International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.1, pp 109-112, Jan-Mar 2013

Characterization Of L-Asparaginase Producing Bacteria From Mangrove Soil

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Abstract: This paper describes the isolation and characterization of bacteria isolated from mangrove soils of Nizampatnam and Chollangi, A.P. India. Bacteria were screened for L-Asparaginase production from mangrove soils. L-Asparaginase activity of bacteria was detected on the basis of formation of pink colour around the colony. L-Asparaginase producers were characterized by morphological ,physiological and biochemical studies and classified to be species belonging to Bacillus sp and Pseudomonas sp. L-Asparaginase from these mangrove microbial strains can contribute to the therapeutic value of this enzyme.
Key words: L-Asparaginase, mangrove soils, *Bacillus, Pseudomonas.*

Introduction:

L-Asparaginase has received increased attention for its ant carcinogenic potential. Anti cancer action of this enzyme is attributed to reduction L-asparagines hence tumor cells unable to synthesis this amino acid are selectively killed by L-asparagines deprivation. In most of the microorganisms L-Asparaginase accumulates as an intra cellular product and exits both in periplasmic and cytoplasmic regions [1]. L-Asparaginase is widely distributed in large number of microorganism. *Erwinea caratovora*, *Pseudomonas aurigenosa*, *Serratia marcescens*, *Vibrio succinogenes* and *staphylococcus* sp. have a potential L-Asparaginase production[2,3,4]. Among fungi- *Aspergillus*, *Fusarium*, and *Pencillum* reported to produce L-Asparaginase. L-Asparaginase enzyme acting on L-asparagine is widely used as anticancer agent .This enzyme converts L-asparagines to aspartic acid and ammonia and hence has been used as chemotherapeutic agent[5].

Mangrove forest in India is productive ecosystem and sensitive to the environmental changes. In the mangrove ecosystem, microorganisms perform various activities such as photosynthesis, Nitrogen Fixation, Methanogenesis, Agarolysis, production of antibiotics and enzymes result in high productivity. Intracellular localization of microbial communities has been studied for the production of alkaline phosphatase deoxyribonuclease[6], cyclic phosphor diesterase 5' nucleatidase, acid phosphatase[7], lipase[8], carboxymethyl cellulase[9] and 1, 7 β- hydroxyl steroid dehydrogenase[10]. Hencethe study of localization of any enzyme plays a vital role in the development of bioprocess.

Biochemical and kinetic properties L-asparaginase vary with the genetic nature of microbial strains used[11] and suggest, isolation and screening of potential microbes which have ability to produce L-asparaginase is indispensable. So far no reports on L-Asparaginase producing soil bacteria of mangrove environment. In present investigation study was focused on characterization of L-Asparaginase producing bacteria isolated from halophilic submerged soil of different mangrove environment.

Material And Methods:

Collection Of Soil Sample:

Soil samples were collected from mangrove environment of Nizampatnam of Guntur district and Chollangi of East coast at a depth of 3 ft and placed in zip locked plastic bags at 4^0 C .The soil contained 3.8% of organic matter and P^H8.8.

Isolation Of Bacterial Strains*:*

1 gm of soil was separately suspended in 9 ml of physiological saline soil in a flask and placed on an orbital shaker (at 100 rpm) at room temperature (28± 2^0C) for 1 hr. At the end of shaking the soil samples were serially diluted up to 10^{-6} with physiological saline. 10^4-10^6 dilutions were placed on modified nutrient agar medium containing Flucanazole (antifungal antibiotic)by pour plate technique and incubated at 28^0C The most prominent colonies were isolated maintained on Nam slants at 4^0c for further studies[12]

Isolation Of L-Asparaginase Producing Bacteria:

After colony count 10-7 dilution was selected. From all samples 0.1ml was inoculated on media containing L-asparagine with phenol red as indicator. L-asparaginase producing colonies were selected on the basis of formation of pink zone around the colonies of the medium. Well isolated colonies from each plate were selected for characterization.

Morpho Physiological And Biochemical Studies: Morphological characters such as shape and color of the colonies were examined. Grams staining and motility were also done. Isolates were biochemically analyzed for the activities of oxidase, catalase, MR-VP test, starch hydrolysis and gelatin hydrolysis, indole production, hydrogen sulphide test, nitrate reduction, sugar fermentation and citrate utilization. The results were compared with Bergey's Manual of Systematic Bacteriology.

Fig 1. L-Asparaginase Producers Isolated From Mangrove Soil

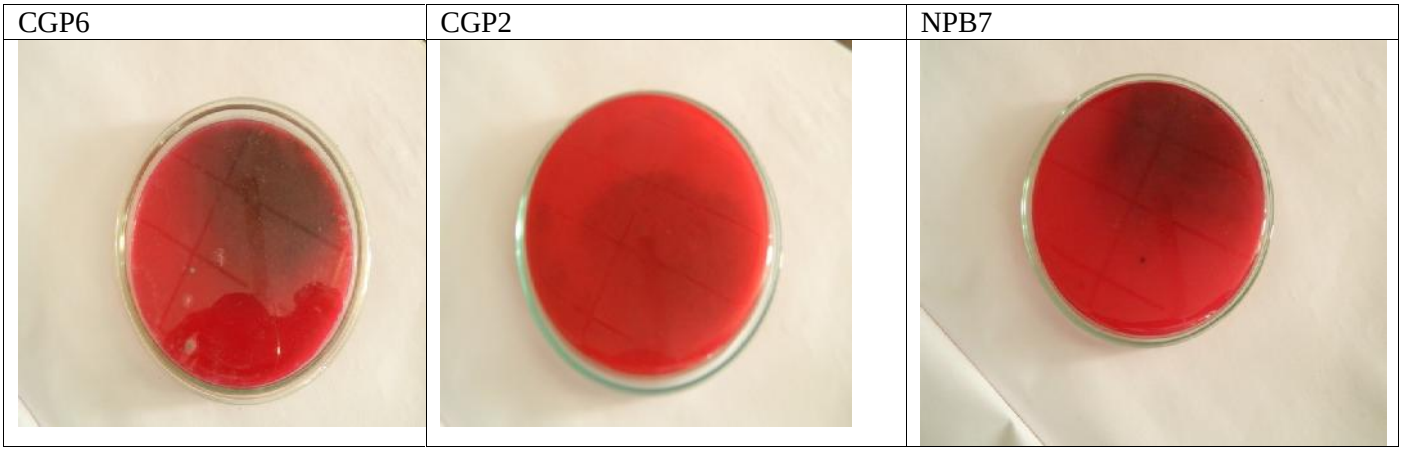

Table -I: Morphological Analysis Of Bacteria Isolated From Different Mangrove Environment

Isolated strain	Gram staining	shape	Organism
NPB7	+ ve	Rods	Bacillus sp.
CGP2	-ve	Short rods	Pseudomonas sp.
CGP6	-ve	Short rods	Pseudomonas sp.

*NP: Nizampatnam, *CG: Chollangi

Table -II: Biochemical Analysis Of Bacteria Isolated From Different Mangrove Environment

Isolated strain	Indole	Methyl red	Vogues Proskeur	Citrate	Catalase	Oxidase	Organism
NPB7	-ve	-ve	-ve	+ve	+ve	+ve	Bacillus sp.
CGP2	-ve	+ve	-ve	+ve	+ve	+ve	Pseudomonas sp.
CGP6	-ve	-ve	-ve	+ve	+ve	+ve	Pseudomonas sp.

*NP: Nizampatnam, *CG: Chollangi

Table -III: Analysis Of Sugar Fermentation Of Bacteria Isolated From Different Mangrove Environment

Isolated strain	Glucose	Lactose	Mannitol	Dextrose	Sucrose	Organism
NPB7	+ve	+ve	-ve	+ve	+ve	Bacillus sp.
CGP2	+ve	+ve	-ve	+ve	+ve	Pseudomonas sp.
CGP6	+ve	+ve	-ve	+ve	+ve	Pseudomonas sp.

*NP: Nizampatnam *CG: Chollangi

Results And Discussion:

Bacterial strains were isolated from soil samples collected from Nizampatnam of Guntur district and Chollangi of East coast. Microbial strains producing L-Asparaginase were identified by pink coloured colony on agar medium with phenol red as an indicator for detection of L- asparaginase producing colonies[13]. Microbial strains NPB7(from Nizampatnam soil sample), CGP2&CGP6 (from Chollangi soil samples) with pink coloured colony were selected (Fig 1)and further characterized by morphological ,physiological and biochemical studies.

The isolated strain from Nizampatmam soil, NPB7 was Gram +ve and rod shaped colony (table 1). The strain was negative to Indole, methyl red and Vogues proskeur tests, able to utilize citrate and exhibited positive catalase and oxidase activity (table II). The strain showed positive growth in media containing glucose, lactose, dextrose and sucrose but negative to mannitol. According to morphological physiological and biochemical characteristics of the strain, NPB7 was classified to be *Bacillus* sp.

The isolated strains from Chollangi soils, CGP2 &CGP6 were Gram -ve and short rod shaped colonies with fluorescent blue colour under UV light (table 1). These strains were negative to Indole, methyl red and Vogues proskeur tests, able to utilize citrate and exhibited positive catalase and oxidase activity (table II). The strain showed positive growth in media containing glucose, lactose, dextrose and sucrose but negative to manifold. According to morphological physiological and biochemical characteristics these strains, NPB7 were classified to be *Pseudomonas sp.*

L-asparagines in the treatment of leukemia and other lympho proliferative disorders has expanded immensely .For these reasons L-asparaginase has established itself to be an indispensable component[14]. Cancer cells differentiate themselves from normal cells in diminished expression of L-asparaginase and hence not capable of producing L-asparaginase and mainly depend on the L-asparagine from circulating plasma pools[15,16]. It catalyses the conversion of L-asparagine to L-aspartate and ammonium ,and this catalytic reaction is essentially irreversible under physiological conditions and attributed to the reduction of L-asparagine ,since tumor cells unable to synthesize this amino acids are selectively killed by L-asparagine deprivation [17,18].Clinical trials indicate that this enzyme is also a promising agent in treating some forms of neoplastic cell disease in man[19]. Microorganisms characterized in present investigation belonged to the genera *Bacillus* and *Pseudomonas* sp. and potential producers of L-asparaginase. Purification and further characterization of L-asparaginase from these halophilic bacteria isolated from mangrove soil can contribute to therapeutic value of this enzyme.

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