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Preliminary Study on L-asparaginase Enzyme Producing Actinomycetes Isolated from Western Ghats of Chikkamagalur

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Abstract— *Western Ghats is a potential ecosystem for actinomycetes diversity. In this study, actinomycetes were isolated from different soil samples from Western Ghats of Chikkamagalur and screened for asparaginase activity. L-asparaginase gained its great importance in clinical research, can used to treat tumours like Acute Lymphoblastic Leukemia (ALL) as effective chemotherapeutic agent. The isolated actinomycetes were primarily screened for L-asparaginase activity by rapid plate assay method. Among 6 soil samples analysed from different regions of the Western Ghats 19 actinomycetes strains were isolated, of which 8 strains were positive for L-asparaginase. The isolated L-asparaginase active strain appears to be a promising agent and requires further investigation of its anti-leukemic activity. Enzymes which degrade amino acids should receive greater attention as potential therapeutic agents.*

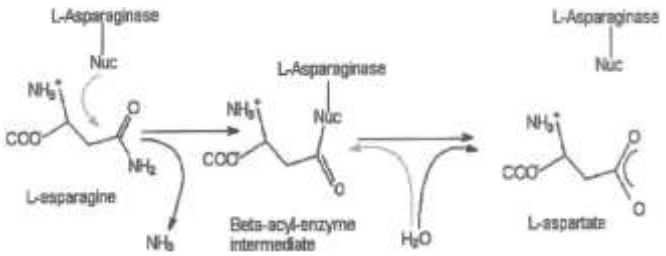
Keywords— Western Ghats; actinomycetes; L-asparaginase; enzyme.

I. INTRODUCTION

Western Ghats of India are known to be an active hotspot region and is one of the eight "hottest hot-spots" of biological diversity in the world. Next to bacteria in abundance, actinomycetes constitute a significant percentage of the soil microbial biomass. These are the most useful prokaryotes in terms of both biotechnology and economy. They are a varied collection of gram-positive bacteria. According to reports, microorganisms produce about 23,000 bioactive secondary metabolites, of which 10,000 are produced by actinomycetes. This means that actinomycetes account for 45% of all bioactive microbial metabolites that have been found (Berdy, 2005). The secondary metabolites of streptomycetes have found industrial applications as antibiotics as well as antifungal, antiparasitic, antitumor and immunosuppressive drugs, among others (Schrempf and Dyson, 2011).

II. ASPARAGINASE ENZYME-THERAPEUTIC AGENT

L-asparaginase is one of the antitumor agents to be intensively studied in individuals. It's used as chemotherapeutic drug for acute lymphocytic leukemia in kids utilized in combination with medical care (Schemer *et al.,* 1981). L-asparagine is an essential aminoacid for the growth of tumour cells whereas the growth of normal cells is independent of its requirement. Most normal tissue synthesizes L-asparagine in amounts sufficient for their metabolic needs with their own enzyme L-asparagine synthetase, but the malignant cells require an external source of L-asparagine for growth and multiplication (Mostafa *et al.*,1979). When L-asparaginase is present, tumor cells lose one of their main sources of development and are unable to endure. This fact showed that this enzyme could be developed into a powerful antitumor or antileukemic medication. Numerous bacteria, fungi, yeasts, actinomycetes, and algae are highly productive L-asparaginase producers. Actinomycetes are also one of good sources for the production of L-asparaginase. (Savitri *et al.,* 2003). Antitumor features of L-asparaginase have been first studied in 1953 by Kidd who observed lymphoma regression in mice and rats in response to the guinea pig plasma (Kidd JG., 1953). L-asparaginase is the first enzyme with antitumour activity to be intensively studied in human beings. It is an enzyme drug of choice for acute lymphoblastic leukemia in children used in combination therapy



ENZYMATIC REACTIONS OF L-ASPARAGINASE

III. METHODOLOGY

A total of 6 Soil samples were collected from different places in Western Ghats region of Chikkamagular. The soil samples were collected from 8-15cm depth using sterile technique and transported to the laboratory. These soil samples were air dried in shady condition at room temperature for 7 days and used for actinomycetes isolation.

Actinomycetes were isolated using the conventional serial dilution technique. A single gram of soil was weighed, suspended in 100 milliliters of purified water, and vigorously agitated. The contents were serially diluted till the dilution of 10^{-6}. 0.1ml of the diluted suspension was inoculated on Starch Casein Agar plates containing cycloheximide (40µg/ml) and Nalidixic acid (100µg/ml). The pH of the media was adjusted to 7.2. Then the plates were incubated at 28°C for 7 to 10 days.

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Various isolates of actinomycetes colonies were taken and subjected to screening for L-asparaginase enzyme production by rapid plate assay method, based on their capability to form a pink zone around the colonies on agar plates of Modified M-9 media supplemented with L-asparagine as a sole source of carbon with phenol red (2.5%) as a pH indicator.

IV. Result & Discussion

As several bioactive secondary metabolites are produced by actinomycetes, screening of these metabolites is increasing now a days. L-asparaginase are widely present and gained its great importance in clinical research. L-Asparaginase can used to treat tumors like Acute Lymphoblastic Leukemia (ALL) as effective chemotherapeutic agent. In recent years L-asparaginase is also gaining an importance as an industrial food-processing agent for reducing or removing acrylamide in processed food products (Pedreschi *et al.* 2008).

The study focused on isolating Asparaginase enzyme producing actinomycetes. L-Asparaginase enzyme produced from bacteria contains glutaminase which have an adverse side effect when given as anti-tumor agent. Hence Actinomycetes were found to produce glutaminase free L-Asparaginase enzyme which does not have side effects on treatment but it has been less explored.

The 6 soil samples collected from different regions of Chikkamagalur were checked for pH and it was found to be in the range of 7.0-8.0 (Table 1). It is well known that in soil from many geographical regions, actinomycetes can grow within this pH range.

TABLE 1

SOIL SAMPLE	PH
CR1	6.8
CR2	7.6
CR3	7.8
CR4	8.1
CR5	6.9
CR6	7.4

A total of 19 strains were isolated from 6 soil samples samples eliminating those that appear close to each other. The colony morphology of the isolates is shown in Table 2.

TABLE 2

Soil Sample	Colony Morphology	Gram Reaction
CR1	Granular pink	Gram positive filamentous
	Velvety white	Gram positive filamentous
	White with yellow	Gram positive filamentous
	Waxy orange	Gram positive cocci
CR2	White dotted	Gram positive filamentous
	Waxy pale pink	Gram positive filamentous
	Black with brown border	Gram positive filamentous
CR3	Pink	Gram positive filamentous
	Ash colour	Gram positive filamentous
CR4	Light Ash	Gram positive filamentous
	Powdery pink	Gram positive cocci
	Green dotted	Gram positive filamentous
	Pink with Green	Gram positive filamentous
	Green	Gram positive filamentous
CR5	Dark Ash	Gram positive filamentous
	White with Ash	Gram positive filamentous
	White with pink	Gram positive filamentous
CR6	Waxy grey	Gram positive filamentous
	Pink with yellow	Gram positive filamentous

The 19 strains on primary screening with asparaginase source 8 isolates shown pink colouration (Fig.1).

Fig. 1. Screening of L-asparaginase enzyme producing actinomycetes using rapid plate assay method

L-asparginase is a medically important enzyme, and it hydrolyses L-asparagine (essential amino acid) to aspartic acid and ammonia. In this present study revealed that Western Ghats soil is a rich source of L-asparaginase active actinomycetes, as 40% of isolates were found to be positive in rapid plate assay method

V. Conclusion

All 8 isolates were considered as active strain for L-asparaginase production based on the formation of zone and analyzed further for L-asparaginase enzyme activity. The LA-5 isolate showing the highest pink colour zone was chosen as the best isolate for the production and optimization of L-asparaginase by submerged fermentation.

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