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Studies on the production and optimization of L-asparaginase by using *Streptomyces spp*

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Abstract

L-asparaginase is receiving the attention because of its potential use as a chemotherapeutic agent in tumour and acute lymphoblastic leukemia. In the present study an attempt has been made to produce L-asparaginase by using *Streptomyces* species isolated from soil. The test isolates were initially screened by rapid plate assay method for L- asparaginase production. *Streptomyces* showed the highest productivity of 45.2 IU/ml with a protein content of 65 µg/ml, and optimum activity at pH 7.5 and 30ÚC. This study clearly indicates that soil can provide a rich source of L-asparaginase producing actinomycetes.

Keywords: *Streptomyces spp*, L-asparaginase, *Actinomycetes*, Rapid plate assay method.

INTRODUCTION

L-Asparaginase,the enzyme which catalyses the hydrolysis of asparaginase to aspartic acid and ammonia,is an important natural enzyme that possesses a broad spectrum of anti-tumour activity. It has been successfully applied to the treatment of cancers such as lymphocyte sarcoma and leukemia. Literally called L-asparaginase aminohydrolase, has been successfully applied to various applications. Clinical studies have demonstrated that the leukemia patients treated with L-asparaginase have shown relatively higher recovery efficiency when compared to the recovery efficiency of leukemia patients treated with IL-2,L-asparaginase, an important chemotherapeutic agent used for the treatment of a variety of lymphoproliferative disorders and lymphomas,acute lymphoblastic leukemia (ALL) in particular. It has been a mainstay of combination chemotherapy protocols used in the treatment of pediatric ALL for almost 30 years.

Hence, L-asparaginase has been included in most contemporary multi-agent regimens for adult ALL.The pioneering work by Clementi in 1922 also revealed the presence of high L-asparaginase activity in the serum of guinea pig, which has led to the development of L-asparaginase as a potential antineoplastic agent. The production of L-asparaginase has been studied in *Serratia marcescens* and *Erwinia carotovora.* Actinomycetes have been shown to be a good source of L-asparaginase. Various Actinomycetes such as *Streptomyces griseus, S. karnatakensis ,S. albidoflavus* and *Nocardia spp* have the abilities to produce the enzymes (Delong,1972; Narayana, 2007; Mostafa and Salama, 1979).The present study deals with the L-asparaginase activity of *Streptomyces spp* isolated from soil.

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MATERIALS AND METHODS:

Microorganism

Soil samples were collected from paddy field in sterile polythene bags to avoid external contamination. Isolation of actinomycetes was performed by plating techniques using starch casein agar medium (starch 10g, casein 0.1g, ferrus sulphate 0.01g, calcium carbonate 0.02g, potassium nitrate 2g, magnessium sulphate 0.05g, sodium chloride 2g, agar 20g, distilled water 1000ml). The soil sample was diluted and spread over the agar plates. The inoculated plates were incubated at 28±2°C for 7-10days. After purification the culture was identified as *Streptomyces spp* on the basis of their morphological and biochemical characteristics.

Screening of L-asparaginase production by rapid plate assay method

The isolates of *Streptomyces* species were subjected to rapid plate assay method for screening of L-asparaginase production as per the method of Gulati *et al*.(1997).The modified Czapedox's medium containing (gL-1)glucose 2g, L-aspargine 10g, KH_2PO_4 1.52, KCl 0.52, $MgSO_4$,$7H_2O$ 0.52, $CuNO_3$.H_2O trace, $ZnSO_4$ $7H_2O$ trace, $FeSO_4$ $7H_2O$ trace and agar 20 was used in the screening process . The medium was supplemented with phenol red dye (2.5% solution prepared in ethanol and pH was adjusted to 7.0), autoclaved and plates were prepared. The control plates were prepared without L-asparaginase (instead containing $NaNO_3$ as the N_2 source). The plates were centrally inoculated 100ml of spore suspension of the *Streptomyces* isolates and incubated at 37°C for one week. The enzyme activity was recorded based on the degree of zone of hydrolytic activity.

Determination of L-asparaginase activity

The active strains were cultured on ADS broth at pH 7.0 and incubated at 28±2°C with shaking at 125 rpm

for 7days. The growth of actinomycetes was measured and expressed as dry weight. L-asparaginase activity was measured following the method of Imelda, (1973).The cultures were centrifuged at 11,000Xg for 15 min. This method utilizes the determination of ammonia liberated from L-asparagines in the enzyme reaction by the Nessler's reaction. Reaction was started by adding 0.5ml supernatant into 0.5ml 0.04 M L-asparagines and 0.5ml 0.05 M tris (hydroxymethyl) amino methane (tris-HCL) buffer pH 7.2 and incubated at 37°C for 30 min.The reaction was stopped by the addition of 0.5 ml.0.5 M trichloroacetic acid (TCA). The ammonia released in the supernatant was determined colorimetrically by adding 0.2 ml Nessler's reagent into tubes containing 0.1 ml supernatant and 3.75 ml distilled water and incubated at room temperature for 10 min, and absobance of the supernatant was read using a UV-visible spectrophotometer at wavelength of 450 nm. One unit of asparaginase is the amout of enzyme which catalysed the formation of 1 mol of ammonia per min at 37°C. The reaction mixture from each strain was assayed in triplicate.

Effect of incubation time

Strain of *Streptomyces* was cultured in 50 ml modified Czapek Dox broth by adding buffer solution ranging from 6.0-9.0 at 30°C with shaking at 125 rpm for 10days. A sample was periodically withdrawn at every 24 h and L-asparaginase activities were measured.

Effect of pH

Strain of *Streptomyces* was cultured in 50ml modified Czapek Dox broth by additing buffer solution ranging from 6.0-9.0 at 30°C with shaking at 125 rpm for 7 days.Then the activity was read by using UV-Vis Spectrophotometer.

Effect of temperature

Strain of *Streptomyces* was cultured in 50ml of modified Czapek Dox broth and incubated at various temperatures (10, 25, 30, 37 and 45°C) with shaking at 125 rpm for 7 days. Then the activity was read by using UV-Vis Spectrophotometer.

RESULTS AND DISCUSSION

Spore suspension of the isolate was grown on starch casein agar medium. The L-asparaginase activity of *Streptomyces* was assessed by rapid plate assay method.The results showed the formation of pink coloured zone around the colony.It indicated the L-asparaginase activity of *Streptomyces*. The L-asparaginase activity of *Streptomyces* was determined by Nesseler's reagent. The yellow colour was observed. Production of L-asparaginase started after 24h and reached a maximum after 168h (7 days) and then decreased. The enzyme activity was optimum at pH 7.0 . The maximum growth and activity of L-asparaginase was produced at 30°C. Soil isolate *Streptomyces* showed the highest productivity of 45.2 IU/ml and optimum activity was at pH7.5 and 30°C. L-asparaginase has been considered as a therapeutic agent against malignant tumours.(Clementi, 1922) .Clinical studies clearly established that the leukemia patients treated with L-asparaginase showed relating higher recovery when compared to the patients treated with chemotherapeutic agents.

L-asparaginase treatment for acute lymphoblastic leukemia is a major break through in modern biology as it induces complete remissions in over 90% children with in four weeks (Table 1-4 and Fig.1-3).

Table 1: Identification chart for Gram staining and biochemical tests

S.No.	Name of Test	*Streptomyces*
1.	Gram Staining	+
2.	Indole Test	+
3.	Methyl Red Test	+
4.	Voges-Prosauer Test	+
5.	Citrate utilization Test	-
6.	Triple Sugar Iron agar Test	+

Table2: Effect of incubation time on L-asparaginase activity

S.No.	Incubation Time	Activity µ mol/ ammonia/ml/hr
1.	24	0.00
2.	72	0.40
3.	120	0.80
4.	168	1.60
5.	216	0.60

Table 3 : Effect of pH on L-asparaginase activity

S.NO	pH	Activity µ mol/ammonia/ml/hr
1.	6	0.00
2.	7	1.20
3.	8	0.80
4.	9	0.60
5.	10	0.20

Table 4: Effect of temperature on L-asparaginase activity

S.No.	Temperature	Activity µ mol/ammonia/ml/hr
1.	10	0.00
2.	25	1.20
3.	30	1.40
4.	37	0.60
5.	45	0.20

Fig 1: Effect of incubation time on L-asparaginase activity

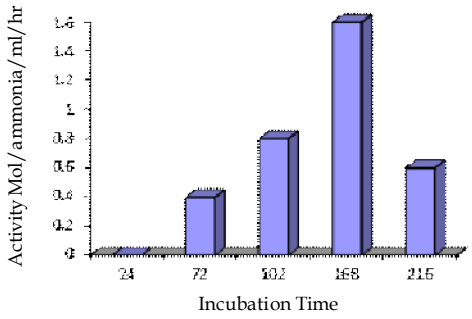


Fig 2 : Effect of pH on L – asparaginase activity

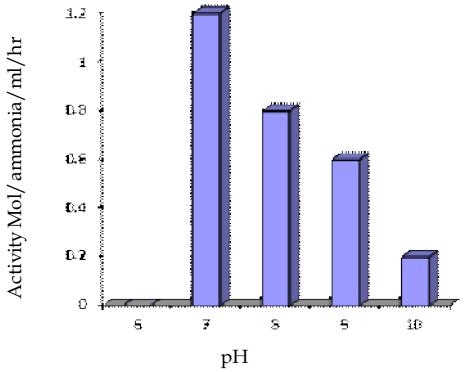
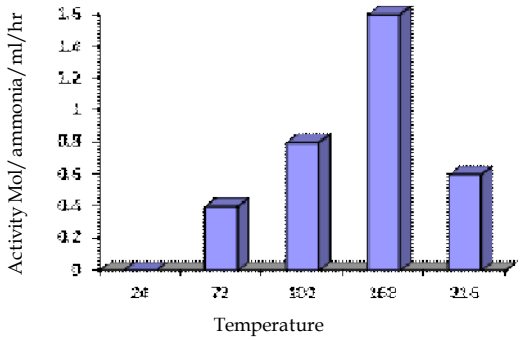


Fig 3 : Effect of temperature on L– asparaginase activity



From this study, it is clearly indicated that paddy field soil can provide a rich source of L-asparaginase producing actinomycetes .The isolate *Streptomyces* showed maximum activity at PH 7,incubation time 168 h and temperature 30°C.The potential of L-asparaginase an accepted act for application in the treatment of childhood acute lymphocytic leukemia and other forms of cancer.

REFERENCES

Clementi ,A.La. 1922 desemidation enzymatique de L-asparaginase chez les differentes especes animals et la signification physiologique de sa presence dans l organisma. *Arch.Int. Physiol.*,;19:369-76.

Delong,P.J.1972. L-asparaginase production by *Streptomyces* griseus. *Appl. Environ, Microbio.*,23(167):1163-1164.

Gulati,R.Saxena,R.K.and Gupta, R.A., 1997 Rapid plate assay for screening L-asparaginase producing micro-organisms. *Lett.Appl.Microbiol.*, 24:23-26.

Imelda A.,1973.Asparaginase and glutaminase activities of microorganism. *J. Gen. Microbiol.*,76:85-99

Mostafa,S .A.,Salama,MS. 1979. L-asparaginase producing *Streptomyces* from soil of *Kuwait.Zentralbl Bakterol Naturwiss.*,134:325-334

Narayana, KJP. 2007. L-asparaginase production by *Streptomyces albidoflavus. Indian J.Microbiol.*,48:331-336.