

## Isolation, Characterization and Screening of Anticancerous L- Asparaginase Producing Microbes from Costal Regions

Sanjotha .G

Department of Biotechnology, Government arts and science college Karwar, Karnataka, India

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**Abstract:** *L-asparaginase is an extracellular enzyme that has attracted much attention because, it has highly potential anti-cancerous activity. In the present study, four soil samples each were collected from different areas in and around the karwar regions, Karnataka, India. Out of 16 isolates three bacterial strains (CB1, CB2, MB) isolated from costal and mangrove ecosystem showed maximum L-Asparaginase production than estuarine and terrestrial ecosystems and three actinomycetes (CA, MA, TA) isolated from costal, mangrove and terrestrial ecosystems showed potential L-Asparaginase production than estuarine ecosystems on modified M9 medium. Selected isolates were characterized by biochemical tests.*

**Keywords:** *L-Asparaginase, Anticarcinogenic, karwar, Bacteria, Actinomycetes*

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### 1. INTRODUCTION

Cancer has emerged as one of the major cause for human suffering with unprecedented morbidity and mortality. There were an estimated 12.7 million cancer cases around the world in 2008, out of these 6.6 million cases were in men and 6.0 million in women. This number is expected to increase to 21 million by 2030 (IAEA Report, 2010). Cancer may be defined as unnecessary tissue growth that results from an imbalance between cell division and apoptosis, (programmed cell death); due to various genetic and epigenetic alterations. Cancer cells are also divided in to benign and metastatic. Leukemia's are neoplasms of hematopoietic cells that proliferate initially in the bone, spleen, lymph nodes and later in other tissues. Acute myeloid leukaemia (AML), chronic lymphoblastic leukaemia (CLL) and hairy cell leukaemia in adults are observed to increase in incidence with age, especially in the sixties and seventies [1].

Many numbers of enzymes have been used as effective therapeutic agent against many kinds of cancer in man [2]. L-asparaginase attracted much attention because, it is highly suitable for treatment of acute lymphoblastic leukemia (mainly in children), Hodgkin disease, acute myelocytic leukemia, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma treatment, reticulosarcom and melanosarcoma [3, 4]and as food processing aid to reduce the acrylamide formation during frying of starchy foods at high temperature. In lymphocytic leukemia cells are not capable of synthesizing L-asparagine and they rely on the exogenous sources to get L-asparagine [5]. On the contrary, normal cells are protected from L-asparagine starvation due to their ability to generate this essential amino acid [6]. L-asparaginase is known to act by hydrolysing the L-asparagine into aspartic acid and ammonia. Hydrolysis proceeds in two steps via a beta- acyl enzyme as intermediate [2], causing deficiency of the amino acid for cancer cells, whereby it limits the growth of cancerous cell [7].

#### 1.1. L-Asparaginase



L-asparaginase is a wide spread enzyme, relatively found in many microorganisms for example, *Enterobacter aerogenes*, *Aspergillus terreus*[8], *Fusarium equiseti*[9], *Pseudomonas aeruginosa*[10], *Bacillus subtilis*[11], *Pyrococcus furiosus*[12], *Enterobacter cloacae*, *serratiamarcescens*[13] etc. The enzymes obtained from bacteria like *E.coli* and *Erwinia* have been used in the treatment of acute lymphoblastic leukemia [14]. However due to prolonged intake of drugs causes an anaphylactic reactions in man. Therefore it is necessary to screen novel strains which are able to produce high yield of L-asparaginase [15].

Marine biosphere is one of the richest of earth's innumerable habitats and yet is one of the least characterized. Because of the diversity and scale, it offers enormous opportunities for non-destructive exploitation within many facets of modern biotechnology [16]. Most of the Marine microorganisms have potential source of bioactive compounds [17, 18]. Among these, Bacteria have gained special importance in production of antibiotics and secondary metabolites [19, 20]. The present study was carried out to explore the soil and marine sources for isolation of microbes. Because, there is a continual need to find out newer microbial sources to obtain high-yielding L-asparaginase producing strains, which could be of use to human.

## **2. MATERIALS AND METHODS**

### **2.1. Sample Collection**

Four soil samples were collected from different areas at a depth of 15 to 20 cm in and around the karwar regions, Karnataka, India. The location of sample collection includes costal, estuarine, mangrove and terrestrial ecosystems. The samples were collected into a sterile polythene bags and carried to laboratory for further microbial analysis.

### **2.2. Isolation of Microorganisms**

All the soil samples were treated for bacterial as well as actinomycetes by serial dilution method. Among four samples each two samples were used for bacterial isolation by using nutrient agar medium [21] with nystatin (50µg/mL) to avoid fungal contamination, inoculated agar plates were incubated at 37<sup>0</sup>C for 24 hours and two samples were used for isolation of actinomycetes by using Starch casein agar (SCA) medium with Streptomycin (20µg/ml) and cycloheximide (50µg/ml) in order to retard the growth of bacteria and fungi. All the plates were incubated at 37<sup>0</sup>C for 6 days. Colonies obtained were further purified and maintained in nutrient agar slants and SCA plates.

### **2.3. Screening for L-Asparaginase Production by Plate Method Assay**

The purified 16 isolates were screened for L-Asparaginase production by rapid plate method by using modified M-9 medium. [22]. The medium contained Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 6.0 g; KH<sub>2</sub>PO<sub>4</sub>, 3.0 g; NaCl, 0.5 g; L-Asparagine, 10.0 g; 1mol-MgSO<sub>4</sub>·7H<sub>2</sub>O, 2.0 ml; 0.1 M solution of CaCl<sub>2</sub>·2H<sub>2</sub>O, 1.0 ml; 20% glucose stock, 10.0 ml; agar 20.0 g. per liter of distilled water. The medium was supplemented with 0.005% phenol red dye (prepared in ethanol) and the pH-6.8. Plates were then incubated at 35°C for 24 hrs. L-Asparaginase producing colonies were selected on the basis of formation of pink color zone around the colonies. Out of 16 isolates three bacterial (CB1, CB2, MB) and three actinomycetes (CA, MA, TA) colonies showed potential L- asparaginase production. However these organisms did not form any pink color zone in control M-9 medium without L-Asparagin. This indicates that the formation of pink zone is due to production of L-asparaginase.

### **2.4. Biochemical Characterization of Isolates**

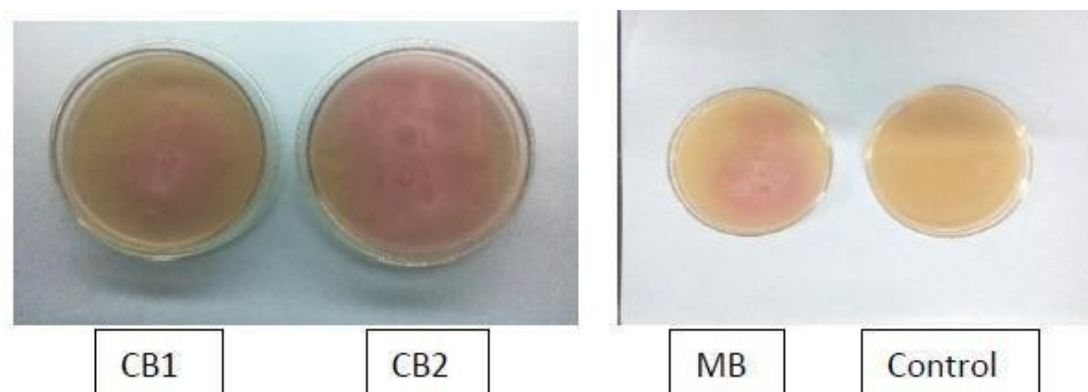
Morphological characters such as shape and color of both bacterial as well as actinomycetes colonies were determined. Grams staining and motility testing by hanging drop method were also performed. Isolates were biochemically analyzed for the activities of oxidase, catalase, MR-VP test, indole production, hydrogen sulphide test, nitrate reduction, citrate utilization, melanoid production test using Waksman medium at an incubation temperature of 37°C for 4 days for the detection of pigment producing property of isolates. Gelatin liquefaction test was carried out in nutrient gelatin at an incubation temperature of 37°C for 24 – 48 hours, acid production test was carried out in glucose nutrient broth at an incubation temperature of 20°C for 15 days. The results were compared with Bergey's Manual of Systematic Bacteriology and standard International Streptomyces Project (ISP) procedure.

## **3. RESULTS AND DISCUSSION**

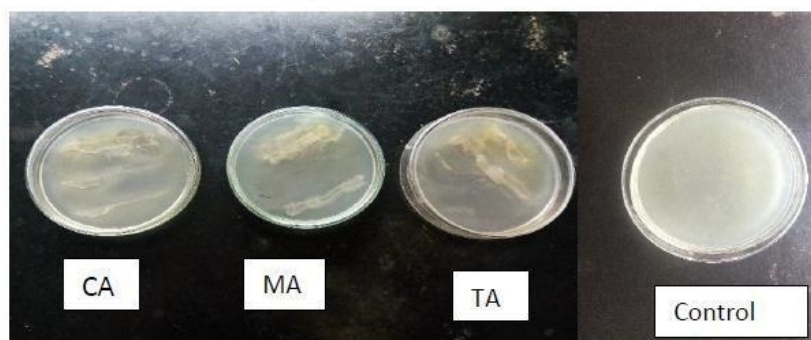
In present study, four soil samples includes costal, estuarine, mangrove and terrestrial ecosystems were collected from different regions of karwar were inoculated on nutrient medium for bacteria and starch casein agar for actinomycetes. Bacterial isolates after incubation showed white, yellow colored colonies on nutrient agar medium and actinomycetes showed grey colored colonies on starch casein agar medium (fig.2). These isolates were used for screening of l-asparaginase production, by using modified M-9 medium. The medium contained asparagine with phenol red as an indicator (fig.3). Out of 16 isolates three bacterial (CB1, CB2, MB) and three (CA, MA, TA) actinomycetes were showed

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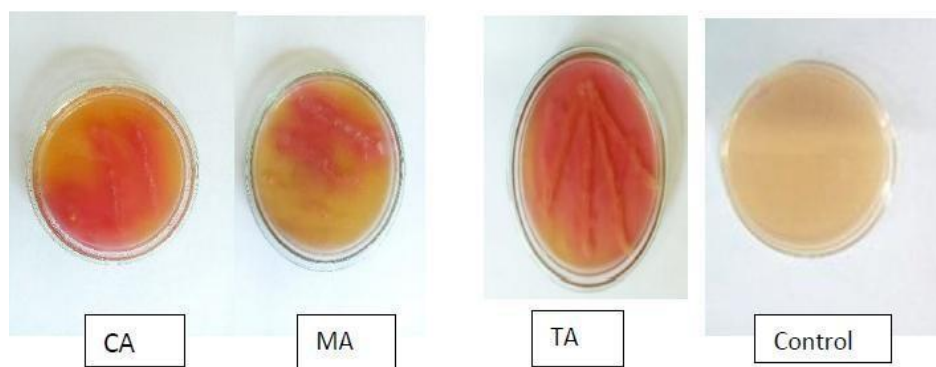
potential L-asparaginase production by forming pink color zone around colonies on the medium (fig.1). These strains were taken for biochemical characterization. Morphology and Biochemical characteristics of both bacteria and actinomycetes are illustrated in table no 1, 2 and 3.



**Figure1.** Isolation and screening of L-Asparaginase producing Bacteria on modified M9 media using L- Asparagine and phenol red indicator



**Figure2.** Isolation and screening of L-Asparaginase producing actinomycetes on starch casein agar (SCA) media, showing gray colored colonies



**Figure3.** Screening of L-Asparaginase producing actinomycetes on modified M9 media, showing pink color zone

**Table1.** Morphology and cultural characteristics Of L-asparaginase producing bacterial isolates

Bacterial isolate designation	Gram staining	shape	Organism
CB1	+ ve	Rods	Bacillus sp
CB2	-ve	Short rods	Pseudomonas sp.
MB	-ve	Short rods	Pseudomonas sp

**Table2.** Biochemical Studies of Bacterial isolates

Bacterial isolate designation	Indole	Methyl red	Vogues Proskeur	Citrate	Catalase	Oxidase
CB1	-ve	-ve	-ve	+ve	+ve	+ve
CB2	-ve	+ve	-ve	+ve	+ve	+ve
MB	-ve	-ve	-ve	+ve	+ve	+ve

**Table3.** Biochemical studies of *Actinomycetes*

S.No	Biochemical Characterization	Isolates		
		CA	MA	TA
1	Melanoid production Test	+	-	+
2	Nitrate reduction test	+	+	+
3	Acid production test	+	+	-
4	Hydrogen sulphide production test	-	-	-
5	Gelatin hydrolysis test	+	+	+

#### 4. CONCLUSION

From this work, it was clearly shows that costal and mangrove ecosystem soil samples can provide a potential source of L-asparaginase producing bacteria when compared to estuarine and terrestrial ecosystem soils samples and costal, mangrove and terrestrial ecosystem soil samples exhibit a potential source of actinomycetese when compared to estuarine soil samples. However in future optimization of these isolates will be done to increase the potentiality to produce L-asparaginase.

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