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Isolation and Characterization of L-asparginase producing isolate from Lonar Lake, Buldhana District, MS, India

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Abstract

L-asparginase has emerged as one of the most important clinically used enzymes as it exhibits chemotherapeutic potential in treatment of acute lymphoblastic leukemia and lymphosarcoma. Increasing reports of Immunological responses limit the utilization of the enzyme and indicate the need of new L-asparginase with new characteristics. Besides its clinical application this enzyme is widely used in food industries to significantly reduce the formation of acrylamide – a potent carcinogen in baked and fried food products. Screening of L-asparginase producing isolate from the highly alkaline and saline Lonar Lake has not been reported yet and an attempt for the same is made in this study. Out of 1592 total colonies four isolates were identified as L-asparginase producers by rapid plate assay. Further, a single isolate with the highest enzyme activity of 16.746 IU/ml was selected and identified. The organisms of the red pigmented, sticky, circular colonies having gram negative cell wall and rod shaped morphology showed positive tests for nitrate reductase, catalase and oxidase. The partial 16S rRNA sequence of the isolate showed maximum similarity with Stenotrophomonas koreensis TR6-01 strain. The alkalitolerant and halotolerant characteristic of this strain was revealed when the growth was observed in the media of pH10 and salt concentration of 4%.

Keywords: L-asparginase, chemotherapeutic potential, acrylamide, *Stenotrophomonas koreensis* TR6-01, Lonar lake.

Introduction

An observation of regression of lymphomas in mice and rat injected with guinea pig serum was a breakthrough in cancer research[1]. The mysterious component of the guinea pig serum responsible for regression was later on revealed to be L-asparginase[2]. L-asparginase or L-aspargine aminohydrolase E.C.3.5.1.1 breaks down L-aspargine into aspartic acid and ammonia. Normal cells possess an enzyme L-aspargine symthase which compensates for the loss of the amino acid unlike in leukemic cells which have negligible quantities or no L-aspargine synthase. Hence leukemic cells are starved of the amino acid and its deficiency causes delayed inhibition of DNA and RNA synthesis, hampers protein synthesis and results in impairment of cell function and cell death[3, 4].

It was observed that the enzyme activity of L-asparginase extracted from *E. coli* and guinea pig serum were comparable[5] and soon microbial sources of this enzyme gained importance. Today L-asparginase from *E. coli* and *Erwinia chyrsanthemi* are used in treatment of leukemia. The application of asparginase from these two sources is restricted because of immunological reactions. Toxicity associated with this enzyme includes allergic reactions, thrombotic diseases, gastrointestinal diseases, pancreatitis, anaphylactic shocks etc[6]. This indicated the need of new asparginase with new characteristics and since then many bacterial, fungal, yeast, actinomycetes were screened for production of this enzymes[7-14]. Besides its clinical uses L-asparginase has its application in food industries where it is used to significantly reduce the amount of acrylamide – a potent carcinogen formed in food stuffs by Maillard reaction[15].

Microbes dwelling in extreme environments like alkaline or saline areas tend to express proteins with different characteristics than those inhabiting in the normal environment. Lonar Lake located in Buldhana district of Maharashtra is like one of the wonders of state and is the world's third largest crater believed to be formed due to a meteor impact. It is a saline soda lake having the pH in the range of 10-12. This provides an extreme environment and serves as an excellent habitat for isolation of halophilic and alkaliphilic organisms. Isolation of L-asparginase producing isolate from Lonar Lake has not been reported yet and an attempt for the same is made in this study.

Material and Methods

Sampling: Soil and water samples were collected in sterile containers from four opposite sides of Lonar Lake. The pH of the lake was noted immediately at the site.

Primary Screening: Primary screening was carried using Rapid plate assay[16] (Gulati et al., 1996) on sterile nutrient agar with 1 % L-aspargine (pH 7) as the growth medium. This method makes the use of few drops of phenol red as a pH indicator. The samples were serially diluted in sterile conditions. The diluted samples were spread on the agar plates. The plates were

incubated for 24 hours at RT. Pure cultures of the colonies identified as L-asparginase producers was prepared and maintained for further use.

Enzyme assay: The quantitative enzyme assay was carried out and enzyme activity was measured using nesslerization method. The standard curve was plotted using varying concentrations of ammonia and the volume is adjusted to 2 ml using distilled water. 1 ml Nessler's reagent was added to each tube and incubated at RT for 15 minutes. Absorbance was measured at 425 nm using UV Spectrophotometer. Standard graph of OD against varying concentrations was plotted.

0.25 ml of enzyme solution was added to 1.25 ml Sodium Phosphate buffer, 1 ml of L-aspargine solution and was incubated at RT for 10 minutes. 0.5 ml TCA was added to inhibit the reaction followed by centrifugation. 0.25 ml of this solution was then subjected to nesslerization and absorbance was measured at 425nm. The recorded absorbance was extrapolated on the standard curve to find amount of ammonia released and enzyme activity was calculated.

A single isolate showing highest enzyme activity was selected for further analysis. To confirm that the enzyme is an extracellular product, the enzyme activity of the broth in which the culture were grown was compared with the enzyme activity of cells.

Identification of the potent isolate: Colony characteristics were observed, general biochemical tests, gram staining and negative staining were performed using standard procedures. Partial 16S rRNA sequence was obtained and compared using BLAST.

Checking pH and Salt tolerance: To check the extent of pH and Salt tolerance agar plates with different pH (4 to 12) and different salt concentrations (2% to 12%) were prepared and the culture was streaked over it. The plates were incubated at RT for 24 hours.

Results and Discussion

Lonar Lake is the world's third largest crater and is believed to be formed due to a meteorite impact. The lake water is highly alkaline and saline. The pH of the lake was noted to be more than 9.5 at the time of sample collection. Out of 1592 total colonies four isolates were found to be L-asparginase producers by Rapid plate assay. After the quantitative assay a single isolate with the highest enzyme activity of 16.746 IU/ml was selected for further analysis. The isolate had rod shaped morphology and gram negative nature of the cell wall. General biochemical tests were performed and positive tests for nitrate reduction, catalase and oxidase were obtained. Partial 16S rRNA sequence of the isolate showed maximum similarity (100%) with *Stenotrophomonas koreensis* TR6-01 strain (Accession no: NR_041019.1). As growth was observed in the medium of pH 8 and 10 and salt concentration of 4% the isolate is said to be alkalitolerant and moderate halotolerant.

Table-1
Observation of the colony characteristics

Colony Characteristics	Observation
Size	1 mm
Elevation	Flat
Transparency	Translucent
Shape	Circular
Boundaries	Regular
Pigmentation	Red
Consistency	Watery/Sticky

Table-2
Results of Biochemical tests

Biochemical Tests	Result +/-
Citrate utilization	-
Lysine utilization	-
Ornithine utilization	-
Urease	-
Phenyalanine deamination	-
Nitrate reduction	+
H2S production	-
Glucose	-
Adonitol	-
Lactose	-
Arabinose	-
Sorbitol	-
MR	-
VP	-
Indole	-
Glucuronidase	-
ONGP	-
Sucrose	-
Oxidase	+
Catalse	+
Motility	-

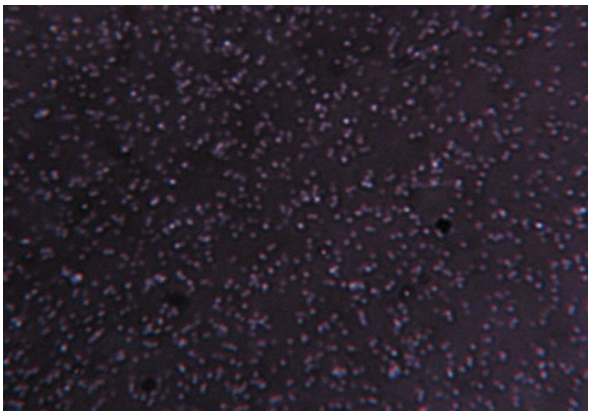

Figure-1
Picture of negative staining

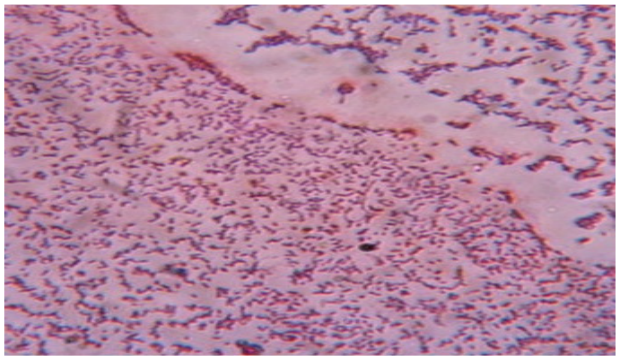

Figure-2
Picture of gram staining

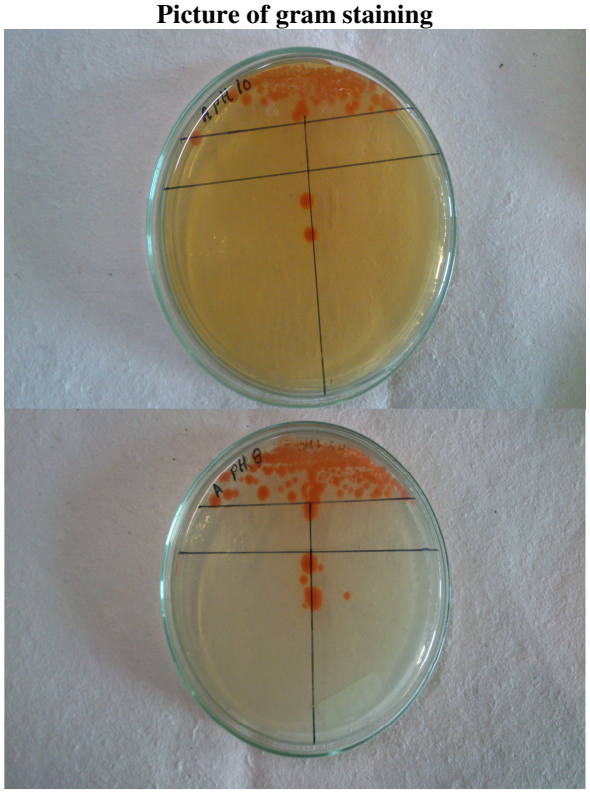

Figure-3
Picture showing growth on medium of pH 8 and 10

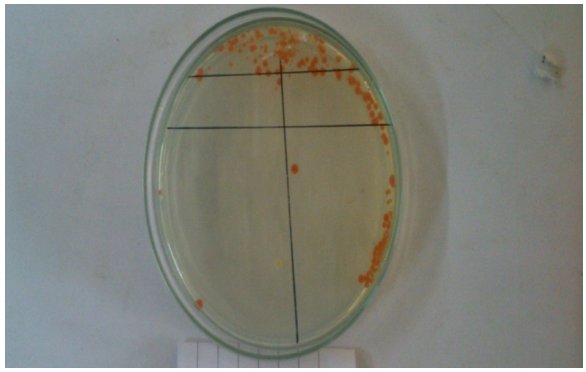

Figure-4
Picture showing growth on medium with salt concentration of 4%

Conclusion

The result of this study imply that the organism *Stenotrophomonas koreensis* TR6-01 isolated from Lonar Lake has a potential of producing L-asparginase enzyme which might have new characteristics and pose a solution to the problems of hypersensitivity exhibited by the enzyme currently in use. Media optimization, purification and characterization of the enzyme are the further milestones of this study.

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