacillus cereus, this fungal enzyme demonstrated greater efficiency as an oncolytic agent against acute lymphocytic leukaemia and hepatic cancer.[23]

Moreover, this fungal enzyme might compensate for bacterial L-shortcomings. When fungal L-glutaminase was compared to bacterial L-glutaminase, it displayed less hypersensitive responses (such as medication neutralization and anaphylactic reactions). This was demonstrated by an in vitro MTT assay test on several cell lines. According to Singh P et al's 2013 study, monovalent cations such Na+, K+, and phosphate ions boosted the enzyme activity more than divalent cations like Pb+2, Hg+2, and Cd+2. Also, this study stated that L-glutaminase enzymes produced mainly from microorganisms were intracellular proteins; however few exhibited extracellular portions.[24] Nevertheless, in this study, Mg+2, K+ ions enhanced L-glutaminase activity. Also, L-glutaminase in the current study was noticed to be extracellular protein with higher yield than most other origins of L-glutaminase. According to a study by Durthi Polta et al. published in 2020, purified L-glutaminase from E. coli or Bacillus cereus had a maximum activity when incubated at PH 8 at 40 0C for 30 minutes;[25] however, in this study, the enzyme's maximum activity was obtained after 50 minutes of incubation at PH 10 at 35 0C. According to Awad H et al 2019 .'s study, L-glutaminase was stable at a broad range of PH between 3.5 and 11, and it demonstrated thermal stability up to 66 0C with Tm value 58 0C.[26] In this study, L-glutaminase was stable at a broad range of PH between 5 and 10, and it demonstrated thermal stability up to 61 0C with Tm value 57.5 0C. According to Fifi et al, 2015.'s work, L-glutaminase had the highest catalytic activity and affinity for L-glutamine (Km 0.129 mM, V max 2.02 U/mg /min), followed by L-asparagine and L-aspartic acid;[27] but in present investigation, it only displayed these characteristics for L-glutamine as a substrate.

Conclusion

This was a promising screening experimental investigation because Aspergillus Niger Strain ATCC 1015 from various soil conditions in Egypt that were cultivated on an MGA selective medium produced L-glutaminase, which has excellent kinetic properties against auxotrophic cancers like hepatocellular carcinoma and acute lymphocytic leukemia. This enzyme showed a more potent anticancer effect when compared to bacterial L-glutaminases produced by Escherichia coli or Bacillus cereus. Compared to bacterial L-glutaminase, it elicited less hypersensitive reactions. It would be wise to do further research to make fungal L-glutaminase a more effective anticancer agent against a wide range of neoplasms.

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