

Biosynthesis of L-Glutaminase by *Streptomyces Variabilis* ASU319 Isolated from Rhizosphere of *Triticum Vulgaris*

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Abstract Out of 210 actinomycetes isolates belonged to the genus *Streptomyces* recovered from rhizospheric soil of *Triticum vulgare*, *Zea mays* and *Vicia faba* cultivated in Assuit and New Valley Governorates of Egypt, ten isolates were capable of producing high amounts of L-glutaminase enzyme. The most potent L-glutaminase producer isolate was *Streptomyces* sp. ASU319 recovered from rhizosphere of *Triticum vulgare*. The higher producer actinomycete isolate was identified by sequencing of 16S rRNA as *Streptomyces variabilis* ASU319 and was deposited in the GenBank nucleotide sequence database under accession number KC145278. Factors affecting L-glutaminase production by the *Streptomyces variabilis* ASU319(KC145278) were examined and the results revealed that the maximum L-glutaminase value was obtained when the isolate cultivated in the production broth medium supplemented by glutamine 8 mg/ml, adjusted at pH 4 and incubated at 35 °C. These results suggest that L-glutaminase-producing *Streptomyces variabilis* ASU319 could be used as a plant growth promoting rhizobacteria by increasing the ammonia content in the rhizosphere soil across degradation of the agricultural wastes. Also this isolate can be used in both pharmaceutical and food industrial application for L-glutaminase production on commercial scale.

Keywords *Streptomyces variabilis*, *Triticum vulgare*, 16S rRNA, L-glutaminase

1. Introduction

Microbial sources like actinomycetes are well recognized to produce a variety of chemical structures, several of which are most valuable pharmaceuticals, agrochemicals and industrial products like enzymes (1). Actinomycetes are considered to be preferred enzymes sources due to their production of extracellular enzymes. They act as decomposers of complex animal and plant materials

resulting in release of simple substances, especially carbon and nitrogen which is easily utilized by other organisms, thus performing a vital role in life cycle. Many enzymes produced by actinomycetes and have been used as drugs like wise L-glutamine amidohydrolase (E.C. 3.5.1.2), commonly referred to as L-glutaminase, has received much attention with respect to its therapeutic and industrial applications. L-glutaminase is used as a flavor enhancer by increasing glutamic acid content in food through hydrolysis of L-glutamine to L-glutamic acid and ammonia. It also used in enzyme therapy for cancer especially for acute lymphocytic leukemia. Another important application of L-glutaminase is in biosensors to monitor the L-glutamine levels in body fluids. This technique is more applicable than previously used methods and characterized by its high specificity compared with cell based sensors in addition to its fast response. This has led to intensive use of glutaminase in clinical purposes especially that is derived from mammalian tissues (2). In 1993, *Pseudomonas* sp., recombinant glutaminase was patented for its activity against cancer and HIV virus therapy (3). L-glutaminase synthesis was well reported from various terrestrial microorganisms such as *Escherichia coli*, *Pseudomonas species*, *Acinetobacter species*, *Bacillus species*, *Hansenula*, *Cryptococcus*, *Candida*, *Aspergillus oryzae* and *Beuveria bassiana* (4). In addition to terrestrial sources, L-glutaminase activity was reported from few marine microorganisms such as *Pseudomonas fluorescens*, *Micrococcus luteus*, *Vibrio cholerae* and *Beuveria bassiana* (5). However, from the available literatures, it is found that information on L-glutaminase production from actinomycetes is still in the stage of infancy (6). Its commercial importance as anticancer and flavor enhancing agent demands not only the search for better yielding viable strains, but also economically viable bioprocesses for its large scale production (7). Different types of organisms were reported to produce glutaminase enzyme. However, the selection of the right organism is very critical to obtain high yield of the required enzyme (8). The purpose of this work was to isolate and characterize L-glutaminase-producing *Streptomyces* and optimized the

culture conditions to increase the production of this enzyme.

2. Materials and Methods

2.1. Isolation of Actinomycetes

Starch casein agar and glycerol asparagine agar media supplemented with griseoflavin (50 µg/ml) and streptomycin (50 µg/ml) were used for isolation of 210 actinomycetes isolates from 100 rhizospheric soil samples were collected from three crop plants (*Triticum vulgare*, *Zea mays* and *Vicia faba*) cultivated in different locations of Assiut and New Valley Governorates, Egypt. The *Streptomyces* isolates were recovered by serially dilution method and the plates were rotated by hand for good dispersion of soil suspension and incubated at 28°C for 7-21 days. Individual colonies were picked up, and sub cultured on fresh agar medium. The actinomycetes cultures were purified and transferred to agar slants of the same medium; incubated at 28°C for 5-7 days to achieve good sporulation, and then stored at 4°C in refrigerator.

2.2. Detection Of L-Glutaminase Producing Actinomycetes

Minimal glutamine agar medium was prepared and used for detection of L-glutaminase producing actinomycetes. Components of MGA (g/L) include 0.5 KCl; 0.5 MgSO₄; 1.0 KH₂PO₄; 0.1 FeSO₄; 0.1 ZnSO₄; 0.5 NaCl; 10.0 L-glutamine and supplemented with 0.012 g phenol red and pH adjusted at 6.8, in which L-glutamine act as the sole carbon and nitrogen source and phenol red as pH indicator. The colour change of the medium from yellow to pink is an indication of the extra cellular L-glutaminase production by the colony. This colour change is due to change in the pH of the medium, as L-glutaminase causes the breakdown of amide bond in L-glutamine and liberates ammonia. All the actinomycetes isolates collected were streaked on minimal glutamine agar medium. After four days of incubation at 30°C, the change of medium colour from yellow to pink around the colony indicating positive response. The secondary screening for the highly producer isolates was done by the same previously method but in liquid medium and the pink colour degree of the supernatant after filtration and centrifugation of the medium at 3000 rpm for 15 min was measured at 540 nm using a visible spectrophotometer.

2.3. Production of L-glutaminase in Submerged Culture

One ml inoculum of the tested actinomycete isolate was inoculated in to 50 ml of production medium which contains (g/L): 1.0 KH₂PO₄; 0.5 MgSO₄; 0.1 CaCl₂; 0.1 NaNO₃; 0.1 tri sodium citrate; 0.5 NaCl; 10.0 L-glutamine and 10.0 glycerol, pH 7. All the flasks were incubated at 28°C on an orbital shaking at 120 rpm for 120 h. The samples withdrawn

were centrifuged at 3000 rpm for 15 min and the clear supernatant was used for enzyme estimation.

2.4. L-Glutaminase Activity

The activity of glutaminase is determined by estimating the amount of ammonia liberated from glutamine. The amount of ammonia was measured by adding 100 µl of the crude enzyme to 200 µl Nessler's reagent and completed to 4 ml by distilled water. Absorbance was measured at 540 nm using a visible spectrophotometer. L-glutaminase activity is expressed in µg ammonia produced per one ml medium. The level of ammonia produced was estimated against the ammonia standard curve using ammonium sulphate.

2.5. Morphological Characterization of *Streptomyces* ASU319

2.5.1. Colony Characterization

The morphology of the strain was studied in tryptone yeast extract agar (ISP-1), oatmeal agar (ISP-3), starch agar with inorganic salts (ISP-4) and glycerol-asparagine agar (ISP-5). The production of the melanin was tested on peptone-yeast extract iron agar (ISP-6) and tyrosine agar (ISP-7). Colony morphology was recorded with respect to colour aerial mycelium, reverse side and diffusible pigment using an Inter-Society Color Council- National Bureau of Standards (ISCC-NBS) color chart (9).

2.5.2. Microscopic Characterization

The actinomycetes were slowly released at the intersection of medium to the cover slip. The plates were inoculated at 28±2°C for 4-8 days. The cover slips were removed from the cultured medium and observed under the light microscope. The photomicroscopy was taken using Nikon microscope. Spore chain and spore surface ornamentation were examined by light and electron microscopy (10).

2.5.3. Gram Staining

The Gram stain was carried out on 3-4 days cultures according to the Hucker method (11).

2.6. Physiological and Biochemical Characteristics

Isomer of diaminopimelic acid was analysed by TLC (12). The physiological and biochemical characteristics were monitored according to standard methods of starch hydrolysis (13), cellulose hydrolysis (14), gelatin hydrolysis (15), casein hydrolysis (16), H₂S production (17), nitrate reduction (18), catalase production (19), milk peptonization and utilization of carbon and nitrogen sources (20).

2.7. Identification of *Streptomyces* ASU319 Using 16S rRNA

2.7.1. DNA isolation

The locally isolated *Streptomyces* ASU319 was grown for 7 days on a starch agar slants at 30°C. Two ml of a spore suspension were inoculated into the starch nitrate broth and incubated for 3 days on a shaker incubator at 200 rpm and 30°C to form a pellet of vegetative cells. The preparation of total genomic DNA was conducted according to the method of Wittenberg (21).

2.7.2. PCR Amplification

The 16S rRNA encoding gene was amplified by the polymerase chain reaction (PCR) from purified genomic DNA using the *Streptomyces* specific PCR primers StrepB, 5'-ACAAGCCCTGGAAACGGGT-3' (forward) and StrepF, 5'-ACGTGTGCAGCCCAAGACA-3' (reverse). The PCR amplification was performed by using Qiagen Proof-Start Tag Polymerase Kit (Qiagen, Hilden, Germany). The following substrates were combined in a total volume of 25 µl including about 50 ng of template DNA, 12.5 µl PCR Master Mix, 5 pmol (0.5 µl) each of forward and reverse primers and the total reaction volume was completed by 11.5 µl of water DNAase free water. This step is performed on the ice. The complete reaction mixture was incubated at automated thermocycler TC-3000 (Applied Bacteriology Laboratory, Botany Department, Assiut University, Assiut). The reaction conditions were: an initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 94 °C for 70 s, annealing at 56 °C for 40 s, and extension at 72 °C for 130 s. A final extension was conducted at 72 °C for 370 s (21). PCR products were analyzed by electrophoresis on 1% (w/v) agarose TBE-gels (Tris-base Boric EDTA-gel) and the gels were visualized and pictured under UV light. PCR products of about (1100 bp) were purified from gel with the QIAquick gel extraction kit (Qiagen, Hilden, Germany).

2.7.3. DNA Sequencing

Purified PCR products were sequenced by cycle sequencing with dideoxy mediated chain-termination (22). Sequencing was done by the Biovision Company, which sent it to the Gene Analysis Unit (Macrogen Inc., Seoul, Korea) Sequencer AB-13730. For sequencing the purified PCR products, the same primers StrepB (forward) and StrepF (reverse) were used. Sequences of the 16S rRNA of isolates were first analyzed using the advanced BLAST search program at the NCBI website: <http://www.ncbi.nlm.nih.gov/BLAST/> in order to assess the degree of DNA similarity. Multiple sequence alignment and molecular phylogeny was evaluated using CLUSTALW program (<http://clustalw.ddbj.nig.ac.jp/top-ehtml>). The phylogenetic tree was displayed using the TREE VIEW program. Phylogenetic tree derived from 16S rRNA gene sequence was generated in comparison to 16S rRNA gene sequences from different standard *Streptomyces* strains obtained from GenBank: *S. variabilis* NRRLB3984, *S. labedae* NBRC15864, *S. griseorubens* NBRC12780, *S. erythrogriseus* LMG19406, *S. griseoincarnatus* LMG19316, *S. griseoflavus* LMG19344, *S. althioticus* KCTC9752, *S. viridodiataticus* IFO13106, *S. longisporuber*

NBRC13488 and *S. paradoxus* NBRC14887.

2.7.4. Nucleotide Sequence Accession Numbers

The nucleotide sequences of the isolate ASU319 was deposited in the GenBank nucleotide sequence database under accession number KC145278.

2.8. Effect of L-glutamine Concentration, Incubation Temperature and pH on L-glutaminase Production by *Streptomyces* ASU319.

The strain *Streptomyces* ASU319, which produced the highest enzyme, was selected for further investigation to identify the optimal conditions for enzyme production. The effect of L-glutamine concentration was studied by cultivating the strain in production medium supplemented with different concentrations of L glutamine (0, 1, 2, 5, 7 and 8 mg/ml). The effect of different temperatures (20, 25, 30, 35, 40 and 45 °C) on enzyme activity was studied by cultivating the strain in the basal medium supplemented with 2mg/ml glutamine and adjusted at pH8. The effect of pH levels (2, 4, 6, 8 and 10) was tested in the basal medium supplemented with 2mg/ml glutamine and incubated at 30°C.

3. Results

3.1. Screening for L-glutaminase Production

A total of 210 actinomycetes isolates were screened for their ability to convert glutamine to ammonia by production of L-glutaminase enzyme. Data presented in Table (1) indicated that a total of 167 (79.52 %) isolates have the ability to produce L-glutaminase but 43 (20.47 %) actinomycetes isolates did not able to produce the enzyme. The producer enzyme isolates were categorized into three groups according to their activity. Among 167 isolates, 132 isolates show low activity, 25 isolates produced moderate activity and the other 10 isolates produced high activity and marked as (+), (++) and (+++), respectively.

Table 1. Primary screening of L-glutaminase biosynthesis by actinomycetes isolates recovered from rhizospheric soil of wheat (*Triticum vulgare*), corn (*Zea mays*) and broad bean (*Vicia faba*)

REMARK*	Level	Number of isolates	Percent	Total Number
-	negative	43	20.47 %	167
+	Low	132	62.85 %	
++	Moderate	25	11.90 %	
+++	High	10	4.76 %	

*The quantitative standard was according to the diameter of pink zone appeared around the actinomycete colony. It marked as +(<5mm), ++(6 – 10mm) and +++(>10mm)

Table 2. Screening of L-glutaminase production by the higher producer actinomycetes isolates

Isolates Code	Location of isolation	Cultivated Plant	O. D. at 540 nm
<i>Streptomyces</i> ASU2	Assiut	Wheat	2.522
<i>Streptomyces</i> ASU37	Assiut-Dairout	Wheat	2.613
<i>Streptomyces</i> ASU58	Assiut-Dairout	Wheat	2.552
<i>Streptomyces</i> ASU147	Assiut-El-Saahell	Corn	3.107
<i>Streptomyces</i> ASU171	Assiut-Manfalout	Wheat	2.832
<i>Streptomyces</i> ASU204	New Valley-Barieess	Corn	3.069
<i>Streptomyces</i> ASU216	New Valley-El-kharga	Corn	2.532
<i>Streptomyces</i> ASU266	New Valley-El-Daakhlai	Corn	2.568
<i>Streptomyces</i> ASU316	New Valley-El-kharga	Wheat	2.582
<i>Streptomyces</i> ASU319	New Valley-El-kharga	Wheat	3.239

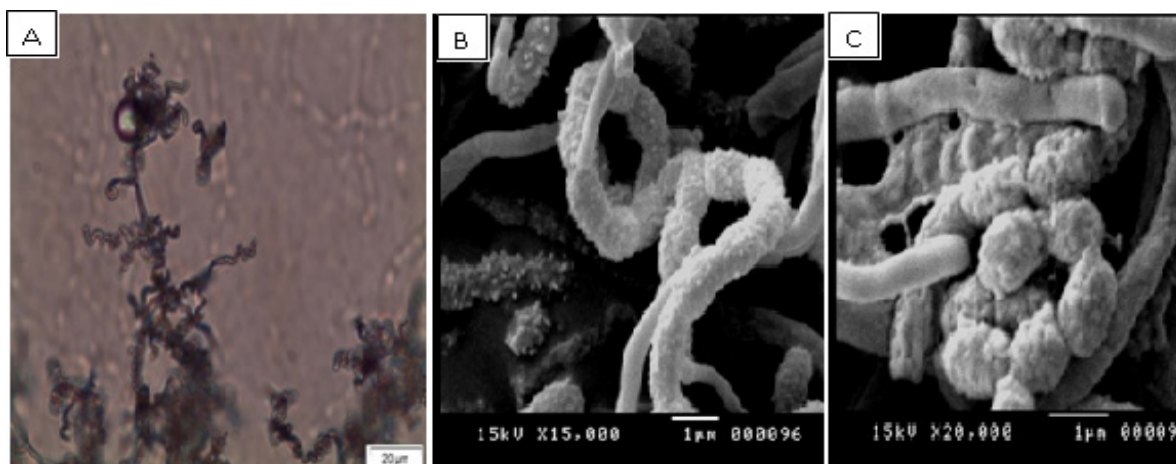
3.2. Secondary Screening of L-Glutaminase Production by the Highly Producer Isolates

Out of 10 most active isolates, five isolates were recovered from Assiut governorate and five from New Valley governorate. It is clearly that the most highest producer isolate *Streptomyces variabilis* ASU319 was isolated from rhizospheric soil sample cultivated by wheat plant in New Valley governorate. This isolate was chosen for further experiments (Table 2).

3.3. Characterization and Identification of the Isolate *Streptomyces* ASU319

3.3.1. Morphological Characteristics

The aerial hyphae of the tested isolate have spore chains of Retinaculum (RA) type with oval spores of spinous surface (Fig. 1). The aerial mass appeared as dark grey, greenish grey, light grey and grey on all the used media, therefore it could be assigned to the grey colour series. The substrate mycelium appeared as dark brown, greenish yellow, reddish brown, pale yellow and pale olive on the tested media. It was evident that this isolate was unable to produce any soluble or melanoid pigments (Table 3).

**Figure 1.** Morphology of the recovered actinomycetes isolate ASU319. A) mycelia and spore chains under light microscope, B&C) scanning electron micrograph showing spore chain and spore surface.**Table 3.** Growth patterns and mycelial colouration of the tested isolate *Streptomyces* ASU319 on different media

Culture Medium	Growth	Aerial mycelium	Substrate mycelium	Soluble pigment
Tryptone malt extract agar (ISP-1)	Abundant	Dark grey	Dark brown	Negative
Yeast malt extract agar (ISP-2)	Fair	Grey	colorless	Negative
Oat-meal agar (ISP-3)	Abundant	Grey	Greenish grey	Negative
Inorganic salts starch agar (ISP-4)	Abundant	Grey	Pale yellow	Negative
Glycerol-Asparagine agar (ISP-5)	Abundant	Light grey	Reddish brown	Negative
Tyrosine agar (ISP-7)	Abundant	Greenish grey	Dark brown	Negative

Table 4. Morphological and biochemical characteristics of *Streptomyces* ASU319

Characteristics	Results
Diaminopimelic acid (DAP)	LL-DAP
Gram's staining	+
Cell morphology	mycelial
Spore chain	retinaculum (RA)
Spore shape	oval
Spore surface	spinous
Starch hydrolysis	++
Cellulose decomposition	++
Gelatin liquefaction	+
H ₂ S production	-
Nitrate reduction	-
Catalase production	+
Milk peptonization	-
Melanin production	-
Utilization of Carbon	
D-Glucose	+
D-Fructose	+
D-Galactose	+
D-Xylose	+
L-Arabinose	-
D-Mannitol	±
Lactose	-
Maltose	±
Meso-Inositol	±
Rhamnose	-
Sucrose	+
Utilization of Nitrogen	
L-Glutamine	+++
L-Tryptophane	++
L-Tyrosine	+
L-Glycine	+++
L-Asparagine	+++
L-Cystine	+
L-phenylamine	++
L-Histidine	+++
L-Hydroxyproline	++
L-Lysine	+++
B-Alanine	+

+ good, ++ very good, +++ excellent, - negative

3.3.2. Physiological Properties

The results presented in Table (4) indicated that the tested isolate was able to hydrolysis starch, decompose cellulose, liquefy gelatin, and produce catalase, but nitrate reduction, milk peptonization and H₂S production was negative by this isolate. The tested isolate utilized D-glucose, D-fructose, D-galactose, D-xylose, and sucrose as carbon source for its growth, but it was unable to grow on L-arabinose, L-rhamnose and lactose. Doubtful growth occurred in case of D-mannitol, maltose and meso-inositol. On the other hand, the tested isolate was able to grow on L-glutamine, L-tryptophane, L-tyrosine, L-glycine, L-asparagine, L-cystine, L-phenyl alanine, L-histidine, L-hydroxyproline, and L-lysine as nitrogen source for its growth. Based on the taxonomic characteristics described above, the isolate ASU319 was assigned to the genus *Streptomyces*.

3.3.3. Identification and Characterization by 16S rRNA Gene Sequences

The phenotypic-based identification was confirmed by phylogenetic analysis. Comparison between 16S rRNA gene sequence of the tested isolate ASU319 and 16S rRNA gene sequences on GenBank database as determined by using Blast search analysis was done. Sequencing of 16S rRNA genes of the tested isolate had 16S rRNA gene with 99 % nucleotides identity to that of *Streptomyces variabilis* NRRLB3984 available in Genbank database (Fig. 2). The phylogenetic tree was inferred from 16S rRNA sequence data by the neighbor-joining method. The tested isolate was identified as *Streptomyces variabilis* belongs to family Streptomycetaceae, order Actinomycetales, class Schizomycetes.

3.4. Optimization of L-Glutaminase Production by *Streptomyces Variabilis* ASU319

3.4.1. Effect of Hydrogen Ion Concentration on L-glutaminase Production

The effect of different pH values (2, 4, 6, 8 and 10) of the culture medium on the biomass and enzyme production by *Streptomyces variabilis* ASU319 was investigated. The result revealed that both mycelial growth and enzyme production by *Streptomyces variabilis* ASU319 was increased gradually with the increase of pH values reaching maximum at pH 4 for ammonia formation (Fig. 3). However the optimum pH for bacterial growth was 8.

3.4.2. Effect of Incubation Temperature

Testing the temperature-dependence on mycelia growth and ammonia formation by *Streptomyces variabilis* ASU319 revealed that 35 °C was the optimum (Fig. 4).

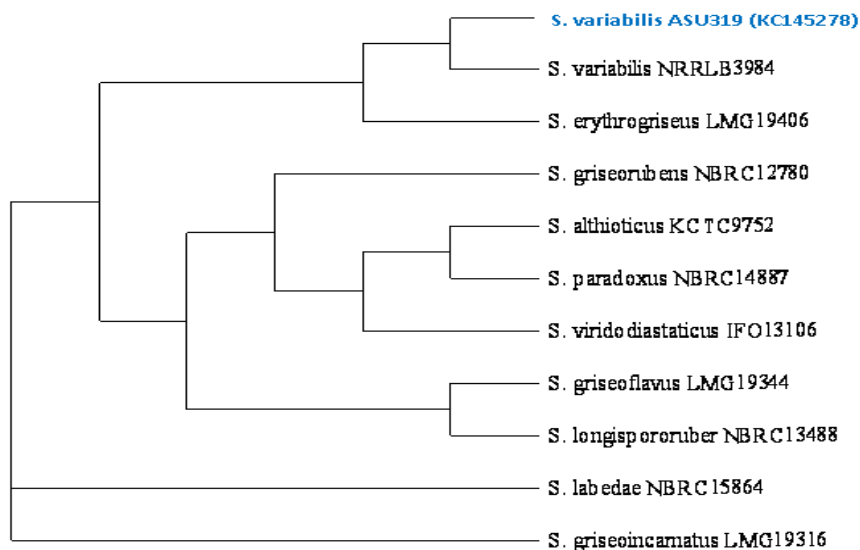


Figure 2. The neighbor-joining tree based on 16S rRNA gene sequences showing the positions of the isolate *Streptomyces* ASU319 and related strains

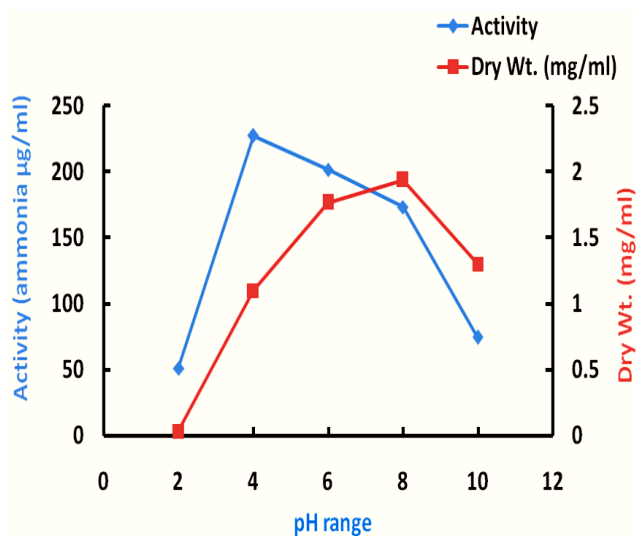


Figure 3. Effect of different pH values on the dry weight (mg/ml) and L-glutaminase activity (µg/ml) by *Streptomyces variabilis* ASU319

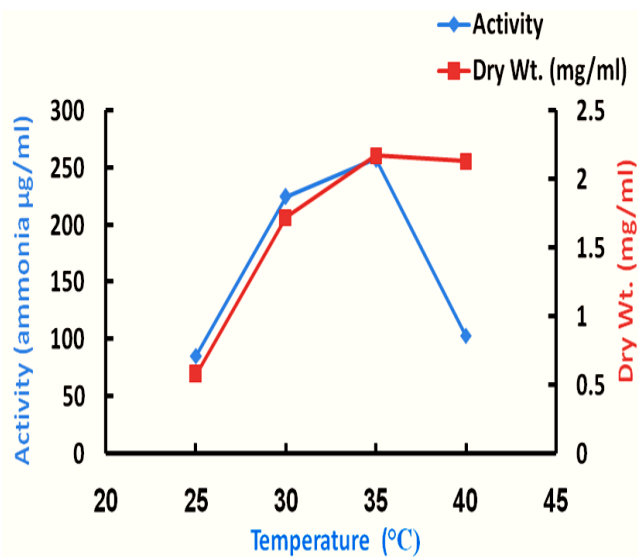


Figure 4. Effect of temperature on the dry weight (mg/ml) and L-glutaminase activity (µg/ml) by *Streptomyces variabilis* ASU319

3.4.3. Effect of Glutamine Concentration on L-glutaminase Production

The effect of various concentrations of glutamine on mycelia growth and L-glutaminase production by *Streptomyces variabilis* ASU319 were investigated and illustrated in Fig. (5). The results indicate that the optimum concentration was 8 mg glutamine per ml of basal medium.

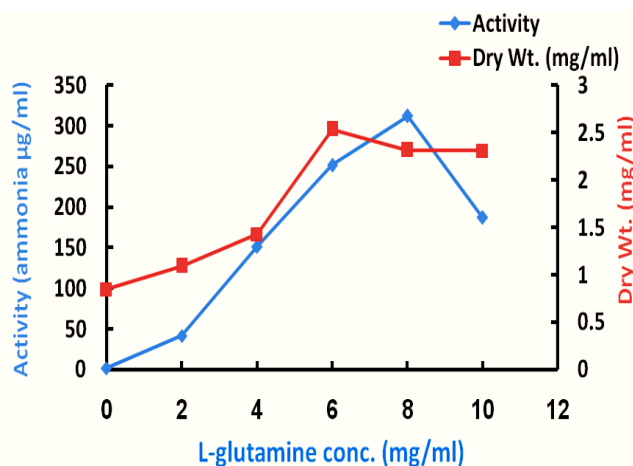


Figure 5. Effect of L-glutamine concentrations on the dry weight (mg/ml) and L-glutaminase activity (µg/ml) by *Streptomyces variabilis* ASU319

4. Discussion

L-glutamate is one of the substances present in the cultivated soil resulted from the degradation of plants debris, and the bioconversion of this substance by action of enzymes produced from microorganisms especially bacteria will lead to the production of ammonia. This community is supported nutritionally by a high input of organic materials derived from the plant roots and root exudates that are necessary for microbial growth (23). However the composition and quantity of root exudates varies depending on the plant species (24) and the physical environment such as humidity and temperature (25). The absorption of the released ammonia by plant roots will increase of the nitrogen content in the plants. L-glutaminase is the enzyme responsible for conversion of L-glutamine to L-glutamic acid and ammonia, so this enzyme is important in agriculture and potentially useful for stimulating plant growth and increasing crop yields. Another important application of L-glutaminase is in biosensors for monitoring the glutamine levels in mammalian and hybridoma cells. But, from the available literature, it is found that information on L-glutaminase production from actinomycetes is still in the stage of infancy (6, 26).

The search for novel L-glutaminase producing actinomycetes and selection of highly producer isolates for commercial and industrial applications are of great interest of the scientists. A total of 210 actinomycetes isolates collected in this study were tested for their ability to produce L-glutaminase in a broth medium supplemented by

L-glutamine as a sole carbon and nitrogen source. L-glutaminase synthesis was well reported from various terrestrial microorganisms such as *Escherichia coli*, *Pseudomonas* species, *Actinobacter* species, *Bacillus* species, *Hansenula*, *Cryptococcus*, *Candida*, *Aspergillus oryzae* and *Beuveria bassiana* (3, 4). In addition to terrestrial sources, L-glutaminase activity was reported from few marine microorganisms such as *Pseudomonas flourescens*, *Micrococcus luteus*, *Vibrio cholerae*, *Vibrio cholerae*, *Vibrio costicola*, *Beuveria bassiana* and *Streptomyces* spp. (5, 26-31).

Out of 210 actinomycetes isolates recovered in this study, 167 (79.52%) isolates showed positive result in rapid plate assay. In most of the microbial screening programs for enzyme production, the microorganisms are the first isolated from certain environments by routine isolation procedures and then screened for enzymatic activity by methods like zone of color change or zone of clearance on agar medium supplemented with suitable substrate (32). Similar screenings of L-glutaminase production by rapid plate assay were reported in actinomycetes strains (26) and in bacterial cultures (27). Also, similar screening of L-asparaginase by the same method was reported in bacterial strains such as *Bacillus circulans* (33), *Streptomyces* sp. PDK7 (34), *Streptomyces* sp. (35).

Apparently, the most highest producer isolate *Streptomyces variabilis* ASU319 which of dark grey color and isolated from rhizosphere soil sample cultivated by wheat plant in New Valley governorate, was selected for the optimization of enzyme production. In general, enzymes are measured by estimation of enzyme activity by qualitative estimation (36, 37).

Balagurunathan *et al.* (26) recorded that in his study it was the first report on L-glutaminase producing *Streptomyces olivochromogenes* in general and marine strains in particular. Also, from the available literatures, we can conclude that it is the first time for the production of L-glutaminase by *Streptomyces variabilis*. The activity of glutaminase by the tested isolate was determined by estimating the amount of ammonia liberated from glutamine. The isolate *Streptomyces variabilis* ASU319 exhibited maximum glutaminase activity of 180 µg/ml among all the tested isolates before optimization of the enzyme production by this isolate. This value is higher than the result recorded by (38), they recorded that the maximum value of ammonia in their study was 12.61 µg/ml produced by *Streptomyces avermitilis*.

Concerning the effect of pH, the results of current research revealed that the optimum pH for L-glutaminase production and mycelia growth by the tested isolate *Streptomyces variabilis* ASU319 was at 4 and 8, respectively. The pH of the medium plays an important role by inducing morphological changes in microbes and enzyme production.

The maximum L-glutaminase production and mycelia growth were achieved at 35 °C. The incubation temperature is characteristic for the organism and vary for each species (39). Balagurunathan *et al.* (26) recorded that the maximal activity of the enzyme by *Streptomyces olivochromogenes*

was obtained at pH 7 and temperature at 30°C. Optimum temperature for L-glutaminase production was found to be in range between 25 and 35°C for the mesophilic microbes (40). Any temperature beyond the optimum range is found to have some adverse on the metabolic activities of the microorganisms and it is also reported by various scientists that the metabolic activities of the microbes become slow at lower or higher temperatures (41).

Concerning the effect of glutamine concentrations, it was found that as the concentration increased the production of L-glutaminase increased till it reached the maximum activity at 8 g/l. Amino acids were common growth factor required for the synthesis of enzyme as major nitrogen source (42). Since Lglutamine is the substrate of L-Glutaminase, the addition to fermentation medium might stimulate enzyme production. It also serves as source of energy and carbon (43).

The result obtained in the present study indicated that *Streptomyces variabilis* ASU319 could be a potential actinomycete strain for L-glutaminase production. Optimization of cultural conditions for attaining the high yields of L-glutaminase is very useful for biotechnological application. This isolate *Streptomyces variabilis* ASU319 exhibited glutaminase production of 180 µg/ml and this value is higher than the other strains. The optimization of growth conditions favor the future large commercial scale production as it maximize the value to more than 300 µg/ml of glutaminase.

5. Conclusion

The present study indicated scope for the use of *Streptomyces variabilis* ASU319 as an ideal actinomycete isolate for the production of extracellular L-glutaminase with maximum activity in the basal medium of 8 g/l glutamine, adjusted at pH 4 and incubated at 35°C that can be used as plant growth promoting rhizobacteria for enriching the soil fertility across the degradation of the agricultural wastes and increase the nitrogen content in the soil. Also this isolate can be used in both pharmaceutical and food industrial application for L-glutaminase production on commercial scale.

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