# STUDIES ON BIOCONTROL POTENTIAL AND OTHER BENEFICIAL TRAITS OF SOIL ACTINOMYCETES

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# STUDIES ON BIOCONTROL POTENTIAL AND OTHER BENEFICIAL TRAITS OF SOIL ACTINOMYCETES

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*By*

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## CERTIFICATE

*This is to certify that the thesis entitled* "**STUDIES ON BIOCONTROL POTENTIAL AND OTHER BENEFICIAL TRAITS OF SOIL ACTINOMYCETES**" *submitted by* **Miss VEENAKUMARI G. B.,** *for the degree of* **MASTER OF SCIENCE (AGRICULTURE)** *in* **AGRICULTURAL MICROBIOLOGY** *to the University of Agricultural Sciences, Dharwad, is a record of research work carried out by her during the period of her study in this university, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.*

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" ... gratitude is the memories of the heart "

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JANUARY, 2005

***( Veenakumari G. B. )***

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# Introduction

# I. INTRODUCTION

It is well known fact that, soil not only harbours myriads of population of microorganisms on the earth, but also serves as an important medium for growth and survival of a majority of them. The soil microorganisms vary considerably both in quality and quantity, depending upon the nature of soil, its depth and physical and chemical properties, the cultural operations it receives and the influence of various environmental factors. Each particle of soil contains myriads of microscopic and sub microscopic organisms. Several of the soil microorganisms are actively involved in cycle of life in nature and hence the soil microorganisms are essential for the well being and progress of mankind on the earth.

Microorganisms live in the soil not in the form of pure cultures, but as complex populations. Many of the organisms depend upon one another, either directly or indirectly for their nutrients. Some compete with others for energy sources as well as nutrients. This results in the formation of numerous relationships of both beneficial and injurious interactions.

Taxonomically, actinomycetes primarily being prokaryote, form a link between bacteria and fungi and occur in abundant quantities in most soils next only to bacteria. The true bacteria are distinctly different from the filamentous fungi and many morphological characteristics separate the two broad types. There is, however, a transitional group between the simple bacteria and fungi, a group with boundaries overlapping its more primitive and its more developed neighbours. These are the actinomycetes which live in soil numerically second only to the bacteria.

Since soil contains a dense population of several thousand microorganisms in a minute area, they live in constant complex association. The antagonistic and mutualistic association among soil dwelling microorganisms have long been recognized. Among the various groups of microorganisms in soil, actinomycetes are of particular interest as they possess relatively more antagonistic activity than the others. Since the dawn of the 'Antibiotic age' in 1939-40 intensive search for antagonistic microorganisms among the soil actinomycetes has been in progress in many parts of the world and most of the present day antibiotics in wide use are obtained from the soil actinomycetes. During the past quarter century, over six hundred antibiotics have been isolated and characterized. Several of the antibiotics are now being used in medicine, animal and crop protection *etc.*

Actinomycetes are the group of gram positive bacteria belonging to class Schizomycetes. They are abundant in all cultivated and uncultivated soils (Waksman and Curtis, 1916). Soil actinomycetes play an important role in decomposition of organic matter, antibiotic production, suppression of soil borne plant pathogens and phosphorus solubilization. Few workers have made attempts to study the role of actinomycetes on the above parameters (Rothrock and Gottlieb, 1984; Crawford *et al.*, 1993; Koretmma *et al.*, 1997; Deshmukh and Godbole, 1998; Lim and Cha, 2000; Youssef *et al.*, 2001).

Recently, Kanavade *et al.* (2002) reported that acidophillic actinomycetes can be easily propagated on whey waste of dairy industry as cheaper substrate for propagation of actinomycetes with phosphate

solubilization and mobilization capacity. Actinomycetes can decompose various organic matter in soil. Actinomycetes can decompose highly resistant lignocellulose organic matter (McCarthy, 1987) and biological degradation of paddy straw by *Streptomyces cyanens* was reported by Berrocal *et al.* (2000).

There are various soil borne plant pathogens which are causing very serious disease to plant which could be controlled by actinomycetes by their antibiotic activity (Whaley and Boyle 1967; Franco *et al.*, 2001).

The actinomycetes are ubiquitous group of microorganisms that, occur in multiplicity of natural and manmade environments. They are found in different habitats such as soil, air, water, food and in a variety of materials like manure, compost and plant residues. Soil is the most common habitat for actinomycetes. These usually dominate next to true bacteria. These usually make up 10-15 per cent of the total microbial community present in all kinds of soil. They perform a number of important activities in soil like mineralization of organic matter and solubilization of inorganic phosphates to maintain soil fertility. Though, a considerable work has been carried out in other countries on actinomycetes, but have received very little attention in India. It is thus becomes evident that, there is a need to study soil actinomycetes in India (Deshmukh and Godbole, 1998, 1999, 2000).

Keeping the above information in view, the investigations were made understand the role of soil actinomycetes with the following objectives.

1. Isolation, purification and characterization of actinomycetes from different soils of Dharwad region,

2. to study the isolated soil actinomycetes for their biocontrol potential against plant pathogens and

3. to find out the ability of soil actinomycetes for organic matter decomposition and also for other beneficial traits

# Review of Literature

# II. REVIEW OF LITERATURE

Actinomycetes are recognised over 100 years primarily on morphological criteria. They are gram positive with the characteristic eubacterial cell wall composition. Morphologically they are different from bacteria, because of the mycelial structure akin to fungi. They form branching hypae at some stage of their development. Because of the bacterial characters and fungal morphological features, they are considered to be evolutionary species between bacteria and fungi.

The actinomycetes are unicellular microorganisms, that produce a slender, branched mycelium which may undergo fragmentation or may subdivide to form asexual spores. The mycelium is, in some genera at least, aerial and typically exhibits a distinct branching habit. The individual hyphae or filaments appear morphologically similar to the fungal filaments but are much less broad, usually 0.5 to 12.4 μm in diameter, a dimension analagous to that of the bacterial cell in addition to proliferation by vegetative means, certain actinomycetes produce asexual spores known as conidia, but there is no known sexual spore stage. The mycelium of the higher actinomycetes has the extensive branching characteristic of the molds, like the fungi, many actinomycetes form an aerial mycelium as well as conidia, the growth of actinomycetes in liquid culture rarely results in the turbidity associated with unicellular bacteria, rather it occurs as distinct clumps or pellets, and the growth rate in unrestricted conditions of at least some strains is not exponential as is the case with bacteria but cubic, a characteristic in common with many of the fungi (Alexander, 1961).

The actinomycetes are a successful group of bacteria that occur in a multiplicity of natural and man made environments. Actinomycetes are commonly believed to have a role in the recyclings of nutrients (Goodfellow and Cross, 1983). Soil actinomycetes play an important role in decomposition of organic matter, antiboitic production, phosphorus, mobilising ability and suppression of soil borne plant pathogens (Rothrock and Gottlieb, 1984; Kortemma *et al.*, 1997). Numerical phenetic and chemical data have revolutionized the classification of several actinomycete genera notably *Actinomadura, Actinomyces, Corynebacterum, Frankia, Mycobacterium, Nocardia* and *Streptomyces* (Williams *et al.*, 1983). Microbial ecologists are not primarily concerned with classification but do require workable diagnostic tests to identify isolates from natural habitats. A combination of morphological, chemical and spores characters can be used to identify actinomycetes to the genus level (Goodfellow and Cross, 1974).

## 2.1 ISOLATION AND CHARACTERIZATION OF ACTINOMYCETES

Actinomycetes constitute a significant component of microbial population in most of the soils and counts over one million per gram are invariably obtained. The soil is a most prolifilic source of actinomycetes which includes to produce antibiotics and other useful metabolites. It is therefore, the most intensively studied habitate but despite this there are still many doubts in our knowledge of the role played by actinomycetes in soil process. Over 20 genera have been isolated with *Streptomycetes* being the ubiqutous and most numerous (Lechevalier and Lechevalier, 1967; Rangaswami *et al.*, 1967; Krassilnikov, 1981; Goodfellow and Williams, 1983; Williams *et al.*, 1983). Isolation of actinomycetes from the soil is

usually made with dilution plate technique using more than half a dozen of selective media (Labeda, 1990). Several substances have been suggested as some of carbon and nitrogen sources favouring growth of actinomycetes (Porter *et al.*, 1960; El Nakeeb and Lechevalier, 1963). Kuster and Williams (1964) who evaluated several carbon and nitrogen source found starch or glycerol + casein and nitrate to be most selective mixture. Use of chitin as a sole source of carbon and nitrogen was reported by Lingappa and Lockwood (1961 and 1962).

Numerous media have been described for the isolation of actinomycetes from soil and other natural materials. Some of these isolation media are rather lean. Since actinomycetes have the ability to survive and grow to some extent on very small amounts of nutrients that they scavenge from non-nutrient substances such as purified agar. Other isolation media have high carbon to nitrogen ratio and contain resistant complex carbon and nitrogen sources, *e.g.*, starch, casein, chitin, humic acid *etc.* Use of these media greatly reduces number of bacteria present on isolation plates, because bacteria, in contrast to actinomycetes grow better on media with low carbon to nitrogen ratios and are usually unable to attack high molecular weight, resistant polymers (Gray and Williams, 1971). Several useful isolation media have been listed containing such wide carbon-to-nitrogen ratio carbon sources with mineral supplementations like Gause No.1 mineral media (Gause *et al.*, 1957), starch-casein agar (Kuster and Williams, 1964), Arginine-glycerol-salts agar (El-Nakeeb and Lechevalier, 1963), Arginine-vitamin agar (Nonomura and Ohara, 1969) and colloidal chitin agar (Hsu and Lockwood, 1975). Use of antibiotics to suppress bacterial contaminations like

cycloheximide, nystatin are also suggested (Corke and Chase, 1956; Williams and Davies, 1965). Reddi and Rao (1972) reported the morphological and cultural characters of isolates which possessed the aerial mycelium on glucose, aspergine agar and on sucrose nitrate agar produced light yellow colour the sporophores were spiral and the spores were spiricle in chains the hypac were weavy and long with extensive branching they did not reduce nitrate and utilize glucose, fructose, mannose, mannitol and starch but not raffinose and xylose. Crawford *et al.* (1993) isolated 267 actinomycetes strains from rhizosphere and non-rhizosphere soil samples in the United Kingdom on various media with poorer organic carbon source. All the isolates grow well *in vitro* at 6.5 to 8 pH majority of species were antagonist toward *Pythium ultimum.*

El-Tarabily *et al.* (1997) screened actinomycetes from carrot rhizosphere for *in vitro* and *in vivo* antagonism to *Pythium coloratum* a causal agent of cavity spot disease of carrots. They demonstrated that seven strains of actinomycetes which belonged to *Streptomyces janthinus, Streptomyces cinerochromogenes, Streptoverticillium netropsis, Actinomadura rubra, Actinoplanes philippinensis, Micromonospora carbonacea* and *Streptosporangium albidum* reduced cavity spot in carrot when inoculated to soil.

## 2.2 THE CONCEPT OF BIOCONTROL

The broad concept of biocontrol of plant diseases include a disease reduction or decrease in inoculum potential of a plant pathogen brought about directly or indirectly by a biological agent excluding the man (Johnson and Carl, 1972; Cook and Baker, 1983). In the recent past use

of biological agent to control plant diseases has attracted great interest. The excessive use of pesticides besides causing resistance development in pathogens also resulted in toxicity in human beings and contamination of natural resources.

## 2.3 ACTINOMYCETES AS A BIOCONTROL AGENT

The biological control of plant disease are achieved generally by use of antagonistic microorganisms or some parasitic forms. Garret (1965) explained antagonistic relationship of soil microbial population and controlling soil borne plant pathogens. The most of the soil borne plant pathogens have high competitive saprophytic ability and able to tolerate the effect of antagonist various microorganisms are used to biological control of plant diseases. Among the various groups of microorganisms in soil, actinomycetes are of particular interest as they posses relatively more antagonistic activity than the others (Rangaswami *et al.*, 1967). The actinomycetes produce various antibiotics which suppress the growth of the fungi and bacteria. The antagonistic action of actinomycetes from soil on soil borne plant pathogens has been reviewed by several workers (Kuster, 1976; Kutzner, 1981; Lacey, 1973; and Williams, 1978).

Reddi and Rao (1972) identified an actinomycete, *Streptomyces ambofaciens*, the most useful antagonist controlling soil borne pathogen *viz.*, *Fusarium oxysporum*, *F. udum*, *F. oxysporum*, *F. vasinfectum*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Macrophomina phaseoli*. *Rhizoctonia bataticola* on PDA was inhibited by actinomycetes isolated from loam soils of Haryana (Paramjit and Mehrotra, 1980). *In vitro* suppression of *Fusarium oxysporum* f.sp. *ciceri*

causing vasicular wilt of *Cicer arietinum* was reported by Dhedi *et al.* (1990) by several soil microorganisms which contained two actinomycetes species. The inhibition of *Fusarium solani* on Czapeck's sucrose nitrate agar medium by *Streptomyces* sp. (Gaur *et al.*, 1991). Jyotsna and Bineeta (1991) conducted study in which a total of 43 fungi, 41 bacteria and 10 actinomycetes isolated from soil infested with *Fusarium solani* under a cucurbit crop. They found that the isolates which showed slow growth of the pathogen or antibiosis were identified as potential inhibitors of *Fusarium solani.* The *Bacillus* spp. and various actinomycetes isolated form maize growing soils showed antagonistic effect against *Fusarium moniliforme* and *Gibberella fujikuriiola in vitro* (Hebbar *et al,* 1992). Rath *et al.* (1992) reported the antagonistic activity of three *Streptomycets* sp. in addition a few bacteria and fungi on *Pythium ultimum.* Crawford *et al.* (1993) classified the antagonist activity of 82 isolates towards *Pythium ultimum* with Difco-corn meal agar assay procedure they grouped them as very strong, strong and weakly antagonist.

Ahmed *et al.* (1987) reported several fungi, bacteria and actinomycetes from the tomato leaves which were found to occur in large number, when the plant reached fruiting stage. The healthy leaves contained *Streptomycetes* species which likely suppressed the growth of *Alternaria solani* on the leaves. They further found that the radicle growth of *Alternaria solani* was inhibited by *Streptomyces* isolate *in vitro.*

Rattikainen *et al.* (1993) reported many isolates of *Streptomyces* spp. from light coloured spagnum peat which showed suppression of fungal growth. The mechanism of suppression was attributed due to

polyene antibiotics. They also showed the significant differences in the suppression of fungal growth by the *Streptomyces* spp.

Walter and Crawford (1995) observed a strong *in vitro* antagonism against various fungal pathogens by *Streptomyces lydicus* in a liquid medium. They found inhibition of *Pythium ultimum* and *Rhizoctonia solani* in presence of *Streptomyces lydicus*. Invasion of seeds by *Pythium ultimum* was protected on inoculation of the actinomycete. They further reported that the actinomycete was not only capable of destroying germinating Oospores but also damaging the cell wall of the fungal hyphae.

Abyad *et al.* (1996) isolated 37 actinomycetes from fertile cultivated soils in Egypt. They were screened for the production of antimicrobial compounds against a variety of organisms. Special attention was given to fungal and bacterial pathogens of tomato on starch-nitrate agar. Fourteen strains were active against *Fusarium oxysporum* f.sp. *lycopersici*. The most active antagonist to the pathogen studied were found to be *Streptomyces pulcher*.

Ouhdouch *et al.* (1996) reported that out of 96 actinomycete strains isolated from Maroccan soil and water samples, 26 showed strong activity against fungi (*Candida albicans, C. tropicalis, Fusarium oxysporum* f.sp. *albedinis* and *Pythium irregulare*).

Using a dual culture method the antagonistic activities of 139 isolates of the actinomycetes on five *Pythium* species were investigated *in vitro*. The actinomycetes which strongly displayed antagonism to all four species of *Pythium* were nos. 129, 199, 251 and 301 (Yoshimoto, 1997).

Castillo *et al.* (2001) in a dual culture method on Czapeck dox agar evaluated 90 actinomycetes from potato rhizosphere. Based on the above study they reported actinomycete strain AC 77 was showing the best inhibition of *Rhizoctonia solani* followed by strain AC12, AC68, AC70, AC66, AC81, AC71 and AC33.

Youssef *et al.* (2001) reported biocontrol of root rot of white lupine by *Streptomyces* sp. isolated from the rhizosphere. They reported that *Streptomyces* sp. was strongly antagonist against *Plectosporium tabacinum in vitro*. Under glass house tests the white root rot of lupine was significantly reduced in presence of *Streptomyces* sp.

## 2.4 MECHANISM OF BIOCONTROL (PRODUCTION OF ANTIBIOTICS) ACTINOMYCETE

It is often assumed that actinomycete play major role in antagonistic interaction because of their greater capacity for antibiotic production *in vitro*. Although several workers have argued that the antibiotics are natural products direct evidence for their presence in natural soil is still lacking (Williams and Khan, 1974; Gottlieb, 1976; Williams, 1982; Goodfellow *et al.*, 1983) thereby indicating that they are not either produced or present because of there short term stability, adsorption to the soil colloids lack of sensitive detection methods (Williams, 1982). Despite the lack of the evidences, literature on effect of actinomycetes on control of fungal root pathogens is enormous. The mechanism of biocontrol through *Streptomyces* is largely ascribed to the antibiosis.

Cooper and Chilton (1949) showed the inhibitory activity of actinomycetes through antibiotic production on root rot fungus *Pythium arrhenomanes*. The inhibitory ability of actinomycetes was observed in soil with increasing pH upto 7.5 above which there was a slight decrease.

Gottlieb *et al.* (1952) reported that actidione and clavacin being produced by soil actinomycetes demonstrated the antifungal activity against *Aspergillus clavatus*. They also reported about Streptomycin, aueramycin, terramycin and chloromycetin being produced by actinomycetes in soil.

Rothrock and Gottlieb (1984) reported the inhibitry growth of *Rhizoctonia solani* in soil by *Streptomyces hygroscopicus* due to production of antibiotics geldanamycin. Amount of geldanamycin produced was 8.8 μg per gram of soil after seven days of incubation. Further they also reported that amending soil with geldanamycin in amounts equivalent to that produced after seven days of incubation, also controlled the disease and reduced saprophytic growth of the pathogen.

According to Roy *et al.* (1989) certain soil fungi and actinomycetes have antagonistic activity on *Rhizoctonia solani* and *Macrophomina phaseolina*.

De and Gupta (1991) found antagonist activity of actinomycetes on phytopathogenic fungi namely *Fusarium solani, Helimenthosporium oryzae* and *Rhizoctonia solani* through agar streak method.

Furumai *et al.* (1993) conducted a study in search of antifungal actinomycetes producing BMS-181184. *Actinomadura* strains isolated from

soil samples were found to produce the antibiotic, BMS-181184 showed a broad spectrum activity against *Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus* and *Trichophyton mentagrophytes.*

Nair *et al.* (1994) reported that gopalamicin an antifungal antibiotic was isolated from two varieties of *Streptomyces hygroscopicus*, MSU-625 and MSU-616. Gopalamicin, which is structurally similar to salbomycin and elaiophylin, completely inhibited the growth of *Aspergillus fumigatus, A. flavus, A. niger, Candida albicans, Alternaria solani, Fusarium oxysporum, F. moniliforme, Pythium ultimum, Phialophora graminicola* and *Leptosphaeria korrae in vitro.* It also partially controlled powdery mildew of wheat, downy mildew of grape and rice blast.

Bhat *et al.* (1996) conducted study in which polyene macrolides were developed from different actinomycetes and screened for antifungal activity against various plant pathogens (*Fusarium oxysporum, Macrophomina phaseolina, Alternaria macrospora, Helminthosporium* sp. *Curvalaria luntana* and *Colletotrichum gleosporioides.*

Hwang *et al.* (1996) reported a manumycin type antibiotic, SW-B, isolated from the solid agar culture of *Streptomyces flaveus* which showed strong antifungal activity against *Phytophthora capisci, Magnoporthe grisea, Cladosporium cucumerinum* and *Alternaria mali.*

Kim *et al.* (1998) found antifungal activity of 16, among the 300 actinomycetes isolated, against plant pathogenic fungi *viz., Magnaporthe grisea, Alternaria mali, Colletotrichum gleosporioides, Phytophthora capsici, Fusarium oxysporum* f.sp. *Cucumerinum* and

*Rhizoctonia solani*. They found the actinomycete isolate NH 50 to possess high broad spectral antifungal activity.

Franco *et al.* (2001) reported production of secondary metabolites produced by *Nocardia gardeneri* in a medium containing molasses and yeast extract or oat broth with flesh extract. The active agent was identified as actinomycin which was similar to cephalosporin and cephomycin which formed the halo inhibition zone against *Fusarium oxysporum* f.sp. *dianthi*.

Three actinomycetes *viz.*, *Pseudonocardia* sp., *Streptomyces* sp. and *Streptosporangium album*, were screened for their antagonistic effect on *Fusarium oxysporum* a causal agent of vascular withering in carnation. *Streptomyces* sp. and *Pseudonocardia* sp. showed antagonistic effect on the pathogen. While no effect was recorded by *Streptosporangium album* on the pathogen (Franco and Valencia, 2001).

## 2.5 DECOMPOSITION OF ORGANIC RESIDUE

For years it has been known that certain fungi belonging to white rot group of basidiomycetes decompose complex lignocellulosic organic wastes (Kirk, 1976). However actinomycetes also are found to decompose such reclacitrant polymers like lignocelluloses in soil through actinomycete al biet slowly (Antai and Crawford, 1981). The actinomycetes also form the component of humic acid in the undecomposable organic residue in the soil (Lacey, 1973; and Eggeling and Sahm, 1981). According to Crawford and coworkers actinomycetes play significant role in degradation of celluloses, as some *Streptomycetes* attack both cellulose and lignin components oxidizing the aromatic ring and side chain carbon

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to $CO_2$ (Crawford, 1978; Crawford and Sutherland, 1979; Phelan *et al.*, 1979). Intact walls douglas fir phloem was degraded by *Streptomyces flavovirens* (Sutherland *et al.*, 1979) catabolism of vanillic acid was demonstrated in *Streptomyces setonii* (Pometto and Crawford, 1985). While *Nacardia* and *Rhodococcus* degraded lignin and related compound (Rast *et al.*, 1980 and Trajanowski *et al.*, 1977). Antai (1985) selected three *Streptomyces* sps. which were lignolytic, out of which on was the most lignocellulosic decomposer which depleted 42 per cent of lignin and 5 per cent carbohydrate at 12 weeks of incubation. Pavlovicha *et al.* (1985) found variability among the species of *Streptomyces* on lignin degradation according to him *Streptomyces fulvovindis* A-42, could degrade upto 45 per cent lignin *Streptomyces endus* could degrade only 14.6 per cent lignin.

Actinomycetes, usually Streptomycetes have been shown to posses the potential to degrade many other polymers occurring in soil and litter, including hemicelluloses (Iizuka and Kowaminami, 1965; Jenkins *et al.*, 1982). Pectin (Kaiser, 1971), Keratin (Young and Smith, 1975) and Chitin (Hsu and Lockwood, 1975; Williams and Robinson, 1981). *Nacardia* have been claimed for many years that streptomycetes, like some fungi can synthesize humic compounds in culture.

A thermoactinomycete degraded upto 70 per cent of cellulose substrate in 24 hours at 55°C and the use of these microbes commercially for the rapid saccharification of cellulose materials has been suggested (Hagerdal *et al.*, 1978).

Pometto and Crawford (1985) compared the ability of *Streptomycetes viridiosporus* and *Pharerochaete chrysosporium* and

reported that an intermediary compound namely APPL was produced during lignin degradation. They demonstrated that *Streptomyces viridosporus* was superior over *Phanerochaete chrysosporium*. On a lignocellulose supplemented medium, it released P-coumaric acid and vanillic acid which are intermediaries of lignin degradation (Donnely and Crawford, 1988).

## 2.6 OTHER BENEFICIAL TRAITS OF ACTINOMYCETES

### 2.6.1 Phosphorus solubilization

The unavailable tricalcium phosphate has to be essentially solubilized to monocalcium phosphate for the plant root to absorb the phosphate ions from the soil solution. Most of the available phosphorus gets bound with the calcium under alkaline condition, aluminium and iron under acidic conditions and is not available directly to plant to absorbed. The solubilization of phosphorus is generally done by certain organic acids produced by several groups of bacteria, fungi and actinomycetes. The capability of the actinomycetes to solubilize the phosphorous is documented as below.

Rao *et al.* (1982) isolated *Streptomyces* sp. in the desertic soils of Rajasthan they reported that the actinomycete could solubilize the tricalcium phosphate in liquid medium with increase in incubation period when the pH dropped from 6.6 to 4.8. They also reported that the actinomycete was thermotolerant and solubilization increased with increase in temperature upto 40°C. Thus recommending utilization of *Streptomyces* sp. in desertic soil.

Deshmukh and Godbole (1998) also reported phosphate solubilization by soil actinomycetes from Karad (Maharashtra). According to them nearly $1/3^{rd}$ of the isolates could solubilize phosphorus.

Chattopadhay (2002) reporting the phosphate solubilization in different soils of West Bengal found that alfisol, entisol and mollisol dominated by the actinomycetes capable of solubilizing triclacium phosphate. They also reported that the rock phosphate was less soluble than calcium phosphate. Alfisols were more effective in phosphate solubilization.

Kanavade *et al.* (2002) reported that whey, a waste of dairy industry can be cheaper medium for propagation of actinomycetes with phosphate mobilizing potential.

### 2.6.2 Plant growth promoting substances

Plant growth promoting substance produced by microorganisms have a potential of inducing plant growth. Evidences of some bacteria and fungi contributing to the production of IAA and GA has been well established. However the actinomycetes are less exploited with in production of IAA and GA.

Katzrnelson *et al.* (1965) repotted the production of gibberllin like substances by bacteria and actinomycetes. Out of the 11 actinomycetes isolated from the rhizosphere of (unknown plant) reported six actinomycetes showing the evidence of production of GA like substance ($A_3$) synthesis. Similarly, Hawa *et al.* (1993) reported actinomycetes among the other microorganisms producing IAA in the laboratory containing

malathion. *Streptomyces chibaensis* was most effective isolate to produce IAA in presence of the insecticide Strzelezyk *et al.* (1984) also isolated from the mycorrhizosphere of pine produced GA like substance.

### 2.6.3 Enzyme activities

A fairly large variety of enzymatic properties are manifested by actinomycetes (Krassilnikov, 1981). Studies have shown that actinomycetes produce enzymes such as proteases (Nomoto and Nasahashi, 1959a, b), amylases (Waksman, 1959; Shimobu, 1958; Cochrane, 1961), keratinases (Acton and McGuire, 1931; Kuchaeva *et al.*, 1963; Noval and Nickerson, 1959; Noval, 1957), chitinase (Lingappa and Lockwood, 1962), phosphatase and urease (Lim and Cha, 2000; Doumbou *et al.*, 2001).

Carrillo and Gomez-Molina (1998) reported mesophillic and themophillic strains of actinomycetes hydrolysing chitin *Streptomyces griscorber* was found to produce chitinase in a colloidal chitin liquid medium at pH 4 and 40°C.

Gomes *et al.* (1999) similarly reported two distinct group of actinomycetes one with capacity to instantly produce chitinase while the other delayed production.

# Material and Methods

# III. MATERIAL AND METHODS

The present investigation were carried out during the year 2002-2004 at the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad. The materials used and the methods followed are presented here under.

## 3.1 COLLECTION OF SOIL SAMPLES

The soil samples were collected from the different ecological conditions of transitional zone of Dharwad district by following standard method described by Jackson (1973). The soils were brought in polythene bags and stored in a cool place to maintain their physiochemical properties for future use.

## 3.2 ISOLATION AND PURIFICATION OF ACTINOMYCETES

Isolation of actinomycetes present in the soil was done by ten fold serial dilution pour plate method. Under absolute sterile conditions using lamina air flow system. Ten g of soil was suspended in 90 ml sterile water blank and shaken on a mechanical shaker for 15 minutes. Further ten fold dilution series was prepared by transferring ten ml of aliquots of soil suspension each time to 90 ml sterile water blanks till $10^{-5}$ dilutions were obtained. The contents in the flasks were shaken between each transfer to ensure an uniform suspension. One ml aliquot from the desired soil dilution was transferred to sterile petriplates and starch-casein agar, Gause No. 1 and Arginine-Glycerol salts agar with cycloheximide (Appendix I) was poured to duplicate of plates were incubated for seven to ten days. The isolated colonies of the actinomycetes under dilutions of

$10^{-3}$ and $10^{-4}$ were counted and the average population was expressed as the number per gram of dry soil. Further the actinomycetes were purified by the streak plate method and well isolated single colonies on the plates were isolated and preserved in starch casein agar slants.

### 3.2.1 Characterization of actinomycetes

Fifty two pure cultures of actinomycete isolates were subjected to the following determinative tests for authenticating their identification.

1. Colony characters,
2. Microscopic observations for mycelial and conidial arrangements,
3. Gram staining,
4. Acid fast staining, and
5. Biochemical tests

#### 3.2.1.1 Colony morphological characters

Colony characters like shape, colour and texture were studied by referring Bergy's Manual of Determinative Bacteriology (John *et al.*, 1994).

#### 3.2.1.2 Microscopic observations

The morphological characters of the actinomycetes were studied by growing them on different agar slides which were incubated in moist chambers following the procedure of Gordon and Smith (1955).

#### 3.2.1.3 Gram staining

Gram staining for the isolates was conducted following the procedures described by Anonymous (1957). Typical gram positive were inferred to be actinomycetes.

### 3.2.1.4 Acid fast staining

Acid fast staining for the isolates was conducted following the procedure described by Conn (1961).

### 3.2.1.5 Biochemical tests

Various biochemical characteristics of the actinomycetes are used in identification of actinomycetes. Reduction of nitrate, starch hydrolysis, gelatin liquefaction, casein hydrolysis and $H_2S$ production were considered important biochemical characteristics useful for the identification of actinomycetes (Gordon and Smith, 1955).

### 3.2.2 Temperature optima for the actinomycetes

All the isolates were tested for temperature optima by inoculating the isolates grown in starch casein broth by spotting over starch-casein agar plate. The plates were incubated at four varying temperature of 25, 35, 45 and 55°C for seven days.

## 3.3 SCREENING OF ACTINOMYCETES ISOLATES FOR ANTAGONISM AGAINST FUNGAL PATHOGENS

### 3.3.1 *In vitro* screening of actinomycetes

The actinomycete isolates were screened for the biocontrol potential against soil borne plant pathogens *viz.*, *Sclerotium rolfsii*, *Rhizoctonia bataticola*, *Fusarium solani* and *Pythium ultimum*. The dual inoculation method as explained by Sakthivel and Gnanamanickam (1986) was followed. The mycelial plug of the pathogen was placed at the centre of PDA plate (Appendix I) using a cork borer. The actinomycetes

cultures were streaked on either sides of the pathogen. A control was maintained without any streak of the actinomycete. There were duplicate plates maintained for each test. The plates were incubated at 30°C for seven days for comparing the extent of radial growth of pathogen in the control plates. The inhibition of fungal growth was qualitatively assessed as strong, medium and weak.

### 3.3.2 Elucidation of mechanisms of biocontrol by actinomycetes

#### 3.3.2.1 Antimicrobial metabolite production

To determine if the actinomycetes elaborate any antimicrobial metabolites, *in vitro* antipathogenic activity was tested on nutrient agar glucose medium (Kraus and Loper, 1992).

The antibiotic activity of the actinomycetes was assessed by extracting and testing the toxicity of metabolites produced by them following the method of Kraus and Loper (1992).

The strains were grown for four days in a 10 ml of nutrient broth amended with glucose (2% W/V). The growth was centrifuged at 10,000 rpm for 10 minutes and the filtrate was collected. The metabolites in the filtrates were extracted with an equal volume of chloroform. The metabolites were also extracted from the pellet and pooled. The upper aqueous layer was discarded and to the remaining chloroform phase, a pinch of sodium sulphate was added to dry off the water. It was centrifuged at 8,000 rpm for eight minutes and sodium sulphate was pelleted. The clear layer was decanted and chloroform was removed by flushing in air. The residue was redissolved in 200 µl acetone and 70 µl

was spotted on to a TLC plate (Sillcagel 60 $F_{254}$, 20 x 20 cm, 0.2 mm thickness, S.D. fine make). The plate was chromotogramed using chloroform: acetone (9:1) as the solvent system. And, the plate was observed under UV light (254 nm). The metabolites were marked and the Rf values were calculated. The metabolites were eluted and redissolved in acetone: water (1:10). The eluted portions were centrifuged to pellet the silica gel and the clear suspension. A sterile paper disc dipped in suspension was placed on the luxuriant lawn of pathogen to study the toxicity of the metaboite against the pathogen.

#### 3.3.2.2 Chitinase activity

The procedure adopted to determine the chitinase activity was by following the procedure (Hsu *et al.*, 1975).

### 3.4 COLLECTION OF SUGARCANE TRASH

The sugarcane trash was collected from the farmer's field at Ramdurga (Belgaum district).

### 3.5 CHARACTERIZATION OF SUGARCANE TRASH

#### 3.5.1 Analysis of sugarcane trash

The sugarcane trash samples were chopped into 1 to 1.5 cm and stored in a gunny bag. The samples, thus prepared were analysed for pH, organic carbon and total nitrogen.

#### 3.5.1.1 pH

Sugarcane trash powder was suspended in distilled water at 1:10 ratio. The pH of the suspension was determined by using glass electrode in a digital pH meter ("Systronics" model 335).

### 3.5.1.2 Organic carbon

The organic carbon in the sugarcane trash samples was estimated by taking known quantities of dried samples in a pre-weighed silica crucible. The samples were kept in a muffle furnace at a temperature of 600°C for two hours. The crucibles were later directly transferred to desicators, cooled and immediately, weighed to a constant weight (ash weight). The total per cent of organic matter was calculated by taking the difference of dry weight of the sample and weight of the ash. Then organic carbon was calculated by dividing the per cent organic matter by the factor 1.724 (Jackson, 1973).

### 3.5.1.3 Total nitrogen

Total nitrogen in the sample was estimated by following the micro kjeldahal method as outlined by (Jackson, 1973). Dried sample (0.5 g) was digested using 10 ml of concentrated sulphuric acid to which was added the potassium sulphate, copper sulphate and selenium powder in the ratio 50:10:1 in the micro kjeldahal digestion unit. The contents were digested in a fume cupboard over a low flame and then over strong flame till a light bluish green colour was obtained. The digested samples were diluted with water and distilled after the addition of sufficient quantities of 4.0 per cent NaOH to make the sample alkaline in the micro kjeldahal distillation unit.

The ammonia evolved was trapped in two per cent boric acid mixed indicator solution and titiated against 0.05 N sulphuric acid. The nitrogen content was calculated from the volume of acid consumed.

$$\% N = \frac{\text{Titer value x N of } H_2SO_4 \text{ x } 0.014 \text{ x dilution factor}}{\text{Weight of the plant sample (g)}} \text{ X } 100$$

**3.5.1.4 C:N ratio**

The C:N ratio was calculated by dividing per cent of organic carbon by per cent total nitrogen.

## 3.6 SCREENING OF MICROORGANISMS FOR LIGNIN DEGRADATION USING INDICATOR MEDIA

### 3.6.1 Identification of production of lignolytic actinomycetes

Fifty-two isolates of actinomycetes grown in starch-casein broth for four days were spotted on to indicator Crawfords medium (Appendix I) with methylene blue (0.02%) (Crawford *et al.*, 1983).

The colonies showing decoloration surrounding Crawford's medium with methylene blue were considered positive for lignin degradation.

## 3.7 COMPOSTING OF SUGARCANE TRASH USING THE ACTINOMYCETES CULTURE

The six lignolytic actinomycetes were selected based on decoloration of methylene blue (0.02%) on Crawford medium for this study.

One kg of sugarcane trash was chopped to 1 to 1.5" length and soaked in water for about 6 to 8 hrs to bring the trash moisture to around 60-70 per cent and filled in 18" x 14" size polyethene bags. The initial C:N ratio of 120:1 was adjusted to 80:1 by the addition of urea to hasten the process of composting. Six actinomycete isolates were selected (A11, A12,

A13, A16, A25, A40, A52) among which five isolates (A11, A12, A13, A16, A25, A40) showed decoloration of methylene blue and isolate A52 which did not shown decoloration of methylene blue was used as negative check. All the six isolates were grown in starch casein broth separately for 15 days.

The cultures in broth were mixed separately with lighite carrier material and inoculated to sugarcane trash @ 1 kg per tonnes. This dose of inoculation was decided based on the recommendations made for *Phanerochrate chrysosporium*. Further two checks *viz.*, *Phanerochrate chrysosporium* and uninoculated control also were maintained. The treatments from $T_1$ to $T_9$ were duplicated one adjusted C:N ratio to 80:1 and other without adjusted C:N ratio *i.e.* 120:1. The whole experimental setup was maintained with frequent mixing to create aeration. The moisture content was maintained at 70 per cent throughout the period of study. In all nine sets for each treatments were maintained and three sets of each were analysed for total carbon and total nitrogen at monthly intervals for three months.

The treatment details were as follows

$T_1$: Sugarcane trash + actinomycete strain A11

$T_2$: Sugarcane trash + actinomycete strain A12

$T_3$: Sugarcane trash + actinomycete strain A13

$T_4$: Sugarcane trash + actinomycete strain A16

$T_5$: Sugarcane trash + actinomycete strain A25

$T_6$: Sugarcane trash + actinomycete strain A40

$T_7$: Sugarcane trash + actinomycete strain A52

$T_8$: Sugarcane trash + *Phanerochrate chrysosporium*

$T_9$: Sugarcane trash + no inoculation (UIC)

## 3.8 OTHER BENEFICIAL TRAITS

### 3.8.1 Phosphate solubilization

All the 52 isolates in duplicates were screened for P-solubilization on Pikovasky's medium (Appendix I) four days old cultures were spotted on the above media and incubated at 37°C for five days. The zone of clearing indicated the solubilization.

#### 3.8.1.1 Quantitative estimation of Pi release from tricalcium phosphate

The isolates showing zone of solubilization on Pikovskaya's agar were further examined for their ability to release Pi form TCP in broth medium. One ml of three days culture of each isolate was inoculated to 50 ml of Pikovskaya's broth (Pikovskaya, 1948) in three replications. All the inoculated flasks were incubated for two weeks at 28±2°C. The amount of Pi released in the broth was estimated on 10 and 20 days of incubation from triplicate flasks at each stage in comparison with a set of uninoculated controls. The broth cultures were centrifuged at 10,000 rpm for 10 minutes in a centrifuge to separate the supernatant from the cell growth and insoluble phosphate. The available P content in the supernatant was estimated by phosphomolybdic blue colour method of Jackson (1973) and expressed mg Pi released per 100 g TCP (mg per ml).

The change in the pH of the Pikovaskay's broth was tested at 10 and 20 days after incubation by using pH meter with combined glass electrode (Jackson, 1973).

### 3.8.1.2 Reagents used

**Chloromolybdic acid**

Chloromolybdic acid reagent was prepared by dissolving 7.5 g of ammonium molybdate in 150 ml distilled water to which 162 ml of concentrated HCl was added. The volume was made upto one litre with distilled water.

**Chlorostannous acid**

Chlorostannous acid reagent was prepared by dissolving 25 g of $SnCl_2$ $2H_2O$ in 100 ml concentrated HCl and making the volume to one litre with distilled water.

Both the reagents were stored in amber coloured bottles in a refrigerator.

**Procedure**

One ml of the culture supernatant was taken in a 50 ml volumetric flask to which 10 ml of chloromolybdic acid was added and mixed thoroughly. The volume was made upto approximately three fourth with distilled water and 0.25 ml chlorostannous acid was added to it. Immediately, the volume was made to 50 ml with distilled water and mixed thoroughly. After 15 minutes, the blue colour developed was read in a spectrophotometer at 610 nm using a reagent blank.

Simultaneously, a standard curve was prepared using various concentrations of standard 2 ppm $KH_2PO_4$ solution. The amount of phosphorous solubilized b the isolates was calculated from the standard curve.

**Preparation of standard curve**

Potassium dihydrogen phosphate was dried at 40°C and 0.2195 g of it was dissolved in 400 ml distilled water. Twenty five ml of $FNH_2SO_4$ was added to it and volume was made upto one liter with distilled water and mixed thoroughly. Twenty ml of this was diluted further to 500 ml with distilled water to obtain two ppm solution and used for preparation of standard curve.

### 3.8.2 Enzyme activity

#### 3.8.2.1 Urease

The procedure adopted to determine the *in vitro* urease activity in cultures of actinomycetes was essentially the same as explained by Pancholy and Rice (1973) except that the ammonia liberated due to hydrolysis of urea in the reaction mixture was determined by nesslerization as described by Jackson (1973).

Ten ml of liquid culture of actinomycetes was placed in duplicates of 100 ml capacity erlenmeyer flasks to which one ml of toluene was added and allowed to stand for 15 minutes to permit complete its penetration in to culture. Each of these flasks were added with 10 ml of phosphate buffer (pH 6.7) and 10 ml of ten per cent urea solution. For control flasks, urea solution was replaced by equal quantity of distilled water. The contents of the flasks were well shaken for five minutes and incubated at 30°C for 24 hrs. After incubation the contents of the flasks were filtered through Whatman No. 42 filter paper. The remaining culture in the flask was added with 15 ml of 1 N KCl solution shaken for five

minutes and filtered. The volume of the total filtrate was made up to 100 ml in a volumetric flask using distilled water.

The amount of ammonia present in the filtrate was determined by nesslerization. One ml filtrate from each sample was transferred to a 20 ml volumetric flask to which two ml of ten per cent sodium tartarate solution and 0.5 ml of Nessler's reagent were added. The volume was made upto 20 ml with distilled water. The yellow colour developed after 30 minutes was measured at 460 nm using systronics – visible range spectrophotometer against reagent blank.

The results obtained were expressed as μg of ammonia liberated per ml of culture per day with reference to standard curve obtained by using graded concentrations (0 to 100 μg $m^{-1}$) of $(NH_4)SO_4$ solution and developing the colour by nesslerization.

**3.8.2.2 Phosphatase**

Phosphatase activity in cultures of actinomycetes was determined by following the procedure of Evazi and Tabatabai (1979). One ml of 7 days old culture broth was placed in duplicates of 50 ml Erlenmeyer flask to which 0.2 ml toluene followed by four ml of modified universal buffer (pH 7.5) were added one ml of p-nitrophenol phosphate solution made in modified universal buffer was added to the flask and contents of the flasks were mixed by swirling for two minutes. The flasks were stoppered and incubated at 37°C for one hour. After incubation one ml of 0.5 $CaCl_2$ and four ml of 0.5 M NaOH were added to the flask. Swirled and filtered through Whatman No. 42 filter paper. The intensity of yellow colour

developed was measured at 420 nm against the reagent blank using systronics spectrophotometer.

Controls were maintained for each samples and were analysed by following the same procedure described above except that the paranitrophenol phosphate solution was added after the addition of 0.5 M $CaCl_2$ and 0.5 M NaOH and just before, filtration. The phosphatase activity in the culture was expressed as µg paranitrophenol formed per ml culture per hour with reference to the standard curve prepared by using graded concentrations of p-nitrophenol phosphate.

## 3.9 PRODUCTION OF GROWTH PROMOTING SUBSTANCES BY THE ACTINOMYCETES

All the 52 isolates were subjected to qualitative analysis for the production of IAA (Bric *et al.*, 1991) and GA (Brown and Burlingham, 1968).

Luria agar (Appendix I) supplemented with 0.06 per cent sodium dodecyl sulphate and one per cent glyceorol was prepared and plated. The surface area of the agar medium was divided into squares of 2 x 2 cm by marking on the bottom of each plate. The three days culture of each isolate was spotted with sterile tooth pick on each square. The spotted plates were overlaid immediately with sterile disc of Whatman No.1 filter paper. Plates were incubated until the colonies reached the size of 0.5 to 2.0 mm in diameter. After an appropriate incubation period, the filter paper discs were removed from the plates and treated with Salkowaski's reagent (2% of 0.5 M fedz in 35% per chloric acid) by soaking in a petridish containing the reagent. The reaction was allowed to proceed

until adequate colour was developed. Actinomycetes producing IAA were identified by the formation of characteristic red halo around the colony on filter paper. The paper discs after treatment with Salkowaski's reagent were viewed under UV light. The spots giving typical green fluorescence were taken as positive for GA production.

The isolates showing IAA and GA production were further examined for the amount of IAA and GA production as detailed below.

### 3.9.1 Quantitative estimation of IAA and GA

Three day old cultures of the isolates which showed the production of IAA and GA in qualitative estimation were inoculated to 50 ml of sterilized Czapeck's solution in duplicates (Appendix I) and incubated at 37°C for seven days in dark. After incubation, the cultures were centrifuged at 6000 rpm for 20 minutes. The supernatant was collected in a conical flask and used for estimation of IAA and GA.

### 3.9.2 Estimation of IAA

Twenty-five ml of the supernatant was collected and the pH was adjusted to 2.8 using 1 N HCl in of 100 ml conical flask. Equal volume of diethyl ether was added to it and incubated in dark for four hours. Extraction of IAA was done at 4°C in a separating funnel using diethyl ehter. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, two ml of methanol was added, pooled and the IAA present in the methanol extract was determined using the method of Gordon and Paleg (1957).

To 0.5 ml of methanol extract, 1.5 ml of distilled water and four ml Sapler's reagent (1 ml of 0.5 M $FeCl_2$ in 50 ml of 35% perchloric acid) were

added and incubated in dark for one hour. The intensity of pink colour developed was read at 535 mm in a UV-visible. Spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity of IAA in the culture filtrate was determined and expressed as µg/25 ml of the medium.

### 3.9.3 Estimation of GA

Twenty five ml of the culture filtrate was taken in a test tube to which two ml of zinc acetate was added. After two minutes two ml of potassium ferropanide was added and centrifuged at 1000 rpm for 154 minutes. To five ml of this supernatant was added five ml of 30 per cent HCl and incubated at 20°C for 75 minutes. The blank sample was treated with five per cent HCl and the absorbence of the samples as well as blank was measured at 254 nm in a UV-visible spectrophotometer. The amount of GA present in the extract was calculated from the standard curve and expressed as µg/25 ml of the medium. The standard curve of GA were prepared by using graded concentrations of $GA_3$ (Paleg, 1965).

## 3.10 STATISTICAL ANALYSIS

The statistical analysis of the data was carried out for completely randomized design (Panse and Sukhatme, 1985).

# Experimental Results

# IV. EXPERIMENTAL RESULTS

Investigations were carried out to isolate actinomycetes from transitional zone of Dharwad region in Karnataka. Fifty-two actinomycetes were isolated and maintained for further study in the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka. The cultures were used to study their biocontrol potential, organic matter decomposition and other beneficial traits. The results obtained during the investigation are presented here under.

## 4.1 ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF ISOLATES OF ACTINOMYCETES

Isolation of actinomycetes in the soil was carried out using three specific media namely starch-casein agar, Gause No. 1 and glycerol arginine-glycerol soils agar with cycloheximide. More than 70 isolations were made from the above media from soils in Dharwad region. Further purification led to obtain 52 well isolated actinomycetes and maintained on starch casein agar. The actinomycetes count of the soil samples on the three different nutrient media employed for enumeration is given in Table 1. As evident from the table that the average actinomycetes count in three different nutrient media *viz.*, starch casein agar, aspergine glycerol salts agar and Gause No. 1 were 18.96, 6.12 and 4.35 thousands/g respectively in the black soils 10.73, 3.57 and 3.03 thousands/g respectively for the red soils. It is thus seen that starch casein agar gave maximum actinomycetes count from the soil samples under investigation, followed by aspergine glycerol salts agar and Gause No. 1.

**Table 1. Actinomycetes population in soils of transitional zone of Dharwad on different growth media**

| Sl. No. | Places | Number of actinomycetes x $10^3$/g soil | | | | | |
|---|---|---|---|---|---|---|---|
| | | Starch casein agar | | Arginine-glycerol salts agar | | Gause No. 1 agar | |
| | | Black soil | Red soil | Black soil | Red soil | Black soil | Red soil |
| 1. | Amminabhavi | 12.0 | 8.3 | 5.0 | 2.3 | 4.0 | 3.6 |
| 2. | Belur | 19.3 | 10.2 | 4.3 | 3.3 | 3.8 | 2.3 |
| 3. | Dharwad | 10.3 | 8.0 | 4.6 | 4.0 | 3.6 | 3.0 |
| 4. | Garag | 27.3 | 17.0 | 6.3 | 3.6 | 2.6 | 2.0 |
| 5. | Hubli | 19.3 | 10.6 | 11.3 | 5.6 | 7.6 | 5.3 |
| 6. | Hulkoti | 30.3 | 15.6 | 3.6 | 3.3 | 4.3 | 2.6 |
| 7. | Kittur | 23.6 | 12.0 | 8.6 | 4.0 | 3.3 | 3.0 |
| 8. | MK Hubli | 12.3 | 5.3 | 4.6 | 3.0 | 4.0 | 2.3 |
| 9. | Mummigatti | 12.6 | 8.3 | 9.3 | 4.0 | 5.3 | 4.0 |
| 10. | Navalgund | 22.6 | 12.0 | 3.6 | 2.6 | 5.0 | 2.2 |
| | Mean | 18.96 | 10.73 | 6.12 | 3.57 | 4.35 | 3.03 |
| | S.Em± | 0.41 | 0.26 | 0.15 | 0.09 | 0.12 | 0.07 |
| | CD at 1% | 1.56 | 0.99 | 0.58 | 0.35 | 0.45 | 0.28 |

### 4.1.1 Colony morphology

The actinomycete colonies were found to be distinctly different from the bacterial colonies on the agar. They formed slight cottony growth with different colourations from white to grey and ash. The colonies were purified on the solid agar which were later identified as *Streptomyces*. Thick leathery colonies were also observed which were characters of *Micromonospora*. White mealy colonies were ascribed to species of *Nocardia*. The morphological and biochemical tests of the 52 isolates were conducted for identification. The results presented in Table 2 indicated that all the isolates were gram positive and none of them were acid fast.

Biochemical tests were carried out to ascertain the generic specificity of all the actinomycetes isolated from the soil (Table 2). All the isolates were capable of hydrolysis of starch but only 23 actinomycetes indicated the casein hydrolysis. Liquefaction of gelatin was demonstrated only in 27 isolates. The $H_2S$ production was demonstrable in 25 isolates. The nitrate reduction was carried out by another 30 isolates of actinomycetes. Based on colony morphology and biochemical charactersitics, the isolates were tentatively assigned to 3 genera as *Streptomyces, Nocardia* and *Micromonospora*. Under microscopic observations 24 *Streptomyces* had open spiral sporophores five had closed spiral sporophores, five were monoverticillus spiral sporophores, four belonged to biverticillus spiral sporophores, one was fasciled sporophores. Nine isolates showed mycelial growth without any sporulation, four isolates showed one spore mycelial growth. The highest per cent of isolates belonged to genus *Streptomyces* (76.9%), next *Nocardia* (17.37%) and 7.6 per cent isolates belonged to genus *Micromonospora*.

**Table 2. Morphological and biochemical characteristics of the soil actinomycetes of transitional zone of Dharwad region**

| Sl. No. | Isolates | Colony morphology | Gram reaction | Acid fast staining | Biochemical tests | | | | | Microscopic observations | | Tentative genera assigned |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | Spore formation | Morphological features | |
| 1. | A1 | A | + | - | + | + | - | + | + | + | Open spiral sporophores | *Streptomyces* |
| 2. | A2 | A | + | - | + | + | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 3. | A3 | B | + | - | - | + | - | - | + | + | Closed spiral sporophores | *Streptomyces* |
| 4. | A4 | A | + | - | - | + | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 5. | A5 | C | + | - | - | - | - | + | + | - | Mycelial growth | *Nocardia* |
| 6. | A6 | B | + | - | + | + | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 7. | A7 | B | + | - | + | + | - | + | + | + | Biverticillus spiral sporophores | *Streptomyces* |
| 8. | A8 | A | + | - | + | - | + | + | + | + | Biverticillus sporophores | *Streptomyces* |
| 9. | A9 | A | + | - | + | + | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 10. | A10 | C | + | - | - | - | - | + | + | + | Mycelial growth | *Nocardia* |
| 11. | A11 | A | + | - | - | + | - | - | + | + | Biverticillus spiral sporophores | *Streptomyces* |
| 12. | A12 | C | + | - | - | + | - | - | + | - | Mycelium growth | *Nocardia* |
| 13. | A13 | B | + | - | + | - | + | + | + | + | Monoverticillus spiral sporophores | *Streptomyces* |
| 14. | A14 | A | + | - | - | + | - | - | + | + | Open spiral sporophores | *Streptomyces* |
| 15. | A15 | A | + | - | - | + | + | + | + | + | Monoverticillus spiral sporophores | *Streptomyces* |
| 16. | A16 | A | + | - | - | + | - | + | + | + | Open spiral sporophores | *Streptomyces* |
| 17. | A17 | C | + | - | - | - | - | + | + | - | Mycelium growth | *Nocardia* |
| 18. | A18 | B | + | - | + | - | - | + | + | + | Closed spiral sporophores | *Streptomyces* |
| 19. | A19 | D | + | - | - | - | + | - | + | + | One spore | *Micromonospora* |
| 20. | A20 | A | + | - | + | - | - | + | + | + | Open spiral sporophores | *Streptomyces* |
| 21. | A21 | A | + | - | + | - | - | - | + | + | Closed spiral sporophores | *Streptomyces* |
| 22. | A22 | C | + | - | - | - | - | + | + | - | Mycelium growth | *Nocardia* |
| 23. | A23 | A | + | - | + | + | - | - | + | + | Monoverticillus spiral sporophores | *Streptomyces* |
| 24. | A24 | A | + | - | + | + | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 25. | A25 | B | + | - | - | + | + | + | + | + | Fasciled sporophores | *Streptomyces* |
| 26. | A26 | C | + | - | - | - | + | + | + | + | Mycelial growth | *Nocardia* |
| 27. | A27 | A | + | - | + | - | - | - | + | + | Open spiral sporophores | *Streptomyces* |

**Table 2. Contd.....**

| Sl. No. | Isolates | Colony morphology | Gram reaction | Acid fast staining | Biochemical tests | | | | | Microscopic observations | | Tentative genera assigned |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | Spore formation | Morphological features | |
| 28. | A28 | A | + | - | + | + | - | - | + | + | Open spiral sporophores | *Streptomyces* |
| 29. | A29 | C | + | - | - | + | + | + | + | - | Mycelial growth | *Nocardia* |
| 30. | A30 | A | + | - | + | - | + | - | + | + | Open spiral sporophores | *Streptomyces* |
| 31. | A31 | B | + | - | - | + | - | + | + | + | One spore | *Micromonospora* |
| 32. | A32 | D | + | - | + | + | - | - | + | + | Biverticillus sporophores | *Streptomyces* |
| 33. | A33 | B | + | - | + | - | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 34. | A34 | A | + | - | + | - | + | + | + | + | Monoverticillus spiral sporophores | *Streptomyces* |
| 35. | A35 | A | + | - | - | - | + | + | + | + | Closed spiral sporophores | *Streptomyces* |
| 36. | A36 | A | + | - | - | - | - | - | + | + | One spore | *Micromonospora* |
| 37. | A37 | D | + | - | - | - | - | + | + | + | Open spiral sporophores | *Streptomyces* |
| 38. | A38 | A | + | - | - | + | - | - | + | + | Closed spiral sporophores | *Streptomyces* |
| 39. | A39 | B | + | - | - | + | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 40. | A40 | A | + | - | - | + | - | - | + | + | Open spiral sporophores | *Streptomyces* |
| 41. | A41 | C | + | - | - | + | - | + | + | - | Mycelial growth | *Nocardia* |
| 42. | A42 | A | + | - | + | + | - | - | + | + | Open spiral sporophores | *Streptomyces* |
| 43. | A43 | A | + | - | + | - | + | - | + | + | Open spiral sporophores | *Streptomyces* |
| 44. | A44 | B | + | - | - | - | + | - | + | + | Monoverticillus spiral sporophores | *Streptomyces* |
| 45. | A45 | A | + | - | + | + | + | - | + | + | Open spiral sporophores | *Streptomyces* |
| 46. | A46 | B | + | - | - | - | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 47. | A47 | D | + | - | - | + | + | - | + | + | One spore | *Micromonospora* |
| 48. | A48 | B | + | - | + | - | - | - | + | + | Open spiral sporophores | *Streptomyces* |
| 49. | A49 | C | + | - | - | - | + | - | + | - | Mycelial growth | *Nocardia* |
| 50. | A50 | A | + | - | - | + | + | + | + | + | Open spiral sporophores | *Streptomyces* |
| 51. | A51 | B | + | - | - | - | + | - | + | + | Open spiral sporophores | *Streptomyces* |
| 52. | A52 | B | + | - | + | + | + | + | + | + | Open spiral sporophores | *Streptomyces* |

**Biochemical tests**

1. Casein hydrolysis
2. Gelatin liquefaction
3. $H_2S$ production
4. Nitrate reduction, 5. Starch hydrolysis

Streptomyces = 76.97%
Nocardia = 17.3%
Micromonospora = 7.6%

A = Ash coloured leathery colony
B = Grey coloured leathery colony
C = Thick leathery colony
D = White mealy colony

## 4.2 TEMPERATURE OPTIMA FOR THE ACTINOMYCETES

Actinomycete isolates were tested for their temperature tolerance at 25, 35, 45 and 55°C incubated for five days. The results thus obtained are presented in Table 3. All the isolates could laxuriantly grow at 25 and 35°C. No difference in their growth was observed at both the mesophilic conditions. However at 45°C, 46 isolates could show growth at the end of five days incubation. Further at elevated temperature of 55°C only nine isolates could grow. It was further observed that at 45°C the growth of colonies on the starch casein solid agar was restricted compared to those of 25° and 35°C. Similarly at 55°C the colony growth was still further reduced compared to the rest of the incubation temperature.

## 4.3 *IN VITRO* BIOCONTROL POTENTIAL OF ACTINOMYCETES

All the 52 actinomycete isolates were tested for their biocontrol potential against four fungal pathogens *viz.*, *Rhizoctonia bataticola*, *Fusarium solani*, *Pythium ultimum* and *Sclerotium rolfsii*. The results are presented in the (Table 4 and Plate 1). Among the isolates tested 15 actinomycetes were found inhibitory on *R. bataticola*, 10 were inhibitory to *F. solani*, 11 were found inhibitory to *P. ultimum*. None of the actinomycete isolate showed inhibition of *S. rolfsii*.

Among the 15 isolates which showed biocontrol potential against *R. bataticola* A23, A24, A29, A38, A48 and A51 showed strong inhibition. A3, A9, A15, A37, A42 A45 and A52 showed moderate inhibition A2 and A43 showed weak inhibition.

Ten isolates inhibited the growth of *F. solani*. Among them the actinomycetes strain A3, A5, A23, A40, A48 and A52 showed strong

**Table 3. Effect of temperature on the growth of actinomycetes***

| Sl. No. | Isolates | Incubation temperature (°C) | | | |
|---|---|---|---|---|---|
| | | 25 | 35 | 45 | 55 |
| 1. | A1 | + | + | + | - |
| 2. | A2 | + | + | + | - |
| 3. | A3 | + | + | + | - |
| 4. | A4 | + | + | + | - |
| 5. | A5 | + | + | + | - |
| 6. | A6 | + | + | + | - |
| 7. | A7 | + | + | + | + |
| 8. | A8 | + | + | + | + |
| 9. | A9 | + | + | + | + |
| 10. | A10 | + | + | + | + |
| 11. | A11 | + | + | + | - |
| 12. | A12 | + | + | + | - |
| 13. | A13 | + | + | + | - |
| 14. | A14 | + | + | + | - |
| 15. | A15 | + | + | + | - |
| 16. | A16 | + | + | + | - |
| 17. | A17 | + | + | + | - |
| 18. | A18 | + | + | + | - |
| 19. | A19 | + | + | + | - |
| 20. | A20 | + | + | - | - |
| 21. | A21 | + | + | + | - |
| 22. | A22 | + | + | + | - |
| 23. | A23 | + | + | + | + |
| 24. | A24 | + | + | + | - |
| 25. | A25 | + | + | + | - |
| 26. | A26 | + | + | + | - |
| 27. | A27 | + | + | + | + |
| 28. | A28 | + | + | + | - |
| 29. | A29 | + | + | + | - |
| 30. | A30 | + | + | + | - |
| 31. | A31 | + | + | - | - |
| 32. | A32 | + | + | + | - |
| 33. | A33 | + | + | + | - |
| 34. | A34 | + | + | + | - |
| 35. | A35 | + | + | + | + |
| 36. | A36 | + | + | + | - |
| 37. | A37 | + | + | - | - |
| 38. | A38 | + | + | - | - |
| 39. | A39 | + | + | - | - |
| 40. | A40 | + | + | + | - |
| 41. | A41 | + | + | + | - |
| 42. | A42 | + | + | + | + |
| 43. | A43 | + | + | + | - |
| 44. | A44 | + | + | + | - |
| 45. | A45 | + | + | + | - |
| 46. | A46 | + | + | + | - |
| 47. | A47 | + | + | - | - |
| 48. | A48 | + | + | + | + |
| 49. | A49 | + | + | + | - |
| 50. | A50 | + | + | + | - |
| 51. | A51 | + | + | + | - |
| 52. | A52 | + | + | + | - |

* Observations made at the end of five days incubation

+ growth, - no growth

**Table 4. Antagonistic effect of soil actinomycetes on plant pathogenic fungi**

| Sl. No. | Isolates | *Rhizoctonia bataticola* | *Fusarium solani* | *Pythium ultimum* | *Sclerotium rolfsii* | Sl. No. | Isolates | *Rhizoctonia bataticola* | *Fusarium solani* | *Pythium ultimum* | *Sclerotium rolfsii* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | A1 | - | - | - | - | 27. | A27 | - | - | - | - |
| 2. | A2 | + | - | + | - | 28. | A28 | - | - | - | - |
| 3. | A3 | + | + | + | - | 29. | A29 | - | - | - | - |
| 4. | A4 | - | - | - | - | 30. | A30 | - | - | - | - |
| 5. | A5 | - | + | - | - | 31. | A31 | - | - | - | - |
| 6. | A6 | - | - | - | - | 32. | A32 | - | - | + | - |
| 7. | A7 | - | - | - | - | 33. | A33 | - | - | - | - |
| 8. | A8 | - | - | - | - | 34. | A34 | - | - | - | - |
| 9. | A9 | + | + | + | - | 35. | A35 | - | - | - | - |
| 10. | A10 | - | - | - | - | 36. | A36 | - | - | - | - |
| 11. | A11 | - | - | - | - | 37. | A37 | + | - | - | - |
| 12. | A12 | - | - | - | - | 38. | A38 | + | - | - | - |
| 13. | A13 | - | - | - | - | 39. | A39 | - | - | - | - |
| 14. | A14 | - | - | - | - | 40. | A40 | - | + | + | - |
| 15. | A15 | - | - | + | - | 41. | A41 | - | - | - | - |
| 16. | A16 | - | - | - | - | 42. | A42 | + | + | + | - |
| 17. | A17 | - | - | - | - | 43. | A43 | + | - | - | - |
| 18. | A18 | - | - | - | - | 44. | A44 | - | - | - | - |
| 19. | A19 | - | - | - | - | 45. | A45 | + | - | - | - |
| 20. | A20 | - | - | - | - | 46. | A46 | - | - | - | - |
| 21. | A21 | - | - | - | - | 47. | A47 | - | - | - | - |
| 22. | A22 | - | - | - | - | 48. | A48 | + | + | + | - |
| 23. | A23 | + | + | - | - | 49. | A49 | + | + | - | - |
| 24. | A24 | + | - | - | - | 50. | A50 | - | - | - | - |
| 25. | A25 | - | - | - | - | 51. | A51 | + | + | + | - |
| 26. | A26 | + | - | - | - | 52. | A52 | + | + | + | - |

+ inhibition and – no inhibition

# LEGEND

**I.**

a. Control (*Rhizoctonia bataticola*)

b. Inhibition of *Rhizoctonia bataticola* by *Streptomyces* A3

c. Inhibition of *Rhizoctonia bataticola* by *Streptomyces* A15

d. Inhibition of *Rhizoctonia bataticola* by *Streptomyces* A45

e. Inhibition of *Rhizoctonia bataticola* by *Streptomyces* A42

**II.**

a. Control (*Fusarium solani*)

b. Inhibition of *Fusarium solani* by *Streptomyces* A3

c. Inhibition of *Fusarium solani* by *Streptomyces* A9

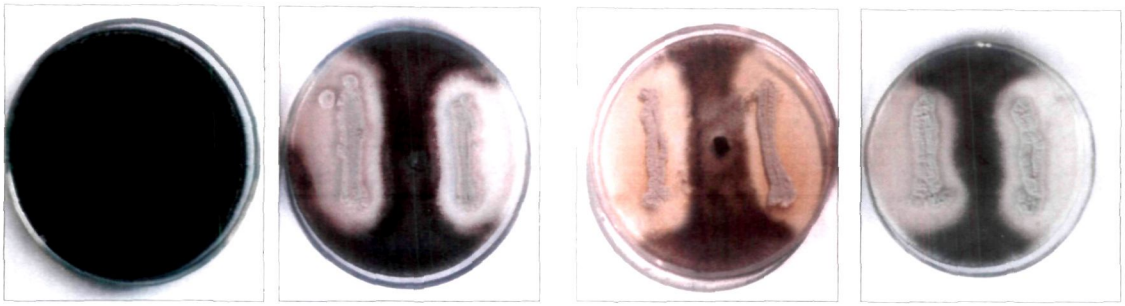
**III.**

a. Control (*Pythium ultimum*)

b. Inhibition of *Pythium ultimum* by *Streptomyces* A40

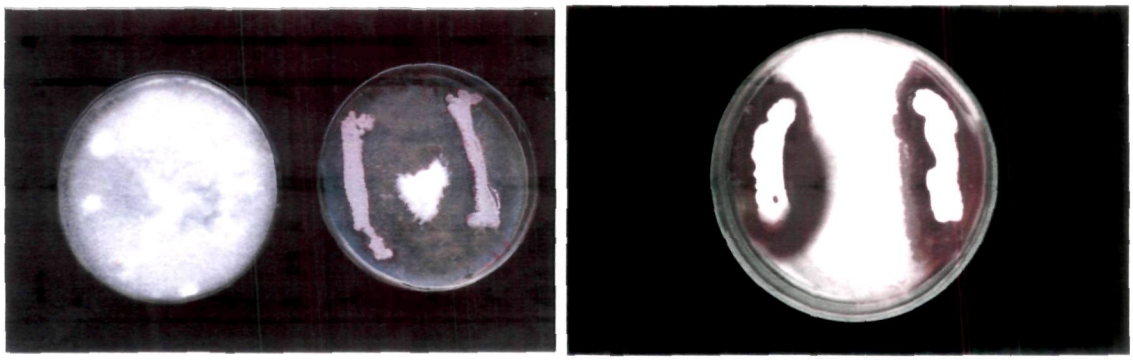
c. Inhibition of *Pythium ultimum* by *Streptomyces* A48

I



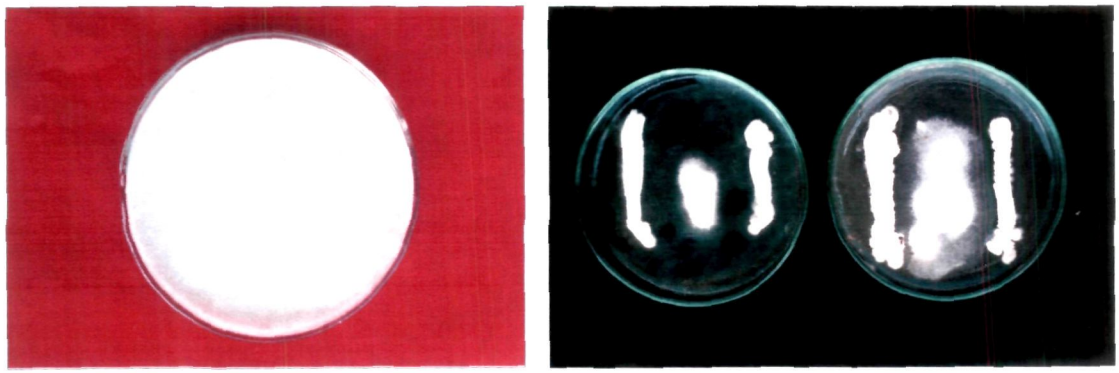
a. b. c. d.

II



a. b. c.

III



a. b. c.

Plate 1: *In vitro* biocontrol potential of soil actinomycetes

inhibition, A42 and A9 showed moderate inhibition and strain A49 showed weak inhibition.

Eleven isolates of actinomycetes inhibited the growth of *P. ultimum*. Among them, the isolates A2, A40, A51 and A52 showed strong inhibition, A9, A15, A26, A32 and A48 showed moderate inhibition and strains A3 and A42 showed weak inhibition (Table 5).

## 4.4 MECHANISM OF BIOCONTROL

### 4.4.1 Production of antimicrobial metabolites

Nineteen isolates as indicated in the Table 6 effective against the fungal pathogens were selected for the analysis of the antimicrobial metabolites produced by them on thin layer chromotographic plates. Invariably all the isolates produced metabolites each of which appeared as dark spots under short wave UV light. The Rf value of the metabolites varied from 0.38 to 0.96.

All the unidentified metabolites on TLC plate were scraped, eluted and redissolved in acetone filter paper discs of each metabolites was transferred on the luxuriant growth of pathogen. Inhibition of the pathogen around the filter paper discs were recorded and presented in Table 7 and Plate 2.

### 4.4.2 Production of chitnase enzyme

All 52 isolates were tested for chitnase enzyme activity. None of the isolates shown chitnase enzyme activity.

**Table 5. *In vitro* biocontrol potential of soil actinomycetes**

| *Rhizoctonia bataticola* | | | *Fusarium solani* | | | *Pythium ultimum* | | |
|---|---|---|---|---|---|---|---|---|
| Sl. No. | Isolates | | Sl. No. | Isolates | | Sl. No. | Isolates | |
| 1. | *Streptomyces* A2 | + | 1. | *Streptomyces* A3 | +++ | 1. | *Streptomyces* A2 | +++ |
| 2. | *Streptomyces* A3 | ++ | 2. | *Nocordia* A5 | +++ | 2. | *Streptomyces* A3 | + |
| 3. | *Streptomyces* A9 | ++ | 3. | *Streptomyces* A9 | ++ | 3. | *Streptomyces* A9 | ++ |
| 4. | *Streptomyces* A15 | ++ | 4. | *Streptomyces* A23 | +++ | 4. | *Streptomyces* A15 | ++ |
| 5. | *Streptomyces* A23 | +++ | 5. | *Streptomyces* A40 | +++ | 5. | *Nocordia* A26 | ++ |
| 6. | *Streptomyces* A24 | +++ | 6. | *Streptomyces* A42 | ++ | 6. | *Micromonosopra* A32 | ++ |
| 7. | *Nocordia* A29 | +++ | 7. | *Streptomyces* A48 | +++ | 7. | *Streptomyces* A40 | +++ |
| 8. | *Micromonosopra* A37 | ++ | 8. | *Nocordia* A49 | + | 8. | *Streptomyces* A42 | + |
| 9. | *Streptomyces* A38 | +++ | 9. | *Streptomyces* A51 | ++ | 9. | *Streptomyces* A48 | ++ |
| 10. | *Streptomyces* A42 | ++ | 10. | *Streptomyces* A52 | +++ | 10. | *Streptomyces* A51 | +++ |
| 11. | *Streptomyces* A43 | + | | | | 11. | *Streptomyces* A52 | +++ |
| 12. | *Streptomyces* A45 | ++ | | | | | | |
| 13. | *Streptomyces* A48 | +++ | | | | | | |
| 14. | *Streptomyces* A51 | +++ | | | | | | |
| 15. | *Streptomyces* A52 | ++ | | | | | | |

Strong inhibition - +++
Moderate inhibition - ++
Weak inhibition - +

- indicates no inhibition, + inhibition

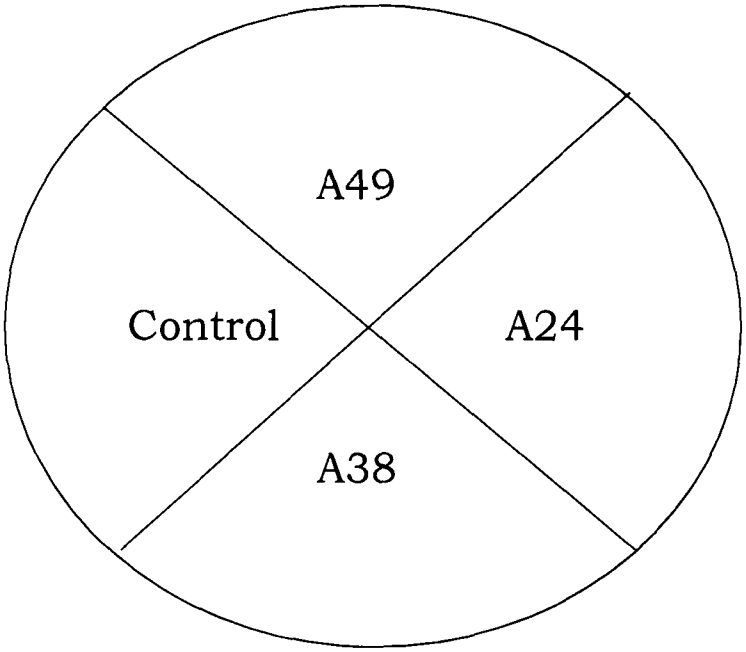
**Table 6. Silica gel thin layer chromotographic migration of the metabolites produced by antagonistic soil actinomycetes**

| Sl. No. | Isolates | Rf value of the metabolite |
|---|---|---|
| 1. | A48, A40, A9 | 0.38 |
| 2. | A24, A26, A42 | 0.48 |
| 3. | A15, A52, A51 | 0.50 |
| 4. | A3, A5 | 0.58 |
| 5. | A43, A32, A45 | 0.60 |
| 6. | A38 A37 | 0.93 |
| 7. | A49, A23, A2 | 0.96 |

**Table 7. Inhibition and fungal growth by the eluted metabolite from actinomycetes**

| *Rhizoctonia bataticola* | | | *Fusarium solani* | | | *Pythium ultimum* | | |
|---|---|---|---|---|---|---|---|---|
| Sl. No. | Isolate | ZOI (mm) | Sl. No. | Isolate | ZOI (mm) | Sl. No. | Isolate | ZOI (mm) |
| 1. | A2 | 12.00 | 1. | A3 | 19.00 | 1. | A2 | 23.00 |
| 2. | A3 | 14.00 | 2. | A5 | 19.00 | 2. | A3 | 13.00 |
| 3. | A9 | 16.00 | 3. | A9 | 14.00 | 3. | A9 | 16.00 |
| 4. | A15 | 13.00 | 4. | A23 | 18.00 | 4. | A15 | 19.00 |
| 5. | A23 | 20.00 | 5. | A40 | 22.00 | 5. | A26 | 14.00 |
| 6. | A24 | 17.00 | 6. | A42 | 15.00 | 6. | A32 | 19.00 |
| 7. | A29 | 19.00 | 7. | A48 | 23.00 | 7. | A40 | 20.00 |
| 8. | A37 | 16.00 | 8. | A49 | 12.00 | 8. | A42 | 10.00 |
| 9. | A38 | 20.00 | 9. | A51 | 16.00 | 9. | A48 | 18.00 |
| 10. | A42 | 17.00 | 10. | A52 | 20.00 | 10. | A51 | 20.00 |
| 11. | A43 | 13.00 | | | | 11. | A52 | 22.00 |
| 12. | A45 | 14.00 | | | | | | |
| 13. | A48 | 19.00 | | | | | | |
| 14. | A51 | 21.00 | | | | | | |
| 15. | A52 | 13.00 | | | | | | |

# LEGEND



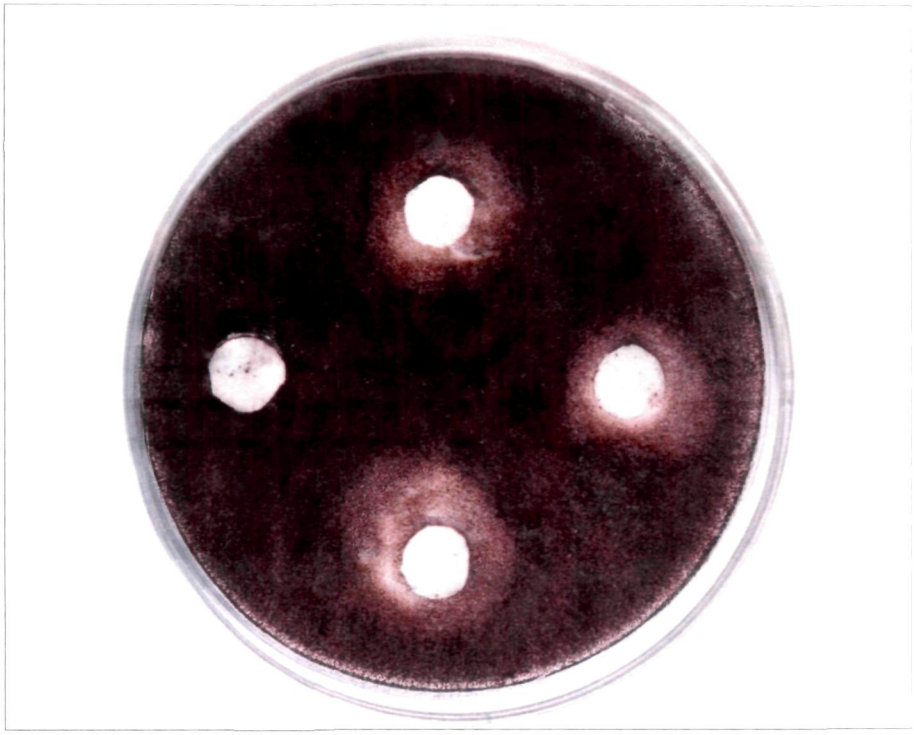


Plate 2: Inhibition of *Rhizoctonia bataticola* by the eluted metabolite from actinomycete

## 4.5 SCREENING OF ACTINOMYCETES FOR LIGNIN DEGRADATION ON INDICATOR MEDIUM

Crawford's agar with 0.02 per cent methylene blue was used to screen the actinomycetes for their potential for lignin degradation. A four day old actinomycete culture was inoculated on to the Crawford's agar plate and incubated for five days. The colonies which decolourized the methylene blue scored for lignin degradation ability. Among the 52 actinomycete isolates only five isolates showed a zone of decoloration around the colonies. Thus actinomycete isolates A11, A12, A13, A16, A25 and A40 exhibited the zone of decoloration. A test strain lignolytic fungi namely *Phanerochrate chrysosporium* also indicated the decoloration of methylene blue on Crawford's agar (Table 8).

### 4.5.1 Biodegradation of sugarcane trash by native actinomycetes isolates

Fresh but dry sugarcane trash was collected from the Ramdurga farmers field for evaluation of ability of the actinomycetes to decompose. Before trash was subjected to decomposition it was analysed for pH, per cent C and N by following the standard procedure. The pH 6.5 to 7.0, C 46.4 per cent and N 0.385 per cent respectively (Table 9). Thus C:N ratio of the trash was 120:1.

Effect of inoculation of actinomycetes isolated from the soil of transitional belt of Dharwad region were tested for their ability to decomposting the sugarcane trash only six actinomycetes which showed decolorization of methylene blue were tested with an efficient lignolytic fungus *Phanerochrate chrysosporium.* Another actinomycete isolate A52

**Table 8. Qualitative testing of actinomycetes for lignolytic activity on indicator medium**

| Sl. No. | Isolates | Result | Sl. No. | Isolates | Result |
|---|---|---|---|---|---|
| 1. | A1 | - | 27. | A27 | - |
| 2. | A2 | - | 28. | A28 | - |
| 3. | A3 | - | 29. | A29 | - |
| 4. | A4 | - | 30. | A30 | - |
| 5. | A5 | - | 31. | A31 | - |
| 6. | A6 | - | 32. | A32 | - |
| 7. | A7 | - | 33. | A33 | - |
| 8. | A8 | - | 34. | A34 | - |
| 9. | A9 | - | 35. | A35 | - |
| 10. | A10 | - | 36. | A36 | - |
| 11. | A11 | + | 37. | A37 | - |
| 12. | A12 | + | 38. | A38 | - |
| 13. | A13 | + | 39. | A39 | - |
| 14. | A14 | - | 40. | A40 | + |
| 15. | A15 | - | 41. | A41 | - |
| 16. | A16 | + | 42. | A42 | - |
| 17. | A17 | - | 43. | A43 | - |
| 18. | A18 | - | 44. | A44 | - |
| 19. | A19 | - | 45. | A45 | - |
| 20. | A20 | - | 46. | A46 | - |
| 21. | A21 | - | 47. | A47 | - |
| 22. | A22 | - | 48. | A48 | - |
| 23. | A23 | - | 49. | A49 | - |
| 24. | A24 | - | 50. | A50 | - |
| 25. | A25 | + | 51. | A51 | - |
| 26. | A26 | - | 52. | A52 | - |
| | | | Check | *Phanerochrate chrysosporum* | + |

+: decoloration and

–: no decolaration of methylene blue

**Table 9. Chemical characteristics of sugarcane trash**

| Sl. No. | Composition | Trash of sugarcane |
|---|---|---|
| 1. | pH | 6.5-7.0 |
| 2. | Per cent C | 46.4 |
| 3. | Per cent N | 0.385 |
| 4. | C : N | 120:1 |

was also included which did not decolorize methylene blue as a negative check. All these inoculations were finally compared with an uninoculated control. The initial C:N ratio of 120 and adjusted C:N ratio 80:1 with urea fertilizer were tested with actinomycete inoculation. The results obtained are presented in Table 10. In general, it may be observed that none of actinomycete were superior in decomposition of sugarcane trash as compared with *Phanerochrate chrysosporium*. However, it is evident that *Streptomyces* sp. isolate no. 16 and 13 were superior among the actinomycete studied. The rate of decomposition as measured by reduction in the C:N ratio indicated decline with the increase in the period of composting further the per cent decline in adjusted C:N of 80:1 with urea ($C_2$) indicated a faster decomposition compared to other *Streptomyces* spp. *Streptomyces* isolate A52 (a methylene blue negative strain) was inferior in decomposition and was comparable to that of an uninoculated control.

### 4.5.2 Biodegradation after four weeks incubation

The mean per cent decline of C:N was superior in $C_2$ that $C_1$. Inoculation of standard check namely *Phanerochrate chrysosporium* was able to decompose significantly higher than the test actinomycete inoculant. Here again *Streptomyces* A13 and A16 were superior among the actinomycetes. The rest of the inoculants namely A11, A12, A25, A40 were also superior to strain A52 and uninoculated control.

The mean decline in the per cent of C:N ratio was almost similar with treatments where C:N ratio were not adjusted.

**Table 10. Influence of inoculation of actinomycetes on sugarcane trash decomposition**

| Sl. No. | Treatments | 4 weeks | | | 8 weeks | | | 12 weeks | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_1$ | $C_2$ | Mean | $C_1$ | $C_2$ | Mean | $C_1$ | $C_2$ | Mean |
| 1. | *Streptomyces* A11 | 93.61 (21.99) | 62.31 (22.11) | 77.96 | 86.29 (28.09) | 58.78 (26.52) | 72.53 | 82.40 (31.33) | 51.93 (35.08) | 67.17 |
| 2. | *Nocordia* A12 | 83.11 (30.74) | 55.28 (30.90) | 69.19 | 77.95 (35.04) | 54.48 (31.75) | 66.22 | 73.33 (38.89) | 46.98 (41.27) | 60.16 |
| 3. | *Streptomyces* A13 | 77.49 (35.42) | 51.56 (35.55) | 64.53 | 72.39 (39.67) | 44.25 (44.68) | 58.32 | 67.39 (43.08) | 39.62 (50.70) | 51.96 |
| 4. | *Streptomyces* A16 | 78.71 (34.40) | 52.36 (34.55) | 65.53 | 73.78 (38.51) | 45.90 (42.62) | 59.84 | 68.30 (43.08) | 40.52 (49.35) | 53.94 |
| 5. | *Streptomyces* A25 | 89.15 (25.70) | 59.34 (25.82) | 74.25 | 83.46 (30.45) | 50.33 (37.08) | 66.89 | 78.46 (34.61) | 44.71 (44.11) | 61.59 |
| 6. | *Streptomyces* A40 | 86.05 (28.29) | 57.90 (27.62) | 71.98 | 80.94 (32.55) | 49.13 (38.58) | 65.03 | 76.73 (36.05) | 42.55 (46.81) | 59.64 |
| 7. | *Streptomyces* A52 | 108.75 (9.37) | 72.52 (9.35) | 90.64 | 101.28 (15.60) | 68.01 (14.98) | 84.65 | 97.25 (18.95) | 62.12 (22.35) | 79.69 |
| 8. | *Phanerochrate crysosporium* (check) | 74.26 (59.69) | 48.37 (39.53) | 61.31 | 66.26 (44.78) | 40.41 (49.48) | 53.33 | 61.19 (49.00) | 30.39 (62.01) | 44.79 |
| 9. | Uninoculated control | 118.95 (0.87) | 78.43 (1.96) | 98.69 | 112.54 (6.21) | 74.51 (6.86) | 93.53 | 109.18 (9.01) | 71.46 (10.67) | 90.32 |
| | Mean | 90.01 (24.98) | 59.78 (25.97) | 74.90 | 83.88 (30.08) | 53.98 (32.52) | 93.53 | 79.36 (33.85) | 45.58 (43.02) | 62.47 |
| | S.Em± | 0.156 | | 0.332 | 0.174 | | 0.371 | 0.397 | | 0.223 |
| | CD at 1% | 0.600 | | 1.277 | 0.671 | | 1.427 | 0.717 | | 1.528 |

$C_1$ –120:1, No adjustment of C:N, $C_2$ – C:N (80:1), Adjustment of C:N, The data in parentheses indicates the C:N ratio reduction over initial C:N ratio

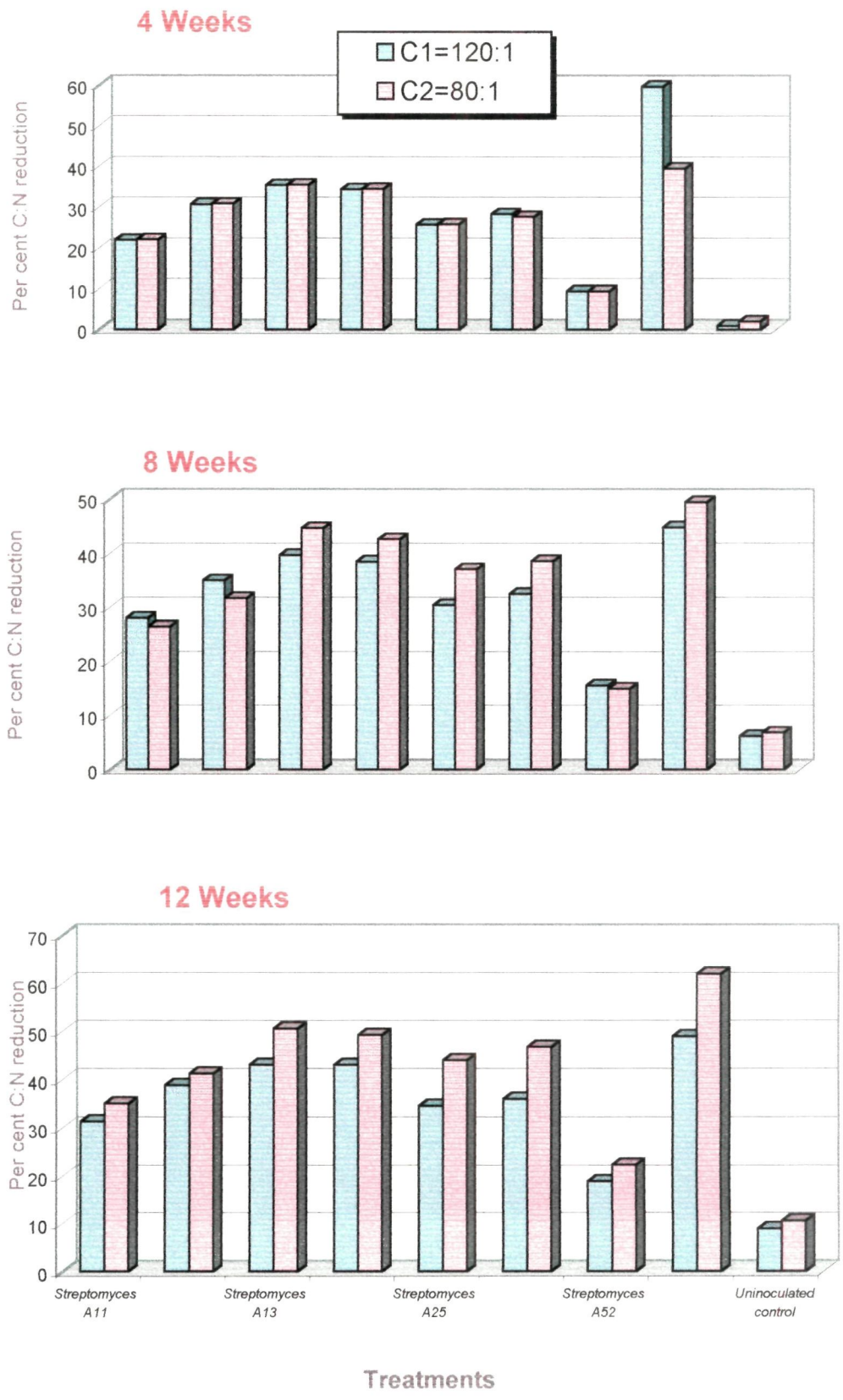



**Fig. 1. Influence of inoculation of actinomycetes on sugarcane trash decomposition**

### 4.5.3 Biodegradation after eight weeks incubation

Similar to the observation made on the fourth week of incubation, isolates A13 and A16 continued to be superior to all rest of the actinomycetes. Here again the *Phanerochrate chrysosporium* was superior to all the other strains of actinomycetes. However, under uninoculated control there was a marginal decline in C:N ratio.

### 4.5.4 Biodegradation after twelve weeks

The check lignolytic fungus namely *Phanerochrate chrysosporium* continued to be superior over all the actinomycetes. In the uninoculated control the decline in C:N was once again very poor. Actinomycete isolates A13 and A16 were once again superior. It also can be seen that inoculation of *Phanerochrate chrysosporium* drastically declined to extent of 49 per cent without adjustment of C:N and 62.01 per cent with adjustment of C:N ratio in general the treatment with adjusted C:N ratio either with inoculation or without inoculation influenced the decomposition.

## 4.6 PHOSPHATE SOLUBILIZATION BY THE ISOLATES

### 4.6.1 Qualitative analysis of the isolates for P solubilization

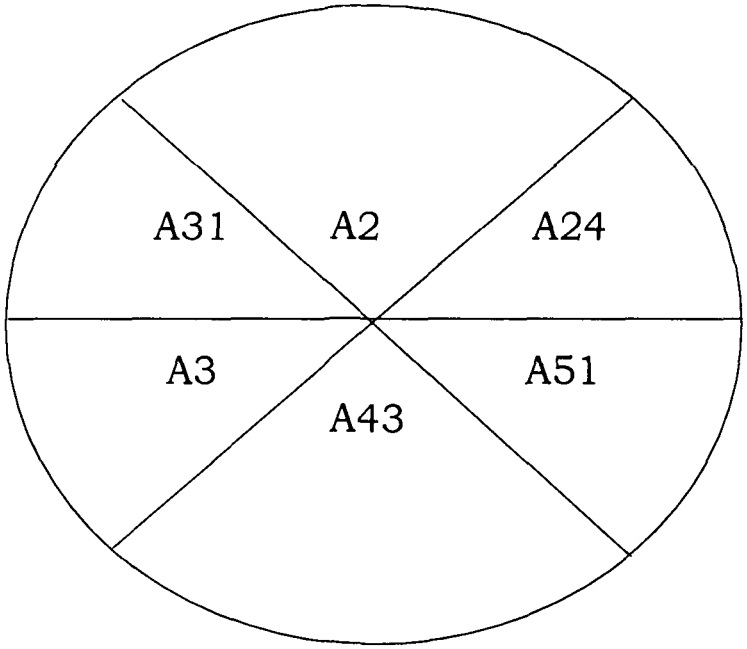
All the 52 actinomycete isolates were examined for their ability to solubilize tricalcium phosphate (TCP) on Pikovskaya's agar medium. Out of 52 only 9 isolates were able to show clear zone of solubilization on Pikovskaya's agar. All other actinomycete isolates failed to solubilize TCP (Table 11 and Plate 3). The diameter of zones of solubilization formed by the 9 isolates on Pikovskaya's agar medium ranged from 0.80 to 1.40 cm on four days of incubation.

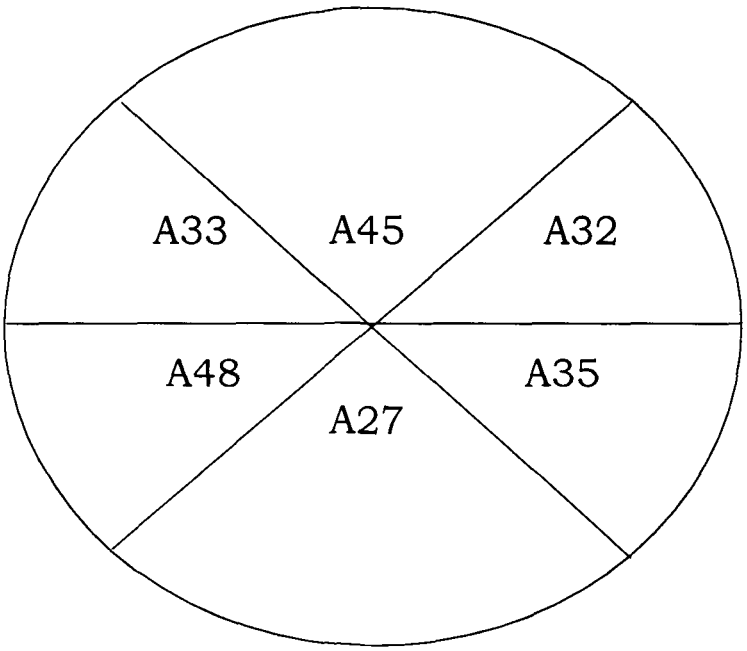
**Table 11. Phosphorus solubilization of soil actinomycetes on TCP medium**

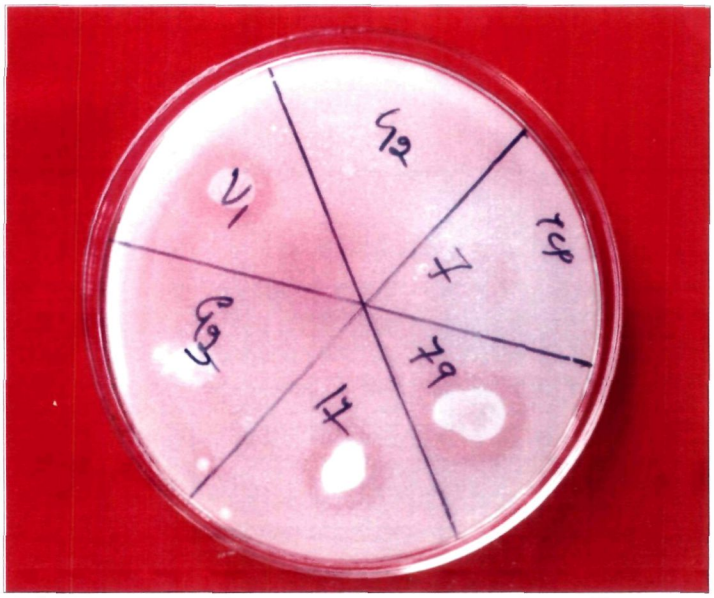
| Sl. No. | Isolates | TCP* | Sl. No. | Isolates | TCP |
|---|---|---|---|---|---|
| 1. | A1 | - | 27. | A27 | + |
| 2. | A2 | - | 28. | A28 | - |
| 3. | A3 | - | 29. | A29 | - |
| 4. | A4 | - | 30. | A30 | - |
| 5. | A5 | - | 31. | A31 | + |
| 6. | A6 | - | 32. | A32 | - |
| 7. | A7 | - | 33. | A33 | - |
| 8. | A8 | - | 34. | A34 | + |
| 9. | A9 | - | 35. | A35 | + |
| 10. | A10 | - | 36. | A36 | - |
| 11. | A11 | - | 37. | A37 | - |
| 12. | A12 | - | 38. | A38 | - |
| 13. | A13 | - | 39. | A39 | - |
| 14. | A14 | - | 40. | A40 | - |
| 15. | A15 | - | 41. | A41 | + |
| 16. | A16 | - | 42. | A42 | - |
| 17. | A17 | - | 43. | A43 | + |
| 18. | A18 | - | 44. | A44 | - |
| 19. | A19 | + | 45. | A45 | + |
| 20. | A20 | - | 46. | A46 | - |
| 21. | A21 | - | 47. | A47 | - |
| 22. | A22 | - | 48. | A48 | - |
| 23. | A23 | - | 49. | A49 | - |
| 24. | A24 | - | 50. | A50 | - |
| 25. | A25 | - | 51. | A51 | + |
| 26. | A26 | - | 52. | A52 | - |

- No solubilization * TCP
+ Solubilization

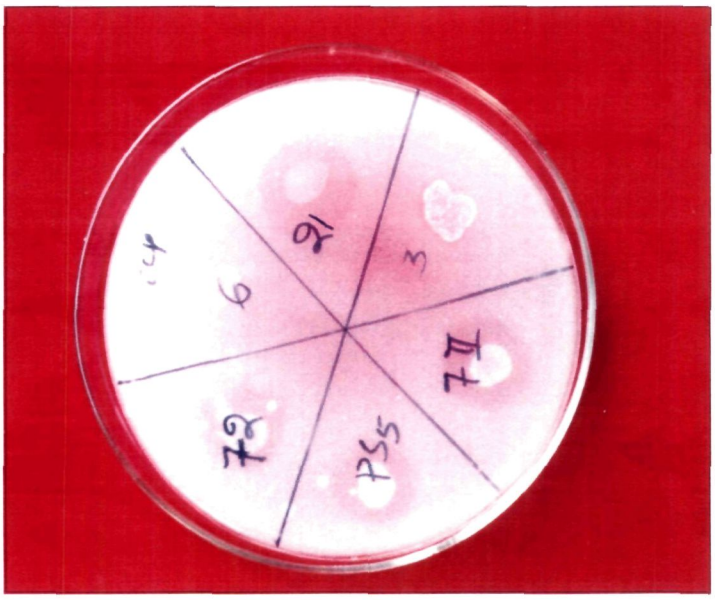
# LEGEND





a.



b.

Plate 3: Phosphorus solubilization of soil actinomycetes on TCP medium

Among the nine actinomycete isolates, isolate A45 and A51 recorded maximum solubilization zone (both with 1.40 cm diameter) followed by A35 (1.30 cm), A19 (1.10 cm diameter), A34 (10 cm diameter). A41, A27, A31 and A43 (with 0.80 cm diameter) solubilization of 0.8 cm diameter was shown by rest of the actinomycete isolates A41, A27, A31 and A43.

### 4.6.2 Quantitative estimation of P solubilizing activity of the isolates

The amount of Pi released from TCP in the Pikovskaya's broth by the 9 isolates was also studied at 10 and 20 days after incubation (DAI). The results presented in Table 12 showed that the amount of Pi released from TCP by all strains increased with increasing incubation time and was maximum at 20 (DAI). The per cent Pi released from TCP by the actinomycetes at 20 DAI ranged from 8.23 to 14.24 per cent. The actinomycete strain A41 was found to release maximum per cent of phosphorus (14.24%) followed by strain A34 (13.23%) and the least amount of per cent Pi was released by the strain A45.

### 4.6.3 pH drop in culture supernatant

The change in the pH of broth medium was tested at 10 and 20 days after incubation (DAI). The results presented in Table 12 indicated that the highest pH reduction was observed in broth inoculated with strain A41 (pH 5.20) followed by A34 (pH 5.41) and A51 (pH 5.60). The lowest pH reduction was observed in the broth inoculated with the strain A45 (pH 6.47).

**Table 12. Per cent Pi release and changes in pH of Pikovskay's broth by actinomycetes**

| Sl. No. | Isolates | Zone of solubilization (cm) | 10 DAI | | 20 DAI | |
|---|---|---|---|---|---|---|
| | | | pH | % Pi released | pH | % Pi released |
| 1. | *Micromonospora* A19 | 1.10 | 5.73 | 4.13 | 5.65 | 8.93 |
| 2. | *Streptomyces* A27 | 0.80 | 6.75 | 5.23 | 5.93 | 10.23 |
| 3. | *Streptomyces* A31 | 0.80 | 6.89 | 4.11 | 6.61 | 9.03 |
| 4. | *Streptomyces* A34 | 1.0 | 5.84 | 9.22 | 5.41 | 13.23 |
| 5. | *Streptomyces* A35 | 1.30 | 5.81 | 7.33 | 5.75 | 9.87 |
| 6. | *Nocordia* A41 | 0.80 | 5.75 | 8.16 | 5.20 | 14.24 |
| 7. | *Streptomyces* A43 | 0.80 | 5.70 | 6.22 | 5.62 | 12.03 |
| 8. | *Streptomyces* A45 | 1.40 | 6.93 | 3.05 | 6.47 | 8.23 |
| 9. | *Streptomyces* A51 | 1.40 | 5.82 | 7.24 | 5.60 | 12.21 |

DAI – Days after incubation

### 4.6.4 Production of plant growth promoting substances (PGPS) by the actinomycetes

All 52 isolates were examined for IAA and GA production on starch-casein supplemented with sodium doedyl sulphate (SDS) (0.06%) and glycerol (1%). Based on the development of red colour on the filter paper on green fluorescence under UV light, 8 isolates were considered as positive for IAA and/or GA production (Table 13). All the 8 isolates were further subjected to quantitative determination of IAA and GA.

#### 4.6.4.1 Quantitative determination of IAA and GA produced by the isolates

The quantity of IAA and GA produced by the 8 isolates was determined at 10 DAI and the results are presented in Table 14. The amount of IAA produced by different strains ranged from 4.0 µg to 17.3 µg/25 ml of broth. Among the isolates examined the actinomycetes strain A17 (17.3 µg/25 ml broth) followed by strain A40 (15.1 µg/25 ml broth) could produce IAA. Least IAA was produced by strain A4 (4.0 µg/25 ml broth) on par with strain A2 (4.2 µg/25 ml broth).

The quantity of GA produced by the strains ranged from 1.4 µg/25 to 6.6 µg/25 ml broth (Table 14). Among all the strains tested, strain A8 was found to produce the highest amount of GA (6.6 µg/25 ml broth) followed by A17 (5.71 µg/25 ml broth). Least GA was produced by actinomycete strain A4 (1.4 µg/25 ml broth).

**Table 13. Qualitative testing of actinomycetes for IAA and GA production**

| Sl. No. | Isolates | IAA | GA | Sl. No. | Isolates | IAA | GA |
|---|---|---|---|---|---|---|---|
| 1. | A1 | - | - | 27. | A27 | - | - |
| 2. | A2 | - | - | 28. | A28 | - | - |
| 3. | A3 | - | - | 29. | A29 | - | - |
| 4. | A4 | + | + | 30. | A30 | - | - |
| 5. | A5 | - | - | 31. | A31 | - | - |
| 6. | A6 | - | - | 32. | A32 | - | - |
| 7. | A7 | - | - | 33. | A33 | - | - |
| 8. | A8 | + | + | 34. | A34 | - | - |
| 9. | A9 | - | - | 35. | A35 | + | + |
| 10. | A10 | - | - | 36. | A36 | - | - |
| 11. | A11 | - | - | 37. | A37 | - | - |
| 12. | A12 | - | - | 38. | A38 | - | - |
| 13. | A13 | - | - | 39. | A39 | - | - |
| 14. | A14 | - | - | 40. | A40 | + | + |
| 15. | A15 | - | - | 41. | A41 | - | - |
| 16. | A16 | - | - | 42. | A42 | + | + |
| 17. | A17 | + | + | 43. | A43 | - | - |
| 18. | A18 | - | - | 44. | A44 | - | - |
| 19. | A19 | - | - | 45. | A45 | - | - |
| 20. | A20 | - | - | 46. | A46 | + | + |
| 21. | A21 | - | - | 47. | A47 | - | - |
| 22. | A22 | - | - | 48. | A48 | - | - |
| 23. | A23 | + | + | 49. | A49 | - | - |
| 24. | A24 | - | - | 50. | A50 | - | - |
| 25. | A25 | - | - | 51. | A51 | - | - |
| 26. | A26 | - | - | 52. | A52 | - | - |

- No production of PGPS
+ Production of PGPS

**Table 14. Production of IAA and GA by actinomycetes**

| Sl. No. | Isolates | IAA (µg/25 ml) | GA (µg/25 ml) |
|---|---|---|---|
| 1. | *Streptomyces* A4 | 4.00 | 1.40 |
| 2. | *Streptomyces* A8 | 14.00 | 6.60 |
| 3. | *Nocordia* A17 | 17.30 | 5.71 |
| 4. | *Streptomyces* A23 | 8.20 | 5.50 |
| 5. | *Streptomyces* A40 | 15.10 | 3.84 |
| 6. | *Streptomyces* A42 | 4.20 | 2.30 |
| 7. | *Streptomyces* A35 | 8.50 | 4.60 |
| 8. | *Streptomyces* A46 | 9.10 | 2.20 |
| | S.Em± | 0.08 | 0.05 |
| | CD at 1% | 0.35 | 0.22 |

## 4.7 ENZYME ACTIVITIES

### 4.7.1 Urease

The *in vitro* urease activity of the test actinomycete isolates are presented in Table 15. The amount of urease activity by different strains ranged from 1.45 to 7.15 μg/25 ml broth. Among the isolates examined, the actinomycete strain A50 was found to produce the highest amount of urease activity (7.15 μg/25 ml broth) followed by A52 (6.91 μg/25 broth). The least urease activity was recorded by actinomycete strain A43.

### 4.7.2 Phosphatase

The data on the *in vitro* phosphatase activity of the test actynomycete isolates are presented in Table 15. The phosphatase activity ranged from 4.66 to 12.20 μg/25 ml broth. Among the isolates examined, the highest amount of phosphatase activity was shown by strain A31. The least amount of activity was recorded by strain A23 (Table 16).

**Table 15. Urease activity by soil actinomycetes**

| Sl. No. | Isolates | mg of $NH_4$ nitrogen released/25 ml broth culture/24 hr |
|---|---|---|
| 1. | *Streptomyces* A1 | 3.94 |
| 2. | *Streptomyces* A2 | 2.98 |
| 3. | *Streptomyces* A4 | 3.43 |
| 4. | *Nocordia* A5 | 2.78 |
| 5. | *Streptomyces* A9 | 2.50 |
| 6. | *Streptomyces* A11 | 3.03 |
| 7. | *Micromonospora* A19 | 4.40 |
| 8. | *Nocordia* A26 | 5.13 |
| 9. | *Streptomyces* A27 | 3.15 |
| 10. | *Streptomyces* A33 | 3.50 |
| 11. | *Streptomyces* A34 | 2.76 |
| 12. | *Streptomyces* A35 | 2.94 |
| 13. | *Streptomyces* A39 | 2.48 |
| 14. | *Micromonospora* A40 | 1.47 |
| 15. | *Streptomyces* A42 | 3.94 |
| 16. | *Streptomyces* A43 | 1.45 |
| 17. | *Streptomyces* A45 | 2.38 |
| 18. | *Nocordia* A49 | 2.82 |
| 19. | *Streptomyces* A50 | 7.15 |
| 20. | *Streptomyces* A51 | 4.06 |
| 21. | *Streptomyces* A52 | 6.91 |
| | S.Em± | 0.055 |
| | CD at 1% | 0.22 |

**Table 16. Phosphatase activity by soil actinomycetes**

| Sl. No. | Isolates | mg of P-nitrophenol released/25 ml broth culture per hour |
|---|---|---|
| 1. | *Streptomyces* A7 | 6.52 |
| 2. | *Streptomyces* A14 | 5.23 |
| 3. | *Micromonospora* A19 | 4.70 |
| 4. | *Streptomyces* A23 | 4.66 |
| 5. | *Streptomyces* A27 | 10.50 |
| 6. | *Streptomyces* A31 | 12.20 |
| 7. | *Streptomyces* A34 | 6.00 |
| 8. | *Streptomyces* A35 | 8.90 |
| 9. | *Nocordia* A41 | 7.40 |
| 10. | *Streptomyces* A42 | 8.30 |
| 11. | *Streptomyces* A45 | 8.20 |
| 12. | *Streptomyces* A51 | 6.20 |
| | S.Em± | 0.05 |
| | CD at 1% | 0.20 |

**Table 17. Categorisation of actinomycete isolates for biocontrol potential and other beneficial traits**

| Sl. No. | Isolate | Biocontrol potential | | | Temperature | Organic matter decomposition | Phosphorus solubilization | IAA and GA production | Urease activity | Phosphatase activity |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Rhizoctonia bataticola* | *Fusarium solani* | *Pythium ultimum* | | | | | | |
| 1. | A1 | | | | | | | | + | |
| 2. | A2 | + | | + | | | | | + | |
| 3. | A3 | + | + | + | | | | | | |
| 4. | A4 | | | | | | | + | + | |
| 5. | A5 | | + | | | | | | + | |
| 6. | A8 | | | | + | | | + | | + |
| 7. | A9 | + | + | + | + | | | | + | |
| 8. | A10 | | | | | + | | | + | |
| 9. | A15 | + | | + | | | | | | |
| 10. | A19 | | | | | | + | | + | + |
| 11. | A23 | + | + | | + | | | + | | + |
| 12. | A26 | | | + | | | | | + | |
| 13. | A27 | | | | + | | + | | + | + |
| 14. | A34 | | | | | | | + | + | + |
| 15. | A35 | | | | + | | + | + | + | + |
| 16. | A40 | | + | + | | + | | + | + | |
| 17. | A42 | + | + | + | + | | | + | + | + |
| 18. | A43 | + | | | | | | + | + | |
| 19. | A45 | + | | | | | + | | + | + |
| 20. | A48 | + | + | + | + | | | | | |
| 21. | A51 | + | + | + | | | + | | + | + |
| 22. | A52 | + | + | + | | | | | + | |

# Discussion

# V. DISCUSSION

Soil is a treasure of microorganisms with great diversity in the quality and quantity from one soil to another. These microorganisms play quite a different role to bring the soil equilibrium with an intention to make it fit for plant growth through supply of nutrients. Among the different groups of microorganisms *viz.*, bacteria, fungi, actinomycetes, algae, protozoa including viruses, actinomycetes play an important role in the soil biological activities right from their recognization as nitrogen fixer (*Frankia*) to mineralization; from producing plant growth regulating substance to antibiotic production. They have a dynamic role in decomposition of organic matter and humus formation. The soil actinomycetes are the less studied species than bacteria and fungi in agricultural microbiology. Their role as antibiotic producers (*Streptomyces* spp.) has a great industrial as well as pharmaceutical applications. Except for the detailed studies conducted by Rangaswami and his group from University of Agricultural Sciences, Bangalore during early part of 60's on the antagonistic activities of soil actinomycetes in South India (Rangaswami *et al.*, 1967), no work has been done in greater detail. In the present research work, an attempt was made to isolate actinomycetes and test their potentialities with respect of antibiotic production, antagonism, growth promotion, soil organic matter decomposition, phosphate solubilization and enzyme activities.

## 5.1 ISOLATION AND CHARACTERIZATION OF ACTINOMYCETES

It is seen that starch casein agar gave maximum actinomycete count followed by arginine-glycerol salts and Gause No. 1. Thus it

indicates that starch casein as the best medium for enumeration of actinomycetes from soils. This result is supported by Kuster and Williams (1964) who had reported starch casein as the best medium for enumeration of actinomycetes. Black soils possessed maximum actinomycetes population followed by red soils. This result is agreement with the result given by (Deshmukh and Godbole, 1999).

In all, large number of isolations were made and only 52 isolates were maintained in pure culture. Based on the morphological, biochemical characters (Bergys manual), most of the soil actinomycetes (76.9%) belonged to *Streptomyces* spp. and very few (17.3%) and (7.6%) belonged to *Nocardia* and *Micromonospora*. Table 2 indicated that *Streptomyces* produced both the substrate and aerial mycelium with different forms of conidiophores and arrangement of conidia. The variations on the morphological features indicated that the *Streptomyces* belonged to various groups which of course could not be identified to the species level. Dominance of *Streptomyces* among the actinomycetes as reported by Alexander (1961) are also supported by Abha *et al.* (1991) and Gesheva and Gesheva (2000).

## 5.2 TEMPERATURE OPTIMA FOR ACTINOMYCETES

The predominance of *Streptomyces* among the other species of the actinomycetes also indicated that, they have diverse adaptability with various ecological situations of soil temperature, moisture, pH and other soil chemical characters. Similar observations have been reported (Waksman and Curtis, 1916; Watson and Williams, 1974 and Rangaswami *et al.*, 1967) and is also supported by the recent

observations of Kurtboke *et al.* (1992), Long and Amphlett (1996) and Lee and Hwang (2002). Both *Nocardia* and *Micromonospora* which contributed only to 17.3 per cent and 7.6 per cent respectively which have less competitive ability and limited nutrient adaptability (Kurtboke *et al.*, 1992 and Long and Amphlett, 1996). Although detailed soil physical and chemical characters could not be made it can be observed that the soil pH, organic matter content and the mineral status influenced their qualitative variability in the soil (Deshmukh and Godbole, 2000). *Streptomyces* which have high antagonistic activity also has an edge of competitive ability to survive by suppressing the other microflora (Raatikainen *et al.*, 1993).

All the isolates were verified for their ability to grow at different temperature conditions from 25 to 55°C. While all the isolates could grow at mesophilic temperatures of 25 and 35°C, their growth retarded with increasing temperature where only eight isolates could grow at 55°C. Transitional soils of Dharwad conditions, where the soil temperature always remains between 25 and 37°C and which rarely cross 40°C, the conditions favoured the mesophilic survival. Table 3 indicates that all the species could tolerate the temperature between 25 to 35°C and only a few could thrive the temperature of 55°C also indicated that they are mesophilic organisms but facultatively thermophilic. Manure piles which during decomposition often cross 55°C and probably such of these actinomycetes which could survive the elevated temperature of 55°C possibly dominate in such ecological conditions. According to Alexander (1961), thermophilic actinomycetes are also common in soil, compost heaps which establish in such warmer situation are also not uncommon. More numerous strains of thermophilic *Micromonospora* classified

generally as thermoactinomycetes often dominate in the manure pits of decaying organic matter. This result was supported by Goodfellow and Williams (1983) and Deshmukh and Godbole (2000) who reported that most of the actinomycetes are mesophilic which can grow at temperature between 25 to 28°C and some of them were thermophilic in nature which could grow at 45°C.

## 5.3 BIOCONTROL POTENTIAL OF ACTINOMYCETES

Actinomycetes have been described as the greatest source of antibiotics and are widely distributed in nature (Raatikainen *et al.*, 1993; Furumai *et al.*, 1993; Nair *et al.*, 1994; Bhat and Narayanan, 1996; Hwang *et al.*, 1996; Kim *et al.*, 1998; Ouhdouch *et al.*, 1996 and Lee and Hwang, 2002). They are found in different habitats such as soil, manure, compost and plant residues (Kulik 1996; Agarwal *et al.*, 1997; Kuster and Williams, 1964). Actinomycetes have established themselves as the most potent group of microorganisms with regard to their capacity of forming antimicrobial substances (Rothrock and Gottlieb, 1984; Roy *et al.*, 1989; Pereira, 1999; Feranco and Valencia, 2001; Lee *et al.*, 2002). More than 6,000 antibiotics have been isolated from microbial source. Our needs for antibiotics is not yet fully met (Jadhav and Deshmukh, 2002). The actinomycetes producing several antibiotics which are used in medicine, animal production, crop protection *etc.* have been reported (Rangaswami *et al.*, 1967). Based on this, 52 actinomycetes isolates were assayed for their *in vitro* antipathogenic activity against the plant pathogenic fungal species like *Rhizocotonia bataticola, Fusarium solani, Pythium ultimum* and *Sclerotium rolfsii.* Among the tested isolates (Table 4 and Plate 1) 15 were inhibitory to *Rhizocotonia bataticola.* Most of *Streptomyces* were effective

in inhibiting various plant pathogenic fungi. The inhibition of *Rhizocotonia bataticola* on PDA agar by *Streptomyces* sp. was reported Paramjit and Mehrota (1980), Walter and Crawford (1995). As many as ten actinomycetes inhibited (Table 4 and Plate 2) *Fusarium solani.* The *in vitro* suppression of *Fusarium oxysporum* f.sp. *Ciceri* causing vesicular wilt of cicer was reported by Dhedi *et al.* (1990) and Rath (1992). Majority of *Streptomyces* were found to suppress the various fungal plant pathogens by the production of antimicrobial metabolites. The suppression of *Fusarium oxysporum* f.sp. *lycopersii* (Abyad, 1996) and *Fusarium oxysporum* f.sp. *phaseoli* (Hamed, 1996) by *Streptomyces* species by way of production antibiotics. The 11 actinomycetes (Table 4 and Plate 3) showed inhibition of *Pythium ultimum.* Similar observations were made by Oudouch *et al.* (1996), Kim *et al.* (1997), El-Tarabily (1997) and Yashimoto (1997). Biocontrol potential against multiple pathogens was observed in many cases. While none of the isolates of actinomycetes were inhibiting *Sclerotium rolfsii.* This may be due to the fact that pathogen was more ineffective to actinomycetes. The pathogen being capable forming resistant structures like *Sclerotium* is immune to the effect of antibiosis (Pushpavati and Chandrasekhar, 1998; Tiwari, 1996). According to Garret (1965) the soil borne pathogen have the superior competitive survival ability because of which they survive in soil among the myriads of soil organisms. In contrary to the present observations, the actinomycetes are also reported to inhibit *Sclerotium rolfsii* (Reddi and Rao, 1972). Most of the antibiotics formed by actinomycetes possess a wide antimicrobial spectrum and suppress the growth and development of various microbial species. There are a few antibiotics with a small range of action. Such antibiotics are found very rare; their action is restricted in the suppression of one, two or

three species of microbes. It is known that some of the antibiotics suppress only some fungi, while others do not (Krassilnikov, 1981).

The analysis of the results of the tests for biocontrol mechanism points out to the fact that the important mechanism involved in the biological control by actinomycetes is by producing antimicrobial metabolities. Over the past 20 years, about 1,000 antibiotic substance have been described which have been produced by actinomycetes, proactinomycetes, Micromonosporas and other members of this group of microbes. Antibiotics have a specific, well expressed specificity of selective antimicrobial action (Krassilnikov, 1981). Of the antibiotics reported in the past few years, a very few have been antifungal agents inhibit one or the other fungi (Gottlieb *et al.*, 1952). The antimicrobial metabolites of the eluted portions have been tested against all the fungal pathogens *in vitro* by dipping filter paper with this antimicrobial metabolites and placed over this fungal pathogens. These results were supported by Franco and Valencia (2001), Kulik (1996), Kim *et al.* (1998), Hwang (1996). Apart from production of antibiotics the actinomycetes inhibit the fungal pathogens by producing chitinase enzyme. The chitinase enzyme produced by actinomycetes degraded the cell wall of fungi which is made up of chitin. Thus the actinomycetes kills the fungal pathogens (Gomes *et al.*, 1999; Okazaki *et al.*, 1995; Taechowisan *et al.*, 2003). However, in the present study none of the actinomycete isolates produced chitinase enzyme. This indicates that mechanism of biocontrol potential of actinomycete against fungal pathogens was probably due to production of antibiotics.

## 5.4 DECOMPOSITION OF ORGANIC RESIDUE BY ACTINOMYCETES

For many years it has been known that certain fungi are capable of decomposing lignocellulose. The white rot fungi have been of particular interest because of their ability to completely oxidize both the cellulose and lignin components of wood to $CO_2$ and water (Kirk *et al.*, 1976). The degradation of various lignolytic substance like jute sticks, wood, sugarcane *etc.* by *Phanerochrate chrysosporum* and reduction in C:N ratio of organic residues over an incubation period was reported by Bhattacharyya *et al.* (1987), Suvarna *et al.* (1992) and Jagadeesh and Geeta (1994). Actinomycetes are thought to be of most significance in the degradation of relatively complex recalcitrant polymers occurring naturally in plant litter and soil (Lechevalier and Lechevalier, 1967; Lacey, 1973). Lignocelluloses are the major components of recalcitrant plant residues and the studies by Crawford and Sutherland (1979) convincingly demonstrated that actinomycetes play a significant role in the degradtaion (Goodfellow and Williams, 1983). The sugarcane trash which is one of the most waste residue which is largely burnt in most of the sugarcane areas is a big concern. Being a residue with an ability to decompose *al-biet* slowly is a challenge to a microbiologist to identify a good inoculants for decomposition. The research in these areas identified *Phanerochrate chrysosporium, Plurotus sarojacaju, Tricoderma viridae etc.* to be good Basidomycetus fungi with an ability to decompose the sugarcane trash. Actinomycetes isolated from the soils of Dharwad region with an ability to decolorize the methylene blue, a test to screen the microorganism for lignin decomposition (Crawford *et al.*, 1983) were tested. The results clearly indicated that only *Streptomyces* spp. A13 and A16 were nearer to

the ability to that of *Phanerochrate chrysosporium* rest of the actinomycetes were marginal in their ability to decompose. The results are supported by the observations of Antai (1985) who reported the decomposition of organic residue by using actinomycetes. Further his observations also supported that the standard strain *Phanerochrate chrysosporium* was superior to *Streptomyces viridosporus*. Further from the Table 11 where the sugarcane trash was preadjustedwith the area to narrow down to C:N ratio to soil hastened the process of decomposition. The percentage or the rapidity of the decline of C:N ratio was superior in the treatment with lower C:N ratio. This is understandable as the lignolytic microorganisms require additional available nitrogen source to decompose the carbon in the organic residues it is therefore clear that the decomposition was fast in the treatments with 80:1 C:N ratio than 120:1 (Bardar and Crawford, 1981).

## 5.5 PHOSPHORUS SOLUBILIZATION BY ACTINOMYCETES

Apart from the biocontrol potential and organic matter decomposition phosphorous solubilization by actinomycetes was also determined. Actinomycetes posses other functional properties like phosphorus solubilization, production of plant growth promoting substances and enzyme activities. Among 52 isolates of actinomycetes only nine were able to solubilize tricalcium phosphate on Pikovaskay's medium. The diameter of the zone of solubilization indicated wide variations among the isolates of actinomycetes to solubilize TCP. The actinomycete isolates were analyzed for the quantity of P solubilized and variation in pH of the broth of at 10 and 20 days of incubation. Invariably, all the 9 isolates showed P solubilization. But, the isolates differed

significantly with respect to the amount of P solubilized. Five isolates recorded more than 10 per cent solubilization and four isolates of actinomycete recorded between eight to 10 per cent of solubilization in 20 days of incubation. It was recorded that the P solubilization was increased at 20 days. It was also recorded that as P release increased with incubation time the pH in medium declined. This result was supported by Rao and Venkateshwaralu (1982) who reported solubilization of TCP by *Streptomyces*. The P release increased with increasing incubation time and with decrease in pH in Pikovaskay's broth. From the Table 12 it was observed that strain A27 and A35 which solubilize the phosphorus and solubilization of phosphorus increased with proportionate decrease in pH. These two actinomycetes strain are thermotolerant in nature. This result was supported by Rao and Venkateshwaralu (1982) reported the solubiliztion of tricalcium phosphate in liquid medium with increase in temperature when the pH dropped by the thermotolerant actinomycetes. Such thermotolerant actinomycetes could further be exploited in inoculating the compost pits to enhance the rate of phosphorus solubilization.

## 5.6 PLANT GROWTH PROMOTING SUBSTANCES

Actinomycetes offer a strong biological system with their ability to promote plant growth directly through production of plant growth promoting harmones and indirectly through control of plant pathogens and deleterious organisms or both. Not much work has been done in this aspect of actinomycetes. Keeping this point in view all the actinomycete isolates were tested for the production of plant growth promoting substances. It was observed that only eight isolates produced both IAA

and GA in significantly varying quantities ranging from 1.46 μg/25 ml to 14.0 μg/25 ml IAA and 1.40 to 6.6 μg/25 ml of GA. The variation in the production of IAA and GA among the isolates could be due to more than one reason. The production of IAA and GA by actinomycetes was reported (Katznelson and Shirley, 1965; Hawa *et al.*, 1993; and Strzelezyk and Pokojska, 1984).

## 5.7 ENZYME ACTIVITY SHOWED BY ACTINOMYCETES

It is reported that large varieties of enzyme activities manifested by actinomycetes (Krassinikov 1981). But much work has not been done on this aspect. Keeping this in mind, 52 actinomycetes isolates were tested for phosphatase and urease activity. Among 52 isolates of actinomycetes only 21 were able to showed urease activity. The large variations among isolates of actinomycetes was noted. The urease activity among isolates ranged from 1.15 μg/25 ml to 6.91 μg/25 ml. Among 52 isolates of actinomycetes only 12 were able to produce phosphatase, activity which ranged from 4.66 to 12.20 μg/25 ml. The urease and phosphatase activity in actinomycetes have been reported by Lim and Cha (2000) and Doumbou *et al.* (2001). None of the actinomycetes isolates showed chitinase activity.

### Conclusion

From the current investigation it may be seen that *Streptomyces* sp. predominated over the other sps of actionmycetes. Among the actinomycetes studied none of them were antagonistic to *Sclerotium rolfsii.* But the other pathogens *viz.*, *Rhizoctonia bataticola, Fusarium solani* and *Pythium oltimum* were sensitive to few of the actinomycetes. The study also indicated that these actinomycetes produced certain antibiotic

substances which may have prevented the growth of these pathogens. The decomposition of organic matter could be hastened by inoculating the actinomycetes. But none of the actinomycetes studied were superior to classical *Phanerochrate chrysosporium*. However, *Streptomyces* sp. no. A16 was found to be good among the microorganisms tested. *Streptomyces* A42 in general was capable of possessing most of the characters like biocontrol potential, phosphorus solubilization, production of IAA and GA and the enzyme. However, it was poor decomposer. The study thus demands that there is need for detailed search to isolate most efficient actinomycetes with multiple functions from soil compost, dump sites *etc.*

# Summary

# VI. SUMMARY

An attempt was made to study soil actinomycetes from the soils of transitional belt of Dharwad for some beneficial traits such as biocontrol potential, organic matter decomposition, phosphorus solubilization, production of plant growth promoting substances and enzyme activities. In all 52 isolates of actinomycetes were isolated and maintained in pure culture. The results obtained from investigation are summarized as below.

Morphological and biochemical properties of the 52 isolates of actionmycetes revealed that, they belonged to different groups of actinomycetes. Among them, *Streptomyces* predominated with (76.97%) of frequency. Other two species were identified as *Nocardia* and *Micromonospora* which contributed to 17.3 and 7.6 per cent, respectively. Among the three different media tested, starch casein agar was found to be efficient in detecting the actinomycetes among the mixed population from the soil. All the isolates could luxuriently grow at 25 and 35°C. However, at 45°C only 46 isolates could grow. Further at elevated temperature of 55°C only eight cultures could show growth.

All the isolates of actinomycetes, subjected to cross streak assay for the biocontrol potential, revealed that, 15 actinomycetes were inhibitory on *Rhizoctonia bataticola*. While 10 actinomycetes inhibited *Fusarium solani* and 11 inhibited *Pythium ultimum*. None of the actinomycetes could inhibit the *Sclerotium rolfsii*. Among the actinomycetes controlling the pathogens six isolates *viz.*, $A_3$, $A_9$, $A_{42}$, $A_{48}$, $A_{51}$ and $A_{52}$ inhibited all the

three fungal pathogens and five actinomycetes inhibited only two pathogens.

Further, it was observed that, all the actinomycetes varied in their degree potential of antagonism. The analysis of mechanism of biocontrol of the actinomycetes revealed that the antibiotic substances produced by the actinomycetes inhibited the fungal pathogens. Further it was observed that the actinomycetes did not produce chitinase.

Only six actinomycetes out of the 52 isolates could indicate the preliminary observation of lignin degradation on Crawfords medium with methylene blue indicator. The C:N ratio reduction as a measure of decomposition of sugarcane trash indicated that on inoculation of different actinomycetes showed decline in the values. However a inoculation of standard fungal inoculant namely *Phanerochrate chrysosprium* was superior over all the actinomycetes. Further the study also revealed that among the actinomycetes studied A13 and A16 were potential in degradation of C:N ratio. The adjustment of C:N ratio with inorganic nitrogen namely urea before subjecting to degradation did not have significant effect on the rate of degradation.

Nine isolates of actinomycetes were able to solubilize the tricalcium phosphate from the Pikovaskay's medium. The Pi release by the isolates ranged from 13.23 to 8.23 per cent. The pH of the medium also decreased with increase in Pi solubilization. Quantitative estimation of IAA and GA

was observed in the eight isolates the quantity of IAA produced ranged from 4.0 to 17.3 mg/25 ml and GA from 1.40 and 6.60 mg/25 ml. The urease and the phosphatase enzyme were produced by 21 isolates and 12 isolates of actinomycetes respectively. The urease enzyme activity ranged from 1.15 mg/25 ml to 6.91 μg/25 ml broth. The phosphatase enzyme activity exhibited by 12 isolates which ranged from 4.66 to 12.20 μg/25 ml of broth.

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# *Appendix*

# APPENDIX I

## Composition of media used

### 1. Arginine glycerol salts (El-Nakeeb and Hechevateir, 1963)

| | |
|---|---|
| Arginine hydrochloride | 1.0 |
| Glycerol | 12.5 |
| $K_2HPO_4$ | 1.0 |
| Nacl | 1.0 |
| $MgSo_4$ 7.$H_2O$ | 0.5 |
| $Fe(So_4)6H_2O$ | 0.010 |
| $CuSo_4$ $5H_2O$ | 1.0 mg |
| $MnSo_4$ $H_2O$ | 1.0 mg |
| $ZnSo_4$ $7H_2O$ | 1.0 mg |
| Agar | 15 g |
| P17 | 67 to 7.1 |

### 2. Crawford's medium (per cent) (Crawford *et al.*, 1983)

| | |
|---|---|
| Glucose | 1.0 |
| Yeast extract | 0.15 |
| $Na_2HPo_4$ | 0.4 |
| $K_2HPo_4$ | 0.1 |
| $MgSO_4$ | 0.02 |
| NaCl | 0.02 |
| $CaCl_2$ | 0.005 |
| Agar | 2.0 |
| pH | 7.0 |

### 3. Czapek's solution (Mahedevan and Sridhar, 1984)

| | |
|---|---|
| $NaNo_3$ | 2 g |
| $K_2HPo_4$ | 1 g |
| $MgSo_47H_2O$ | 0.5 |
| Kcl | 0.5 |
| $FeSo_4.H_2O$ | 0.01 |
| Sucrose | 3.0 |
| D.W | 1000 |

### 4. Gause No.1 mineral medium (grams/liter) (Gause *et al.*, 1957)

| | |
|---|---|
| Soluble starch | 2.0 |
| $KNo_2$ | 1.0 |
| $K_2HP_4$ | 0.5 |
| NaCl | 0.5 |
| $FeSo_4$ | 10 mg |
| Agar | 20 |
| pH | 7.3 |

### 5. Starch casein agar (Kuster and Williams, 1964)

| | |
|---|---|
| Soluble starch | 10.0 |
| $KNO_3$ | 2.0 |
| Casein (vitamin free) | 0.3 |
| $K_2HPO_4$ | 2.0 |
| $MgSO_47H_2O$ | 0.05 |
| NaCl | 2.0 |
| $FeSO_4\ 7H_2O$ | 0.01 |
| $CaCo_3$ | 0.02 |
| Agar | 1.8 |
| pH | 7 to 7.2 |

**6. Pikovskaya's medium (Pikovskaya, 1948)**

| | |
|---|---|
| Glucose | 10 g |
| $Ca_3(PO_4)_2$ | 5 g |
| $(NH_4)_2SO_4$ | 0.5 g |
| Yeast extract | 0.5 g |
| $MgSO_4 . 7H_2O$ | 0.2 g |
| NaCl | 0.1 g |
| $FeSO_4 . 7H_2O$ | Traces |
| Distilled water | 1000 ml |
| Agar | 15 g |
| pH | 6.8 to 7.0 |

**7. Luria agar medium (Sambrook *et al.*, 1989)**

| | |
|---|---|
| Tryptone | 10.00 g |
| Yeast extract | 5.0 g |
| Sodium chloride | 5. g |
| Agar | 18.00 g |
| Distilled water | 1000 ml |
| pH | 7.2 |

**STUDIES ON BIOCONTROL POTENTIAL AND OTHER BENEFICIAL TRAITS OF SOIL ACTINOMYCETES**

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**ABSTRACT**

From the transitional zone of Dharwad, as many as 52 isolates of actinomycetes were isolated and purified. Their morphological and biochemical properties revealed that they belonged to different genera. Among them, *Streptomyces* was found to be predominant, followed by *Nocardia* and *Micromonospora.* All the isolates could luxuriantly grow at 25° and 35°C, and 46 isolates could grow at 35°C. Only eight cultures could show grow at an elevated temperature of 45°C. In the *in vitro* dual culture assay, out of 52 isolates, 15 were found to be inhibitory to *Rhizoctonia bataticola,* 10 actinomycetes to *Fusarium solani* and 11 to *Pythium ultimum.* None of the actinomycetes could inhibit *Sclerotium rolfsii.* Among actinomycetes studied with biocontrol potential of the pathogens, six isolates inhibited all the three fungal pathogens and five actinomycetes inhibited only two pathogens. When assessed for mechanism of biocontrol, all the potent antagonists produced antifungal antibiotics. None of the actinomycetes tested produced chitinase. Only six actinomycetes, out of the 5 isolates, could indicate the preliminary observation of lignin degradation on Crawfords medium with methylene blue indicator. The C:N ratio reduction, as a measure of decomposition of sugarcane trash, brought about by these isolates indicated that they have a role in rapid decomposing of lignocelluloses. Nine isolates of actionmycetes were able to solubilize tricalcium phosphate in the Pikovskaya's medium. The Pi released by the isolates ranged from 8.23 to 13.23 per cent. Quantitatively, only eight isolates were found to produce IAA and GA. IAA ranged from 4.20 to 17.3 µg/25 ml and GA from 1.40 to 6.60 µg/25 ml. The urease and the phosphtase enzymes were produced only by limited isolates. The urease activity ranged from 1.45 µg/25 ml to 7.15 µg/25 ml broth and phosphatase activity ranged from 4.66 to 12.20 µg/25 ml broth.