

Full Length Article

Screening and Characterization of L-Asparaginase Producing *Streptomyces* Isolated From Soil Samples of Periyar Lake, Kumily

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ABSTRACT

This study attempted for the isolation and screening of an anti-cancer enzyme L-asparaginase from actinomycetes isolated from Periyar lake, Kumily. Eight actinomycetes strains were isolated on Starch Caesin Agar (SCA) medium. They were identified as *Streptomyces spp.* by slide culture, morphological and physiological characteristics. The isolated strains were screened for L-asparaginase activity. The L-asparaginase activity was detected on Czapekdox agar medium supplied with L-asparagine and 3 drops of phenol red indicator dye (pH-7). L-asparaginase activity was detected on the basis of pink color around the colony. Out of eight colonies isolated only four strains shows L-asparaginase activity. Likewise efficient L-asparaginase producing *streptomyces* were screened.

Key words: L-asparaginase, characterization, *Streptomyces*, screening.

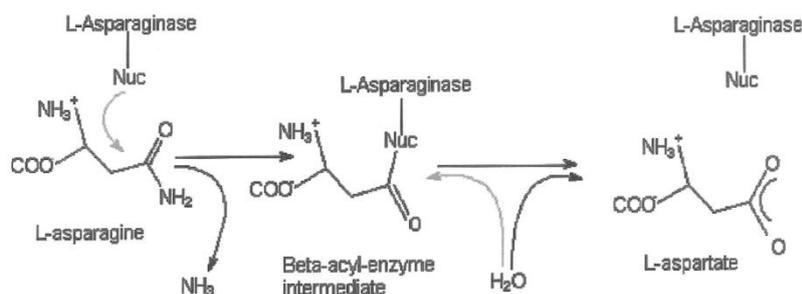
INTRODUCTION

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. L-Asparaginase received increased attention in recent years for its anticarcinogenic potential (Siddalingeshwara and Lingappa, 2010). Leukemic cells are unable to synthesize the non essential aminoacid asparagine their own, which is very essential for the growth of tumor cells, whereas normal cells can synthesize their own asparagine; thus leukemic cells require high amount of asparagines.(Kumar *et al.*,2012) So the tumor cells take L-asparagine from blood circulation or body fluid as it cannot synthesize L-asparagines. The presence of L-asparaginase enzyme as chemotherapeutic agents may degrade the L-asparagine present in blood circulation and indirectly starve tumor cells and lead to cell death. i.e., L- asparaginase catalyses the conversion of L-

asparagine to aspartic acid and ammonia and thereby prevents the tumor cells from rapid malignant growth. L – asparaginase (L – asparagine aminohydrolase EC 3.5.1.1.) is an enzyme that catalyses the hydrolysis of asparagine to aspartic acid and ammonia. The precise mechanism of its action is still unknown although hydrolysis proceeds in two steps via a beta- acyl enzyme as intermediate (Fig.1) (Siddalingeshwara and Lingappa, 2010).

L-asparaginase activity was widely reported in plants, animals and microorganisms (bacteria, fungi and actinomycetes) and also in serum of certain rodents but asparaginase was not isolated from human source (Abha Mishra 2006).

Actinomycetes are the group of gram positive bacteria with high G+C (guanine + cytosine) content which form branching filaments or hyphae and asexual spores (Balagurunathan *et al.*, 2007). Actinomycetes are threadlike bacteria that look like fungi.



Enzymes such as amylase, lipase, protease, cellulase, chitinase, pectinase, insulinase and glucose isomerase are well reported from actinomycetes in particular *Streptomyces*. In general actinomycetes are recognized as less explored source for L-asparaginase. Several species of *Streptomyces* such as *S. karnatakensis*, *S. venezuelae*, *S. longsporusflavus* and a marine *Streptomyces* sp. PDK2 are reported to produce detectable amount of L-asparaginase (Narayana *et al.*, 2007). Though there are many enzymes reported from actinomycetes, there are only few reports on the production of L-asparaginase from actinomycetes in our country (Mohana Priya *et al.*, 2011). Although L-asparaginase from bacteria has been extensively characterized, a similar attention has not been paid to actinomycetes. With this view the present study is initiated for the isolation and screening of an anti-cancer enzyme L-asparaginase from actinomycetes isolated from Periyar lake, Kumily. Isolates were inoculated on SCA plates out of 8 isolates only 4 were found potential for L-asparaginase activity.

MATERIALS AND METHODS

Sample Collection

The soil samples were collected from Boat area of Periyar lake, Kumily at a depth of 10cm in August 2013. 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.

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 plates in triplicate plates. Streptomycin (20µg/ml) and cycloheximide (50µg/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 plates.

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.

SCREENING FOR L-ASPARAGINASE PRODUCTION (RAPID PLATE ASSAY)

The L-asparaginase activity of eight actinomycetes isolates was screened by using Czapekdox agar medium supplied with L-asparagine and 3 drops of phenol red indicator dye (pH-7). L-asparaginase producing colonies were selected on the basis of formation of pink zone around the colonies of the medium.

Fig. 1: Isolation of actinomycetes in Starch Caesin Agar Medium

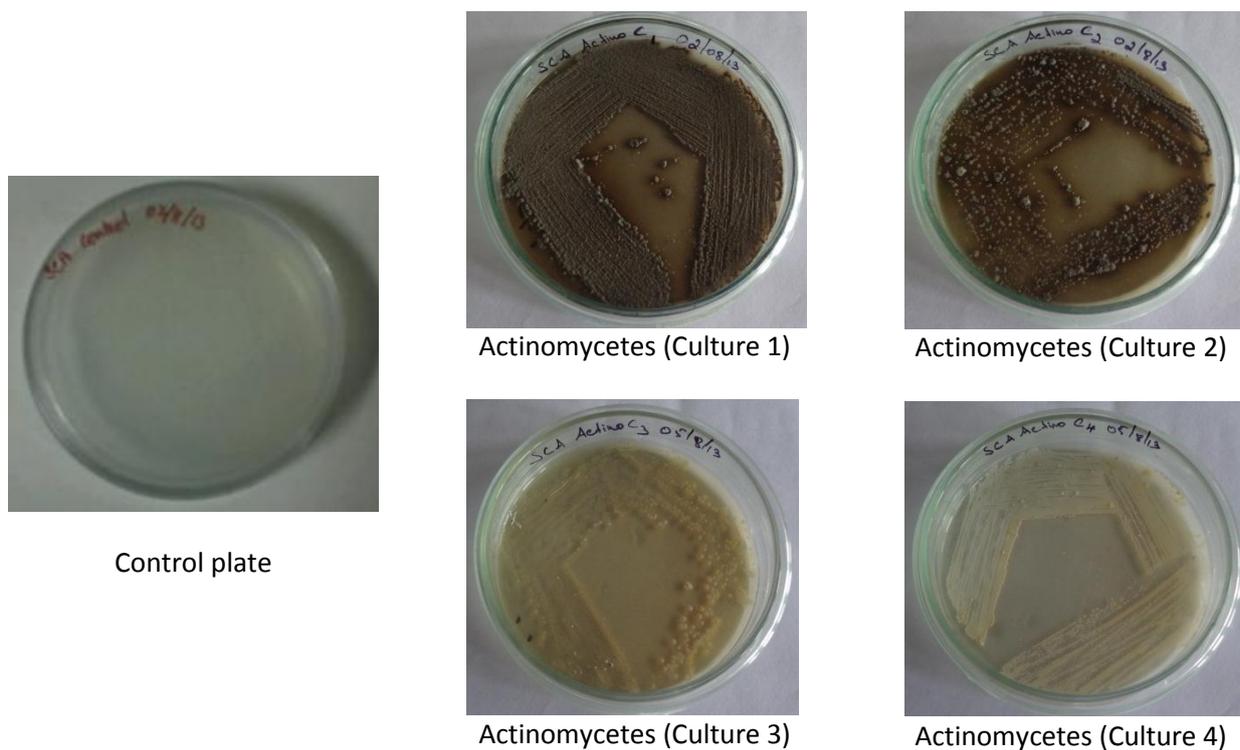
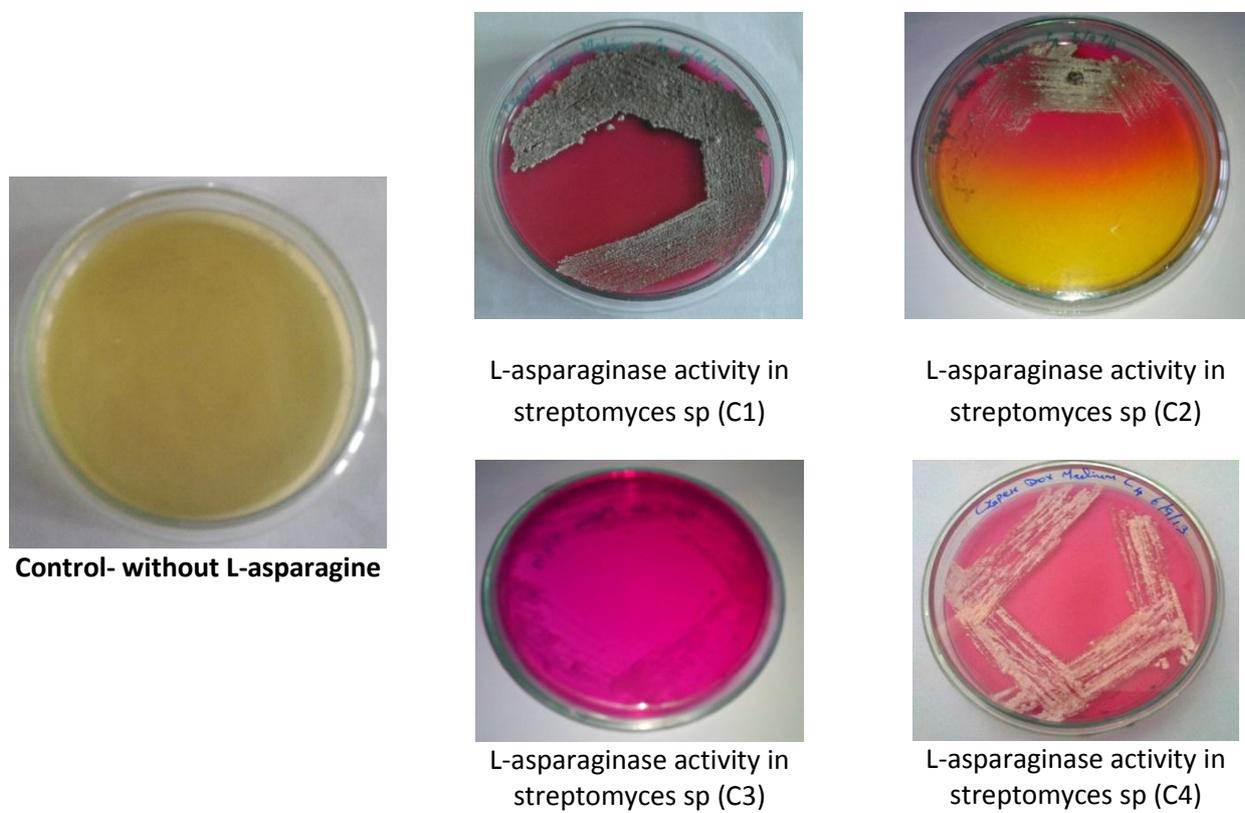


Fig. 2: Screening of L-asparaginase production by *streptomyces* spp.



RESULTS AND DISCUSSION

Samples were inoculated on Starch Caesin agar plates. 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 isolated *streptomyces* species screened for L-asparaginase activity, only four 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. These colonies were taken for further studies. The L-asparaginase producing positive colonies were identified by formation of pink zone around the medium. It indicates deamination with release of ammonia. For the conformation the L-asparaginase activity was detected by spot inoculation on L-asparaginase producing medium. After incubation at 28°C for 5days zone around inoculated spots were observed. The biochemical characteristics of the soil isolates are summarized in table 1.

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

S.No	Biochemical characterization	Soil Isolate							
		C1	C2	C3	C4	C5	C6	C7	C8
1.	Melanoid Production test	+	+	-	+	-	-	-	-
2.	Nitrate Reduction test	+	+	+	-	-	+	+	+
3.	Acid production test	+	+	+	-	+	+	+	-
4.	Hydrogen sulphide production test	-	-	+	+	-	-	-	-
5.	Gelatin hydrolysis test	+	-	-	-	+	-	-	+

CONCLUSION

Although L-asparaginase from bacteria has been extensively characterized, a similar attention has not been paid to actinomycetes. To date, antibiotics are the major bioactive compounds obtained from actinomycetes. However, the ability to produce a variety of enzymes may attract research interest in these prokaryotes. Out of 8 colonies isolated from Periyar lake, Kumily only 4 isolates possess L-asparaginase activity. However the organism did not produce any pink zone in control czapekdox agar plate incorporated without L-asparagine. This indicates that the formation of pink zone is only due to L-asparaginase production. The characterized actinomycetes (*streptomyces*) isolated from Periyar lake, Kumily are potential source for high yield of producing L-asparaginase enzyme and high substrate specificity. The actinomycetes (*streptomyces*) produces enzyme optimally at 28°C and at pH 7.

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How to Cite this Article:

Dhanam Jayam G and Kannan S, 2014. Screening and Characterization of L-Asparaginase Producing *Streptomyces* Isolated From Soil Samples of Periyar Lake, Kumily. *Biosci. Disc.*, **5**(1):50-54.