Optimization and characterization of l-asparaginase production by a novel isolated *streptomyces* spp. strain

Abeer A. El-Hadi, Hanan Mostafa Ahmed, Rania A. Hamzawy

Department of Chemistry of Natural and Microbial Products, National Research Center, Dokki, Giza, Egypt

Correspondence to Hanan M. Ahmed, PhD, Department of Chemistry of Natural and Microbial Products, National Research Centre, 33 St El-Behoos, Dokki, Giza 12311, Egypt
Tel: +20 2335 4974; fax: +20 2337 0931;
e-mail: hananmostafa56@yahoo.com

Received 22 May 2018
Accepted 9 September 2018

Egyptian Pharmaceutical Journal 2019, 18:111–122

Introduction
and purpose Microbial L-asparaginase (L-asparagine amino hydrolase, E.C.3.5.1.1) has been applied as the most important chemotherapeutic agent in the treatment of certain human cancers, especially in acute lymphoblastic leukemia. Actinomycetes are recognized as a comparatively less explored source for L-asparaginase and therefore act as candidates for the production of L-asparaginase. The possibility of using novel actinomycete as a source of production of some industrially important microbial L-asparaginase was investigated in this study.
Materials and methods
Genomic DNA of the actinomycetes isolate from soil samples was extracted using the Gene JET Genomic DNA purification kit (Thermo scientific #k0721). The actinomycete isolate was identified by 16S rDNA. The identified actinomycete isolate was inoculated on starch casein agar slants and incubated for 7–10 days at 28°C, and then it was maintained at 4°C until further use. Inoculum was prepared from a 7-day old culture of the strain. Production of L-asparaginase was initially tested in four different media. The actinomycete strain was used further for the optimization of cultural conditions, namely, L-asparagine substrate concentrations, pH, temperature, and incubation conditions. The pH of the medium was varied from pH 3.0 to pH 9.0; the incubation temperature was varied from 30 to 50°C. The effect of carbon source and nitrogen source on L-asparaginase production was studied. Modified Czapex Dox broth was supplemented with different carbon and nitrogen sources such as starch, mannitol, mannose, sucrose, cellulose, and fructose at a concentration of 1% (w/v) and ammonium sulfate, beef extract, yeast extract, and peptone at a concentration of 0.2% (w/v), respectively, keeping other components constant. The properties of *Streptomyces* spp. L-asparaginase were also studied such as pH, assay temperature, and thermal stability.
Results
and discussion Genotypic characterization of the most promising unknown actinomycete isolate showing the maximum production was identified by 16S rDNA. PCR amplified the 16 s rDNA region using primers. Genotypic characterization of the most promising unknown actinomycete isolate showing the maximum production was identified by16S rDNA. The 16 s rDNA region was amplified by PCR (about 1000 bp) using primers. According to sequencing similarities and multiple alignments, the isolate was found to be closely related to *Streptomyces griseoplanus* strain NRRL-ISP 5009 16 s ribosomal RNA gene with 85% identity. Higher enzyme activity was observed in modified Czapex Dox broth as compared with other media used. Modified Czapex Dox broth was supplemented with different concentrations of L-asparagine; the enzyme production was maximum at 1.5% L-asparagine (126.20 U/ml). Analysis of the culture supernatant showed that the enzyme activity rise from 126.20 U/ml on the fifth day to 141.11 U/ml on the 10th day which its peak enzyme production activity. Different carbon sources such as starch, mannitol, lactose, sucrose, and glucose were amended in asparagine-modified Czapek Dox broth to determine their impact on L-asparaginase production. Biosynthesis of L-asparaginase by *S. griseoplanus* strain has been reported to be higher when the basal medium was supplemented with starch. For the production of L-asparaginase by *S. griseoplanus* strain, yeast extract has been reported as a good nitrogen source. According to the properties of the enzyme, the maximum activity was achieved at 45°C. The half-lives of the free enzyme were calculated to be 521 min (8.5 h) at 50°C, 312.6 min (5.2 h) at 55°C, and 195.2 min (3.25 h), at 60°C.

Keywords:
16s rDNA identification, culture optimization, L-asparaginase, *Streptomyces* spp

Egypt Pharmaceut J 18:111–122

1687-4315

Introduction

Enzymes are catalytic proteins. In enzymatic reactions, substrates are the molecules that begin the reaction and they are converted into different molecules called the products. In a biological cell, almost all processes need enzymes to occur at significant rates. Since a few reaction enzymes are selective for their substrates and speed up only from among many possibilities, metabolic pathways occur in that cell are made by the set of enzymes. One of the important facts of today's pharmaceutical industry is the manufacture of an enzyme and its use as a drug [1].

Microbial L-asparaginase (L-asparagine amino hydrolase, E.C.3.5.1.1) has been applied as a most important chemotherapeutic agent in the treatment of certain human cancers, especially in acute lymphoblastic leukemia [2]. The discovery of L-asparaginase, a medical agent for the treatment of malignant tumors, was made in 1922. Clementi showed that the guinea pig serum contained a high activity of L-asparaginase. L-asparaginase catalyses the conversion of L-asparagine to L-aspartate and ammonium, and this catalytic reaction is essentially irreversible under physiological conditions. Supplementation of L-asparaginase results in continuous depletion of L-asparagine. Under such an environment, cancerous cells do not survive. This phenomenal behavior of cancerous cells was exploited by the scientific community to treat neoplasias using L-asparginase [3]. Microorganisms such as bacteria, fungi, yeast and actinomycetes are very efficient producers and a better source of L-asparaginase, because they can be cultured easily and the extraction and the purification of L-asparaginase from the mare are also convenient, facilitating large-scale production [4]. Actinomycetes are recognized as a comparatively less explored source for L-asparaginase and therefore act as candidates for the production of L-asparaginase [5,6]. Actinomycetes, like *Streptomyces griseoluteus* [7], *Streptomyces karnatakensis* [8] and *Streptomyces venezuelae* [9] are recognized as a comparatively less explored source for L-asparaginase and therefore act as candidates for the production of L-asparaginase [5,6].

The present investigation deals with the isolation and characterization of L-asparaginase from soil sample isolates of actinomycetes as a novel source of enzyme which is effective against leukemia. The isolate was identified as *Streptomyces griseoplanus* strain NRRL-ISP 5009 by 16s rDNA sequencing studies. Further analysis deals with the optimization of different parameters for L-asparaginase production.

Materials and methods

Microorganism and culture conditions

Actinomycete strain used in this study was isolated from various soil samples collected from different localities in Egypt. Actinomycete from the soils had been isolated using the standard dilution plate method procedure on Petri plates containing starch nitrate agar medium of the following composition (g/l): starch, 20; KNO_3, 2; K_2HPO_4, 1; $MgSO_4.7H_2O$, 0.5; NaCl, 0.5; $CaCO_3$, 3; $FeSO_4$ ⊠$7H_2O$, 0.01; agar, 20, and distilled water up to 1 l; then the plates were incubated for a period of 7 days at 30°C. *Streptomyces* spp. isolate was purified and maintained as spore suspensions in 20% (v/v) glycerol at −20°C for subsequent investigation.

L-asparaginase production by plate assay

It is generally observed that L-asparaginase production is accompanied by an increase in pH of the culture filtrates. The plate assay was based on the Mostafa [8] method with the incorporation of pH indicator phenol red (prepared in ethanol) in a medium containing L-asparagine (sole nitrogen source). Phenol red at acidic pH is yellow and at alkaline pH turns pink; thus a pink zone is formed around the microbial colonies producing L-asparaginase. L-asparaginase-producing actinomycete was carried out with the use of asparagine dextrose salts agar (g/l: asparagine, 10; dextrose, 2; K_2HPO_4, 1; $MgSO_4$, 0.5; and agar, 20); pH was adjusted to 6.8 and supplemented with phenol red as a pH indicator (0.009% final concentration) [8] and sterilized at 1.5 atmospheric pressure for 20 min. The inoculated plates were incubated at 30°C for 7 days. Plates were examined for the change in color of the medium from yellowish to pink due to the change of pH indicating the positive asparaginase activity. Control plates were prepared as inoculated medium without dye and as uninoculated medium with dye.

Inoculum preparation

Inoculum culture was prepared by inoculating 1 ml of thawed spore suspension (25.8×10^6 CFU/ml) into 50 ml starch casein broth (contained g/l: starch, 10; casein, 0.3; KNO_3, 2; NaCl, 2; K_2HPO_4, 2; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 0.02; $FeSO_4.7H_2O$, 0.01; pH 7) in a 250-ml Erlenmeyer flask. The flask was then incubated at 28±2°C for 7 days at 200 rpm. Two milliliters of the grown culture were used to inoculate 50 ml of fermentation medium [10].

Polymerase chain reaction 16s rDNA and electrophoresis

Genomic DNA of the bacterial isolates was extracted using the Gene JET Genomic DNA purification kit

(Thermo Scientific # k0721). The 16s rRNA fragments were partially amplified by PCR using Maxima Hot Start PCR Master Mix (Thermo K1051) in Sigma Company of Scientific Services, Egypt (www.sigma-co-eg.com). A region of ~1000 bp from the 16s rRNA gene was amplified using the primers: forward primer AGA GTT TGA TCC TGG CTC AG and the reverse primer GGT TAC CTT GTT ACG ACT T. The PCR program consisted of one cycle of DNA initial denaturation at 95°C (10 min), 35 cycles of 95°C (30 s), 65°C (1 min), and 72°C (1 min and 30 s), plus one additional cycle of a final chain elongation at 72°C (10 min). Finally sequencing was accomplished to the PCR product on GATC Company by use of an ABI 3730xl DNA sequencer by using forward and reverse primers.

DNA sequencing

PCR products were purified by Qiagen extraction kit according to the manufacturer's instructions before applying the DNA sequencer. Sequencing was performed by automated the florescent dye terminator sequencing method originally developed by using (ABI 3130, DYEnamic ET Terminator Cycle Sequencing Kit; Amersham Pharmacia Biotech).

Production of L-asparaginase by submerged fermentation media

Fermentation was carried out in duplicate 250-ml Erlenmeyer flasks, each containing 50 ml production medium, and they have been incubated at 25±2°C for 7 days at 150 rpm. Production of L-asparaginase was initially tested in four different media. The most suitable medium underwent further detailed studies for maximum L-asparaginase production. The composition (g/l) of the four tested media was as follows:

(1) Medium I (modified starch casein broth) it contains g/l: starch, 10; casein, 0.3; KNO_3, 2; NaCl, 2; K_2HPO_4, 2; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 0.02; $FeSO_4.7H_2O$, 0.01; supplied with L-asparagine (1.5%) pH 7.0 [11].
(2) Medium II (modified Czapek Dox medium supplied with L-asparagine 1.5%) it contained g/l: sucrose, 30.0; sodium nitrate, 3.0; K_2HPO_4, 2; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 0.02; $FeSO_4.7H_2O$, 0.01, pH of the medium was adjusted to 7.0 [12].
(3) Medium III (asparagine dextrose salt broth, containing 1% dextrose, 0.05% L-asparagine, 0.05% di-potassium hydrogen phosphate, and 0.2% meat extract with initial pH 7. The inoculated flasks were then incubated at 30°C for 7 days [10].
(4) Medium IV: the fermentation medium contained (g/L): glucose, 20; peptone, 1; yeast extract, 1; KH_2PO_4, 0.74; L-asparagine, 0.7; and $MgSO_4.7H_2O$, 1.0; pH of the medium was adjusted to 6.5 [8].

Preparation of cell-free extract

The cells from 100 ml of culture broth were harvested by centrifugation and the pellet was suspended in lysis buffer (9 g glucose, 3 g Tris, 20 ml of 0.25 MEDTA per 1000 ml distilled water). After autoclaving, it was stored at 4°C with 0.02% lysozyme. After overnight incubation this cell-free extract was used for the assay of L-asparaginase.

Assay of L-asparaginase activity

L-asparaginase activity was measured by the following method [13,14]. The cultures in the modified Czapek Dox medium supplied with L-asparagine 1.5% broth were centrifuged at 8000 rpm for 30 min and the resultant supernatant, the crude extract, was used to determine the L-asparaginase activity. Reaction was started by adding 0.5 ml of crude cell-free extract into 0.5 ml of 0.04 M L-asparagine solution and 0.5 ml of 0.05 M Tris-HCL buffer pH 8 and incubated at 37°C for 30 min in a water bath. The reaction was stopped by the addition of 0.5 ml of 1.5 M trichloroacetic acid. The precipitated proteins were removed by centrifugation at 8000 rpm for 15 min at 4°C, and the ammonia was determined spectrometrically at 425 nm by nesslerization (by Nessler's method). Tubes kept at zero time incubation served as control. Enzyme activity was determined on the basis of liberation of ammonia calculated with reference to a standard curve of ammonium sulfate. In the concentration range 0.1 ml of the supernatant was added to 3.8 ml of distilled water, followed by addition of 0.2 ml of Nessler's reagent and was incubated at room temperature for 10 min after which the absorbance was taken at 425 nm. One unit of asparaginase is the amount of enzyme which catalyzed the formation of 1 μmol of ammonia per minute at 37°C.

Protein estimation

The protein content was determined according to the Lowry method [15] using bovine serum albumin as the standard.

Optimization of fermentation parameters for L-asparaginase

The production of L-asparaginase from actinomycete strain mainly depends on factors like L-asparagine substrate concentrations, temperature, pH, carbon,

and nitrogen source. Hence these parameters must be optimized in order to achieve higher yields of L-asparaginase.

Initial pH and temperature optimization

Impact of pH on the production of L-asparaginase was examined by culturing the strain in a modified Czapek Dox medium supplied with L-asparagine 1.5% broth adjusted to various pH levels ranging from 3.0 to 9.0. The optimal pH achieved at this step was used for further study. To determine the optimum temperature for L-asparaginase production, the strain was cultured in a modified Czapek Dox medium supplied with L-asparagine 1.5% broth at different temperatures ranging from 25 to 50°C for an incubation period of 10 days and the supernatant was used as crude enzyme to calculate the L-asparaginase activity [3].

Effect of carbon source

In the present study, different carbon sources were added to modified Czapek Dox's liquid media at an equivalent weight. Various sources of carbon such as mannitol, fructose, mannose, cellulose, and lactose were supplemented with L-asparagine (1.5%) as nitrogen source in the growth media. Thereafter, L-asparaginase production was investigated. The inoculum was added in the medium and incubated at 30°C for 10 days under static conditions [14,16].

Effect of nitrogen source

As a fact, the nitrogen sources consider the secondary energy source after carbon sources and they play a vital role in the growth of organisms and enzyme production. In microorganisms, amino acids, nucleic acids, proteins, and cell wall components are metabolized by nitrogen (both organic and inorganic forms). In the present experiment, the supplementation of additional nitrogen sources (either organic or inorganic) such as urea, peptone, yeast extract, beef extract, ammonium sulfate was used to determine the maximum enzyme activity [16].

Characterization of native *Streptomyces* spp. L-asparaginase

Optimum pH and temperature

The optimum pH was determined by measuring enzymatic activity at 37°C using different buffers (0.1 M) with various pH values as: citrate–phosphate buffer (pH 5.0–7.0) and Tris-HCl buffer (pH 8–9). Assay temperatures of 30–55°C were studied by measuring activity at this range of temperatures for 30 min using 0.04 M L-asparagine as substrate and at the optimum pH values.

Activation energy (Ea)

The activation energy was determined using the slope of a linear plot of the log of the enzyme activity versus 1/T. The enzyme activity (v) was expressed in U (mg protein)–1, the temperature (T) in Kelvin (K), the gas constant (R)=1.987 cal/K/mol, and the activation energy (Ea) in kcal/mol [17].

Thermal stability of L-asparaginase

The thermal stability of L-asparaginase was investigated by incubating the native enzyme at various temperatures (30–65°C) with different incubation periods (15, 30, and 60 min) in the absence of the substrate. Then, the enzyme was incubated with 0.5 ml asparagine in Tris buffer (1 M, pH 8) at 40°C for 30 min. The relative activities were plotted against different temperature values for each incubation time.

Results and discussion

Isolation and detection for L-asparaginase production by plate assay of actinomycete strain

The isolate obtained was screened for the production of the enzyme L-asparaginase by using the plate assay method (qualitative method). The results obtained showed a high intensity of pink coloration for the tested culture (Fig. 1).

Identification and phylogenetic analysis

The isolated strain was determined by the 16 s rRNA gene sequence (1000 bp). It was found that the 16 s rRNA gene sequence of the isolated strain was similar to that of many species of the genus *Streptomyces* spp. according to a BLAST search [18] of the GenBank database. The costruction of a phylogenetic tree (Fig. 2) based on 16 s rRNA gene sequences of members of the genus *Streptomyces* spp. was attained according to the bootstrap test of the neighbor-joining algorithm method of Saitou and Nei [19] with MEGA4 [20].

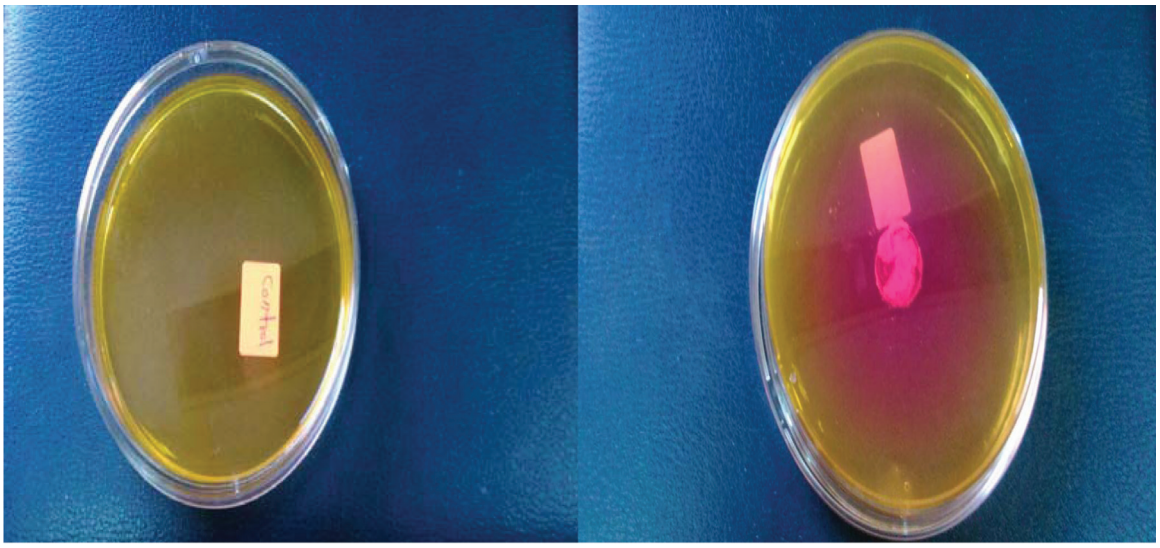
According to sequencing similarities and multiple alignments, the isolate was found to be closely related to the *S. griseoplanus* strain NRRL-ISP 5009 16s ribosomal RNA gene with 85% identity.

Identification and phylogenetic analysis

Use of different media for growth and production of the enzymes

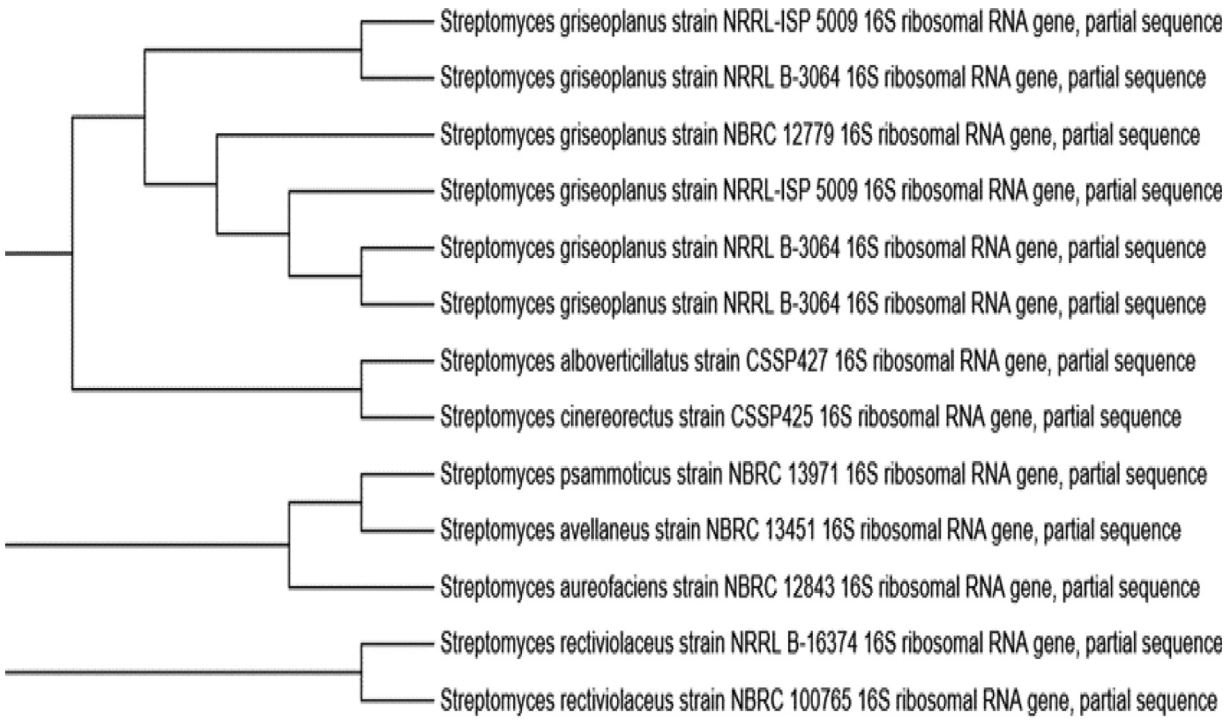
Comparative analysis was carried out by inoculating the culture in different media in order to obtain increased growth and production of the enzyme. Results have shown that higher enzyme activity was observed in modified Czapex Dox broth (medium II)

Figure 1



Results of plate assay method for qualitative determination of L-asparaginase production by *Streptomyces griseoplanus.* (a) Control without inoculation. (b) Positive asparaginase activity by qualitative plate assay method.

Figure 2



Phylogenetic tree showing the relationships of the organism and their closest relatives according to the 16 s rRNA gene.

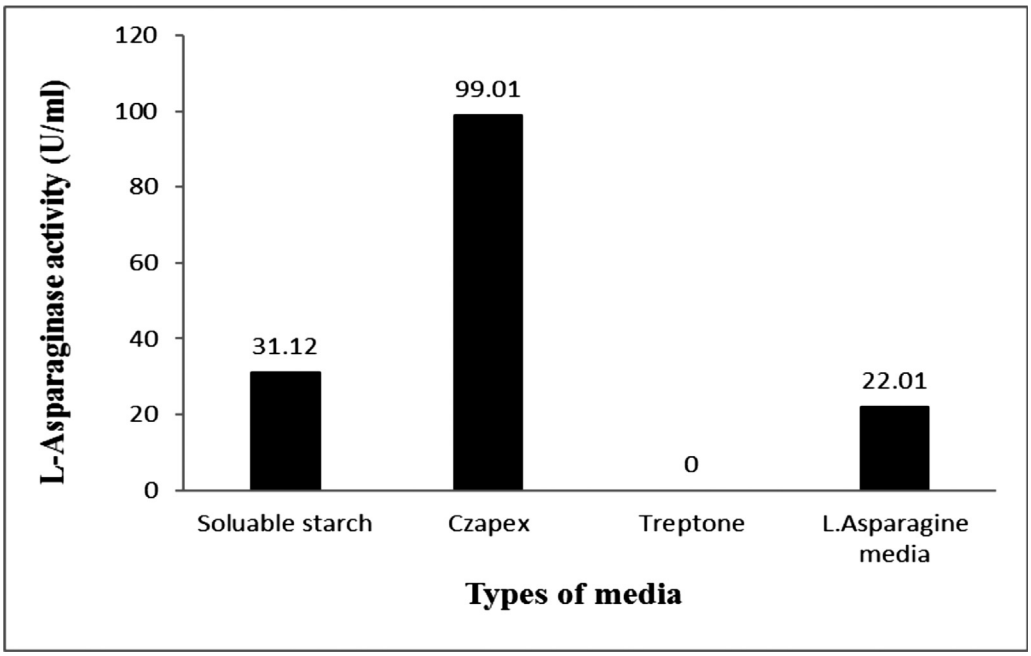
as compared with other media used (Fig. 3). Medium II showed better enzyme production (99.01 U/ml); this might be attributed to the fact that sucrose has an inductive effect as it makes a great stabilization of enzyme [21]. Medium II was selected for all further experiments.

Optimization of L-asparaginase production

Effect of different L-asparagine concentrations

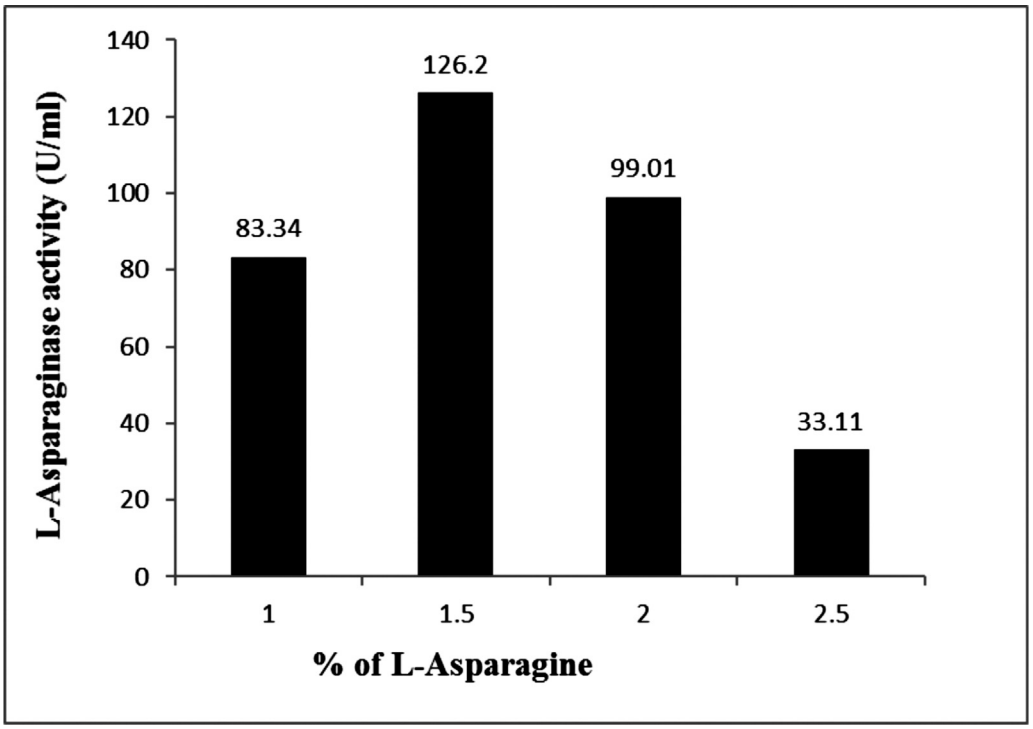
L-asparaginase production is induced by the addition of L-asparagine. Individual addition of different concentrations (0.5, 1, 1.5, 2, and 2.5%) of L-asparagine was done to the modified Dox medium to

Figure 3



Effect of different media on *Streptomyces griseoplanus* Pseudomonas aeruginosa-asparaginase production.

Figure 4



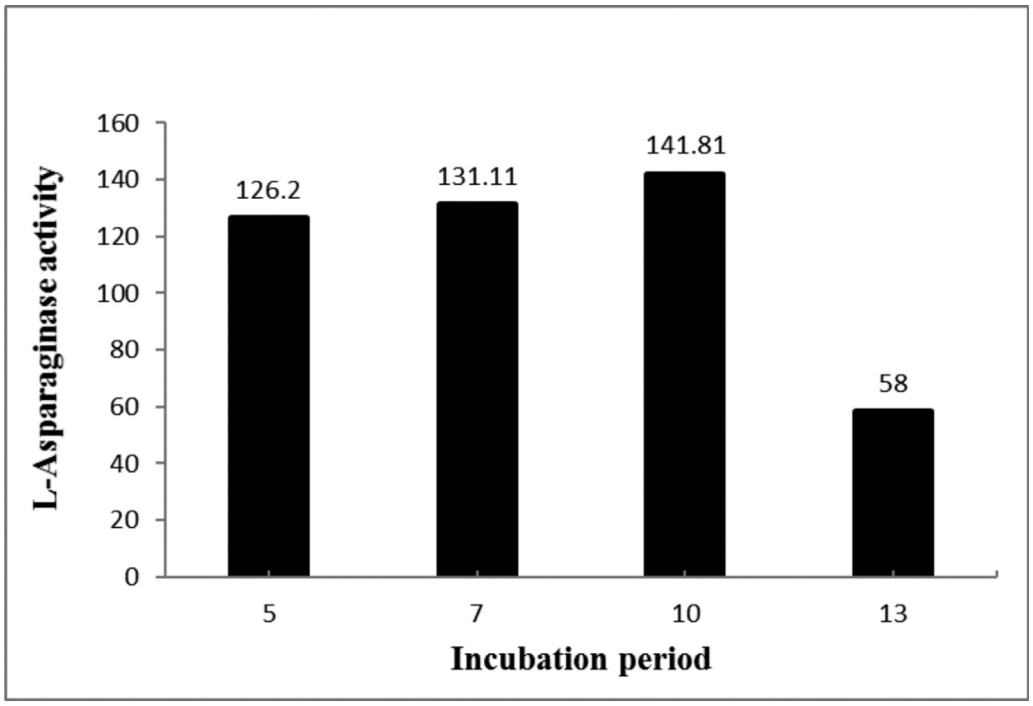
Effect of different L-asparagine concentrations on the production of *Streptomyces griseoplanus* L-asparaginase.

determine the best concentration of L-asparaginase production. 1.5% L-asparagine was the optimum concentration for L-asparaginase production (126.20 U/ml) (Fig. 4). Higher concentrations (2 and 2.5%) of L-asparagine decreased the productivity (99.01 and 33.11 U/ml, respectively). Niharika and Supriya [22] have also suggested that maximum enzyme production depends on the nature of the substrate.

Effect of incubation period

An important criterion for the potency assessment is the time course of L-asparaginase production. The

Figure 5



Effect of incubation time on *Streptomyces griseoplanus* L-asparaginase production.

highest peak activity of the enzyme production (141.11 U/ml) was attained after 10 days of fermentation and then decreased (Fig. 5). Analysis of the culture supernatant showed that the enzyme activity started with an initial productivity of 126.20 U/ml on the fifth day and ended with a productivity of (58 U/ml) on the 13th day.

Initial pH and temperature optimization

The enhancement or inhibition of the enzyme can be influenced by the change in pH and can affect the growth of microorganisms [23]. The microbial enzyme activity can be influenced by the charges present on the surface of the amino acids. A decrease in enzyme activity of the microorganisms could be affected by the modifications in their pH optima. The aim of this study is to reach optimum conditions such as the optimum pH for maximum L-asparaginase production. Different pHs (3–9) will be studied using 1N HCl or 1N NaOH.

At pH 6, the maximum pH 6.5 gives a maximum activity of 141.81 U/ml (Fig. 6). The enzyme activity decreased may be due to the inhibitory effect of both alkaline and acidic pHs on enzyme production. Change of the shape and properties of an enzyme and/or the substrate is related to the change in pH values and consequently prevents the binding of a substrate to the enzyme [18].

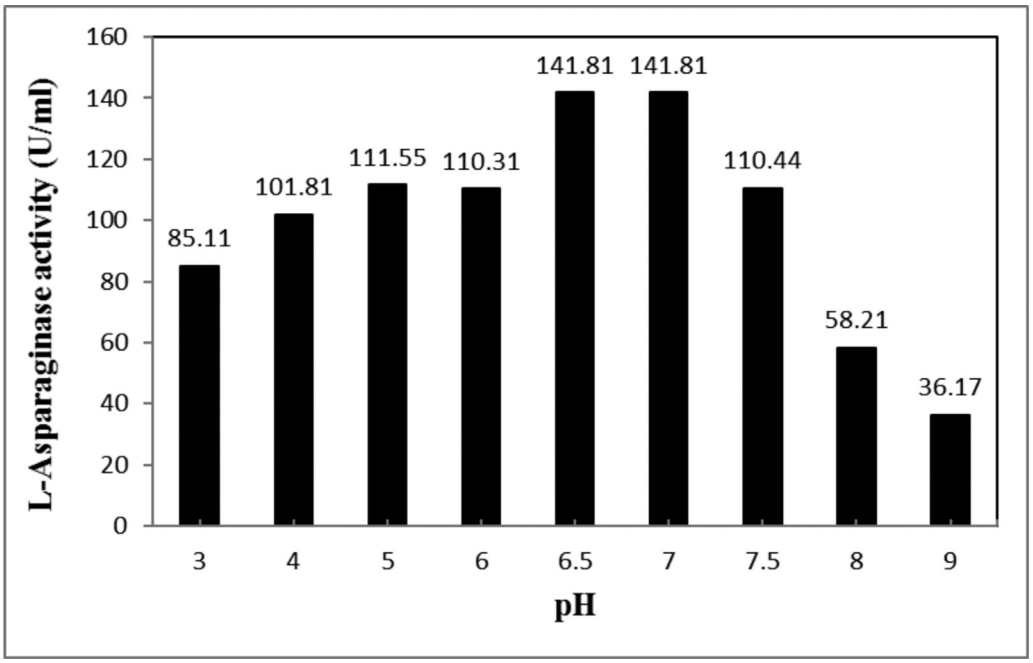
L-asparaginase production

One of the most critical factors for L-asparaginase production is the incubation temperature which affects the microbial growth, enzyme secretion, rate of the chemical reaction, which in turn affects the rate of enzymatic activity. The effects of inhibition, cell viability, and death could be determined by the incubation temperature. In the present study, the maximum activity obtained was 158 U/ml at 30°C (Fig. 7). By increasing the incubation temperature, a gradual decrease in enzyme production was obtained. This may be due to the heat accumulation in the medium during the process.

Effect of carbon sources on L-asparaginase production

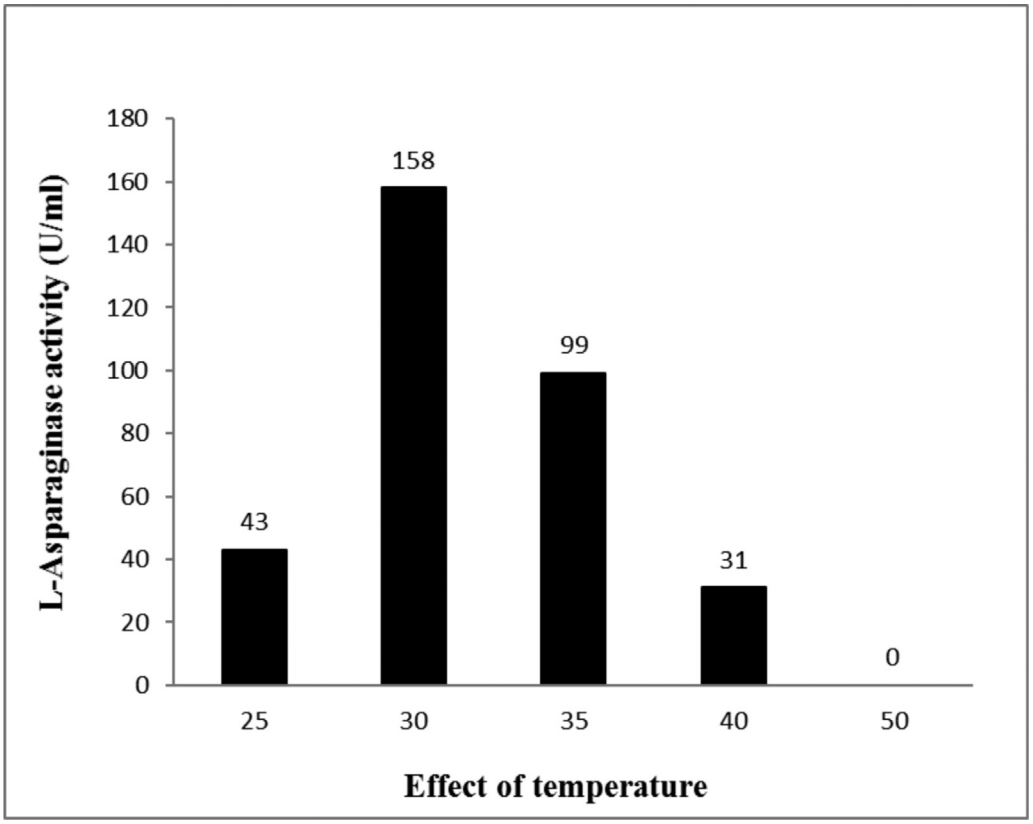
Generally, the most significant carbon source used in the fermentation medium is the carbohydrates. During industrial fermentation process, the growth energy of the microorganism is achieved either from the oxidation of medium components or from light. The enzyme activity obtained was 405 U/ml when lactose was used as a carbon source. On the other hand, mannitol recorded the lowest L-asparaginase production (22 U/ml) (Fig. 8). When sucrose is added to the basal medium, it acts as a good carbon source for L-asparaginase production by actinomycetes isolated from estuarine fishes [24].

Figure 6



Effect of different pH values on *Streptomyces griseoplanus.*

Figure 7

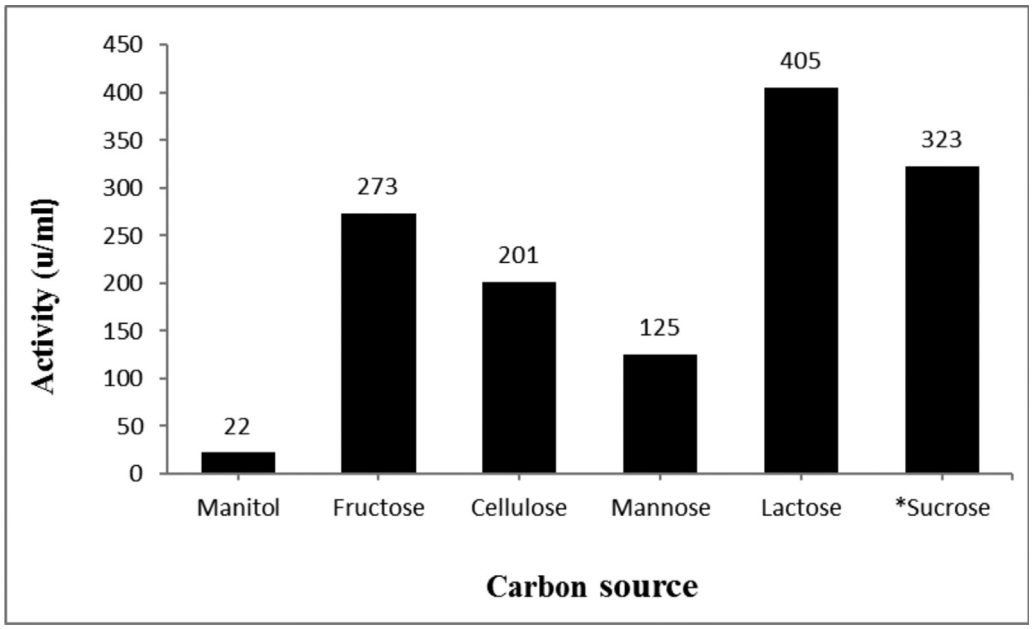


Effect of temperature on *Streptomyces griseoplanus* L-asparaginase production.

Effect of nitrogen source on L-asparaginase production

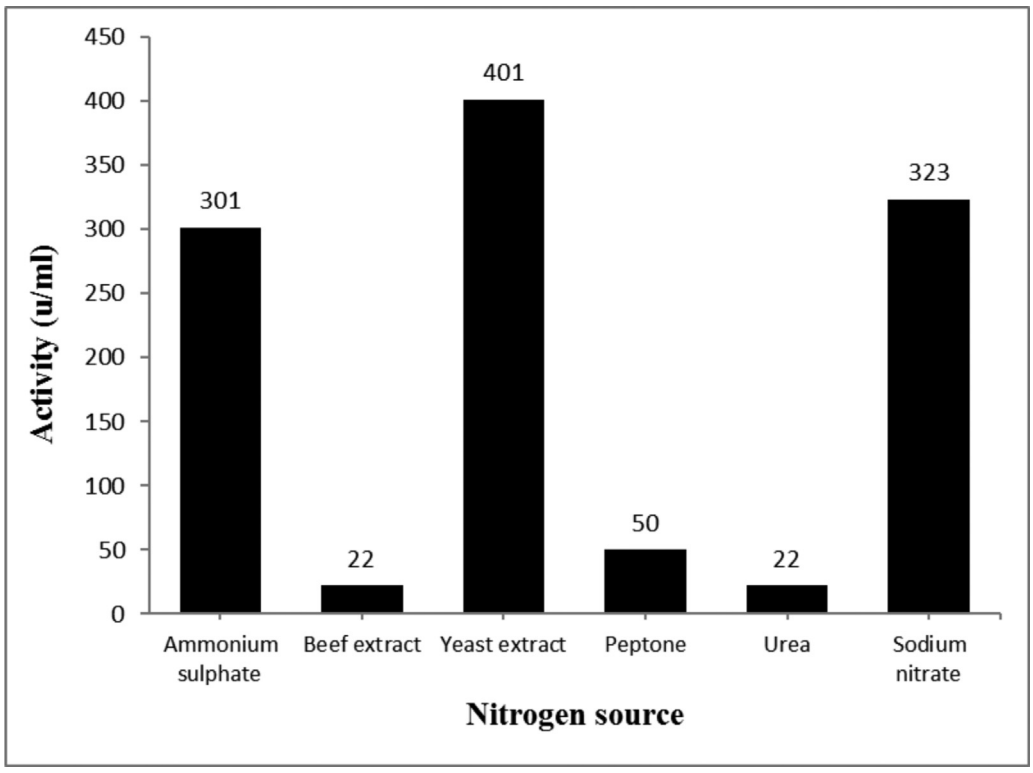
Enhancing the production of L-asparaginase is highly affected by the nitrogen sources either the organic form or inorganic form. Both of them are utilized by most of the industrial enzymes and the growth of the most industrial fermentation process will be faster with a supply of organic and inorganic nitrogen source.

Figure 8



Effect of different carbon sources on *Streptomyces griseoplanus* L-asparaginase production.

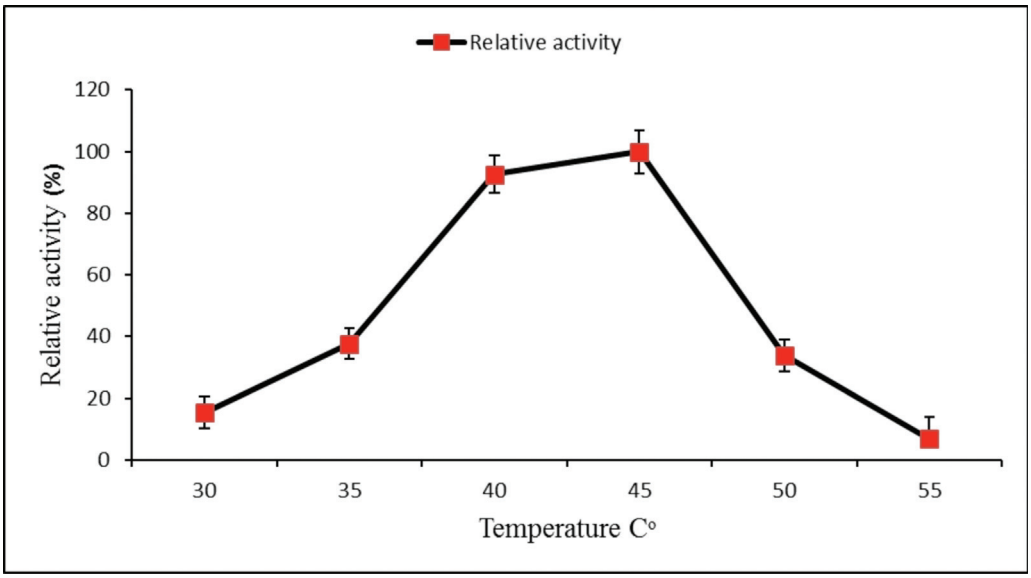
Figure 9



Effect of different nitrogen sources on *Streptomyces griseoplanus* L-asparaginase production.

Yeast extract in the medium enhanced L-asparaginase production (401 U/ml) (Fig. 9) as compared with the other tested nitrogen sources. These results are in good agreement with those reported for the production of L-asparaginase by other microorganisms, *Streptomyces albidoflavus*, yeast extract has been reported as a good nitrogen source [14]. Similar results have been reported by Khamna *et al.* [13] in their studies associated with the production of L-asparaginase from isolates obtained from rhizosphere soil of a Thai medicinal plant.

Figure 10



Effect of temperature on the activities of the native *Streptomyces griseoplanus* L-asparaginase.

Properties of *S. griseoplanus* L-asparaginase

Effect of assayed temperatures on Streptomyces asparaginase activity

When profiling the reaction temperature against the relative activity, the maximum activity was achieved at 45°C for the enzyme. These results were similar to that of L-asparaginase *Aspergillus terreus* KIS2, which exhibited an optimum temperature at 45°C [25]. Low L-asparaginase activity observed at higher temperatures may be attributed to partial denaturation [26]. The relative activity of L-asparaginase was 6.77% (Fig. 10), when the reaction temperature rose to 55°C. The Arrhenius plots of the enzyme were linear and the activation energy (Ea) was calculated to be 2.884 Kcal/mol. At higher temperatures, low L-asparaginase activity was observed and this may be attributed to the partial denaturation of the enzyme [26].

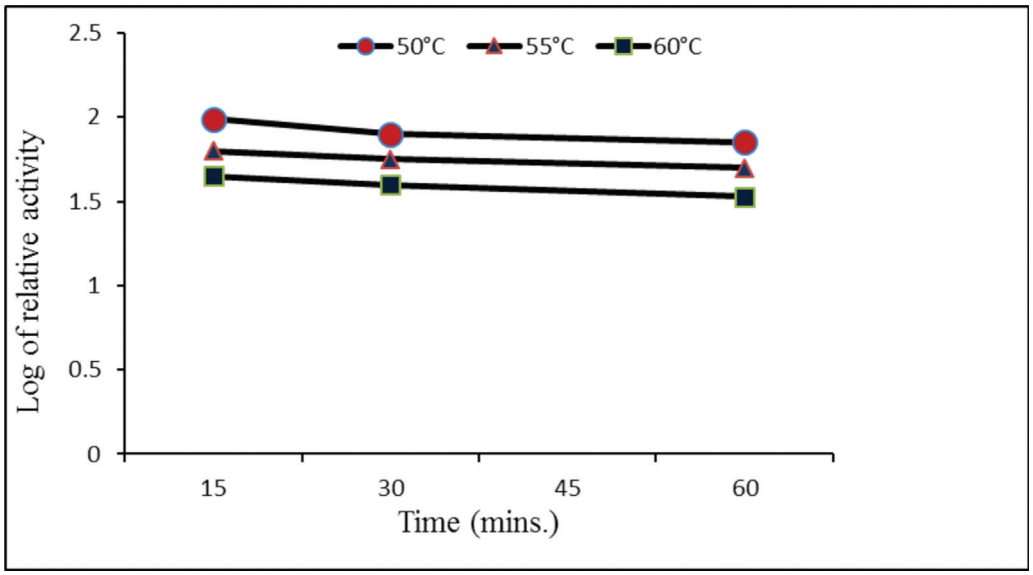
Thermal stability for the native Streptomyces spp. L-asparaginase

Thermal stability for the native *Streptomyces* spp. L-asparaginase native *Streptomyces* spp. was studied in terms of residual activities. At a temperature of 45°C, the enzyme retained its residual activity (100%) for 1 h. In this experiment the free enzyme was incubated, in the absence of the substrate, at different temperatures (30–65°C) for different incubation periods (15, 30, and 60 min). Thereafter, the relative activities were determined under standard conditions. The results showed that the relative activities of the enzyme was completely stable (100%) when incubated at 30–45°C. However, the enzyme, was slightly affected when heated to a temperature of 50°C (it lost about 20% for 30 min). It is more or less resistant to high temperature (50–65°C). It lost 40% of its relative activity when exposed to a temperature of 55°C for 30 min On heating the enzyme at 60°C, the adverse effect of the temperature was observed. The enzyme lost 65% of its relative activity for 1 h. At a temperature of 65°C, it has completely lost its activity [27]. The enzyme preparations give straight lines when the relative activities were plotted against time at different temperatures (50, 55, 60°C) (Fig. 11). This means that the theoretical curves of the first-order reaction can be applied on the thermal inactivation process of the enzyme form. The half-lives of the enzyme were calculated to be 521 min (8.5 h) at 50°C, 312.6 min (5.2 h) at 55°C), and 195.2 min (3.25 h), at 60°C. The deactivation rate constants were calculated at temperatures of 50, 55, and 60°C for the native enzyme to be 1.33×10^{-3}, 2.22×10^{-3}, and 3.55×10^{-3}, respectively (Table 1).

Table 1 Activation energy, half-life, and deactivation rate constant of *Streptomyces griseoplanus* L-asparaginase

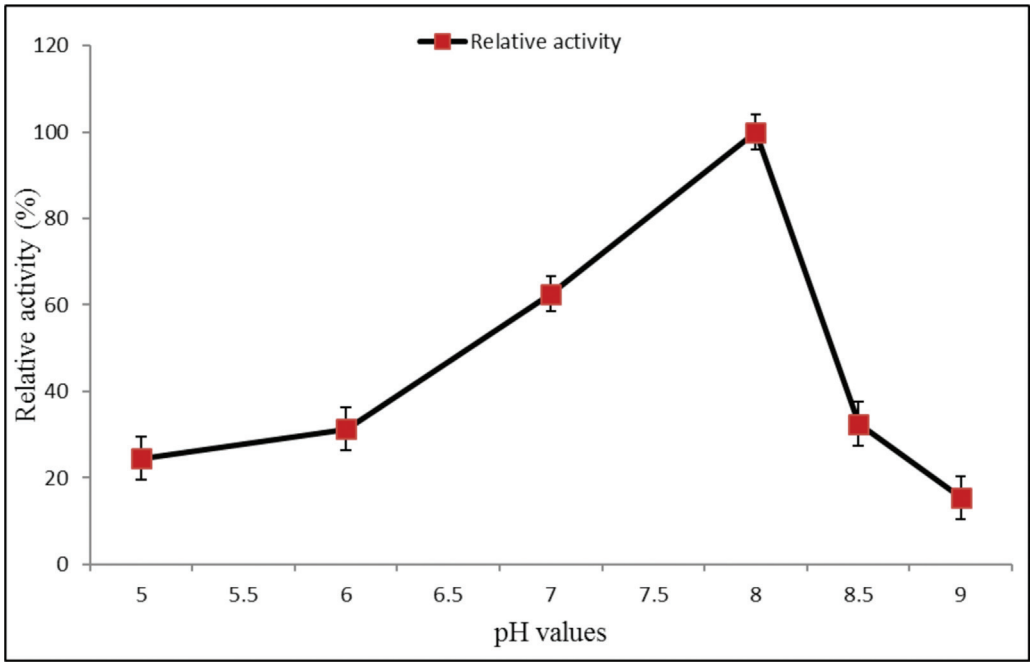
Kinetic parameters	Native enzyme
Activation energy (Kcal/mol)	2.844
Half-life time (min)	
50°C	521
55°C	312.16
60°C	195.2
Deactivation rate constant	
50°C	1.33×10^{-3}
55°C	2.22×10^{-3}
60°C	3.55×10^{-3}

Figure 11



First-order plots of thermal inactivation for the native *Streptomyces griseoplanus* L-asparaginase activity.

Figure 12



Effect of pH values on the activities of native *Streptomyces* spp. L-asparaginase.

Optimum pH for the native enzyme

The maximum relative activity of native L-asparaginase was achieved at pH 8. It retained only 32.5% and 15.48% of its relative activity at pH 8.5 and 9, respectively. The optimum activity of L-asparaginase is at an alkaline pH of 8 (Fig. 12). This is reported by Tabandeh and Aminlari [28]. This can be explained by the fact that L-aspartic acid is produced and inhibit the enzyme under acidic conditions. Dhevagi and Poorani [29] found that pH 8 and pH 8.5 were the most optimum pHs for L-asparaginase activity of *Streptomyces* spp. PDK7 and pH 9 was the optimum for L-asparaginase activity extracted from *S. gulbargensis*. Similar results were found by El-shafei *et al.* [30], who revealed that pH 8.0 was the optimal for L-asparaginase from *Penicillium brevicompactum*. Ohshima *et al.* [31] found that, in general, L-asparaginase is active at neutral and alkaline pHs.

Moreover, in 2009 Basha *et al*. reported that the maximum activity of asparaginase by marine actinomycetes between pH 7.0 and 8.0 with 80% activity at physiological pH [31].

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Hosamani R, Kaliwal BB. Isolation, molecular Identification and optimization of fermentation parameters for the production of an anticancer agent by *Fusarium equiseti*. Int J Microbiol Res 2011; 3:108–119.

2 McKnight S. Gene switching by metabolic enzymes – how did you get on the invitation list? Cell 2003; 114:150–152.

3 Poorani E, Saseetharan MK, Dhevagi P. L-asparaginase production and molecular identification of marine *Streptomyces sp* strain EPD 27. Int J Integ Biol 2009; 7:150–155.

4 Patro KKR, Satpathy S, Gupta N. Evaluation of some fungi for L-asparaginase production. Ind J Fund Appl Life Sci 2011; 1:219–221.

5 Bhat MR, Nair JS, Marar T. Isolation and identification of L-asparaginase producing *Salinicoccus Sp*. M KJ997975 from soil microbial flora. Int J Pharm Sci Res 2015; 6:3599–3605.

6 Jain R, Zaidi KU, Verma Y, Saxena P. L-asparaginase: promosing enzyme for treatment of acute lymphoblastic leukemia. People's J Sci Res 2012; 5:29–35.

7 Kumar KPV, Girija SG, Prabhakar T. Optimization of L-asparginase production by *Streptomyces griseoluteus* WS3/1 using experimental methods. J Pharm Biomed Sci 2011; 10:1–6.

8 Mostafa SA. Activity of L-asparaginase in cells of *Streptomyces karnatakensis*. Zentralbl Bakteriol Naturwiss 1979; 134:343–351.

9 Mostafa SA. Production of L-asparaginase by *Streptomyces karnatakensis* and *Streptomyces venezuelae*. Zentralbl Bakteriol Naturwiss 1979; 134:429–436.

10 Noura EE, Hassan M, Nancy ME, Sara ME. Optimization of culture conditions for production of the anti-leukemic glutaminase free L-asparaginase by newly isolated *Streptomyces olivaceus* NEAE-119 using response surface methodology. BioMed Res Int 2015; 2015:627031.

11 Deshpande N, Choubey P, Agashe M. Studies on optimization of growth parameters for L-asparaginase production by *Streptomyces ginsengisol*. Sci World J 2014; 014:895167.

12 Abeer AE, Heba AE, Mona SS, Rania Z, Hanan M. Statistical optimization of L-asparaginase production by using Fusarium solani. Egypt Pharm J 2017; 16:16–23.

13 Khamna S, Yokota A, Lumyong S. L-Asparaginase production by actinomycetes isolated from some Thai medicinal plant rhizosphere soils. Int J Integ Biol 2009; 6:22–26.

14 Narayana KJP, Kumar KG, Vijayalakshmi M. L-Asparaginase production by *Streptomyces albidoflavus*. Ind J Microbiol 2008; 48:331–336.

15 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193:265–275.

16 Amena S, Ishalakshi NV, Prabhakar M, Dayanand A, Lingappa K. Production, purification and characterization of L-Asparaginase from *Streptomyces gulbargensis*. Braz J Microbiol 2010; 41: 173–178.

17 Han D, Rhee JS. Characteristics of lipase-catalyzed Hydrolysisolive oil in AOT-Isoactane reversed micelles. Biotech Bioeng 1986; XXVIII: 1250–1255.

18 Altschul SF, Madden TL, Schäffer AA. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997; 25:3389–3402.

19 Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987; 4:406–425.

20 Tamura K, Dudley J, Nei M, Kumar S. MEGA4:molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 2007; 24:1596–1599.

21 Datar R. Economics of primary separation steps in relation to fermentation and genetic engineering. Process Biochem 1986; 21:19–26.

22 Niharika YC, Supriya S. Production of L-asparaginase by Fusarium oxysporum using submerged fermentation. Int J Pharm Sci Invention 2014; 3:32–40.

23 Jalgaonwala RE, Mahajan RT. A review: bacterial endophytes and their bioprospecting. J Pharm Res 2014; 4:795–799.

24 Sahu MK, Sivakumar K, Poorani E, Thangaradjou T, Kannan L. Studies on L-Asparaginase enzyme of actinomycetes isolated from estuarine fishes. J Environ Biol 2007; 28:465–474.

25 Siddalingeshwara KG, Lingappa K. Key fermentation factors for the synthesis of L-asparaginase – an anti-tumor agent through solid state fermentation methodology. Int J Pharm Sci 2010; 1:103–112.

26 Dange VU, Peshwe SA. Production, purification and characterization of fungal L-asparaginase. Bion Fron 2011; 4:162–167.

27 Kumar S, Kikon K, Upadhyay A, Kanwar SS, Gupta R. Production, purification and characterization of lipase from thermophilic and alkaliphilic *Bacillus coagulans* BTS-3. Protein Express Purif 2005; 41:38–44.

28 Tabandeh MR, Aminlari M. Synthesis, physicochemical and immunological properties of oxidized inulin-l-asparaginase bioconjugate. J Biotech 2009; 141:189–195.

29 Dhevagi P, Poorani E. Isolation and characterization of L-Asparaginase from marine actinomycetes. Ind J Biotechnol 2006; 5:514–520.

30 El-shafei AM, Hassan MM, Abd-Elmontasr M, Mahmoud DA, Lghonemy DH. L-asparaginase from *Penicillium brevicompactum* NRC 829. Brit Microbiol Res J 2012; 2:158–174.

31 Ohshima M, Yamamoto T, Soda K. Further characterization of glutaminase isozymes from *Pseudomonas aeruginosa*. Agri Biolog Chem 1976; 40:2251–2256.

32 Basha NS, Rekha R, Komala M, Ruby S. Production of extracellular anti-leukaemic enzyme l-asparaginase from marine actinomycetes by solid state and submerged fermentation: purification and characterization. Tropical J Pharm Res 2009; 8:353–360.