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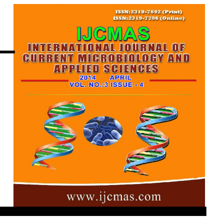


Original Research Article

Optimization of Media Components and Cultural conditions for L-asparginase production by a potent isolate from the unusual habitat of Lonar Lake, India

Chande Ashish* and Bhat Manish

Department of Biotechnology and Bioinformatics,
Padmashree Dr. D. Y. Patil University, C.B.D Belapur, Navi Mumbai, India
**Corresponding author*

A B S T R A C T

Keywords

L-asparginase. Chemo-therapeutic agent; *Stenotrophomonas koreensis;* Acute lymphoblastic leukemia; Lonar lake

L-aspraginase, a protein encrypted with chemotherapeutic potential is used to treat Acute lymphoblastic leukemia and Lymphosarcoma. However, increasing reports of toxicity and resistance limit the application of the clinically approved enzyme. L-asparginase with different characteristics, extracted from a novel source implies promising application in case of resistance and hypersensitivity. With this interest, an L-asparginase producing microorganism was isolated from the highly alkaline and saline Lonar Lake and its partial 16s rRNA sequence had high similarity with *Stenotrophomonas koreensi* TR6 01 strain. In this article the effect of Nitrogen sources (L-aspargine, yeast extract, peptone), Carbon sources (Glucose, Fructose, Sucrose, Maltose, Lactose) and growth affecting parameters like initial pH, incubation temperature and inoculum size on the extracelluluar production of L-asparginase from the same isolate using Nesslerization assay is presented. L-aspargine 1% w/v, Yeast extract 0.6% w/v, Peptone 0.8% w/v and Glucose 0.4% w/v. were found to be optimum concentrations with optimum initial pH of 7, inoculum size of 2% and incubation at room temperature. The maximum L-Asparginase activity of 19.79 IU/ml was obtained at optimized conditions.

Introduction

L-asparginase, an aminohydrolase, cleaves its substrate L-aspargine into L-aspartate and ammonia. L-aspargine can be synthesized by normal cell by the virtue of the enzyme L-aspargine synthetase, making it a non-essential amino acid. On contrary, it proves to be an essential amino acid for tumor cells as they lack or have low levels of L-aspargine synthetase and depend on the serum levels of this amino acid. Depletion of l-aspargine by asparginase starves the tumor cells of their essential amino acid, hampers their protein synthesis and leads to apoptosis (Becker F, Broome, 1969; Bon et al., 1997).

Enzyme produced from *E.coli* and *Erwinia chrysanthemi*, used under the clinical names of Elspar® and Erwinaze™ respectively, has shown increasing cases of hypersensitivity reactions, both, in children and adults, leading to toxicity like liver disturbances, central nervous disturbances, thrombocytopenia, hyperlipidemia, intracranial hemorrhage, renal failure (Broome, 1981; Cairo Mitchell, 1982; Chande Ashish and Bhat Manish, 2014; El-Bessoumy ET AL., 2004) and even resistance in some cases. Administration of l-asparginase from a different source independently or combination with the already used ones can reduce the allergic responses (Gaffar S, Shethna, 1077). Production of this enzyme from various fungi, bacteria, yeast and actinomycetes has been reported (Haskell, et al., 1969; Howard and James, 1968; Kil *et al*. 1995 Mishra, 2006; allergic responses (Muller and Boos, 1998); renal failure (Oettgen et al., 1970; Priest John et al., 1982 Gaffar S, Shethna Y 1977, El-Bessoumy AA et al. 2004, Mishra A 2006, Sahu M et al. 2007, Khamna S et al. 2009, Kil J. O. *et al*. 1995, Bon E. P. *et al*. 1997, Howard C, James S 1968).

Microbes from unusual habitats tend produce proteins with novel or different characteristics than those in usual habitats. Isolation of an l-asparginase producing isolate from the highly saline and alkaline Lonar Lake was reported for the first time. The partial 16s rRNA sequence of this isolate has shown maximum similarity with *Stenotrophomonas koreensis TR6 01* strain (Chande Ashish, Bhat Manish 2014). In this article Optimization of Carbon/Nitrogen sources and cultural conditions is presented for the same.

Materials and Methods

Micro-organism

The microbe used for this study was isolated from Lonar Lake, Maharashtra, India. This lake is believed to be formed by a meteor impact and is known for its extreme salinity and alkalinity, an attraction for microbiology research to discover novel species and their metabolites. The partial 16s rRNA sequence of this isolate had similarity with *Stenotrophomonas koreensis TR6 01* strain (Chande Ashish, Bhat Manish 2014.).

Optimization of Carbon/Nitrogen sources

Optimization was started by adding varied concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 1.2%) of l-aspargine in Sterile M 9 broth (Na_2HPO_4 – 6 gms/ Lit , KH_2 PO_4 – 3 gms/ Lit, NaCl – 0.5 gms/ Lit, $CaCl_2$ – 0.001 gms/ Lit , $MgSo_47H_2O$ – 0.12 gms/ Lit . Quantitative Nesslerization Assay(Chande Ashish, Bhat Manish, 2014) was performed for four days in the intervals of 24 hours and a concentration yielding the highest enzyme activity was selected and included as an additional component of M9 medium thereafter. M 9 broth without L-aspargine was used as a control every time. This process was similarly carried forward by using nitrogen sources yeast extract and peptone. For optimizing Carbon sources different sugars glucose, fructose, sucrose, maltose, lactose were added separately to the sterile M9 media with optimized concentration of Nitrogen sources. A carbon source showing high enzyme production was selected and the effect of its different concentration of 0.2% - 0.6% was then studied.

Optimization of growth affecting parameters

The effect of growth affecting parameters on the production of L-asparginase in the nutrient optimized media was also studied. Production was subjected in different conditions like pH (3-7), temperature (Room temperature and 37° C) and inoculum size (0.5% - 2.5%). The activity of the enzyme produced under the optimized conditions was calculated.

Results and Discussion

The optimum Concentration of L-asparginse, yeast extract and peptone was found out to be 1%, 0.6% and 0.8% respectively (table 1, fig 1-3). Out of all the other sugars used, enzyme activity with glucose was observed to be the highest with its concentration of 0.4% (table 2 and fig. 4, 5). The optimum pH was found out to be 7 (fig.6), the optimum inoculum size was 2% of the total volume of media (fig.7) and Room temperature was found out to be an optimum temperature of incubation for the production of L-asparginase (fig. 8).

Yeast extract when added to modified M9 media including 1% L-aspargine, elevated the enzyme activity from 10.06 I.U/ml to 19.54 I.U/ml while addition of Peptone as an extra Nitrogen source and Glucose as a Carbon source decreased the overall activity to some extent due to catabolite repression.

Table.1 Effect of various Nitrogen sources and their different concentration on Enzyme activity calculated in the intervals of 24 hrs

Concentration % (w/v)	Enzyme activity after 24 hrs (I.U/ml)	Enzyme activity after 48 hrs (I.U/ml)	Enzyme activity after 72 hrs (I.U/ml)	Enzyme activity after 96 hrs (I.U/ml)	Enzyme activity after 120 hrs (I.U/ml)
L-aspargine					
0.2 %	4.94	5.88	6.82	7.08	2.13
0.4 %	4.01	4.35	5.97	5.80	4.01
0.6 %	5.71	6.65	9.55	8.10	7.85
0.8 %	4.86	6.40	8.10	7.33	7.25
1.0%	7.25	7.59	10.06	8.44	3.84
1.2%	5.03	5.29	6.40	6.40	5.88
Yeast extract					
0.2 %	2.73	3.41	10.41	10.49	10.32
0.4 %	3.07	3.32	10.83	10.49	9.98
0.6 %	7.25	13.99	19.54	19.37	18.51
0.8 %	7.42	12.88	18.00	18.00	17.92
1.0%	5.54	10.49	15.01	14.84	14.08
1.2%	6.31	8.87	13.90	13.90	13.90
Peptone					
0.2 %	5.71	6.65	7.50	7.16	6.99
0.4 %	4.52	4.94	6.65	6.40	6.05
0.6 %	6.57	7.33	10.75	10.41	10.06
0.8 %	5.41	7.16	11.34	10.92	10.58
1.0%	6.05	7.93	10.41	10.24	10.06
1.2%	5.37	5.63	6.74	6.74	6.22

Table.2 Effect of various Carbon sources (0.4%) on Enzyme activity calculated in the intervals of 24 hrs.

Sugars (0.4%)	Enzyme activity after 24 hrs (I.U/ml)	Enzyme activity after 48 hrs (I.U/ml)	Enzyme activity after 72 hrs (I.U/ml)	Enzyme activity after 96 hrs (I.U/ml)	Enzyme activity after 120 hrs (I.U/ml)
Glucose	1.28	11.776	17.49	17.06	16.64
Maltose	1.19	1.96	8.96	8.70	8.53
Sucrose	0.68	2.04	8.10	7.68	7.50
Fructose	1.10	10.83	15.78	15.53	15.36
Starch Sol	0.08	1.53	7.25	6.65	6.48
Lactose	0.85	1.45	7.50	7.25	7.08

Fig.1 Graph showing the effect of different conc. of L-aspargine on enzyme activity.

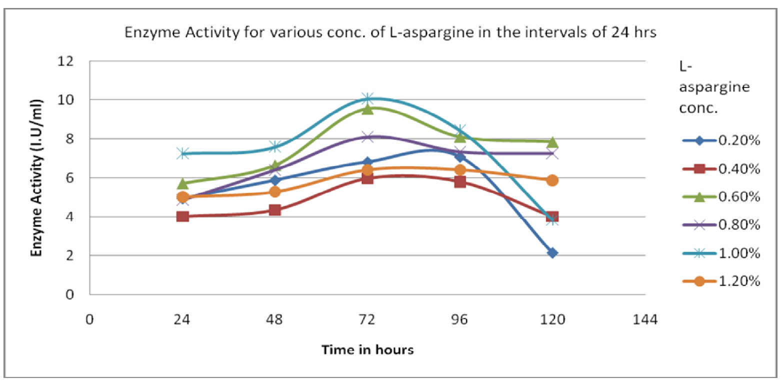


Fig.2 Graph showing the effect of different conc. of Yeast extract on enzyme activity

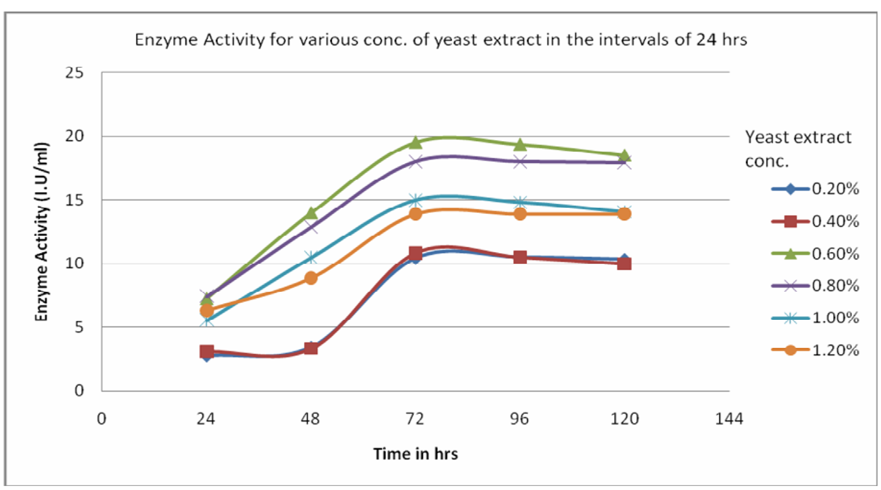


Fig.3 Graph showing the effect of different conc. of peptone on enzyme activity

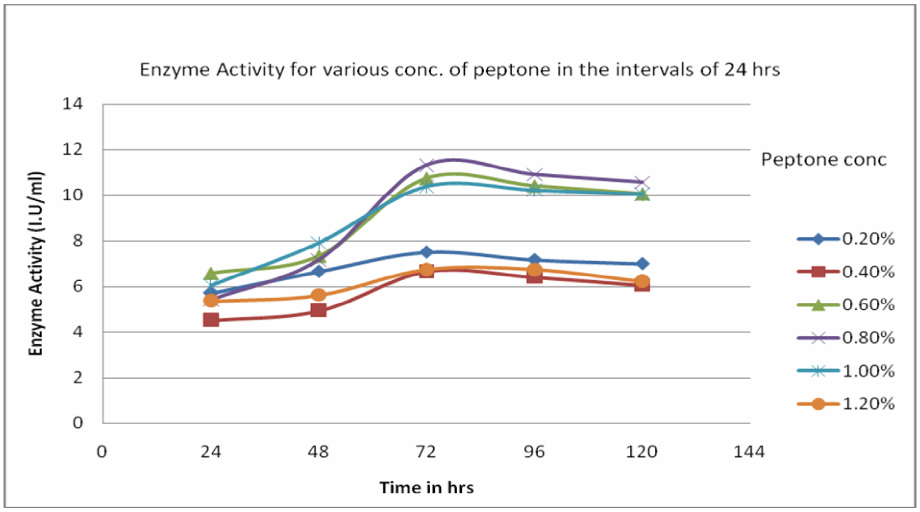


Fig.4 Graph showing the effect of different Sugars on enzyme activity

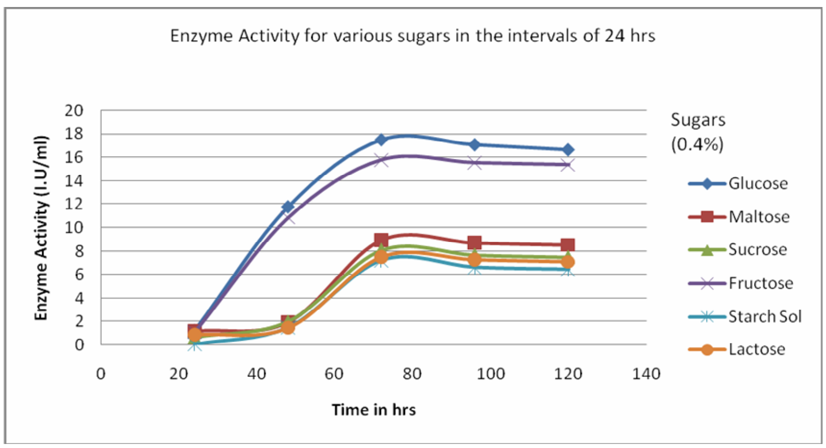


Fig.5 Graph showing the effect of different concentrations Glucose on enzyme activity

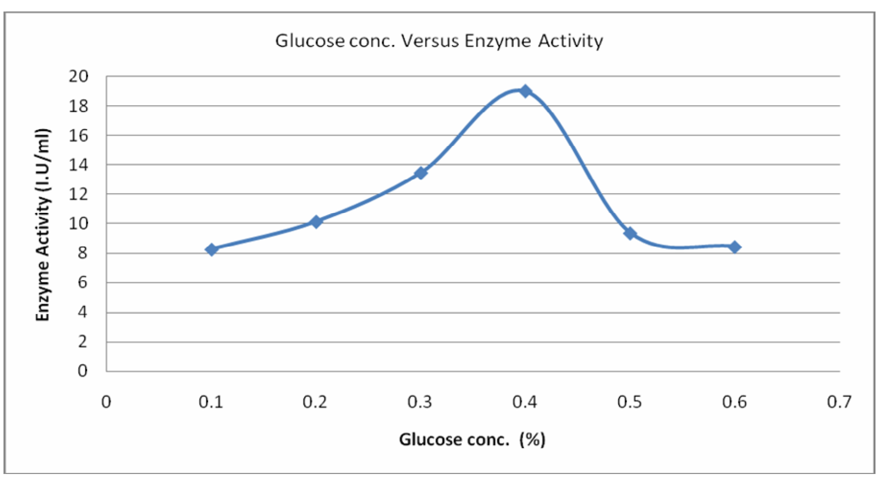


Fig.6 Graph showing the effect of different pH on enzyme activity

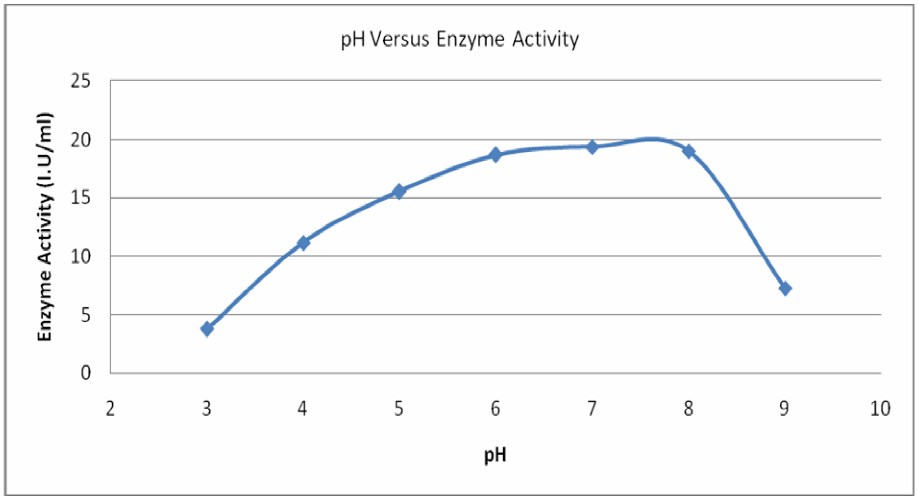


Fig.7 Graph showing the effect of different inoculums size on enzyme activity

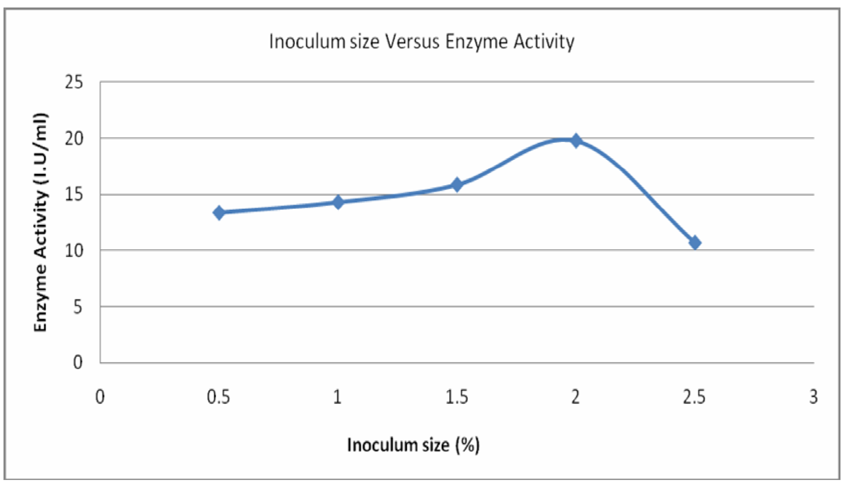
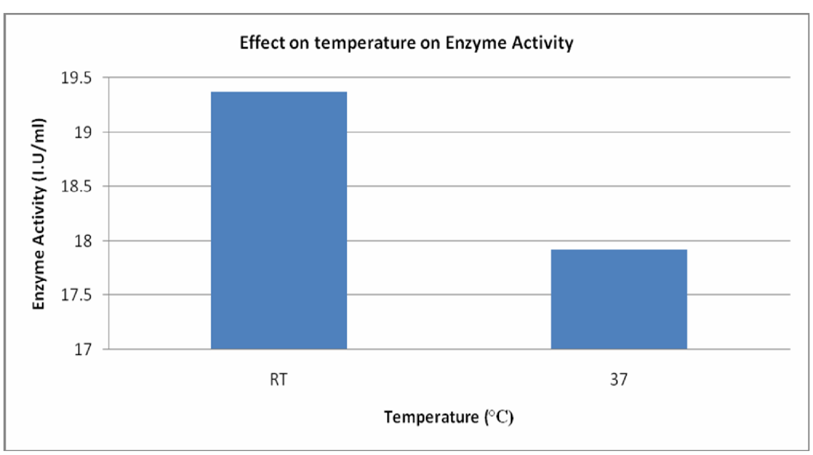


Fig.8 Graph showing the effect of temperature on enzyme activity



The increase in temperature from RT leads to denaturation of protein because of which high activity 19.37 I.U/ml was found at RT and 17.92 I.U/ml at 37°C. Increase in H^+ ion concentration above the neutral pH tends to change the secondary and tertiary structure of proteins, changes the charge interactions between the polypeptides and result in low activity. Purification and characterization of enzyme, its effect on cell lines form the future prospects of the study.

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