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Research Article

MICROBIAL L-ASPARAGINASE: THE ANTICANCER SOLUTION HIDDEN IN LAND OF MANGROVES

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ABSTRACT

Mangroves represent a vast and productive ecosystem for a wide range of organisms. The peculiar characteristics like scarcity of oxygen, salinity of the water make this environment unique to support the growth of specific organisms found in this ecosystem. India is well known for its richness of biodiversity and has 7516.6 km coastline. The wide range of bacteria, fungi and actinomycetes are not only unique to the mangrove environment but also are very diverse in their enzyme activities. However, this diversity of marine ecosystems has not been much explored as far as the microbial communities and their interactions are concerned. With this background in view, the current study was undertaken to explore the variety of organisms inhabiting land of Mangroves in Maharashtra with special reference to their ability to produce an enzyme L-asparaginase.

L-Asparaginase is the unique enzyme that can be a potential anticancer agent. Geographically versatile land of mangroves was chosen for obtaining highly efficient L-Asparaginase producer. Soil and water samples were collected from the rhizosphere of the mangroves. These samples were then subjected to incubation in ADS broth for enrichment of the desired type of organisms. The isolates were then tested semi-quantitatively for production of the enzyme. Six out of total twenty isolates were found to be highly efficient producers of the enzyme. This enzyme can not only be used in treatment of lymphoproliferative disorders and lymphomas but also be used in food industry to reduce acrylamide levels in fried foods, proving their potential in industrial application.

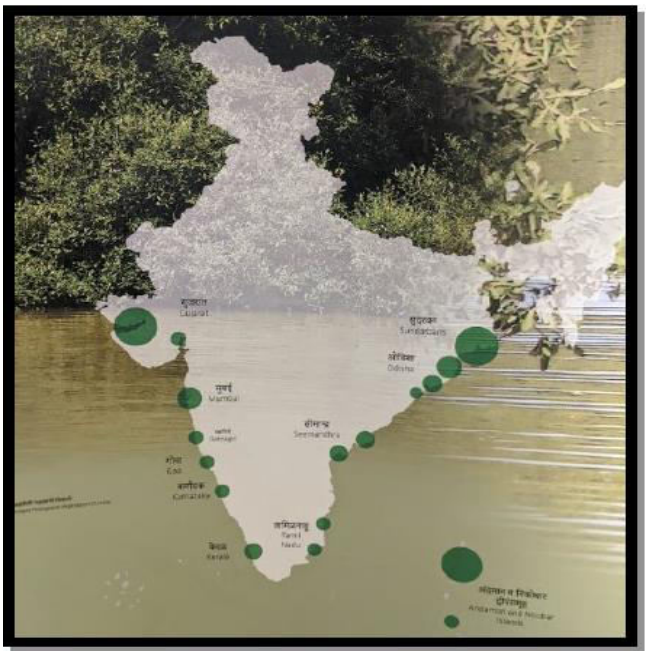
Keywords: Mangrove, Microbial L-asparaginase, Anticancer.

1. INTRODUCTION

India is well known to possess variety and richness in biodiversity in different states. Indian land is also surrounded by large water bodies from three sides making it unique to possess a coastline of 7516.6 km. Microbiologists have always tried to explore the novel microorganisms in unique sites for commercial applications. Mangroves is one of such sites where microbial diversity is found to be abundant. [1] Mangroves represent environmentally peculiar characteristics like high salinity of water, scarcity of oxygen etc. These characteristics make the microbial flora of the land very unique. Many studies have shown that number of microorganisms possessing specific property like nitrogen fixation, phosphate solubilization, siderophore production, plant growth promotion, antibiotic production, enzyme production etc. [2].

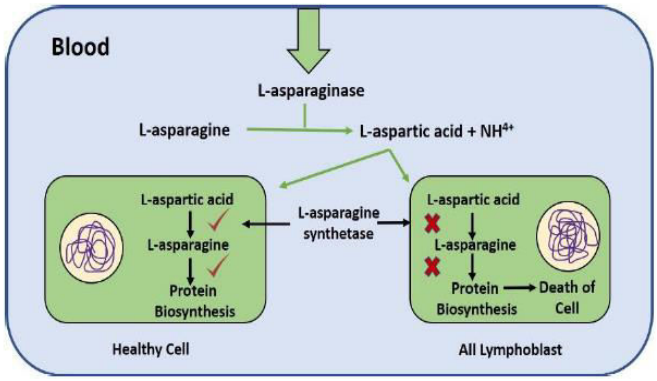
Microorganisms are known to be natural factories of various enzymes that can be commercially employed for various applications. These enzymes include cellulases, lipases, proteases, amylases, ligninases, Laccases, galactosidases, glutaminases and many more. One of these enzymes is L-asparaginase which has been proved to be a promising solution in treatment of cancer. [3, 4] For several years cancer was essentially being treated with antiproliferative agents or radiotherapy. Both of these had their own drawbacks making it necessary to have a more promising, specific treatment. Therapeutic enzymes like L-asparaginase have been used in cancer treatment. These enzymes can be obtained from bacteria, plants, fungi and even animals. Bacterial sources are preferred for their ease in handling, high yield and fast growth. L-asparagine is an essentially required by certain tumour cells through an external source for their growth and multiplication. This is because they lack or have very low levels of asparagine synthetase enzyme which plays important role in synthesis of asparagine. The enzyme L-asparaginase

hydrolyzes the L-asparagine available in serum into aspartic acid and ammonia, making it unavailable for tumour cells and thus proving to be a potent anticancer therapeutic enzyme. [5, 6] Various studies have isolated variety of organisms capable of synthesizing this enzyme from various sources. The commonly obtained organisms include *Escherichia spp., Bacillus spp., Erwinia spp., Streptomyces spp., Aspergillus spp.* etc. [7, 8] Despite having richness of innumerable habitats, mangroves in India remains much less explored for organisms capable of synthesizing this enzyme. With this background in view, the current study was undertaken to isolate industrially important bacterial L-asparaginase producers from mangroves in Mumbai.



(Courtesy: Mangrove Foundation)

Fig. 1: Mangroves of India



https://doi.org/10.1016/j.bjm.2016.10.004

Fig. 2: Mechanism of working of L-asparaginase

2. METHODS AND MATERIALS

2.1. Sample Collection

Soil and water samples were collected from the four different sites at a depth of 25 cm near the rhizosphere of Vikhroli Mangroves managed by Godrej in Mumbai Maharashtra. The sites were Palm Garden (N 19° 05' 53.1" E 072° 56' 03.3"), BMC Canal Road (N 19° 05' 38.1" E 072° 55' 56.3"), Nature trail (N 19° 06' 01.3" E 072° 56' 13.4") and Medicinal Garden (dominated by *Rhizophora spp.*) (N 19° 05' 53.16" E 72° 56' 25.24"). Samples were also collected from Bhandup bird watching site, Mumbai Maharashtra and from Sundarbans in West Bengal. The soil samples were collected using a sterile spatula and were immediately transferred to a previously unopened zip lock polythene bag. Water samples were collected in a screw capped alcohol sterilized bottles and labelled immediately. Both the samples were transported to the laboratory for microbiological analysis. In case of any delay in processing, the samples were refrigerated until processed.

2.2. Enrichment & Isolation

Suspensions were prepared from soil samples. The soil sample (1 gm) was added and mixed thoroughly in 9 ml of sterile saline. The suspension was allowed to settle down and supernatant was used as soil sample. Water samples were used directly. 1 ml of these samples were subjected to enrichment in 15 ml sterile asparagine dextrose broth in duplicates. The composition of media included L-Asparagine (1%), Dextrose (0.2%), K_2HPO_4 (0.1%), $MgSO_4$ (0.05%), Phenol Red (0.006%). The pH of the medium was adjusted to 7.4 ± 0.2. Hydrolysis of L-asparagine by extracellular L-asparaginase produced by organisms released ammonia which was detected visually by pH indicator phenol red added in the medium. Colour change of the medium from orange to pink due to alkaline conditions indicated the presence of asparaginase producers. All such tubes showing positive result were then subjected to further processing.

Each positive tube was then subjected to the tenfold serial dilution and 0.1 ml of each dilution tube was then used for surface spreading on the sterile Asparagine Dextrose Salt (ADS) agar plate in order to get a well isolated viable bacterial colony from the population. Each bacterial colony was streak isolated separately on an ADS agar plate and preserved on ADS slant too. All individual bacterial colonies were recorded for their colony characteristics and gram nature. The isolates

were then subjected to semiquantitative analysis of enzyme production to identify the best asparaginase producers from the isolates.

2.3. Semiquantitative analysis

The isolates thus obtained were individually enriched in asparagine dextrose broth for 24-48 hours and then centrifuged to obtain cell free extract of extracellular enzyme produced by them. Sterile 1 % asparagine agar plates lacking dextrose were prepared and wells were bored into it to carry out the semiquantitative analysis of this extract, using agar cup method. Each well was loaded with 50 µl of the extracellular enzyme extract.
The plates were incubated at room temperature for 10-20 hours. Absence of dextrose ensured no growth of bacterial cells or any contamination due to lack of carbon source. The enzyme diffused across the agar and showed a pink colored zone around the well. The diameter of the well directly proportional to the amount of enzyme produced. The isolates showing highest zones of diameter can be taken for further processing.

3. RESULTS & DISCUSSION

3.1. Sample Collection

Following are the sites from where the soil and water samples were collected.

3.2. Enrichment and Isolation

The Enrichment broth showed colour change after 48-72 hours showing presence of delayed production of enzyme in some samples. Upon isolation on agar plates, only bacterial colonies were chosen for further analysis. A total of 18 isolates were obtained out of which 15 were from Vikhroli, 3 from Bhandup bird watching site and 5 from that of Sundarbans. Most of the isolates were Gram positive rods while some were Gram negative coccobacilli. One of the isolates was also found to have presence of capsule.

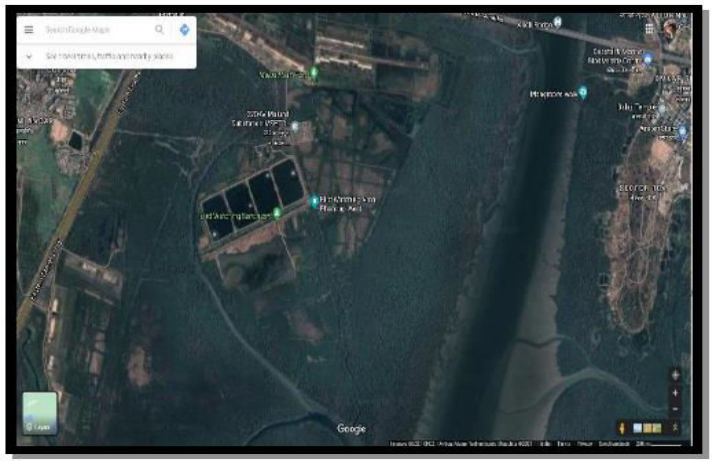


Fig. 3: Bhandup Bird watching site (Google maps)

Fig. 4: Nature Trail site at Vikhroli Mangroves

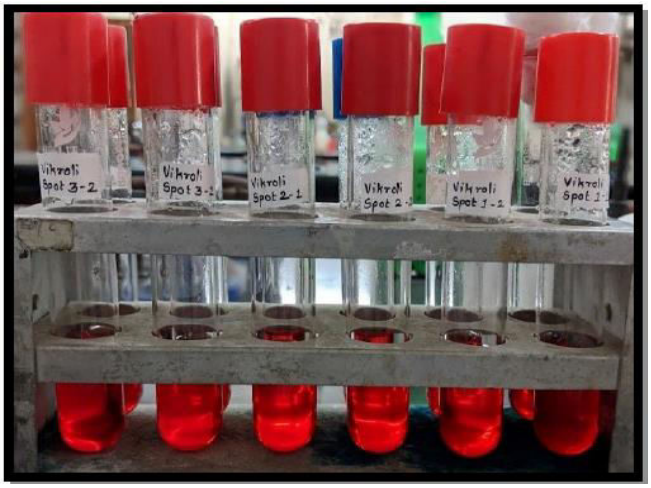


Fig. 5: Enrichment of samples in sterile ADS broth

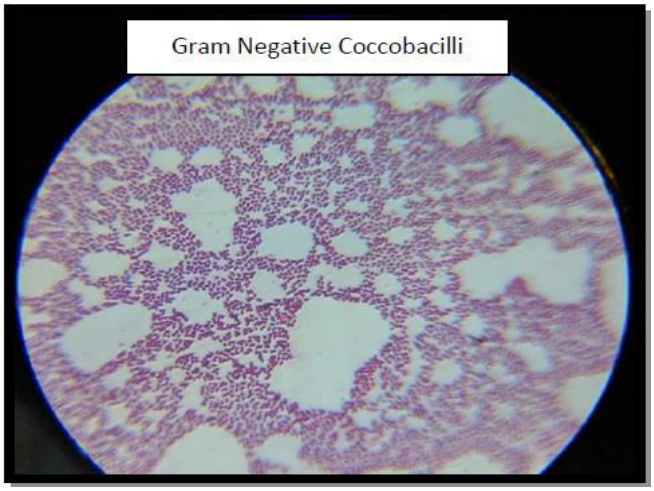


Fig. 6: Result of Gram staining of an isolate

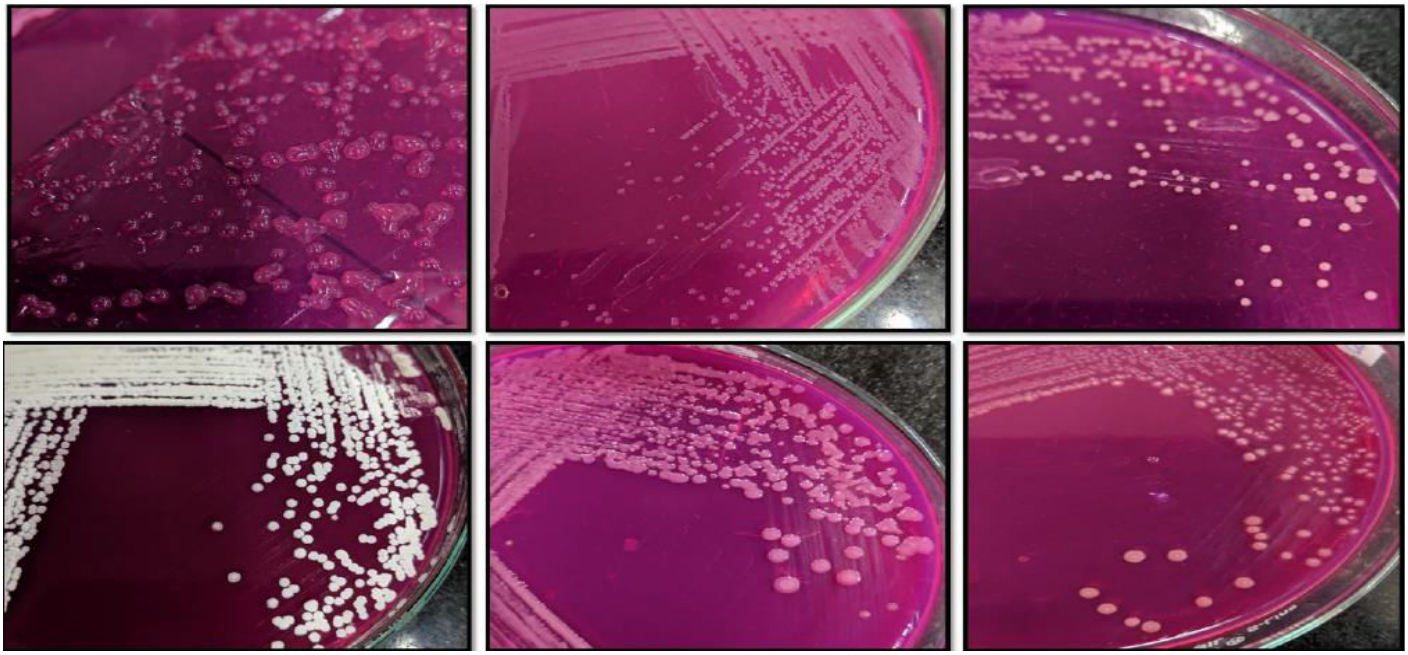


Fig. 7: Isolates on sterile ADS agar

3.3. Semiquantitative analysis

Among all, there were five isolates that showed promising results. These were V7, V10, V13, V15, B2, S4. Results are depicted in picture below:

Isolate	Diameter of Zone (cm)
V7	2.8
V10	4.2
V13	4.9
V15	3.7
B2	3.2
S4	2.8

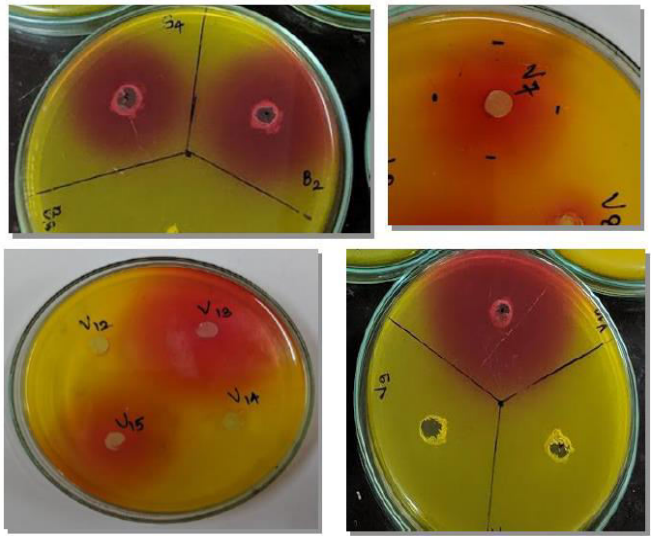


Fig 8: Results of Semiquantitative analysis of isolates

4. DISCUSSION AND CONCLUSION

The current study has given six bacterial isolates that can prove to be industrially important as source of therapeutic anticancer enzyme called L-asparaginase. The sites chosen for the study were unique and no research to isolate asparaginase producing bacteria have been conducted at these sites before. Many scientists have found L-asparaginase producing strains of actinomycetes and fungi from different environment. [2, 6, 9-20] Moreover, studies in establishing the optimum conditions required by the organisms for growth and enzyme production have also been carried out. Enzyme can also be extracted purified and checked for its molecular weight by SDS-PAGE analysis. Many review articles have stated the possible positive results of immobilization of purified enzyme in treatment of cancer. With advancements in nanobiotechnology, there have been reports that nano-immobilization of this enzyme would yield best results. This research was at halt due to lockdown imposed across nation during pandemic. It is now being funded by Mangrove foundation to carry out for wider analysis at three different sites that represent mangroves in Maharashtra. Studies can also be conducted with respect to the organisms associated with specific species of mangroves. Nevertheless, it can be surely concluded that Mangroves can prove to be the best source for organisms producing this anticancer enzyme.

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