

Isolation, Characterization and Screening of L-Asparaginase Producing Actinomycetes (Streptomyces) from the Soil Samples of Gulf of Mannar Region

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Abstract: *L-asparaginase (L-asparagine amido hydrolase, E.C.3.5.1.1) is an extra cellular enzyme that has received considerable attention since it is used as an anticancer agent. In the present study, the 17 actinomycetes strains were isolated from the soil samples of Gulf of Mannar region (Rameshwaram) using starch casein agar medium and ISP-5 medium. The isolated strains were screened for the L-asparaginase production by using Modified M9 Medium containing L-asparagine and phenol red as indicator. L-asparaginase activity was detected on the basis of pink color around the colony. Out of 17 strains screened for L-asparaginase activity only five strains shows positive result.*

Keywords: Soil, actinomycetes, Characterization, L-asparaginase, Screening

1. Introduction

Cancer is defined as uncontrolled division of cells. Acute lymphoblastic leukemia is cancer of WBC, characterized by the excessive multiplication of malignant and immature WBC (lymphoblast) in bone marrow. Many enzymes have been used as drugs like wise L-asparaginase attracted much attention because of its use as effective therapeutic agent against lymphocytic leukemia and other kinds of cancer in man [7]. L-asparaginase (EC 3.5.1.1), a medically important enzyme, hydrolyze L-asparagine (essential amino acid) to aspartic acid and ammonia. Since several types of tumour cells require L-asparagine for protein synthesis, they are deprived of an essential growth factor in the presence of L-asparaginase, thus, resulting in cytotoxicity of leukemic cells [3]. i.e., L-asparaginase interferes with the growth of malignant cells, which do not have the capacity of synthesizing L-asparagine for their metabolism[13]. L-asparaginase is a relatively wide spread enzyme, found in many microorganisms such as *Pyrococcus furiosus*[22], *Enterobacter cloacae*, *Serratia marcescens*[19], *Enterobacter aerogenes*, *Aspergillus terreus*[21], *Fusarium equiseti*[18], *Pseudomonas aeruginosa*[20], *Bacillus subtilis*[23], etc. The enzymes isolated from *E.coli* and *Erwinia carotovora* are now being used in the treatment of acute lymphoblastic leukemia [4]. However due to prolonged administration of L-asparaginase, the corresponding antibodies are produced in man, which causes an anaphylactic shock or neutralization of the drug effect. Therefore there is a continuing need to screen newer organisms in order to obtain strains capable of producing high yield of L-asparaginase [5].

Marine actinomycetes are a profile source of secondary metabolites and the vast majority of these compounds are derived from the single genus *Streptomyces* [16]. Actinomycetes are the group of gram positive bacteria with high G+C (guanine + cytosine) content which form branching filaments or hyphae and asexual spores[2]. Actinomycetes are threadlike bacteria that look like fungi.

Streptomyces species are distributed widely in marine and terrestrial habits[17] and are of commercial interest due to their unique capacity to produce novel metabolites. Enzymes such as amylase, lipase, protease, cellulase, chitinase, pectinase, insulinase and glucose isomerase are well reported from actinomycetes in particular *Streptomyces*. Among the actinomycetes several terrestrial streptomyces like *S.karnatakensis*, *S.venezulae*, *S.longsporusflavus* and *S.albidoflavus* are capable of producing detectable amounts of L-asparaginase [6]. But there are only limited reports on the production of L-asparaginase from marine *Streptomyces* like *S.aurantiacus* [14], *Streptomyces* sp PDK 2 & PDK 7[4] and *Streptomyces* sp. S3, S4 & K8 [15]. Although L-asparaginase from bacteria has been extensively characterized, a similar attention has not been paid to actinomycetes. Considering the above facts, an attempt was made for the first time on the isolation and screening of an anti-cancer enzyme L-asparaginase from actinomycetes isolated from soil samples of Gulf of Mannar region (Rameshwaram). Isolates were inoculated on Starch Caesin agar and ISP-5 Medium. Out of 17 isolates screened for L-asparaginase activity only 5 isolates found potential for L-asparaginase activity.

2. Materials and Methods

2.1 Sample Collection

The soil samples were collected from Gulf of Mannar region (Rameshwaram) at a depth of 15cm in January 2014. The samples were collected using alcohol rinsed Peterson grab and were transferred to new polythene bags using sterile spatula.

The samples were transported to the laboratory for the isolation of actinomycetes.

2.2 Isolation of Actinomycetes

Sample was air dried aseptically. After a week sample was incubated at 55°C - 5 min in order to facilitate the isolation of actinomycetes. Then tenfold serial dilution was prepared with one gram of soil sample using distilled water. Samples were inoculated on Starch Caesin agar and Glycerol Asparagine agar (ISP-5 Medium) in triplicate plates. Streptomycin (20 µg/ml) and cycloheximide (50 µg/ml) were added to the medium in order to retard the growth of bacteria and fungi respectively. All the plates were incubated at 28 ± 2 °C for 7 days. Colonies with suspected Actinomycetes morphology (i.e., isolates with grey and white colonies) were purified using yeast-extract malt extract agar medium. The pure cultures of the actinomycetes were streaked on SCA and ISP-5 Medium plates (Fig 1 & 2).

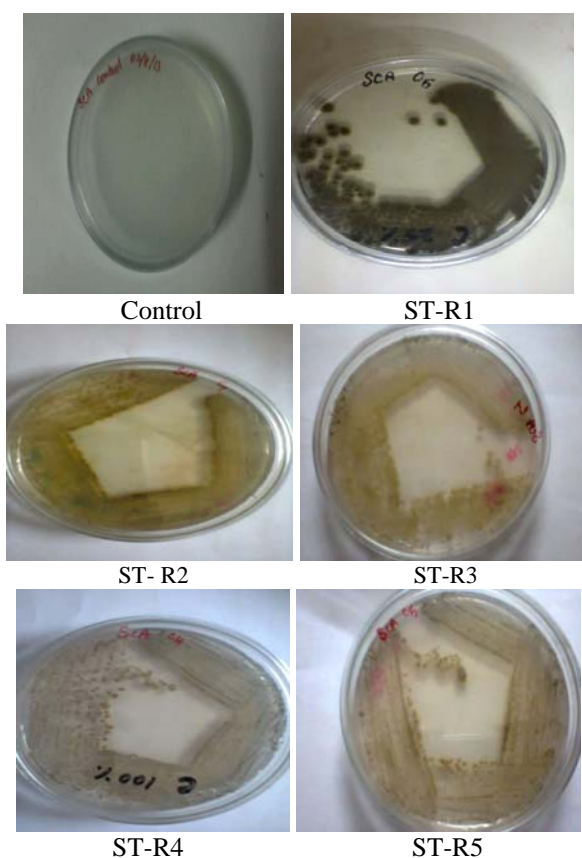


Figure 1: Isolation of Actinomycetes in Starch Caesin Agar Medium

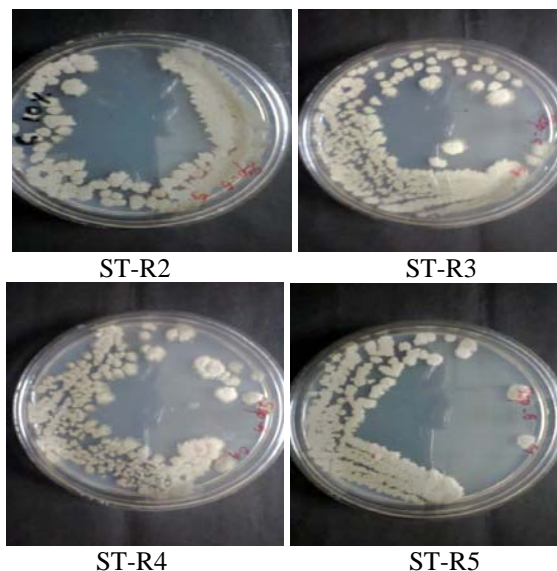
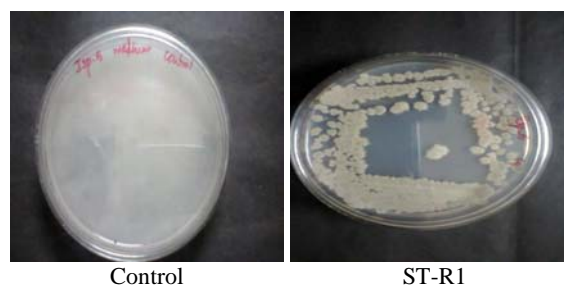


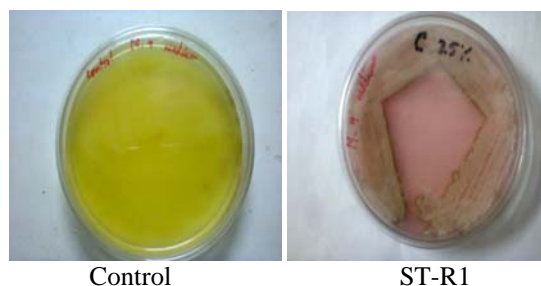
Figure 2: Isolation of Actinomycetes in ISP-5 Medium

2.3 Identification of Soil Isolates

Isolated colonies were identified using standard International Streptomyces Project (ISP) procedure. Morphological identification of isolated colonies was carried out by simple staining, Grams staining and motility testing by hanging drop method. Biochemical characterization was by melanoid production test using Waksman medium at a incubation temperature of 37 °C for 4 days for the detection of pigment producing property of isolates; organic nitrate reduction test was carried out in organic nitrate broth at an incubation temperature of 37 °C for one week for the detection of nitrate reducing property of isolates; acid production test was carried out in glucose nutrient broth at an incubation temperature of 20 °C for 15 days for the detection of glucose fermentation leading to the production of acid; hydrogen sulphide production test was carried out in SIM Agar at an incubation temperature of 37 °C for 5 days, while gelatin liquefaction test was carried out in nutrient gelatin at an incubation temperature of 37 °C for 24 – 48 hours for the detection of gelatin hydrolyzing properties of isolates.

2.4 Screening For L-Asparaginase Production (Rapid Plate Assay)

The L-asparaginase activity of 17 isolates was screened by using Modified M-9 Medium supplied with L-asparagine and 3 drops of phenol red indicator dye (pH-6.8). L-asparaginase producing colonies were selected on the basis of formation of pink zone around the colonies of the medium (Fig:3).



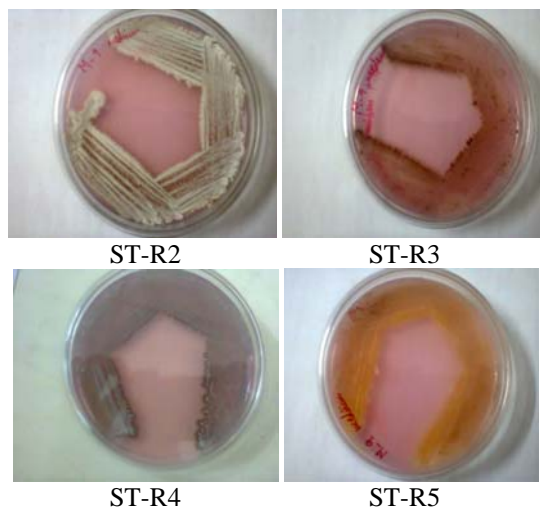


Figure 3: Screening of L-asparaginase production in M-9 Medium

Table 1: Biochemical characterization of the isolated organism. (Note: '+' indicates positive, and '-' indicates negative)

S.No	Biochemical Characterization	Soil Isolates				
		ST-R1	ST-R2	ST-R3	ST-R4	ST-R5
1	Melanoid production test	+	-	+	+	-
2	Nitrate reduction test	+	+	+	-	+
3	Acid production test	+	+	-	+	+
4	Hydrogen sulphide production test	-	-	+	-	+
5	Gelatin hydrolysis test	+	-	-	+	-

3. Result and Discussion

Soil Samples from Gulf of Mannar region (Rameshwaram) were inoculated on Starch Caesin agar and ISP-5 medium. Colonies with suspected actinomycetes morphology (i.e., isolates with grey and white colonies) were subcultured and used for further studies. They were identified as Streptomyces spp. by slide culture, morphological and physiological characteristics. Of the 17 isolated streptomyces species screened for L-asparaginase activity, only five isolates showed positive result in rapid plate assay method. The medium employed contained asparagine with phenol red and after incubation pink zone around the colonies were observed. It indicates deamination with release of ammonia. These strains were taken for biochemical characterization. The biochemical characteristics of the soil isolates are summarized in table 1.

4. Conclusion

Most of the colonies that grew on SCA and ISP-5 Medium plates belong to Streptomyces, since the colonies were slow growing, aerobic and chalky of different colors. In addition all colonies possessed an earthy odour (Suneetha et al., 2011). Microbial strains producing L-asparaginase were identified by a pink colored colony on Modified M-9

medium with Phenol red as an indicator for detection of L-asparaginase producing colonies. Out of 17 strains screened, only 5 strains were selected as potential strain for the production of L-asparaginase. However the organism did not produce any pink zone in control M-9 agar plate incorporated without L-asparagine. This indicates that the formation of pink zone is only due to L-asparaginase production.

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