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Research Article

Enrichment and Screening of Potential L -Asparaginase Producing Bacillus Sp. from Hyper saline and Alkaline Habitat.

Shivraj B. Patil* and S. M. More

Department of Microbiology, Yeshwant Mahavidyalaya,
Nanded- 431602. (Maharashtra), India
E-mail Id of corresponding author:patilsp1@hotmail.com

ABSTRACT:
Microorganisms isolated from extreme habitat can be used as alternate source for metabolites to existing one's having certain limitations. Alkalophiles, have been studied and used for production of different enzymes, antibiotic's, amino acids etc. Alkalophiles have wide spread application. The aim of present study was to isolate a potential L-asparaginase producer, that can be utilised for production of L- asparaginase that is widely used as antineoplastic agent, acrylamide reducer in fried foods & in biosensors. Water & sediment Samples collected from Lonar Lake were subjected to physicochemical analysis and used for isolation of potential L-ase producers. More than 45 strains were isolated and screened for possible L-asparaginase producer by primary and secondary methods. Among 45 isolated strain 5 strains were identified as potent L-asparginase producers. The asparginase producer strain were further identified by morphological test and Biochemical test, By using Biolog (Microlog^{TM}). Among the identified species the potential l-asparaginase producers were *Bacillus Licheniformis*, *Bacillus Subtilis* , & *Bacillus Megaterium* .

Key words: Screening, L –Asparaginase, Hyper saline, Alkaline Habitat, *Bacillus Licheniformis*, *Bacillus Subtilis*, & *Bacillus Megaterium.*

INTRODUCTION:

L-Asparaginase (E.C.3.5.1.1) is an enzyme that catalyzes the hydrolysis of asparagine to aspartate and ammonia. L-Asparaginase is synthesized by plants ,microorganisms, and some animals, it does not naturally occur in humans. After the assess that antileukemic activity of guinea pig serum was due to its l-asparaginase activity(Broom1996) and isolation of this enzyme from *Escherichia coli* that had similar activity(Roberts et al 1996) a considerable attention was attracted to this area of research. L-asparaginase is also used in reduction of acrylamide formation in fried and baked foods processed at high temperature. Acrylamide is a neurotoxin and has been classified as probably carcinogenic to humans (Medeiros Vinci et.al 2012). The Main formation pathway of acrylamide is via a Millard reaction between the free amino acid L-asparagine and a carbonyl-containing compound at high temperature (>120^{o} C)(Zyzak et.al 2003). An effective mitigation measure is the removal of asparagine an important precursor of acrylamide, by addition of l-asparaginase (Capuano et.al. 2009). Microorganisms are considered the most important source of Lasparaginases, and various L-asparaginase s have been discovered in bacteria, fungi,

Actinomycetes ,and algae. The bacterial lasparaginase have been classified as type I and type II isoenzyme based on their subcellular location and properties. The type II enzymes have shown beneficial pharmacological effects in acute lymphoblastic leukemia. Commercially available asparaginase includes Colapase, Crasnitin, Crisantas, Pasum, Kidrolase, Elspar, Erwinaze PEG-asparaginase, and Pegasparagasum. Most of the commercially available LAse are produced by using *E. coli and E. carotovora* as producer strain. L-asparaginase causes certain side effects, and despite its potential antileukemic activity, utilization of L-asparaginase by leukemic patients causes lethality to normal cells. L-asparaginase produces a broad range of symptoms such as edema, skin rashes, fever, hepatic dysfunction, diabetes, leucopenia, pancreatitis, neurological seizures, and hemorrhage (Nelken, et al. (2002).

The aim of the present investigation is to search for new and potential sources of L-asparaginase producers from extreme habitat. Extremophilic bacteria are considered important groups of extremophilic organisms studied in the past few years. They are considered as a source of enzyme due to great diversity. Microbial enzymes of extreme habitat are active and stable under extreme conditions. These enzymes pose new opportunities for new line research, and can be efficiently used as alternate to existing enzymes available having certain limitations.

MATERIALS & METHODS: All chemical used in the present study were of analytical grade purchased from Himedia, Mumbai, India.

Sample collection:

Water and sediment samples from Lonar lake were collected from different Sampling points at a depth of 15 to 20 cm. The samples were collected into a sterile Screw capped bottles and sterile polythene bags and carried to department research lab for further microbiological analysis and isolation.

Enrichment and Isolation of Microorganism's:

10 ml Sample was enriched with media shown in table.1, in 100ml conical flasks and incubated in rotatory orbital shaker for 48 hrs**.** The enriched sample was used for isolation of microorganisms on same media containing 2.00% agar. The isolated colonies were sub-cultured and used further for screening of l-asparaginase production.

Table:1. Composition of Enrichment Media.

Horikoshi I	**gms/litre**	**Horikoshi II**	**gms/litre**	**Nutrient broth pH-9.5**	
Glucose	10.000	Starch	10.000	Peptone	10.000
Yeast extract	5.000	Yeast extract	5.000	Beef extract	10.000
Polypeptone	5.000	Polypeptone	5.000	Sodium chloride	25.000
K2HPo4	1.000	K2HPo4	1.000	pH	9.00
Mg2SO4	0.2	Mg2SO4	0.2		
Na2Co3	10.000	Na2Co3	10.000		
pH	**9.00**	**pH**	**9.00**		

Screening of L-asparaginase producers:

The isolated subcultures were spot inoculated on modified m9 medium (Gulati et.al)containing (g/l): Na2HPO4.2H2O, 6·0 g; KH2PO4, 3·0g; NaCl, 0·5 g; L-asparagine, 5·0 g; 1 mol l^{-1} MgSO4.7H2O,2·0 ml; 0·1 mol l^{-1} CaCl2.2H2O, 1·0 ml; 20% glucose stock,10·0 ml; agar 20·0 g.,pH-7.5(adjusted), dye phenol red (conc.0.001-0.009%). Control plates without dye and without asparagine (Na_2NO_3 as nitrogen source) were used. The plates were incubated at 37^{o}C for 24 hrs. The zone and colony diameter

was measured by plate assay method. Modified M9 medium(broth) without dye was used for cultivation of isolates, further the filtrate was used for used for screening.

Identification of Potential L-asparginase producer:

Potential L-asparaginase producer's were identified on the basis of morphological character's and using Biolog Microbial Identification system software (Microlog^{TM}).

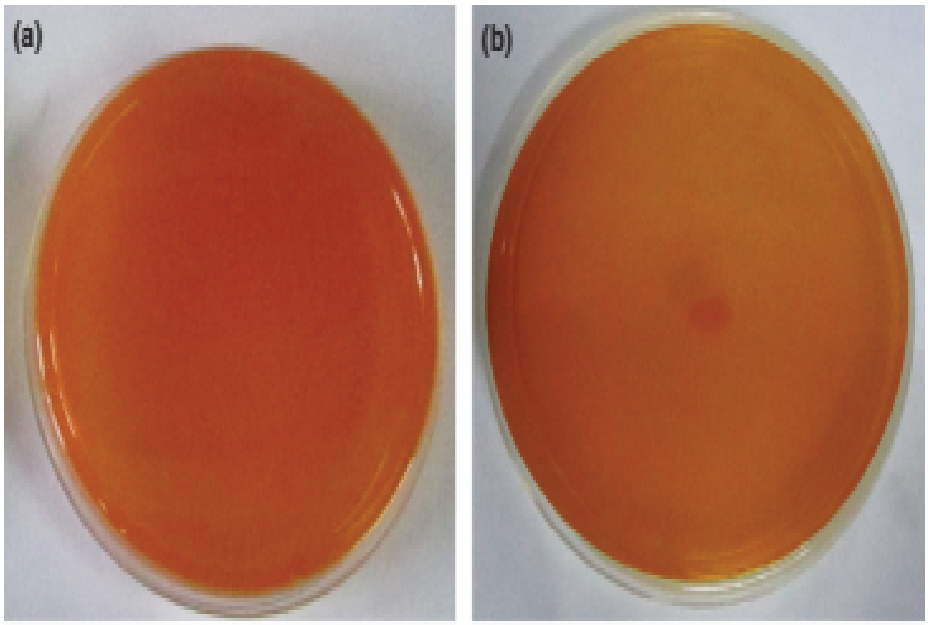
RESULT AND DISCUSSION:

A total of 45 isolated bacterial culture were used for Screening of L-asparaginase production, Out of which 5 isolates (C5,C6,C13,C14,C30) were found to be potent l-asparaginase producers**.** The isolates surrounded by pink zone were considered as l-asparaginase producers. The pink zone attributed was due to the release of ammonia during hydrolysis of asparagine which increased the pH, whereas no colour change was observed in control plates without dye and aspargine. Among the five the potent l-Ase producers identified by Bilog were *Bacillus Licheniformis, Bacillus Megaterium*,

Future investigations are aimed at production and molecular characterization of LAse and its producer.

Table2. L-asparaginase activity of isolate after 24hrs

Sample Id	Colony diameter (mm)	Zone diameter (mm)
C5	**5.50**	**0.98**
C6	**5.00**	**0.87**
C13	**5.33**	**0.80**
C14	**6.00**	**0.75**
C30	**5.00**	**1.00**



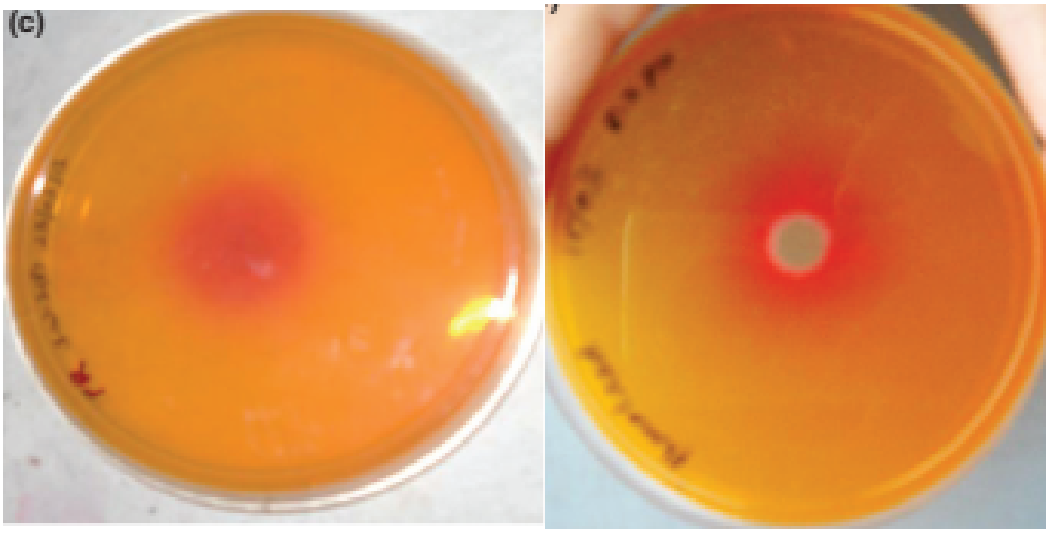


Fig.1. photograph of Screening of l asparaginase producer on Modified M9 medium plates.

a) Plate without inoculation
b) Plate without l-aspargine inoculated with isolate.
c) Plates inoculated with culture isolate.
d) Plates inoculated with culture filtrate from broth.

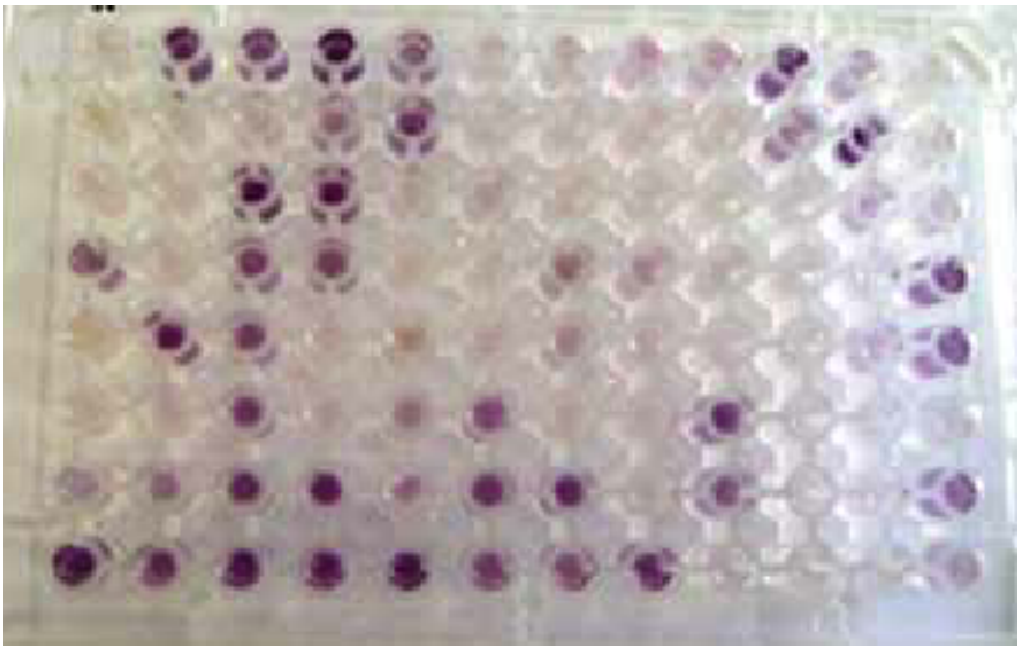


Fig.2 Photograph of Biolog Microplate inoculated with isolated culture.

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