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RESEARCH ARTICLE

MICROBIOLOGY

ISOLATION, PURIFICATION & CHARACTERIZATION OF ASPARGINASE FROM *ESCHERESHIA COLI* .

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ABSTRACT

Asparginase is an enzyme that catalyzes the hydrolysis of aspargines to aspartic acid. It is marketed under the brand name Elspar to treat acute lymphoblastic leukemia. *Eschereshia coli,* capable of producing asparginase was isolated from local habitats and screened for its production on corn steep liquor. The extracted enzyme was purified by ammonium sulphate precipitation,dialysis and anion exchange chromatography and was estimated quantitatively by Lowry method. Effect of temperature and pH were also studied on the purified enzyme and the enzyme activity was found to be optimum at pH 5 and 37 ° C temperature.

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KEY WORDS

Asparginase, *Eschereshia coli,* anion exchange chromatography.

INTRODUCTION

L-asparaginase is known for its potent ability to be used as a therapeutic agent for the treatment of leukemia since many years. Microbial L-asparaginase has attracted considerable attention since the demonstration that L-asparaginase from *E.coli* has anti-tumor activity[1]. L-asparaginase is now known to be a potent antineoplastic agent in animals and has given complete remission in some human leukemia's [1-3] found L-asparaginase to be responsible for the antitumor activity of guinea pig serum. Subsequently, it was identified as an effective antitumor agent in human clinical trials, and today it is regarded as one of the useful components of the antitumor therapy. Some of the L-asparaginase enzyme derived from *E.coli* was also proved as an effective antitumor activity similar to guinea pig serum [4]. This event opened up the possibility of large scale production of the enzyme for ultimate clinical trials. As a result, nowadays more attention has been given to isolate the L-asparaginase enzymes by microorganisms. Different microorganisms were used for the screening; some of the Pseudomonas species were also proved for the production of L-asparaginase enzyme [5]. Several terrestrial Streptomyces like S. karnatakensis, S. venezualae, S. longsporusflavus and S. albidoflavus are capable of producing detectable amount of L-asparaginase [6]. There are limited reports on production of L-asparaginase from marine Streptomyces like S. aurantiacus [7], Streptomyces sp. PDK2 and PDK7 [8]and Streptomyces sp. S3, S4 and K8 [9]. The enzyme is produced throughout the world by both submerged and solid-state cultures. Extra-cellular asparaginases are more advantageous than intracellular since they could be produced abundantly in the culture broth under normal conditions and can be purified economically.

MATERIALS AND METHODS

Isolation of microbes

Soil samples were subjected to 10 fold serial dilution and aliquots of (0.1 ml) were plated on Nutrient agar for the isolation of *Eschereshia coli* .Plates were incubated for 24hrs at optimal conditions. The isolated strains were kept in slant cultures at 4°C for further investigations.

Characterization of isolated strains

The isolated strains were studied for morphological, cultural and biochemical characteristics. The morphology was identified by high resolution microscope.

Screening of the strains for the production of Asparginase

Eschereshia coli was inoculated separately in a 50 ml conical flask containing production media (corn steep liquor) and incubated at 37°C for one week. The culture was centrifuged at 6000rpm for 10 min. Supernatant was collected and filtered by Whatman filter paper aseptically. The supernatant obtained was the crude asparginase.

Purification of asparginase

Ammonium sulphate precipitation

The supernatant was taken in a clean beaker. The beaker was kept in cool condition with the help of ice bags. Appropriate amount of

ammonium sulfate was added into the beaker, stirred, incubated overnight and the pellet was collected by centrifugation for 10 min at 10000 rpm. The pellet was dissolved in 10 ml of 10mM Tris Hcl followed by dialysis in dialysis bags with sodium phosphate buffer at 4° C overnight.

Anion exchange chromatography

Six elutes were prepared with 25mM HCl and different concentrations of Sodium chloride ranging from 25mM to 150mM in six different tubes. Column washed with ethanol and distilled water, followed by the addition of DEAE Cellulose .After eluting the column with the buffer, enzyme was added and left for 15min for settling. Elution was carried out by the prepared elutes and the collected samples O.D was taken at 436nm.

Quantitative estimation

A series of BSA solution were set up in 6 test tubes using 0.2 to 1.0 ml of the stock solution and distilled water was added to make up the volume to 1 ml. 5ml of alkaline copper solution was added and incubated for 10min at room temperatute.0.5 ml of Folin-ciocalteau reagent was added and incubated in dark for 30min. Distilled water was used as blank. Absorbance was taken at 660 nm after setting to zero absorbance with the blank.

Enzyme kinetics

Effect of pH and temperature was studied on the purified enzyme.

RESULTS & DISCUSSION

The present study revealed that the isolated strain ***Eschereshia coli*** was circular, Gram negative rod with entire margin and tested positive for lactose fermentation , MR-test and indole production test (Table-1) . The enzymeactivity was observed optimum at pH 5 and 37°C temperature (Fig- 1 & 2).Table -2 shows the asparginage assay by ion exchange chromatography and Table-3 shows the quantitative estimation of asparginage.

Table-1
Biochemical test results for Eschereshia coli

Biochemical tests	Result
Gram staining	-ve rods
Lactose fermentation	+ve
Indole production test	+ve
Vogus proskauer	-ve
Methyl red	-ve
Citrate utilization	-ve

Table-2
Assay of the enzyme by ion exchange chromatography

Elutes	Units/ml of enzymes
1.	0.02
2.	0.08
3.	0.07
4.	0.05
5.	0.02
6.	0.03

Test tube no:	Vol. of BSA(ml)	Vol.of Distilled water (ml)	Vol.of Copper sulphate(in ml)	Incubation 10min (room temp.)	Vol.of FC reagent (in ml)	Incubation 10 min (dark)	OD at 660nm
00	Blank	1.0	5		0.5		0.000
01	0.2	0.8	5		0.5		0.008
02	0.4	0.6	5		0.5		0.011
03	0.6	0.4	5		0.5		0.018
04	0.8	0.2	5		0.5		0.020
05	1.0	----	5		0.5		0.025

Table-3
Quantitative estimation of asparginase by Lowry method

Test tube No.	Vol.of enzyme	Vol.of Distilled water(ml)	Vol. of Copper sulphate(ml)	Incubation 10 min(room temp.)	Vol.of FC reagent	Incubation 10 min (dark)	O.D at 660 nm
Crude	0.1	0.9	5		0.5		0.266
Elute 2	0.1	0.9	5		0.5		0.180
Elute 3	0.1	0.9	5		0.5		0.149
Elute 4	0.1	0.9	5		0.5		0.130
Dialysis	0.1	0.9	5		0.5		0.122

Fig-1
Effect of pH on enzyme activity

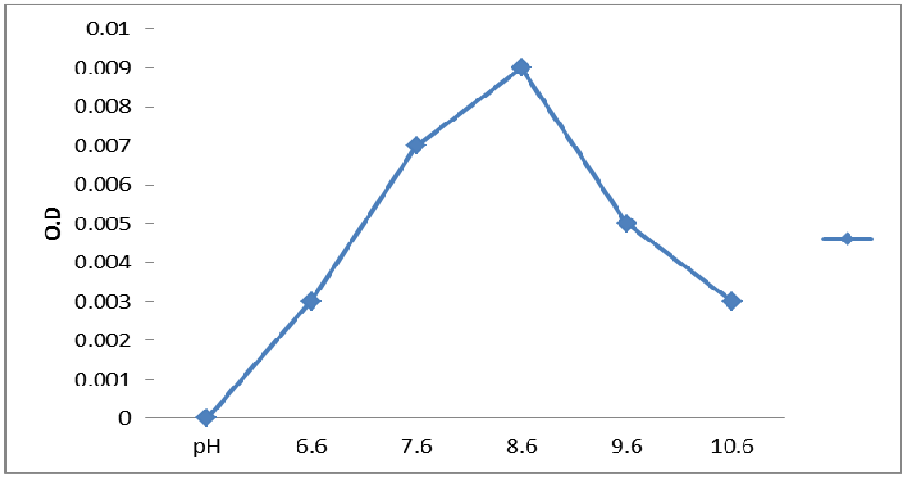
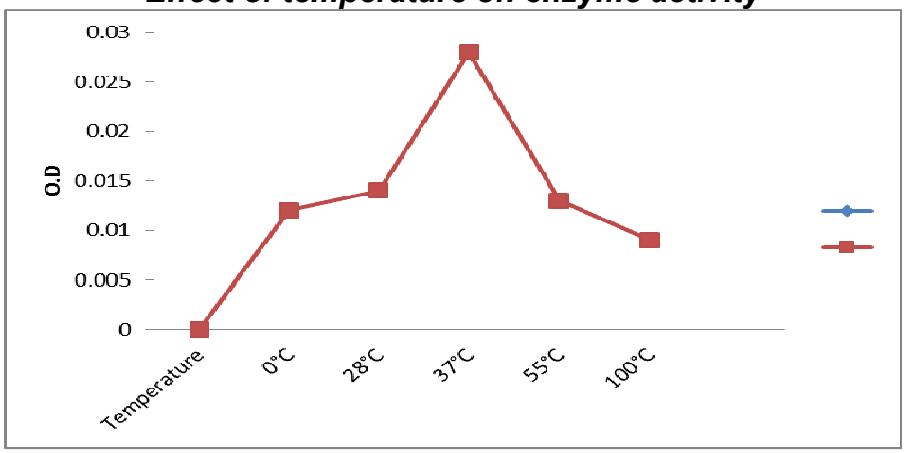


Fig-2
Effect of temperature on enzyme activity



CONCLUSION

The enzyme L-Asparginase is obtained from the bacteria Escherichia coli. It also contains the asparginase without the antileukemic activity. This asparginase is removed during the purification of the enzyme. The enzyme is also obtained from plant and animal tissues, fungi and yeast in pure form. This enzyme catalyses the hydrolysis of L-aspargine to L-aspartate and ammonia. It is present as white and crystalline powder. It is soluble in water. Each mg of L-asparginase contains 250 units. L-asparginase interferes with the growth of malignant cells, which do not have the capacity of synthesizing L-aspargine for their metabolism. Thus this enzyme is used in chemotherapy of acute lymphocytic leukemia in sequential combination with other drugs. It is also used for induction of remission in children with relapse of acute lymphocytic lymphoma. It also shows immuno-suppressant activity.

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