Marine Environment: A Potential Source for L-Asparaginase Producing Microorganisms

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Soil samples from Muthupet mangrove environment were collected and fungal and bacterial strains were isolated using PDA and nutrient agar medium respectively. A total of 30 fungal strains and 20 bacterial strains were isolated, and out of these, 17 fungi and four bacterial strains were found to produce L-asparaginase enzyme. L-asparaginase enzyme production was detected by the color change (yellow to pink) in the medium used for the screening of the enzyme producers. Modified Czapek dox and modified M9 medium were used for the screening of L-asparaginase producing fungi and bacteria respectively. Thus the present work was undertaken for screening of the L-asparaginase enzyme from the bacterial and fungal strains of Muthupet mangrove environment.

Keywords: L-asparaginase, Mangrove environment, Bacteria, Fungi, Rapid plate assay method

Introduction

L-asparaginase is receiving attention because of its potential use as a chemotherapeutic agent in tumor and acute lymphoblastic leukemia. It is an intracellular enzyme produced by different microorganisms. The enzyme catalyzes the hydrolysis of L-asparagine into L-aspartic acid and ammonia, resulting in the depletion of serum L-asparagine. Plasma asparagine is essentially undetectable throughout the entire period in which asparaginase is present. Leukemic lymphoblasts and certain other tumor cells, which lack or have very low level of L-asparagine synthetase, do not synthesize L-asparaginase *de novo* and rely on L-asparagine supplied in the serum for survival (Patil and Sawant, 2007).

In the recent years, considerable interest has been shown in the secondary metabolites of marine fungi and bacteria because of their importance (Kirk and Catalfomo, 1970) in medicine (Kirk *et al.*, 1973; Cuomo *et al.*, 1995; Kobayashi *et al.*, 1996; Varoglu *et al.*, 1997; Crews, 1998; Hoeller *et al.*, 1999; Jenkins, 1999; and Byun *et al.*, 2003).

L-asparaginase was isolated and characterized from various microorganisms, including many gram-ve bacteria, *mycobacterium*, yeast, and molds, as well as from plant and plasma of certain vertebrates. The enzyme L-asparaginase has the ability to control tumor and leukaemia diseases in animals and humans. The role of L-asparaginase for these diseases impels the need for a survey on the screening of microbes. This work on screening implies a greater scope for obtaining potent bacterial and fungal strains for the production of L-asparaginase enzyme from marine soil environments.

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Materials and Methods

Collection of Sample

The soil samples (100-500 g) were collected in sterile ploythene bags to avoid the external contamination from Muthupet mangrove environment situated in the Thiruvarur district of Tamil Nadu (10°20' N; 79°35' E), covering an area of about 20,000 ha. Paminiyar, Koraiyar, Kilaithangiyar and Maraka Koraiyar flow through Muthupet and form a lagoon, before they enter into the Palk Strait. The northern and western border of the lagoons are occupied by dense mangrove vegetation. The southern side of the lagoon is devoid of mangroves and remains as sandspit.

Isolation and Identification of Bacteria and Fungi

One gram of the sediment was serially diluted in sterile sea water (10^3 – 10^6). From the dilutions, 0.1 mL was transferred aseptically into the 50% sea water sterile nutrient agar plates and potato dextrose agar plates and were spread with a sterile glass rod and incubated at room temperature (24 h) for five days for bacteria and fungus isolation respectively. The bacterial strains were identified based on the gram staining technique and biochemical tests, and the fungi were identified based on their morphology and staining techniques. Standard manuals of Gillman (1957), Ellis (1971 and 1976) and Subramanian (1971) were referred to identify the species.

Screening of L-Asparaginase Production by Bacteria and Fungi

Rapid Plate Assay Method

To screen the L-asparaginase production from bacteria and fungi, the media used was modified M9 agar medium and modified Czapek dox medium respectively. A loopful of bacterial inoculum was taken and inoculated into the modified M9 medium containing plates with the help of simple streak method. The plates were then incubated at 37 °C for 24-48 h. For fungal enzyme screening, medium was prepared and fungal blocks were cut with the help of cork borer, and the fungal blocks were placed into the Czapek dox medium containing plates. The inoculated plates were incubated at room temperature (25 °C) for 24-96 h. After incubation, the plates were examined. The color change around the colony (yellow-pink) indicated the production of L-asparaginase in both modified M9 medium and modified Czapek dox medium.

Results

A total of 30 fungal and 20 bacterial strains were isolated from marine soil denoted by the code names, SSF1-SSF30 and SSB1-SSB20. From this, 11 bacterial strains and 21 fungal strains (Plates 1 and 2) were found to produce L-asparaginase enzyme (Tables 1 and 2). Based on gram staining and biochemical tests, the organisms-produced L-asparaginase were identified as SSB2-*Escherischia coli*, SSB3 *P. vulgaris*, SSB6-*Bacillus* sp SSB14-*Pseudomonas* sp, and SSB19-*Proteus* sp.

Discussion

The present study is aimed at screening for novel L-asparaginase producing microorganisms in Muthupet mangrove soil. Out of 30 fungal strains, 21 were found to produce

Table 1: L-Asparaginase Production by Bacterial Isolates		
Isolate Code	Name of the Bacterial Isolates	L-Asparaginase Production
SSB2	*E. coli*	+ + +
SSB3	*P. vulgaris*	+ + +
SSB6	*Bacillus* sp	+ + +
SSB14	*Pseudomonas* sp	+ +
SSB19	*Proteus* sp	+
SSB20	*Escherischia coli*	+ + +
Note: + Low production; + + High production; + + + Very high production.		

Table 2: L-Asparaginase Production by Fungal Isolates		
Isolate Code	Name of the Bacterial Isolates	L-Asparaginase Production
SSF1	*Aspergillus niger*	+
SSF3	*Aspergillus terrus*	+
SSF4	*Aspergillus flavus*	+ +
SSF5	*Aspergillus fumigatus*	+ + +
SSF7	*Aspergillus ochraceous*	+
SSF8	*Aspergillus luchensis*	+ +
SSF9	*Pencillium janthinellum*	+
SSF10	*Pencillium citrinum*	+
SSF11	*Curvularia palase*	+ +
SSF12	*Trichoderma ressi*	+
SSF13	*Trichoderma koningii*	+ + +
SSF14	*Trichoderma harzianum*	+ + +
SSF16	*Trichoderma virens*	+
SSF17	*Trichoderma viridae*	+ +
SSF18	*Trichoderma hamatum*	+ +
SSF19	*Fusarium oxysporum*	+ + +
SSF20	*Drecheriella ellssi*	+
Note: + Low production; ++ High production; +++ Very high production.		

Plate 1: Photo Showing L-Asparaginase Enzyme Production by *T. harzianum*

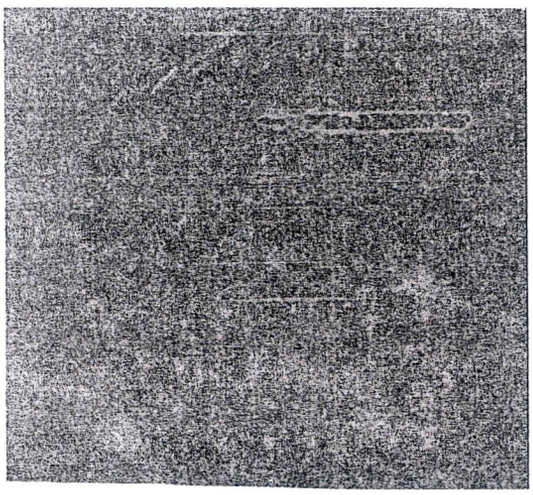
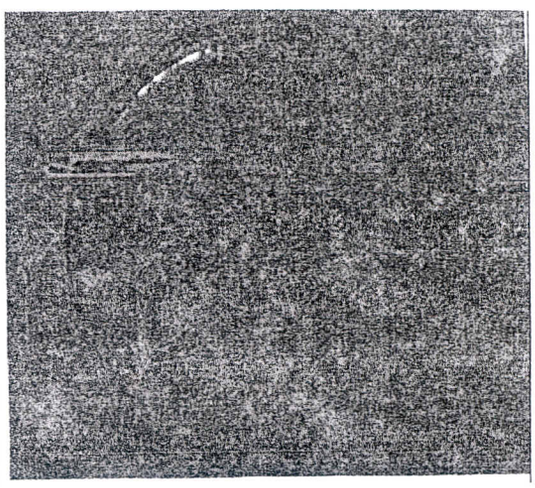


Plate 2: Photo Showing L-Asparaginase Enzyme Production by *T. viridae*



L-asparaginase enzyme. Out of 20 bacterial strains, 11 strains were found to produce L-asparaginase enzyme. *A. fumigatus, T. koningii, T. harzianum, F. oxysporum* and *Bacillus* sp were found to be good producers of L-asparaginase. The present plate assay (Rapid Plate Assay Method) is advantageous, as the method is quick and L-asparaginase production can be visualized directly from the plates without performing time-consuming assays (Wade *et al.*, 1971; Arima *et al.*, 1972; and Imada *et al.*, 1973). The incubation period required prior to selection of an L-asparaginase producer is significantly reduced. The incubation period for bacteria and fungi was 18 h and 48 h and at times exceeds 96 h for fungi (Barnes *et al.*, 1977).

In this screening procedure, 70% of the fungal strains from marine soil sample have the ability to produce the enzyme L-asparaginase and 55% of the bacterial strains too have the ability to produce the same. The potential of L-asparaginase is an accepted fact for application in the treatment of childhood acute lymphoblastic leukaemia and other forms of cancer. This study is a prerequisite for further optimization for enzyme production.

Conclusion

Enzymes are the biocatalysts synthesized by living cells. They are complex protein molecules that bring about chemical reactions concerned with life. They are produced in small amounts by microorganisms and have vital applications which include their use in food production, food processing and preservation, washing powders, textile manufacture, leather industry, paper industry, medical applications, improvement of environment and in scientific research. Commercial enzymes can be produced from a wide range of biological sources. At present, a great majority of about 80% of them are from microbial sources (Gulati *et al.*, 1997).

A total of 50 strains, which include 30 fungal and 20 bacterial strains were isolated. When the screening for L-asparaginase producing microorganisms were done, 21 fungal strains and 11 bacterial strains were found to produce L-asparaginase enzyme. Among the

various fungi and bacteria tested by the rapid plate assay method, *A. fumigatus, T. koningii, T. harzianum, F. oxysporum* and *Bacillus sp, E. coli* were found to be good producers of L-asparaginase.

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