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Screening Of L-Glutaminase Produced By Actinomycetes Isolated From Different Soils In Egypt

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Abstract: Screening of L-glutaminase producing actinomycete strains from some soil samples collected from different places along Egypt was done. Among the one hundred and two isolates, only six showed L-glutaminase activity from which two strains identified as *Streptomyces avermitilis* (GLU1) and *Streptomyces labedae* (GLU2) having the highest activity reaching 8.41 and 12.23 (U/ml) respectively. Effect of physicochemical factors namely, incubation period, temperature, initial pH, inoculum size, NaCl concentration, supplementary carbon source and nitrogen sources and metal ions on the production of L-glutaminase produced from the two identified streptomycetes were studied and accordingly optimum conditions were determined.

The L-glutaminase produced from the two strains was purified by ammonium sulphate precipitation and ion exchange chromatography. The molecular weight of enzyme was 50 kDa. The purified enzyme exhibited optimal activity at 7-8 pH and 30^{o}C. The purified enzyme was more stable at 4% NaCl and its activity increased when NaCl and $MgSO_4$ were added as metal salts and showing high stability in the presence of different oxidizing agents.

Key Words: L-glutaminase, screening, *Streptomyces avermitilis*, *Streptomyces labedae*, selective isolation, purification.

Introduction

Actinomycetes are aerobic gram positive filamentous bacteria with high G+C content which form asexual spores and which are widely distributed in both terrestrial and aquatic habitats. Actinomycetes are well recognized to produce a variety of chemical structures, which are most valuable for pharmaceuticals, agrochemicals and industrial products like enzymes [1]. The value of actinomycetes to society in terms of providing useful drugs especially antibiotics and anticancer agent and to the pharmaceutical industry for revenue generating discovery platform, is indisputable [2].

L-glutaminase (L-glutamine amido hydrolase EC 3.5.1.2) enzyme has attracted significant attention owing to its potential

application in medicine as an anticancer agent and could be of significance in enzyme therapy of acute lymphocytic leukemia [3]. The enzyme L-glutaminase can be derived from plant source, but microbial enzymes are significantly meeting the industrial demands. Bacteria and fungi are the potential candidates for the production of L-glutaminase enzyme [4 and 5]. L-glutaminase production from terrestrial actinomycetes is still scanty [6]. In recent years, L-glutaminase has attracted much attention in both pharmaceutical and food industrial applications. In food industry, 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 for monitoring the glutamine levels in mammalian and hybridoma cells.

The present investigation designed for selective isolation of L-glutaminase producing actinomycetes from some soil samples and characterization of the most active species. In addition, optimization of media composition for maximizing the L-glutaminase production was examined as well as purification and characterization of produced enzyme.

Experimental

Selective isolation of L-glutaminase producing actinomycetes

Soil samples collected from different cultivated soil in Egypt including El Fayoum and El Sharkia Governorates as well as El Kanater were dried at room temperature for a week. Ten grams of soil samples were transferred to sterile Petri plates and kept at 55°C for 10 minutes. The pretreated samples were used for the isolation of actinomycetes [7].

Minimal glutamine agar (MGA) medium was prepared and used for the selective isolation of L-glutaminase producing actinomycetes. Components of MGA (g/L) include 0.5 KCl, 0.5 $MgSO_4$, 1.0 KH_2PO_4, 0.1 $FeSO_4$, 0.1 $ZnSO_4$, 25 NaCl and 10 L-glutamine in which L-glutamine act as carbon and nitrogen source. The MGA medium was supplemented with 0.012 (g/L) of 2.5 % of phenol red as pH indicator. After sterilization, the MGA medium was supplemented with two filter sterilized antibiotics viz., cycloheximide (20 µg/ml) and nalidixic acid (100 µg/ml), in order to retard the growth of fungi and gram negative bacterial populations respectively [8]. All the plates were incubated at 28°C for 7 days.

L-glutaminase activity was identified by formation of a pink zone around colonies. Colonies formed pink zone were picked and maintained on the MGA slants at 4°C [9].

Molecular identification of actinomycetes isolates

Extraction of DNA was performed adopting the method given by [10]. Then, 1 µl of extracted DNA was used as template for PCR reaction. PCR was achieved using Premix Taq (Ex *Taq* Version, Takara, Japan) according to instruction manual. A pair of flanking sequences was used for primer binding sites to partially amplify target 16S rRNA genes from the actinomycetes isolates 16S-1F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16S-517R (5'- ATTACCGCGGCTGCTGG -3'). PCR was attained in genius model FGENO2TD thermal cycler (Techne, England). The PCR conditions were adjusted to 5 min for initial denaturation at 94°C and then 35 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C, and finally 10 min at 72°C. PCR was terminated after the program was completed and amplified genes were electrophoresed out on a 1% agarose gel with size marker [11].

Direct sequencing of PCR product of 16S rDNA was carried out using model 3100 genetic sequence analyzer. Then Obtained sequences were analyzed by Genetyx-Win MFC application software version 4.0. Nucleotide sequences were identified using BLAST search program, National Center for Biotechnology Information [12]. Sequence alignments were performed by Clustal W1.83 XP software [13]and phylogenetic tree was constructed using neighbor-joining method [14] using MEGA4 software.

L-glutaminase production from actinomycetes by submerged fermentation

For the preparation of actinomycetes spores, all the actinomycetes strains were inoculated on sporulation agar medium and incubated for 7 days at 28ºC. After incubation, actinomycetes spores were scrapped and inoculated into 50 ml of mineral salt glutamine (MSG) medium (pH 7) in 250 ml conical flask. Components of MSG medium include (g/L) 1.0 KH_2PO_4; 0.5 $MgSO_4$; 0.1 $CaCl_2$; 0.1 $NaNO_3$; 0.1 $Na_3\ C_6\ H_5\ O_7$; 25 NaCl; 10 glucose. All the flasks were incubated at 28ºC for 72 hours in a rotary shaker at 120 rpm. Each 100 ml of MSG medium with phenol red (0.012 %) at pH 7 was prepared in 500 ml Erlenmeyer flask and used for the production

of L- Glutaminase enzyme. After sterilization by autoclaving, 5% of actinomycete inoculums was transferred into MSG production medium and incubated at 28ºC in rotary shaker for 120 hours. The above procedure was applied for all the actinomycetes isolates. After incubation, fermentation medium was removed from shaker a centrifuged by using cooling centrifuge at 10,0 rpm for 30 minutes at 4ºC. The clear supernata was collected in screw cap tube and stored at 4ºC to use as a crude enzyme [5].

For L-glutaminase assay, minimal glutamine agar medium was prepared and a hole with 5 mm diameter was made at the centre of the agar medium. About 50 µl of crude enzyme was added into the hole and plates were incubated at 37°C for 24 hours. One uninoculated plate was kept as control [15]. Actinomycetes strains with its crude L-glutaminase enzyme showed maximum zone of colour change (from yellow to pink) on minimal glutamine agar medium, was selected as potential strain for further studies. Crude L-glutaminase enzyme was produced in large quantities from potential actinomycetes strains by submerged fermentation method described earlier.

Determination of L-glutaminase activity and enzym protein

L-glutaminase activity was determined using L-Glutamine as substrate and the released ammonia product during the catalysis was measured by using Nesseler's reagent [16]. One unit of enzyme activity was defined as the amount of enzyme that liberates 1 µ Mol of ammonia under optimal assay conditions. Protein concentration was determined using bovine serum albumin as the standard [17].

Effect of various physicochemical factors on L-glutaminase production:

Factors influencing the production of L-glutaminase enzyme were optimized by a single factor of varying the parameters one at a time. Experiments were conducted in Erlenmeyer flasks (250 ml) containing MSG broth. After sterilization of the broth, the strains were inoculated (5ml) and incubated for 5 days (except for incubation period experiment) separately as described. Fermentation was carried out in triplicate at different temperatures (20, 28, 37, 40 and 45°C), pH (3-11), carbon sources (1% of glucose, fructose, maltose, lactose, mannitol, galactose, sucrose and starch), nitrogen sources (0.08% of sodium nitrate, peptone, yeast extract, urea, malt extract, casein, ammonium nitrate and ammonium sulfate), sodium chloride concentrations (0, 1, 2, 2.5, 3, 3.5 and 4%) and incubation periods (1-9 days). Different inoculum size ranging from 2 to 10 ml /100 ml medium was tested. Optimum condition identified for one parameter was used for optimizing the other parameters one by one.

Enzyme purification

A- Ammonium sulfate precipitation

Solid ammonium sulfate was slowly added to the crude enzyme filtrate with gentle stirring to bring 40% saturation (fraction I). The mixture was allowed to stand overnight at 4ºC. It was centrifuged at 10,000 rpm at 4°C for 20 minutes to remove the precipitate while the resulting supernatant was subjected to the addition of ammonium sulfate until reached to the concentration 50% saturation (fraction II), then it was allowed to the same previous conditions. The resulting supernatant was further subjected to ammonium sulfate precipitation to bring 80% saturation (Fraction III) in a sequential manner as previously described. The enzyme precipitate obtained from each saturation was dissolved in a minimal volume of 0.01M phosphate buffer (pH 8) and dialyzed against 0.01M phosphate buffer (pH 8) for 48-72 hours at 4°C and the buffer were changed occasionally [18].

B- Ion-exchange chromatography

The L-glutaminase solution from ammonium sulfate precipitation (80%) of *Streptomyces avermitilis* was loaded onto the DEAE-cellulose chromatography column pre-equilibrated with 0.02 M Tris HCl buffer (pH 8.0). The column was washed with four to five bed volumes of 0.02M Tris HCl buffer (pH 8.0). The bound protein was eluted with a discontinuous gradient of NaCl (from 0.1 to 0.25 M) prepared in the same buffer. The flow rate was maintained at 0.5 ml/min. with a fraction volume of 5 ml. The fractions eluted at each NaCl concentration were separately pooled and tested for protein and L-glutaminase activity determinations as mentioned before. The active fractions were used for testing the purity by polyacrylamide gel electrophoresis [19].

The chromatographic fractions were electrophoretically analyzed on SDS-PAGE. Bio-Rad vertical slab gel with size of 0.75 mm x 14cm x 14cm apparatus was used.

Some properties of the partial purified L-glutaminase

pH stability

One hundred µl of partial purified enzyme was incubated with different pH values of Tris-HCl

buffer (0.1M) ranging from 3 to 11 at 37ºC for one hour. The residual activity was measured [20].

Thermostability

For thermostability, 100 µl of partial purified enzyme was incubated at 20, 30, 40, 50 and 60ºC for one hour before the L-glutaminase assay [21].

Effect of different NaCl concentrations

The effect of different NaCl concentrations on L-glutaminase activity was examined by incubating 100 µl of partial purified enzyme with 100 µl of each concentration (0, 1, 2, 3, 4, 5, 10, 15 and 20%) for one hour and the activity was then measured with standard enzyme assay [22].

Effect of different metal salts

The effects of various metal salts ($MgSO_4$, $CuSO_4$, $ZnSO_4$, EDTA and NaCl at 1 mM) on L-glutaminase activity were examined by incubating 100 µl of partial purified enzyme with 100 µl of each metal ion for one hour, and the residual activity was then measured with standard enzyme assay [23].

Stability in oxidizing agents

One hundred µl of the two oxidizing agents, hydrogen peroxide and sodium hypochlorite at 1% (v/v) were incubated with the same volume of partially purified enzyme for one hour, and the residual activity was then measured with standard enzyme assay [24].

Results And Discussion

Selective isolation of L-glutaminase producing actinomycetes

Out of 102 actinomycete isolates, only 6 exhibited L-glutaminase activities which designated as GLU1, GLU2, GLU3, GLU4, GLU5 and GLU6. Based on the cultural and morphological appearance all strains were suspected as streptomycetes. Cultural characteristics of all the six isolates were given in Table (1). The isolates GLU1 and GLU2 exhibited the two largest zone size when compared with the others (40 and 38 millimeter respectively). The two isolates were subjected to species level identification.

Identification of the two active actinomycete isolates by sequence based 16S rDNA molecular typing

Around 500 bp of extracted 16S rDNA genes was amplified by PCR and the products were sequenced. The obtained sequences for the streptomycete isolates were cross-matched with standard ones using Blast search program (NCBI). A phylogenetic tree was constructed based on 16S rDNA sequences obtained from both strains and comparison with analogues sequences from Gene Bank (Figure 1). Strain GLU1 shared 99% sequence similarity to *Streptomyces avermitilis*. Similarly, a glutamine hydrolyzing streptomycete strain was isolated and identified as *Streptomyces avermitilis* by Omura *et al.* [25]. Strain GLU2 shared 98% sequence similarity to *Streptomyces labedae* [26].

Effect of various physicochemical factors on L-glutaminase production

The various physicochemical factors which influence L-glutaminase production by both *Streptomyces* species were optimized. The obtained results revealed that the L-glutaminase produced by *Streptomyces avermitilis* increased as the inoculum size increased until it reached its maximum (12.61 U/ml) at 5 ml (Table 2). The productivity remained unchanged with inoculums size 6 and 7 ml, after that it decreased as the inoculum size increased. In case of *Streptomyces labedae* the highest L-glutaminase productivity (12.23 U/ml) was recorded at inoculum size 7 ml and it was decreased with any increase in inoculum size (Table 2). It could be concluded that high inoculum concentration allows a rapid L-glutaminase production because of the reduction of the lag phase yielding a maximum L-glutaminase productivity [27].

Table 1: Cultural characteristics of L-glutaminase producing streptomycetes

Strain	Source of isolation	Aerial mycelium	Substrate mycelium	Mycelial colour	Reverse side pigment	Soluble pigment	L-glutaminase activity (zone size in mm)
GLU1	El Sharkia (Corn)	+	+	White	+	-	40
GLU2	Fayoum (Sorghum)	+	+	White	-	-	38
GLU3	Fayoum (Sorghum)	+	+	Gray	+	+	30
GLU4	Kanater (Taro)	+	+	Black	+	+	26
GLU5	Fayoum (Corn)	+	+	Gray	+	-	31
GLU6	Kanater (Corn)	+	+	Gray	-	-	19

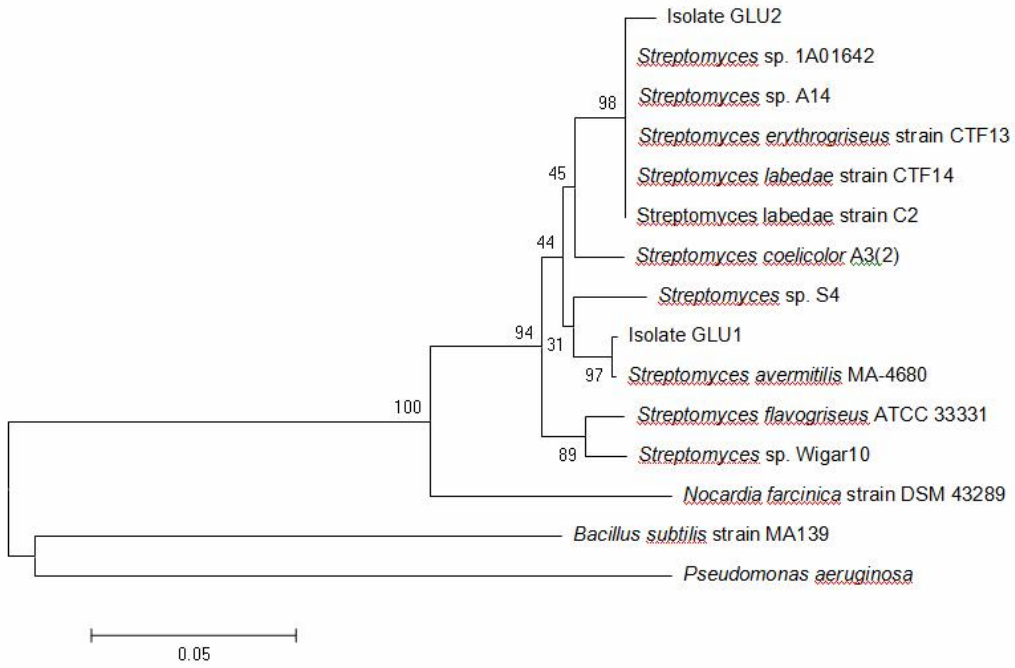

Fig. (1) Phylogenetically ordered tree of the selected strains clustered together with their closest relatives

Maximum L-glutaminase production by *Streptomyces avermitilis* (8.41 U/ml) was obtained after 5 days of incubation and remained at its maximum values till the 7th day then the enzyme productivity was decreased (Table 2). In case of *Streptomyces labedae* the maximum L-glutaminase productivity (13.39 U/ml) was obtained after 5 days and gradually decreased thereafter (Table 2). Incubation period was the most important physical variable in the fermentation process. The incubation period varies with enzyme production. Krishnakumar *et al*. [28] mentioned that the highest production of L-glutaminase by marine alkalophilic *Streptomyces* sp. SBU1 which was isolated from Cape Comorin Coast, India was after 4 days of incubation. The L-glutaminase production increased during the logarithmic phase up to 5 days followed by decline on extended incubation [29].

Incubation at 28^{o}C enhance the L-glutaminase production (12.48 U/ml) and (12.10 U/ml) in case of *Streptomyces avermitilis* and *Streptomyces labedae* respectively compared to the other temperatures (Table 2). The incubation temperature is characteristic for the organism and vary for each species [30]. Optimum temperature for L-glutaminase production was found to be in range between 25 and 35^{o}C for the mesophylic microbes [31]. 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 [32].

Concerning the effect of pH, maximum L-glutaminase production by *Streptomyces avermitilis* (13.47 U/ml) was recorded at pH 8 (Table 2). The pH 7 was the optimum for maximum L-glutaminase productivity (9.39 U/ml) by *Streptomyces labedae*. No L-glutaminase production was detected at pH 3, 4 and 11 for both species. Balagurunathan *et al.* [5] stated that the optimum pH for L-glutaminase production by *Streptomyces olivochromogenes* was 7. The pH of the medium plays an important role by inducing morphological changes in microbes and enzyme production. Neutral to alkaline pH is required for maximal enzyme production by actinomycetes [33] which is in accordance with the present study.

Extracellular enzyme production depends greatly on the composion of the medium. Among the carbon sources tested, glucose exhibited maximum L-glutaminase production (7.12 and 8.15 U/ml) by *Streptomyces avermitilis* and *Streptomyces labedae* respectively. The obtained results were confirmed by that reported by Sivakumar *et al.* [6] who observed that L-glutaminase produced by *Streptomyces rimosus* that was isolated from estuarine fish, *Chanos chanos* showed the highest activity when glucose used as carbon source. Such enhanced production of L-glutaminase by supplementation of carbon sources could be attributed to the positive influence on biosynthesis [34].

Table 2: Effects of different parameters on L-glutaminase activity from *Streptomyces avermitilis* and *Streptomyces labedae*

Parameter	*Streptomyces avermitilis*	*Streptomyces labedae*
	Enzyme activity	
Inoculum size		
2	5.83	3.04
3	6.22	5.75
4	8.15	5.75
5	12.61	11.58
6	12.61	11.84
7	12.61	12.23
8	9.01	7.03
9	8.24	4.03
10	3.86	2.10
Incubation period (day)		
1	0.0	0.0
2	1.28	2.14
3	3.0	5.83
4	6.35	9.39
5	8.41	13.39
6	8.41	11.37
7	8.41	8.24
8	5.75	8.24
9	4.12	7.46
Incubation Temperature (ºC)		
20	5.92	3.69
28	12.48	12.10
37	11.58	10.60
40	9.52	8.24
45	4.03	2.16
Initial pH		
3	0.0	0.0
4	0.0	0.0
5	2.70	1.24
6	10.60	8.15
7	12.48	9.39
8	13.47	7.21
9	9.01	5.57
10	5.49	2.06
11	0.0	0.0
Carbon sources		
Glucose	7.12	8.15
Fructose	6.99	5.83
Maltose	3.30	3.13
Lactose	0.98	1.97
Mannitol	4.09	5.06
Galactose	6.00	5.06
sucrose	1.71	2.66
Starch	0.68	0.85
Nitrogen sources		
Peptone	9.52	12.53
Malt extract	4.37	12.53
Yeast extract	4.03	7.59
Casein	1.35	2.70
Urea	0.55	0.94
Sodium nitrate	10.30	10.30
Ammonium nitrate	2.10	5.06
Ammonium sulphate	3.04	3.34
NaCl concentration (%)		
0	6.0	4.3
1	8.06	5.23
2	10.55	8.24
2.5	11.45	9.65
3	12.36	7.46
3.5	7.21	6.09
4	4.1	3.0

Nitrogen sources could be considered as important limiting factor in the production enzymes. In case of different nitrogen sources used, peptone and sodium nitrate gave maximum L-glutaminase production of 9.52 U/ml and 10.30 U/ml respectively by *Streptomyces avermitilis* (Table 2). Peptone and malt extract were found to be the most suitable organic nitrogen sources for L-glutaminase production from *Streptomyces labedae* with maximum productivity of 12.53 U/ml. Moreover, sodium nitrate is considered to be the best inorganic nitrogen source for L-glutaminase production by *Streptomyces labedae* with productivity reached 10.30 U/ml (Table 2). The enhanced production of L-glutaminase might be due to rapid growth accomplished by the variability of additional carbon sources along with the L-glutaminase. Sivakumar *et al.* [6] mentioned that malt extract support L-glutaminase production by *Streptomyces rimosus*. Urea inhibited L-glutaminase production for both *Streptomyces* species.

Concerning the effect of NaCl concentrations, it was found that as the concentration increased the production of L-glutaminase increased till it reached the maximum values of 12.36 U/ml at 3% for *Streptomyces avermitilis* and 9.65 U/ml at 2.5% for *Streptomyces labedae*. This would indicate that both species are not halophilic, but could be halotolerant. Krishnakumar *et al.* [28] found that the maximal L-glutaminase activity produced by marine alkalophilic *Streptomyces sp.*-SBU1 was observed in a medium supplemented with 2% NaCl (w/v).

Enzyme purification

A- Ammonium sulfate precipitation and dialysis

Partial purification of L-glutaminase produced by *Streptomyces avermitilis* using ammonium sulfate precipitation showed that the best fraction was (80%) with respect to the crude enzyme and other fractions (Table 3). It gave the maximum values of total activity, specific activity and yield of the L-glutaminase enzyme which reached (122.3 U, 9.7 U/mg and 6.6%) respectively. The purification fold of the purified enzyme was 2.0 when 80% ammonium sulfate was used. The previous findings were identical to that reported by Balagurunathan *et al.* [5]who found that 80% ammonium sulfate was best fraction which gave the highest yield of L-glutaminase activity from *Streptomyces olivochromogenes*.

B- DEAE-cellulose chromatography

The L-glutaminase from *Streptomyces avermitilis* precipitated by 80% ammonium sulfate was applied to anion exchange chromatography (DEAE-cellulose). The L-glutaminase enzyme eluted at 0.25 M NaCl showed the highest specific enzyme activity and yield (39.3 U/mg and 4.3%) respectively with 8.02 purification fold (Table 4). The purified enzyme showed a single band on SDS-PAGE . The molecular weight of L-glutaminase enzyme was estimated to be 50 kDa. the purification of L-glutaminase from *Lactobacillus reuteri* by DEAE chromatography was described by Jeong-Min *et al.* [22] who determined its molecular weight as (70 KDa).

Table 3: Ammonium sulfate precipitation of L-glutaminase from *Streptomyces avermitilis*

Fraction (%)	Total volume (ml)	Total protein (mg)	Total activity (U/mg)	Specific enzyme activity (U/mg)	Yield (%)	Purification fold
Crude enzyme	100.0	373.0	1840	4.9	100.0	1.0
40	5.0	10.3	56.4	5.4	3.06	1.1
50	5.0	12.4	87.0	7.0	4.7	1.4
80	5.0	2.6	122.3	9.7	6.6	2.0

Table 4: Purification profile of L-glutaminase from *Streptomyces avermitilis*

Fraction (%)	Total volume (ml)	Total protein (mg)	Total Activity (U/mg)	Specific enzyme activity (U/mg)	Yield (%)	Purification fold
Crude enzyme	100.0	373	1840	4.9	100.0	1.0
Ammonium sulphate (80%)	5.0	12.6	122.3	9.7	6.6	2.0
DEAE cellulose (0.25 M)	5.0	2.0	78.6	39.3	4.3	8.02
DEAE cellulose (0.2 M)	5.0	3.9	42.0	10.8	2.3	2.2
DEAE cellulose (0.1 M)	5.0	5.3	0.0	0.0	0.0	0.0

Table 5 : Effect of different parameters on L-glutaminase stability from *Streptomyces avermitilis*

pH value	3	4	5	6	7	8	9	10	11
Enzyme activity (U/ml)	5.7	5.9	7.2	9.8	19.0	19.0	16.3	8.0	6.1
Residual activity (%)	31.0	36.9	39.1	53.3	103.3	103.3	88.6	43.5	33.2
Temperature (ºC)	20	30	40	50	60				
Enzyme activity (U/ml)	18.6	22.3	17.2	14.9	9.4				
Residual activity (%)	101.1	121.2	93.5	81.0	51.1				
NaCl (%)	1	2	3	4	5	10	15	20	
Enzyme activity (U/ml)	19.6	19. 8	20.1	22.4	20.9	20.9	13.9	9.4	
Residual activity (%)	106.5	107.6	109.2	121.7	113.6	113.6	75.5	51.1	
Metal salts (1mM)	$MgSO_4$	$CuSO_4$	$ZnSO_4$	EDTA	NaCl				
Enzyme activity (U/ml)	19.3	16.2	15.5	13.9	19.8				
Residual activity (%)	104.9	88.0	84.2	75.5	107.6				

Some properties of the partial purified L-glutaminase enzyme

The partially purified enzyme from *Streptomyces avermitilis* exhibited maximum L-glutaminase activity at pH 7 and 8 (19.0 U/ml). The enzyme retained 53.3 and 88.6% of the maximum activity when assayed at pH 6 and 9 respectively. The pH 7 was also the optimum for maximum L-glutaminase activity from marine *Micrococcus luteus* as reported by Moriguchi *et al.* [35].

The enzyme was optimally active (22.3 U/ml) at a temperature range of 20 to 30ºC. Incubation above 40°C promoted remarkable inactivation of L-glutaminase from *Streptomyces avermitilis*, while about 93.5% of the optimum activity remained at 40ºC. Dura *et al.* [19] found that maximum activity of L-glutaminase from *Debaryomyces* spp was at 40°C.

The results revealed that in *Streptomyces avermitils*, as NaCl concentration increase the activity of L-glutaminase enzyme increase until it reach its maximum activity (22.4 U/ml) at 4%. The glutaminase from *Lactobacillus rhamnosus* which showed increased activity in the presence of 5% (w/v) salt was reported by Alexandra *et al.* [36].

The L-glutaminase activity from *Streptomyces avermitilis* was enhanced by $MgSO_4$ and NaCl with 19.3 and 19.8 (U/ml) activities and 104.9 and 107.6% residual activity respectively. On the other hand, $CuSO_4$, $ZnSO_4$ and EDTA decreased the activity. These results are in accordance to those reported by Lu *et al.* [37] who indicated that the activity of glutaminase from *Actinomucor taiwanensis* was enhanced by addition of $MgSO_4$ and NaCl.

L-glutaminase from *Streptomyces avermitilis* showed high stability with the used oxidizing agents. It was highly stable in the presence of sodium hypochlorite and hydrogen peroxide with activity (21.7 and 21.4 U/ml) and (116.3 and 117.9%) residual activity respectively.

Conclusions

The important of L-glutaminase in the biotechnological industries to produce in large scale were crafted to search the high potential bacteria. This study has been taken up with a view of searching the actinomycetes producing L-glutaminase from different soil samples. The strains of *Streptomyces avermitilis* and *Streptomyces labedae* isolated by selective isolation possess remarkable capacity to produce L-glutaminase. The enzyme from *Streptomyces avermitilis* was purified and showed good activity and stability over a wide range of physiological conditions. Also, it possesses the positive property of salt-tolerance which is often required and highly advantageous for food fermentation processes.

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