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Production, purification and characterization of L-glutaminase enzyme from *Streptomyces avermitilis*

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Since the discovery of its anti-tumor properties, L-glutaminases have been in prime focus and microbial sources of the enzyme are sought. In the present study L-glutaminase from *Streptomyces avermitilis* was optimized and the results revealed that the optimum pH, temperature, inoculum size, incubation period and NaCl concentration for enzyme production were pH 8, 28°C, 5 ml/100 ml media v/v, 5 days and 3% NaCl respectively. Glucose and sodium nitrate proved to be the best carbon and nitrogen sources respectively. The enzyme was purified 8.02 fold and the apparent molecular weight of the enzyme was found to be 50 kDa. The optima pH and temperature for the enzyme were (7.0 and 8.0) and 30°C respectively. The enzyme was more stable at 4% NaCl and its activity increased when NaCl and $MgSO_4$ were added as metal salts. The enzyme also showed high stability in the presence of different oxidizing agents.

Key words: L-Glutaminase, *Streptomyces avermitilis*, anti-tumor, purification.

INTRODUCTION

Enzymes are biocatalysts produced by living cells to bring about specific biochemical reactions generally forming parts of the metabolic processes of the cells. Enzymes are highly specific in their action on substrates and often many different enzymes are required to bring about, by concerted action, the sequence of metabolic reactions performed by the living cell. All enzymes which have been purified are protein in nature and may or may not possess a nonprotein prosthetic group (Underkofler et al., 1957).

L-Glutaminase (EC.3.5.1.2) is an amidohydrolase which catalyses the hydrolytical deamidation of L-glutamine resulting in the production of L-glutamic acid and ammonia. L-Glutaminases are ubiquitous in the biological world (Ohshima et al., 1976a; Iyer and Singhal, 2010) and organisms ranging from bacteria to human beings have the enzyme. L-Glutaminase has a central role in mammalian tissues (Errera and Greenstein, 1949). These are generally categorized as the kidney type and liver type glutaminases and both types have been purified and characterized (Svenneby et al., 1973; Curthoys et al., 1976; Heini et al., 1987). Interest on amidohydrolases started with the discovery of their antitumor properties (Broome, 1961; El-Asmar and Greenberg, 1996; Santana et al., 1968; Roberts et al., 1970) and since then, a lot of efforts have gone into extensive studies on microbial L-glutaminases with the intention of developing them as antitumor agents. A parallel interest on microbial L-glutaminases stemmed from its applications in food of biotechnology, microbial L-glutaminases found newer applications in clinical analysis and even in manufacture of metabolites. This led to the extensive studies on L-gluta-

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minase despite the fact that modern biotechnological techniques suggest alternative and specific methods for the treatment of cancers, where amidohydrolases used to be employed.

Actinomycetes are the main source of clinically important antibiotics, most of which are too complex to be synthesized by combinatorial chemistry and which are ecofriendly filamentous bacteria. 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 (Okami, 1986).

Since the sources for L-glutaminases are limited, the search for potential microbial strains that hyper produce the enzyme with novel properties for their industrial production is being pursued all over the world (Prabhu and Chandrasekaran, 1995).

In this paper, the production of extracellular L-glutaminase by *Streptomyces avermitilis* was reported under submerged culture and also attempts were made to study the optimization of L-glutaminase production, its purification and characterization from *Streptomyces avermitilis.*

MATERIALS AND METHODS

The strain *Streptomyces avermitilis* was obtained from the Department of Microbiology, Faculty of Science, Ain Shams University, Cairo, Egypt. The isolate was maintained on starch casein agar slants (pH 7.2) containing (gL-1) Starch 10, K_2HPO_4 2.0, KNO_3 2.0, NaCl 2.0, Casein 0.3, $MgSO_4.7H_2O$ 0.05, $CaCO_3$ 0.02, $FeSO_4.7H2O$ 0.01 and agar 20 at a temperature of 4°C. Regular sub culturing of the isolate was performed at an interval of every 4 weeks.

Streptomyces avermitilis showed ability to produce L-glutaminase when cultured by using Minimal glutamine agar (MGA) medium. 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; 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. L-Glutaminase activity was identified by formation of a pink zone around *S. avermitilis* colonies due to accumulation of ammonia which is resulted in change in pH indicator color from yellow to pink due to the increase in pH value which is caused by L-glutamine utilization (Hymavathi et al., 2009).

Inoculum preparation and production of L-glutaminase

For the preparation of *S. avermitilis* spores, the strain was inoculated on sporulation agar medium and incubated for 7 days at 28°C. After incubation 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 tri sodium citrate; 25 NaCl; 10 glucose. All the flasks were incubated at 28°C for 72 h 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 the inoculum was transferred into MSG production medium and incubated at 28°C in rotary shaker for 120 h. After incubation, fermentation medium was removed from shaker and centrifuged by using cooling centrifuge at 10,000 rpm for 30 min at 4°C. The clear supernatant was collected in screw cap tube and stored at 4°C to use as a crude enzyme (Balagurunathan et al., 2010).

Assay of L-glutaminase

L-Glutaminase activity was determined using L-Glutamine as substrate and the product ammonia, released during the catalysis was measured by using Nesseler's reagent. An aliquot of 0.5 ml of the sample was mixed with 0.5 ml of 0.04 M L-glutamine solution in the presence of 0.5 ml of distilled water and 0.5 ml of phosphate buffer (0.1 M, pH 8.0). Then the mixture was incubated at 37°C for 15 min and the reaction was arrested by the addition of 0.5 ml of 1.5 M Trichloro Acetic Acid. To 0.1 ml of the mixture, 3.7 ml of distilled water and 0.2 ml of Nessler's reagent were added. The absorbance was measured at 450 nm using a Visible spectrophotometer. Then a standard graph was plotted using ammonium chloride as the standard for computation of the concentration of ammonia, liberated due to enzyme activity (Imada et al., 1973).

One international unit of L-glutaminase was defined as the amount of enzyme that liberates one µMol of ammonia under optimum conditions. The enzyme yield was expressed as Units/ml (U/ml). Protein concentration was determined (Lowry et al., 1951) using bovine serum albumin as the standard.

Effect of various parameters on L-glutaminase production by *S. avermitilis*

Factors influencing the secretion 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 (5 ml) 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%), incubation periods (1-9 days) for inoculum size experiment; different inoculum size ranging from (2-10 ml /100 ml medium) were tested. Optimum condition identified for one parameter was used for optimizing the other parameters one by one.

Purification of L-glutaminase

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 4°C. It was centrifuged at 10,000 rpm at 4°C for 20 min 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 stand at 4°C and the resulting precipitate was obtained by centrifugation at 10,000 rpm at 4°C for 20 min. 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 h at 4°C and the buffer were changed occasionally.

Ion-exchange chromatography

Anion-exchange DEAE-cellulose (Diethylaminoethyl-cellulose) chromatography was performed for further purification of the L-glutaminase enzyme obtained from the previous ammonium sulfate precipitation.

DEAE cellulose chromatography

Resin preparation

Four grams of DEAE cellulose were mixed with 500 ml of 0.02 M Tris HCl an equilibration buffer. The mixture was left for one hour and the buffer was poured off with the small particles into the column. The column was incubated at room temperature overnight to ensure complete swelling (Dura et al., 2002).

Sample application

The L-glutaminase solution from ammonium sulphate precipitation (80%) of *S. 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 0.02 M 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 purity by polyacrylamide gel electrophoresis (Dura et al., 2002).

Analysis of protein pattern by polyacrylamide gel electrophoresis (SDS-PAGE)

The materials used were glass plates, 0.75 mm spacers and Teflon comb. The monomer solution (solution A) was of analytical grade acrylamide (29.2 g) and 0.8 g Bis-acrylamide was dissolved in 50 ml distilled water and completed to 100 ml. The resolving gel buffer (solution B, 1.5 M Tris-base, pH 8.8) was Tris-base (18.2 g) which dissolved in 50 ml distilled water and completed to 100 ml. The pH was adjusted at 8.8 with HCl. The stacking gel buffer (solution C, 0.5 Tris-base, pH 6.8) was prepared with 6 g Tris-base dissolved in 50 ml distilled water and completed to 100 ml. The pH was adjusted at 6.8 with HCl. The sodium dodecyle sulfate 10% (SDS) (solution D) was used as 10 g SDS dissolved in 50 ml distilled water and completed to 100 ml. This solution was kept at room temperature to avoid precipitation. The ammonium persulphate 10% (AP) (solution E) was ammonium persulphate (0.5 g) dissolved in 5 ml distilled water as a polymerization catalyst. The TEMED (N, N, N, N tetramethylenediamine) was 50 µl of TEMED added for stacking the resolving gels (TEMED is a strong polymerizing agent). The resolving gel was 10 ml of solution A, 7.5 ml of solution B, 0.3 ml of solution D and 12 ml distilled water were mixed and shaked well. Freshly prepared solution E (300 µl) was added and shaked well. Finally, 300 µl TEMED were added just before gel casting. The stacking gel was solution A (1.33 ml), 2.5 of solution C, 0.1 ml of solution D, 100 µl of solution E and 6.1 ml distilled water were added, respectively and shaked well. Just before casting, 50 µl TEMED were added. The sample buffer was Tris-HCL (pH 6.8), 2% SDS, 10% sucrose, 0.1% β- mercaptoethanol and 0.5% bromophenol blue. The overlay isopropyl alcohol was 50 ml of isopropyl alcohol and 5 ml distilled water. The tank buffer was 6 g Tris-base, 28.8 g glycine and 20 ml of solution D dissolved in 500 ml distilled water and completed to 2 liters. The staining solution (1% COBB) was 10 g of Coomassie Brilliant Blue (COBB, R-250) were dissolved in 200 ml distilled water. The staining solution was prepared as follows: 62.5 ml of stain, 250 ml of methyl alcohol and 50 ml of acetic acid glacial were added then, completed to 500 ml with distilled water. The destaining solution was 45 ml methyl alcohol, 10 ml acetic acid glacial added and completed to 100 ml with distilled water (Iwasa et al., 1987).

Experimental procedures

The chromatographic fractions were electrophoretically analyzed on SDS-PAGE (SDS-Polyacrylamide gel electrophoresis) according to the study of Dura et al. (2002). Bio-Rad vertical slab gel with size of 0.75 mm x 14 cm x 14 cm apparatus was used. Aliquots (10-15 µl), from each fraction, were mixed with an equal volume of sample buffer and denatured by heating in a boiling water for 2-5 min then, loaded in equal amounts (20 µl). Biometra multigel-long is the device which was used in protein electrophoresis. Resolving gel was poured in-between the two plates leaving 2 cm beneath the plates end. A layer of 90% isopropyl alcohol was added over the resolving gel to prevent corrugation of the gel surface. After gel solidification, the alcohol was poured off. Stacking gel mixture was added over the solid resolving gel till the top of the glass plates. A comb consisted of 12 wells and 1.0 mm dimension was added. Once the solidification took place, the comb, clips and the silicon rubber were removed. The two plates were installed in the electro-phoretic chamber. The tank buffer was added to immerse the wells completely. The protein samples were pipette in wells by automatic variable micropipette. The run was carried out at 30 volt till the loaded samples passed stacking gel, after so, the volt was raised to 70. The gels were stained for 24 h in the prepared staining solution. To obtain a clear background, the gels were destained by the prepared destaining solution. The destained gels were finally photo-graphed.

L-Glutaminase characterization

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 37°C for one hour. The residual activity was measured (Peter, 1972).

Thermostability

For thermostability, 100 µl of partial purified enzyme was incubated at 20, 30, 40, 50 and 60°C for one hour before the L-glutaminase assay (John and Arthur, 1971).

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 (Jeong-Min et al., 2010).

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 (Gaffer and Shethna, 1975).

Stability in oxidizing agents

One hundred microliter 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 (Kumar et al., 2009).

Table 1. Effect of various parameters on L-Glutaminase production by *Streptomyces avermitilis.*

Inoculum size (ml)	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Enzyme activity (U/ml)	5.83	6.22	8.15	12.61	12.61	12.61	9.01	8.24	3.86
Incubation period (day)	1	2	3	4	5	6	7	8	9
Enzyme activity (U/ml)	0.0	1.28	3.0	6.35	8.41	8.41	8.41	5.75	4.12
Incubation Temperature (ºC)	20	28	37	40	45				
Enzyme activity (U/ml)	5.92	12.48	11.58	9.52	4.03				
Initial pH	3	4	5	6	7	8	9	10	11
Enzyme activity (U/ml)	0.0	0.0	2.70	10.60	12.48	13.47	9.01	5.49	0.0
Carbon source	Glucose	Fructose	Maltose	Lactose	Mannitol	Galactose	Sucrose	Starch	
Enzyme activity (U/ml)	7.12	6.99	3.30	0.98	4.09	6.00	1.71	0.68	
Nitrogen source	Peptone	Malt extract	Yeast extract	Casein	Urea	Sodium nitrate	Ammonium nitrate	Ammonium Sulphate	
Enzyme activity (U/ml)	9.52	4.37	4.03	1.35	0.55	10.30	2.10	3.04	
NaCl concentration (%)	0	1	2	2.5	3	3.5	4		
Enzyme activity (U/ml)	6.0	8.06	10.55	11.45	12.36	7.21	4.1		

RESULTS AND DISCUSSION

The present study revealed that, all the selected parameters examined, showed a considerable impact on L-glutaminase production by the isolate, *S. avermitilis* as shown in Table 1. Further, the high catalytic activity of the enzyme at physiological pH and temperature and its considerable stability over a wide range of pH and temperature makes it highly favorable to be exploited as a potent anticancer agent.

Effect of various parameters on L-glutaminase production by *S. avermitilis*

The results reveal that in *S. avermitilis* the L-glutaminase productivity increased as the inoculum size increased until it reached its maximum productivity (12.61 U/ml) at 5 ml. Then the productivity remained unchanged with inoculums size 6 and 7 ml, after that it decreased as the inoculum size increased. Interestingly similar results were reported by Shirling and Gottlieb (1966) as maximum L-glutaminase productivity from *S. avermitilis* was detected at 5 ml inoculum size. 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 (Tobin et al., 2001).

Maximum L-glutaminase productivity by *S. 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 decreased. These results reveal that the relation between enzyme productivity and incubation period is variable depending on the organism. Krishnakumar et al. (2011) 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.

Also the maximum values of L-glutaminase production (12.48 U/ml) was obtained at temperature 28°C. Then the enzyme productivity gradually decreased at 37, 40 and 45°C. Such results are in accordance to glutaminase from *Lactobacillus rhamnosus* reported by Alexandra et al. (2003) who found that 30°C was the best incubation temperature for maximum glutaminase production by Lactobacillus rhamnosus.

Concerning the effect of pH, maximum enzyme productivity (13.47 U/ml) was recorded at pH 8 and no production was detected at pH 3, 4 and 11. Balagurunathan et al. (2010) stated that the optimum pH for L-glutaminase production by *Streptomyces olivochromogenes* was 7. The medium contain glucose as sole carbon source gave the highest yield of L-glutaminase production (7.12 U/ml). The obtained result was confirmed by that reported by Sivakumar et al. (2006) who observed that L-glutaminase

Table 2. 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 3. 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

produced by *Streptomyces rimosus* that was isolated from estuarine fish, Chanos chanos showed the highest activity when glucose was used as carbon source.

Peptone, malt extract and yeast extract were found to be the most suitable organic nitrogen sources for L-glutaminase production by *s. avermitilis*, with maximum productivity reached (9.52 U/ml) for peptone, while sodium nitrate is considered to be the best inorganic nitrogen source for enzyme production with productivity reached (10.30 U/ml). Urea inhibited the enzyme production by *S. avermitilis*. Malt extract was found to support L-glutaminase production by *S. rimosus* as described by Sivakumar et al. (2006).

As the concentration of NaCl increased the production increased till it reached the maximum value (12.36 U/ml) at 3% then the productivity decreased. Krishnakumar et al. (2011) 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

Partial purification of L-glutaminase produced by *S. avermitilis* using ammonium sulfate precipitation showed that the best fraction was (80%) with respect to the crude enzyme and other fractions (Table 2). 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. (2010) who found that 80% ammonium sulfate was best fraction which gave the highest yield of L-glutaminase activity from *Streptomyces olivochromogenes.*

The enzyme precipitated by 80% ammonium sulfate was applied to anion exchange chromatography (DEAE-cellulose). Results (Table 3) showed three fractions were collected at different NaCl concentrations (0.1, 0.2 and 0.25 M). 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. 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. (2010) who determined its molecular weight as (70 KDa).

Properties of the partially purified enzyme

The partially purified enzyme exhibited maximum activity at pH 7 and 8 (19.0 U/ml). pH 7 was also the optimum for maximum L-glutaminase activity from marine *Micrococcus luteus* as reported by Moriguchi et al. (1994). The enzyme was optimally active (22.3 U/ml) at a temperature range of 20 to 30 °C for 60 min and pH 7. Incubation above 40°C promoted remarkable inactivation of L-glutaminase from *Streptomyces avermitilis*. Dura et al. (2002) found that maximum activity of L-glutaminase from Debaryomyces spp was at 40°C.

Also results revealed that as the NaCl concentration increase the activity of the enzyme increase until it reach its maximum activity (22.4 U/ml) at 4% (w/v) NaCl, 30°C and pH 7 after that any increase in NaCl concentration lead to decrease in enzyme activity. Such results indicate the high salt-tolerance of the enzyme as the glutaminase from *Lactobacillus rhamnosus* which showed increased activity in the presence of 5% (w/v) salt (Alexandra et al., 2003).

Table 4. Effect of different parameters on L-Glutaminase stability.

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				

L-Glutaminase from *Streptomyces avermitils* was affected by all metal salts tested at 30°C and pH 7. The activity was 19.3 U/ml when $MgSO_4$ was added and 19.8 U/ml when NaCl was used. The activity ranged from 13.9 to 16.2 U/ml with the other metal salts. This indicate that the activity was enhanced by $MgSO_4$ and NaCl. The activity decreased when $CuSO_4$, $ZnSO_4$ and EDTA were used. These results are in accordance to glutaminase from *Actinomucor taiwanensis* reported by Lu et al. (1996) who indicated that the activity of glutaminase from Actinomucor taiwanensis was enhanced by the addition of $MgSO_4$ and NaCl. It was also highly stable in the presence of sodium hypochlorite and hydrogen peroxide at 30°C and pH 7 with activity (21.7 and 21.4 U/ml) respectively (Table 4).

Conclusion

The strain *Streptomyces avermitilis* showed remarkable capacity to produce L-glutaminase enzyme and could, therefore, be potentially useful for industrial production for L-glutaminase enzyme. The enzyme from *Streptomyces avermitilis* possesses the positive property of salt-tolerance which is often required and highly advantageous for food fermentation processes.

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