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Bioactive L-asparaginase production by *E.coli* strains

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ABSTRACT

L-asparaginase is an anticancer agent, especially for acute lymphoblastic leukemia. Nine *E.coli* strains were screened forits ability to produce an extracellular L-asparaginase enzyme. The optimum culture conditions for L-asparaginase production was found at pH 8.0, Incubation time 48 h, and 37^{o}C temperature. Highest yields of L-asparaginase(140.5 and 96.4 IU/ml) by *E.coli* strains using glucose and beef extract as sole carbon and nitrogen sources respectively. 0.6 fold of higher L-asparaginase activity (168.4 IU/ml) was recorded using pUC18 UV60 than the parent strain. 0.54 fold increased L-asparaginase activity (220.6 IU/ml)was observed using pUC18 NTG90.The molecular mass of L-asparaginase was determined by SDS-PAGE and it was found to be 29KDa.

Keyword: L-asparaginase, *E.coli*, UV, NTG and SDS-PAGE

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INTRODUCTION

Bacterial L- asparaginase are enzymes of high potency used in treating various kinds of cancers, mainly acute lymphoblastic leukemia. Current clinical studies indicate that this enzyme is also a promising agent in treating some forms of neoplastic cell diseases in man. The antineoplastic activity is attributed to the depletion of L-asparagine by the action of L-asparaginase from *Erwinia carotovora*[1].

The L-asparaginase enzyme produced by *E.coli* strain and its tumour activity was reported earlier[2].Many enzymes have been used as drugs, like wise L-asparaginase attracted much attention because of its use as effective therapeutic agent against lymphocytic leukemia and other kinds of cancer in man[3]. This enzyme is widely distributed, being found in animal, microbial and plant sources as well as large number of microorganisms that include *Erwiniacarotovora*, *Pseudomonas stutzeri*[4], *Pseudomonas aeruginosa*[5]and *Escherichia coli*[6,7].

The primary goal of my research is to investigate various *E.coli* strains for its ability to produce L-asparaginase by submerged fermentation. The focus of my research is the enhanced production of biomedically valuable L-asparaginase production by strain improvement through UV and NTG treatment. The influence of various carbon and nitrogen sources was optimized with respect to differences in pH, temperature and incubation time.

MATERIALS AND METHODS

All the chemicals and reagents were of analytical grade and procured from Galaxo India Limited, Hi-Media, SD fine chemicals, Sigma, Sisco Research Laboratories and Merck.

Bacterial Strains

The bacterial strains used throughout the study for the production of L-asparaginase were *E.coli* strains such as *E.coli*226; *E.coli* DH5α; *E.coli* CSH57; *E.coli*HB101; pUC18, pBR322, pBR329, *E.coli* KL16, and *E.coli* KL12.They were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India.

Growth Medium and Culture Conditions

The microbial strains were grown in the specified Luria Bertani (LB) media having composition: Tryptone (1% w/v), yeast extract (0.5% w/v) and NaCl (1% w/v), pH: 7.2and temperature 37 ^{0}C. It was maintained by further sub-culturing after every 30 days and stored at 4 ^{0}C. The primary inoculum was grown overnight in 10 ml LB medium and was inoculated in 50 ml of LB medium and grown at 37 ^{0}C. Cells were grown to an optical density of 0.25-0.3 at 600 nm for 24 hours in shake flask (200rpm).

Culture media selection for L-asparaginase production

Four different media[8] were selected for the production of L-asparaginase by *E.coli* strains. The composition of four different media is presented in Table 1. The ingredients were weighed and dissolved in distilled water. Then the media were autoclaved. After sterilization, the prepared liquid media was used in the present study.

Table 1 : Culture Media composition for the production of L-asparaginase enzyme by selected *E.coli* strains (Bilimoria, 1969)

Ingredients	Culture Media (g/L)			
	Medium A	Medium B	Medium C	Medium D
Peptone	1.0	0.5	1.0	0.2
Beef Extract	0.5	0.5	0.5	0.2
Yeast Extract	-	0.5	-	-
KH_2PO_4	0.33	-	0.33	0.2
$MgSO_4.7H_2O$	-	-	-	0.05
$FeSO_4$	-	-	-	0.0006
$MnSO_4$	-	-	-	0.0005
$CaCl_2$	-	-	-	0.01
NaCl	-	-	-	1.0
L-asparagine	0.1	0.1	0.5	0.5

Screening of L-asparaginase producing strains

Modified M9 medium was supplemented with different concentrations of the phenol red dye. A 2.5% stock of the dye was prepared in ethanol and the pH was adjusted to 7.0 using mol/lNaOH. The stock solution of the dye, ranging from 0.04 ml to 0.3 ml, was added to 100 ml of modifiedM9 medium, giving final dye concentrations of 0.001-0.009% respectively. The media was autoclaved and plates were prepared. Control plates were prepared using modified M9 medium (i) without dye and (ii) without asparagine (instead containing$NaNO_3$ as nitrogen source). The plates were inoculated with 24-h culture of *E.coli* strains as reference organism. The dye concentration was optimized. Then the zone and colony diameter of the selected isolates and reference organism were measured by the plate assay after 24 h incubation at 37ºC[9]. The selected isolates and reference organism were then subjected to L-asparaginase assay.

Enzyme Extraction

For extraction of the intracellular enzyme, Liquid Nitrogen Method was used. The biomass was separated out by centrifugation (5000 rpm, 20 min and 4 ^{0}C); the pellet was put into a pestle and crushed by a mortar after adding liquid nitrogen. The obtained powder was dissolved in 1 ml of 0.1 M PBS (pH 8.0). The contents were micro centrifuged 5000 rpm, 5 min and 4ºC. The supernatant was used as the enzyme source.

Enzyme Assay

L-asparaginase activity was assayed by a modified method[10]. A 0.1 ml purified enzyme solution, 0.9 ml sodium borate buffer (0.1M, pH 8.5) and 1ml L-asparagine(0.04M) solution were combined and incubated for 10 minutes at 37°C. The reaction was stopped by the addition of 0.5ml of 15%w/v trichloro acetic acid. The reaction contents were centrifuged at 8000 rpm. The supernatant was collected and 0.2ml supernatant was diluted to 8ml with distilled water. The resulting mixture was treated with 1.0ml of Nessler's reagent and 1.0ml of 2.0M NaOH. The color reaction was allowed to proceed for 15 minutes before the absorbance at 500nm was determined. The absorbance was then compared with a standard curve prepared from solutions of ammonium sulfate as the ammonia source. One international unit of L-asparaginase is the amount of enzyme which liberates 1 μ mole of ammonia in 1 minute at 37ºC[11].Determination of protein concentrations were made on whole cell suspension or crude enzyme preparations[12].

Effect of Incubation time

The effect of incubation time on L-asparaginase production was carried out with varying incubation time such as 12, 24, 36, 48, 60 and 72 h. After incubation, the enzyme was extracted and assayed.

Effect of carbon sources

To determine the effect of different carbon sources on L-asparaginase activity, various alternative carbon compounds (glucose, sucrose, maltose, xylose, mannose and starch) were substituted in the medium. The carbon sources were studied at a concentration of 0.1% w/v in the LB medium. The enzyme was extracted and assayed.

Effect of nitrogen sources

To study the effect of different nitrogen sources on L-asparaginase activity, various alternative nitrogen sources such as beef extract, tryptone, yeast extract, peptone, glycine at a concentration of 0.5% for 48 hrs incubation. After incubation, the enzyme was extracted and assayed.

Effect of pH:

Different pH ranges such as2, 4, 6, 8, and 10 for 48 h incubation period was tested for maximal enzyme production.

Effect of temperature

Different temperatures like 25, 30, 37, 40, and 45^{o}C for 48 hrs incubation were tested for favorable L-asparaginase production.

Mutagenesis of L-asparaginase producing *E.coli* strains by UV

The UV treatment for L-asparaginase production was done according to the method[13]. The L-

asparaginase producing microorganisms are exposed to UV for 30, 60, 90, and 120 seconds. After exposing the plates to UV, the plates were kept at room temperature for 24 h. After incubation, the L-asparaginase activity and protein concentration were determined.

Mutagenesis of L-asparaginase producing *E.coli* strains by NTG

The effect of NTG treatment for L-asparaginase production was determined followed by the method[13]. The culture was pelleted by centrifugation, then washed it by using citrate (0.1M; pH-5.5) and Phosphate (0.1 M; pH- 7.0) buffers separately. 0.2 ml NTG (10mg/10 ml) was added at different time intervals such as 0, 30, 60, 90, and 120 seconds. After that, the plates were incubated at 37^{o}C for 24h. After incubation, the L-asparaginase activity and protein concentration were determined.

Purification of L-asparaginase

The purification of produced enzyme was carried out at 4°C on the crude extract according to the modified method[14].

Ammonium sulphate precipitation

Finely powdered ammonium sulphate was addedupto80% saturation level. The mixture was left for 12 h at 4^{o}C followed by centrifugation at 8000 rpm for 20 min at 4°C. The precipitate was dissolved in 0.01 M phosphate buffer pH- 8.5 and dialyzed overnight against the same buffer at 4^{o}C. The dialyzed sample was collected in a sterile container and stored at -20°C for further studies. The protein content and L-asparaginase activity was assayed.

Molecular weight determination

The molecular weight of the purified asparaginase enzyme was determined[15]. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a 3 mm slab gel of 12%. The gels were stained with 0.025 Coomassie brilliant blue R-250 and destained with destaining solution. The standard proteins were used as marker protein for the molecular weight determination (Bangalore Genei).

The molecular weight of partially purified L-asparaginase enzyme was determined by plotting the values in standard graph with log molecular weight versus the mobility. The distance travelled by each protein was measured & the mobility was calculated relative to the tracking dye[16].

$$\text{Mobility} = \frac{\text{Distance travelled by the sampl protein}}{\text{Distance travelled by bromop henol blue}}$$

RESULTS AND DISCUSSION

Screening of L-asparaginase producing strains

The preliminary screening study showed that among the nine *E.coli* strains only five strains produced L-asparaginase enzyme (Table 2). Among the five strains pUC18 produced better L-asparaginase activity followed by DH5α and HB101 strains. Next to this strain,CSH57 as a moderate producer and 226 strain of *E.coli* as a weak producer. The other four strains of *E.coli* were non producers. The present plate assay is advantageous as the method is quick and L-asparaginase production can be visualized directly from the plates without performing time consuming assays[17,18,19]. Similar findings are observed in the present experiment.

Effect of pH on L-asparaginase production

Data on the effect of pH on L-asparaginase enzyme production by *E.coli* strains in submerged fermentation is given in Figure 1. The maximum L-asparaginase activity (84.6 IU/ml) was recorded at pH 8.0 by pUC18 strain of *E.coli*. The produced L-asparaginase enzyme was ranged from 28.4 to 84.5 IU/ml. High and low levels of pH from the optimal level (pH 8.0) the L-asparaginase activity was decreased.

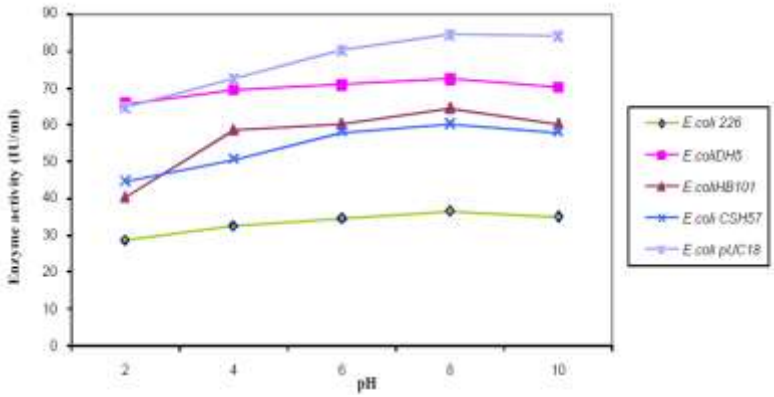

Figure .1: Effect of pH on L-asparaginase production (IU/ml) by various *E.coli* strains in submerged fermentation

The statistical one way ANOVA test for the data on L-asparaginase activity revealed that there was no significant difference (F=0.59; P>0.05) between pH and *E.coli* strains. Followed by ANOVA test, we analyze the pair of mean difference significantly, the data was again analyzed by Tukey test.

The Tukey test also showed that there was no significant difference between the different *E.coli* strains and pH. The present data showed the optimum pH of purified L-asparaginase from *E.coli* was found to be 8.0. El-Bessouomy*et al.* (2004) suggested that maximum L-asparaginase activity occurred when it was incubated with an optimum substrate concentration at pH 9.0. Similar pH values were obtained for *E.coli*[20,21] *Pseudomonas aeruginosa* 10143[22] and many other microbial asparaginase activity[23].

In contradiction to the previous report, the present experiment showed maximum L-asparaginase activity recorded at pH 8. The optimum pH for L-asparaginase production was found to be 8.0 and temperature optimum was 37°C[24]. This finding is in agreement with the present result. Similar results were recorded earlier for asparaginase from *Pseudomonas aeruginosa* 50071[15], *Pseudomonas stutzeri*MB-405[4], *Erwinia carotovora*[25], and *Staphylococcus*[26].

Table 2 : L-asparaginase activity of the selected *E.coli* strains

***E.coli* strains**	**L-asparaginase activity (IU/ml)**
226	-
DH5α	+++
HB 101	+++
CSH 57	++
pUC18	++++
pBR322	-
pBR329	-
KL 16	-
KL 12	-

++++ Very strong L-asparaginase activity

+++ Strong L-asparaginase activity

++ Moderate L-asparaginase activity

\+ Weak L-asparaginase activity

\- Non L-asparaginase activity

Effect of temperature on L-asparaginase production

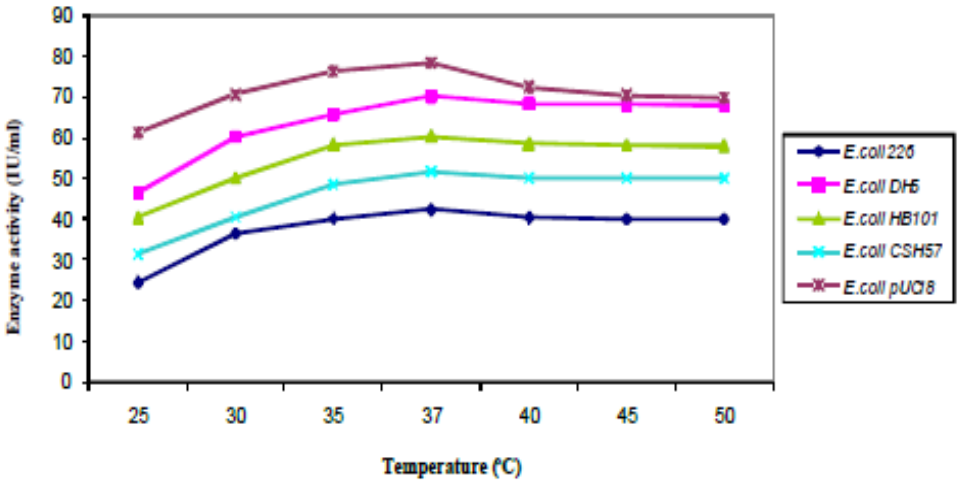

Figure .2: Effect of temperature on L-asparaginase production (IU/ml) by various *E.coli* strains in submerged fermentation

Figure 2 explains the data on the effect of temperature on L-asparaginase enzyme production by *E.coli* strains in submerged fermentation. The maximum L-asparaginase activity (78.5 IU/ml) was recorded at 37°C by pUC18 strain of *E.coli*. The produced L-asparaginase enzyme was

ranged from 42.4 to 78.5 IU/ml. Increased and decreased level of temperature from the optimal level (37°C) the L-asparaginase activity was proportionately decreased. The temperature as a function of L-asparaginase production using *E.coli* strains was checked through statistical analysis of variance, the result was statistically significant ($F=26.2$; $P<0.05$). Next, to ANOVA, the data was again analyzed by Tukey test. The Tukey test revealed that the overall conclusion was $\mu1 \neq \mu2, \mu3, \mu5, \mu2 \neq \mu4, \mu3 \neq \mu5, \mu4 \neq \mu5$. The maximum L-asparaginase production at 35°C was reported ealier[27]. More or less similar observation was recorded in the present experiment.

Effect of Incubation time on L-asparaginase production

The data for the effect of incubation time on L-asparaginase production by *E.coli* strains in submerged fermentation is shown in Figure 3. It revealed that the highest L-asparaginase production was 86.5 IU/ml at 48 hrs of incubation by pUC18 *E. coli* strain. Next to this strain, 80.2, 68.4, 58.5, and 36.5 IU/ml of L-asparaginase were produced by DH5α, HB101, CSH57 and 226 strains of *E.coli*, respectively. Increased and Decreased level from the optimum incubation time (48hrs) showed less L-asparaginase enzyme activity. The statistical data for L-asparaginase production using different *E.coli* bacterial strains as a function of incubation time revealed that there is a significant difference ($F=15.7$; $P<0.05$). Followed by ANOVA, the data was again analyzed by Tukey test. The Tukey test revealed that the overall conclusion was $\mu1 \neq \mu4 = \mu2 = \mu3 \neq \mu5$. The incubation period for bacteria and fungi was 18 h and 48 h, while in broth studies it is 24-48h for bacteria and often exceeds 96 h for fungi[19]. This concept is inagreement with the present data. The present result showed that the incubation period for better production of L-asparaginase was 48 h.

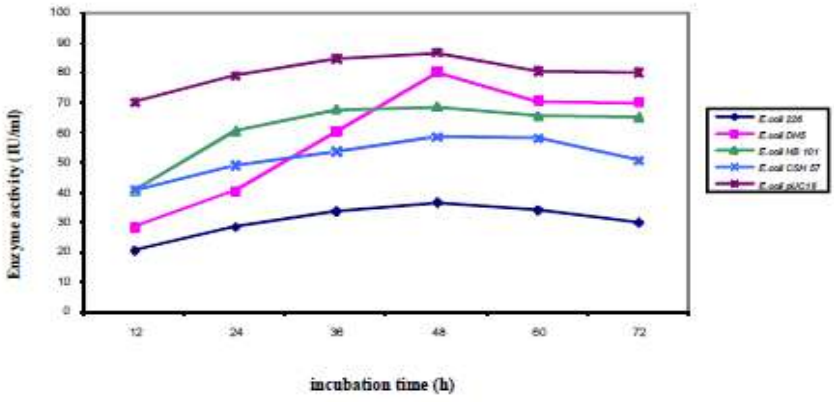

Figure .3: Effect of incubation time on L-asparaginase production (IU/ml) by various *E.coli* strains in submerged fermentation

Effect of carbon source on L-asparaginase production

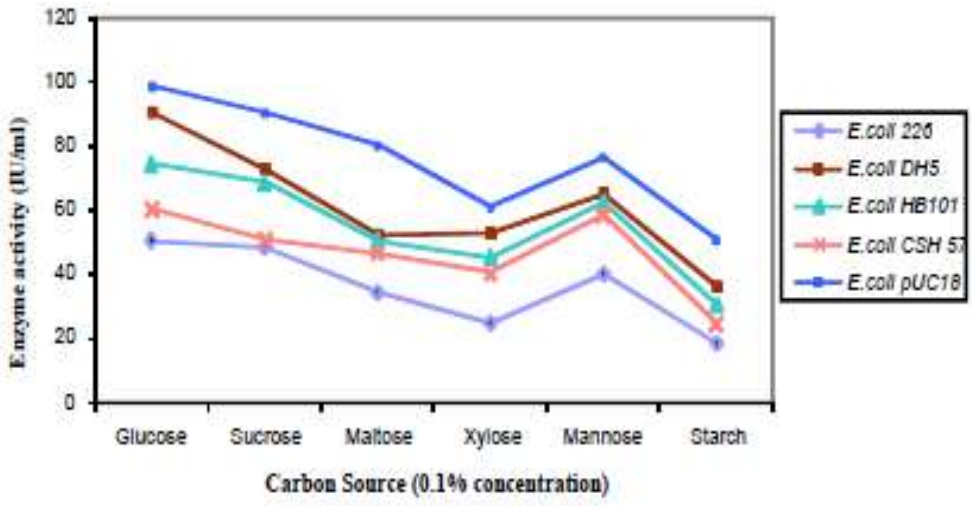

Figure .4: Effect of carbon source on L-asparaginase production (IU/ml) by various *E.coli* strains in submerged fermentation

Figure 4 depicts the data for the function of carbon source on L-asparaginase production by *E.coli* strains in submerged fermentation. It revealed that maximum L-asparaginase production was 98.6 IU/ml by pUC18 *E. coli* strain using glucose as sole carbon source. Next to this strain, 90.4, 74.5, 60.2, and 50.6 IU/ml of L-asparaginase were produced by DH5α, HB101, CSH57 and 226 strains of *E.coli*, respectively. Compared to glucose, the other carbon sources showed decreased level of L-asparaginase enzyme activity. Statistical analysis of the carbon source was performed to evaluate the analysis of variance (ANOVA) it showed highly significant ($F=5.4$; $P<0.05$). Followed by ANOVA test, the data was again analysed by Tukey test. The Tukey test revealed that the overall conclusion was $\mu 5 \neq \mu 1, \mu 4$. Glucose at 1% caused almost total inhibition of enzyme activity, while at 0.1% it showed a slightly stimulatory effect on enzyme production, compared with glucose free medium[28] .This finding is in agreement with the present result. In the present experiment 0.1% of glucose as carbon source showed a stimulatory effect on L-asparaginase production.

Effect of nitrogen source on L-asparaginase production

The data for the effect of nitrogen source on L-asparaginase production by *E.coli* strains in submerged fermentation is presented in Figure 5. It revealed that the highest amount of L-asparaginase production was 92 IU/ml by PUC18 *E. coli* strain using beef extract as sole nitrogen source. Next to this strain, 78.6, 70.1, 52.6, and 50.2 IU/ml of L-asparaginase were produced by DH5α, HB101, CSH57 and 226 strains of *E.coli* respectively. Compared to beef extract, the other nitrogen sources showed decreased levels of L-asparaginase enzyme activity.

Statistical One way ANOVA test revealed that the data between nitrogen source and bacterial strains used in the study was statistically significant (F=5.1; P<0.05). Followed by ANOVA, the data was further analyzed by Tukey test. It revealed that the overall conclusion was $\mu 5 \neq \mu 1, \mu 2$. Similar finding was reported earlier[24].

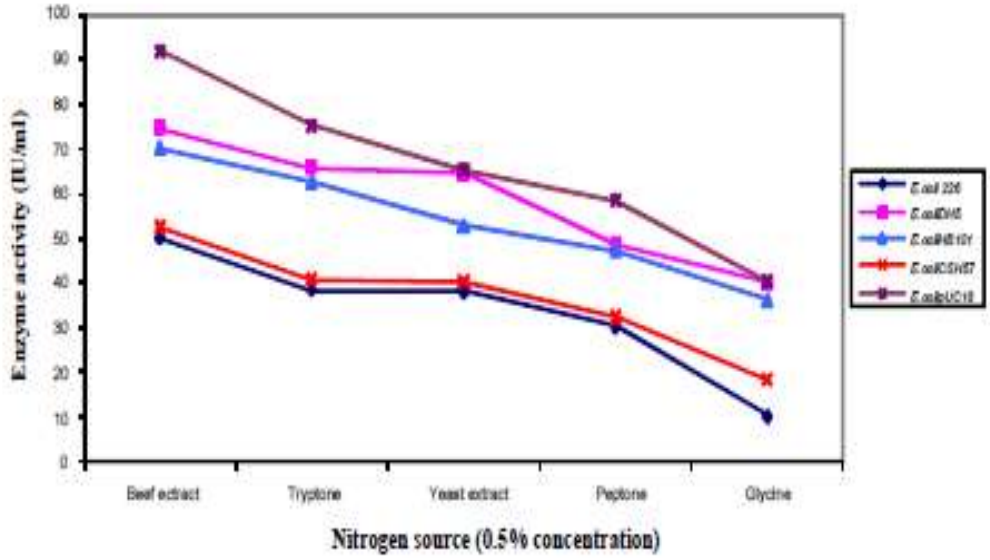

Figure .5: Effect of nitrogen source on L-asparaginase production (IU/ml) by various *E.coli* strains in submerged fermentation

Medium composition on L-asparaginase production in submerged fermentation

Table 3 represents the data for the medium composition and its effect on L-asparainase enzyme production by *E.coli* strains. It showed that the best medium for better L-asparaginase enzyme production was Medium B. The maximum activity recorded was 78.5 IU/ml by PUC18 strain of *E.coli*. Followed by this medium, medium C (72.4 IU/ml), medium A (68.2 IU/ml), and medium D (44.6 IU/ml) produced less amount of L-asparaginase. The protein concentration of the produced L-asparaginase enzyme was ranged from 23.10 mg/ml to 55.47 mg/ml. The statistical oneway ANOVA for the data on L-asparaginase production of different media as a function of various bacterial strains was statistically more significant (F=74.88; P<0.05). The four production media are not equal for L-asparaginase production. Followed by ANOVA test, the data was again analyzed by Tukey test. It revealed that medium A and B, A and D, B and D,C and D showed significant difference.

Table 3: Production of L-asparaginase by shake cultures of selected *E.coli* strains in different media.

Media	*E.coli* Strains	Growth (OD 600 nm)	Cell yield (mg/ml) of culture	L-asparaginase activity (IU/ml)	Protein (mg/ml)
Medium A	226	0.52	0.54	58.6	41.4
	DH5α	0.6	0.62	62.5	44.2
	HB 101	0.57	0.59	60.4	42.3
	CSH 57	0.55	0.56	59.8	41.9
	pUC18	0.65	0.74	68.2	48.2
Medium B	226	0.65	0.70	68.8	48.6
	DH5α	0.75	0.88	77.4	54.7
	HB 101	0.72	0.84	74.3	52.5
	CSH 57	0.69	0.76	70.5	49.8
	pUC18	0.8	0.94	78.5	55.5
Medium C	226	0.61	0.67	67.8	47.9
	DH5α	0.72	0.79	70.2	49.6
	HB 101	0.68	0.76	67.3	47.6
	CSH 57	0.65	0.72	65.8	46.5
	pUC18	0.75	0.82	72.4	51.2
Medium D	226	0.45	0.43	32.7	23.1
	DH5α	0.52	0.50	42.4	29.9
	HB 101	0.50	0.54	40.2	28.4
	CSH 57	0.48	0.44	35.6	25.2
	pUC18	0.56	0.48	44.6	31.5

UV Mutant on L-asparaginase production

The data for the UV mutants on L-asparaginase production exhibited highest L-asparaginase activity (178.4 IU/ml) was observed by using pUC18 UV60 (Table 4). It was 0.6 fold higher from the Parent strain. Similarly all the mutant strains showed enhanced L-asparaginase activity. The specific activity observed was ranged from 0.873 to 1.691 IU/mg. The percentage increase of L – asparaginase activity was higher while using mutant strain than the parent strain. The highest percentageincrease(61.45%) was recorded by pUC18 UV 60 *E.coli*. Similarly all the other strains also showed high percentage increase of L-asparaginase activity.The UV induced mutant AUV 4 showed highest enzyme activity and it was 1.64 fold higher than the parent strain[13]. Similarly the present experiment also showed higher enzyme activity by using mutant strains than the parent strains. An increasedlipase enzyme production of 3.25 fold moreby using *Pseudomonas* mutant of UV and NTG[29]. This finding is in agreement with the present report it also showed an increase in L-asparaginase production by using various *E.coli* mutants of UV & NT

NTG mutant on L-asparaginase production

Table 4: L-asparaginase activity (IU/ml) by UV mutant strains of *E.coli*

***E.coli* strains**	**L-asparaginase activity (IU/ml)**	**Protein (mg/ml)**	**Specific activity (IU/mg protein)**	**% increase of activity**
Parent strain CSH 57	60.5	64.5	0.938	-
CSH 57 UV30	64.5	78.4	0.873	13.2
CSH 57 UV60	69.8	70.6	0.974	15.4
CSH 57 UV90	62.5	63.75	0.980	3.31
CSH 57 UV120	-	-	-	-
Parent strain 226	60.5	48.6	1.245	-
226 UV30	61.5	43.46	1.415	1.7
226 UV60	68.5	40.5	1.691	13.2
226 UV90	63.5	54.2	1.172	4.9
226 UV120	-	-	-	-
Parent strain DH5œ	90.0	85.4	1.054	-
DH5α UV30	95.5	78.6	1.215	6.1
DH5α UV60	140.6	86.5	1.625	56.2
DH5α UV90	110.4	78.01	1.415	22.7
DH5α UV120	-	-	-	-
Parent strain HB 101	68.6	69.5	0.987	-
HB 101 UV30	74.2	69.5	1.068	8.2
HB 101 UV60	84.6	74.6	1.134	23.3
HB 101 UV90	70.5	68.0	1.036	2.8
HB 101 UV120	-	-	-	-
Parent strain PUC 18	110.5	98.6	1.120	-
pUC 18 UV30	120.5	95.6	1.260	9.05
pUC 18 UV60	178.4	128.5	1.386	61.45
pUC 18 UV90	130.5	102.0	1.279	18.09
pUC 18 UV120	-	-	-	-

The data for NTG mutant on L-asparaginase production is given Table 5. It revealed that the L-asparaginase activity of the mutant pUC18 NTG 90 showed increased activity (220.6 IU/ml). It showed 0.52 fold greater activity was recorded using mutant strain than the wild E.coli pUC18 strain. The specific activity was ranged from 0.878 to 1.861. The percentage increase of L – asparaginase activity was high (99.6%) while using pUC18 NTG 90 strain of *E.coli.*

Table 5: L-asparaginase activity (IU/ml) by NTG mutant strains of E.coli

***E.coli* strains**	**L-asparaginase activity (IU/ml)**	**Protein (mg/ml)**	**Specific activity (IU/mg protein)**	**% increase of activity**
Parent strain CSH 57	60.5	58.5	1.034	-
CSH 57 NTG30	68.5	78.0	0.878	13.2
CSH 57 NTG 60	84.5	65.8	1.284	39.7
CSH 57 NTG 90	110.5	78.08	1.415	82.6
Parent strain 226	60.5	48.6	1.245	-
226 NTG 30	62.5	72.0	0.868	3.31
226 NTG 60	78.2	68.5	1.142	29.3
226 NTG 90	88.6	62.6	1.415	46.4
Parent strain DH5α	90.0	85.4	1.054	-

DH5α NTG30	98.7	75.0	1.36	9.7
DH5α NTG 60	140.6	124.0	1.134	56.2
DH5α NTG 90	170.7	113.56	1.503	89.7
Parent strain HB 101	68.6	69.5	0.987	-
HB 101 NTG 30	70.5	68.0	1.036	2.8
HB 101 NTG 60	85.6	60.49	1.415	24.8
HB 101 NTG 90	128.4	69.0	1.861	87.2
Parent strain PUC18	110.5	98.6	1.120	-
pUC18 NTG 30	120.6	115.0	1.049	9.14
pUC18 NTG 60	180.7	168.0	1.076	63.5
pUC18 NTG90	220.6	210.0	1.050	99.6

Purification profile of L-asparaginase

Purification steps of L-asparaginase enzyme is given in Table 6. The L-asparaginase enzyme of all *E.coli* strains were recovered following 80% saturation of the culture supernatant with ammonium sulphate showed an increase of specific activity ranged from 0.07 to 1.7 IU/mg protein.The ten fold increased L-asparaginase enzyme activity with a specific activity of 55 IU/mg protein and recovery of 54%[30]. Similarly in the present study showed 1.7 fold purification of the L-asparaginase enzyme with a specific activity of 0.53 IU/mg protein and the recovery of 80.5%.

Table 6: Purification profile of L-asparaginase activity (IU/ml) by *E.coli* strains

***E.coli* strains**	**Purification Steps**	**Collected Volume (ml)**	**Total activity (IU/ml)**	**Total Protein (mg/ml)**	**Specific activity (IU/mg protein)**	**Purification factor**	**Recovery**
226	Crude extract	500	365000	475	1.5	1	100
	80% Ammonium sulphate	25	5500	234	0.94	0.63	49.3
	Ethyl alcohol	2.5	31.5	56	0.23	0.24	11.8
DH 5α	Crude extract	500	355000	430.6	1.6	1	100
	80% Ammonium sulphate	25	5250	240.8	0.87	0.54	55.9
	Ethyl alcohol	2.5	26.5	48	0.22	0.25	11.1
HB 101	Crude extract	500	320000	380	1.7	1	100
	80% Ammonium sulphate	25	4500	230.4	0.78	0.45	60.6
	Ethyl alcohol	2.5	21.5	46	0.19	0.24	12.1
CSH 57	Crude extract	500	160000	280	1.14	1	100
	80% Ammonium sulphate	25	3000	225.4	0.53	1.7	80.5
	Ethyl alcohol	2.5	17	37	0.18	2.5	13.2
pUC18	Crude extract	500	48000	180	0.53	1	100
	80% Ammonium sulphate	25	2000	118.6	0.67	1.3	65.9
	Ethyl alcohol	2.5	6	35	0.07	0.10	19.4

Molecular mass determination

All the tested *E.coli* strains produced relatively higher amount of L-asparaginase enzyme. The molecular mass of partially purified L-asparaginase sample was run in SDS –PAGE gel using coomassie brilliant blue staining method. The molecular mass of the purified L-asparaginase seemed to be 29,000 dalton(Table 7).

Table 7: Determination of molecular weight of purified L-asparaginase enzyme with Known molecular weight standard Protein markers.

Protein Markers	Distance moved by proteins (cm)	Mobility of protein	Molecular weight of protein (kda)	Log. Molecular weight of standard protein
Myosin, Rabbit muscle	0.6	0.12	2.05×10^5	5.3118
Phosphorylase b	1.9	0.36	9.74×10^4	4.9886
Bovine serum Albumin	2.5	0.48	6.6×10^4	4.8195
Ovalbumin	4.1	0.79	4.3×10^4	4.6335
Carbonic anhydrase	4.5	0.87	2.9×10^4	4.4624
Soyabean Trypsin – inhibitor	5.0	0.96	2.01×10^4	4.3032
E.coli 226	4.4	0.85	2.9×10^4	4.4624
E.coli DH 5	4.5	0.87	2.9×10^4	4.4624
E.coli HB 101	4.5	0.87	2.9×10^4	4.4624
E.coli CSH 57	4.4	0.85	2.9×10^4	4.4624
pUC18	4.5	0.87	2.9×10^4	4.4624

$$\text{Mobility} = \frac{\text{Distance moved by protein (x cm)}}{\text{Distance moved by bromophenol blue dye (5.2cm)}}$$

Molecular weight (kda) = Antilog of Log. Molecular weight

CONCLUSION

On the basis of the present data, it can be concluded that the highest catalytic activity of the enzyme at physiological pH and temperature and its considerable stability over wide range of pH and temperature makes it highly favourable to act as a potent anticancer agent. Studies on the enzyme relating to purification and characterization would open new avenues in the application of the enzyme in the healthcare industry. Consequently we suggest that the enzyme which degradeaminoacids should receive greater attention as potential therapeutic agents.

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