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ISOLATION AND SCREENING OF CELLULOSE DEGRADING ACTINIMYCETESFROM SOIL

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ABSTRACT :

Cellulose is a fibrous, tough, water insoluble substance found in cell wall of plants. In present study, soil samples collected from few selected area in Ambajogai, Maharashtra. The soil samples were serially diluted was spread inoculated on sterile glycerol asparagine agar plates and isolated total 279 actinomycetes. The typical, dry, tough and powdery colonies of actinomycetes were selected. Out of which, 59 isolates shown positive results for cellulose degradation. All isolates were screened for their cellulose degrading potential. Among them 4 actinomycetes CA-6, CA-34, CA-35, and CA-39 were possessing remarkable cellulase activity. Secondary screening of these 4 actinomycetes was carried out. It was confirmed that CA-34 was showing maximum zone of hydrolysis for cellulase activity. The colony on carboxy methyl cellulose medium using 1% Congo red. Actinomycetes plates were screened for their ability to produce the hydrolytic enzymes.

KEY WORDS: *Cellulose, Actinomycetes, Congo red.*

1.INTRODUCTION:

In the developing countries like India, biodegradable Municipal Solid Waste accounts for more than 60% of the total MSW generation. It is the source of major vector borne diseases like Malaria, Cholera, Dengue, Yellow fever, Dysentery etc. Cellulose is the most abundant organic polymer found in the nature and in the MSW as well. Generally 40-50% of the MSW composed of cellulose material .Employing cellulolytic microorganisms in the waste management will lead to the efficient and cost effective management of the MSW as well as it could generate derive economic benefits from the end products like compost and biofuels.The cellulolytic system of aerobic fungi and bacteria consists of three types of cellulases i.e. endoglucanases, exoglucanases and B- glucosidases. Cellulase is a consortium of hydrolytic enzyme capable of hydrolyzing cellulose in to simple glucose units. Several studies were carried out for the isolation and identification of the cellulolytic fungi possessing activity .The potential of cellulose being a renewable source of energy was only recognized after the identification of cellulose degrading enzymes such as cellulases (Bhat and Bhat 1997).

Actinomycetes are widely distributed in soil and they play an important role in degrading lignocellulose components of plant cell walls (Lacey 1973). Most of the studies done were largely attributed to fungi, and the ability of actinomycetes in degrading the lignocellulose was neglected (Li 1997). Study done by Alam et al. (2004) showed that isolates of *Streptomyces omiyaensis* are able to produce cellulolytic enzymes. Bioconversions of cellulosic materials to desirable products involve complex processes which require a number of different enzymes (Alam et al. 2004). Actinomycetes constitute a significant component of microbial population in different soil types and are widely distributed in terrestrial ecosystems. They are Gram-positive bacteria and exist as saprophytes (Takizawa et al., 1993). Plant rhizosphere soils are a major habitat for actinomycetes, where they aid plant growth by decomposing soil organic matter or fixing atmospheric nitrogen (Goodfellow and Williams 1983).They produce antibiotics which are thought to be

effective against fungal infections of plants (Weller et al 2002).Most soil living actinomycetes belong to the genus Streptomyces (Lazzarini et al., 2000).They have the potential to produce a wide range of secondary metabolites and extracellular enzymes that are economical and beneficial to human beings.Roughly 60% of biologically active compounds that have developed for agriculture use originated from Streptomyces sp. with other genera such as Saccharopolyspora, Amycolatopsis, Micromonospora and Actinoplanes producing less (Challis and Hopwood 2003).

Various groups of bioactive compounds such as macrolides, benzoquinones, amyloglycosides, polyenes and nucleoside antibiotics are examples of agriculturally useful metabolites produced from Streptomyces sp. Agroindustries have a marked interest in actinomycetes as a source of agroactivecompounds,of PGPR and of biocontrol tools (Behal 2000; Tanaka and Omura 1993). Actinomycetes can protect roots against invasion by root pathogenic fungi either by producing enzymes which degrade fungal cell wall or by producing antifungal compounds. Streptomyces griseoviridis strain K61 has been reported to be antagonistic to a variety of plant pathogens, including Alternariabrassicola, Botrytis cinerea and Fusariumoxysporum (Mohammadi and Lahdenpera 1992). Mycostop TM is biofungicide that contains Streptomyces griseoviridis as the active ingredient which can control some root rots and wilt diseases caused by Pythium sp., Fusarium sp., Rhizoctonia and Phytophthora sp. (Mahadeven and Crawford 1997).

MATERIALS AND METHODS

Collection of samples

Soil samples collected from few selected area in Ambajogai, District BeedMaharashtra. These soil samples were transported to research laboratory for isolation and screening.

Isolation of actinomycetes

Soil samples were collected from Ambajogai region from two inches deep using a sterilized spatula in sterile containers [4]. The collected soil samples were suspended and 1g of sample was serially diluted in 10ml of sterile distilled water and mixed properly up to 10^{-7} dilutions. 100 µl (10^{-7}) of each dilution was spread inoculated on sterile glycerol aspargine agar plates and then incubated at room temperature for 3-7 days.These plates were observed for the growth of actinomycetes. The typical, dry, tough and powdery colonies of actinomycetes were selected and transferred on glycerol asparagine agar slants.

Primary Screening of actinomycetes:

Isolatedactinomyceteswere spotted onnutrient agar medium containing 1% carboxy methyl cellulose. The plates were incubated at room temperature for 3-7 days. After complete growth, the plates were flooded with 0.1% Congo redsolutionfor 15–20min and then the congo red solution was removed. 1% of Nacl solution to distain the plates. The zone of cellulose hydrolysis was appeared as a clear area around the colony.

Secondary Screening for cellulose degrading

The primary screening was repeated only for four selected actinomycetes. The diameter of zone of hydrolysis and diameter of colony were measured.

RESULTS AND DISCUSSION

Isolation of actinomycetes

Soil samples collected from few selected area in Ambajogai, district BeedMaharashtra.Enrichment of soil sample take place for the isolation of actinomycetes. Isolated actinomycetes were spotted onRees and Mendel agar medium containing 1% carboxy methyl cellulose.The plates were incubated at room temperature for 3-7 days. The typical, dry, tough and powdery colonies of actinomycetes was shown in plate no. 01

Plate no. 01: Isolated colony of actinomycetes

A total of 279 isolates were isolated from the soil samples of nine different area of Ambajogai region. The number of actinomycetes isolated from each site is given in Table no. 01.

Site	1	2	3	4	5	6	7	8	9
Number of isolates	38	28	30	35	24	32	38	32	22

Table no.01 : Number of isolates vs site

Screening of cellulose degrading actinomycetes

Although a large number of microorganisms are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of cell-free enzymes capable of completely hydrolyzing crystalline cellulose in vitro. 279 isolates were screened for cellulolytic activity, only 59 showed shown zone of hydrolysis around the colony. Among these 59 only 4 isolates showed significant cellulolytic activities.The actinomycetes colony having the largest clear zone was shown in plate no. 02

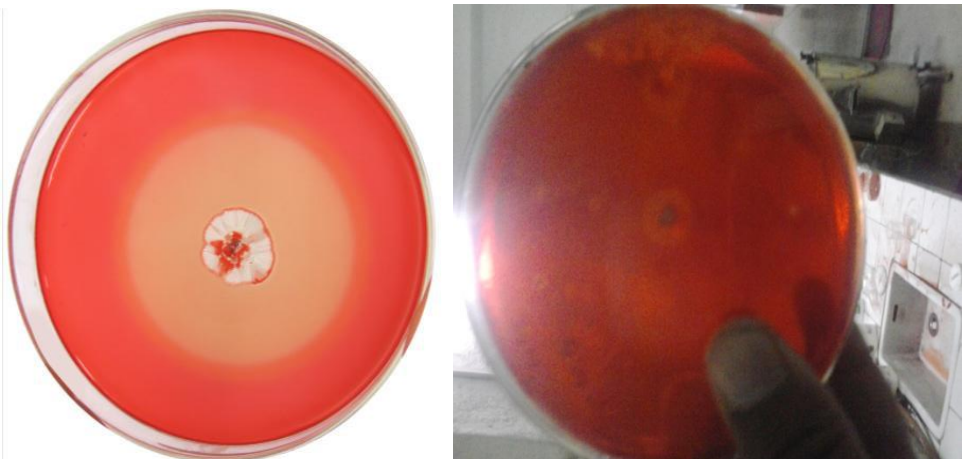

Plate no. 02: Colony showing zone of hydrolysis

The activity of these actinomycetes strains diameter zone of hydrolysis and diameter of colony were measured is given in the Table no.02.

Sr. No	Isolate Code	Diameter of colony (mm)	Diameter of the Zone of hydrolysis (mm)
1	CA-06	5 mm	30 mm
2	CA-34	4 mm	40 mm
3	CA-35	6 mm	29 mm
4	CA-39	4 mm	30 mm

Table no.02: Zone of hydrolysis around the colony

Actinomycetes plates were screened for their ability to produce the hydrolytic enzymes. The Actinomycetes CA-34 shows maximum cellulose degrading activity.

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