

“Variability Mapping of Actinomycetes,
Sulphur oxidiser and Plant Growth Promoting
Rhizobacteria from Soils of Maharashtra”

A THESIS

SUBMITTED TO

MAHATMA PHULE KRISHI VIDYAPEETH

RAHURI – 413 722, DIST – AHMEDNAGAR

MAHARASHTRA, INDIA

**In partial fulfillment of the requirements for
the degree of**

DOCTOR OF PHILOSOPHY

in

AGRICULTURAL MICROBIOLOGY

by

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(Reg. No. 08/056)

**DEPARTMENT OF PLANT PATHOLOGY
AND AGRICULTURAL MICROBIOLOGY,
POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,
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2011

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has not been submitted
to any other university
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LIST OF ABBREVIATIONS

@	:	At the rate of
°C	:	Degree Celsius
A	:	Actinomycetes
BOD	:	Biological oxygen demand
bp	:	Base pair
CP	:	Commercial <i>Pseudomonas</i>
C.D.	:	Critical difference
CFU	:	Colony forming unit
Cm	:	Centimeter (s)
cm ²	:	Square centimeter
CRD	:	Completely randomized design
CV	:	Coefficient of variation
DNA	:	Deoxyribonucleic acid
dNTP	:	Deoxynucleotide triphosphate
EDTA	:	Ethylene diamine tetra acetic acid
<i>et al.</i>	:	Etalia (and other)
etc.	:	Et cetera (and so forth)
fig	:	Figures
GPS	:	Global positioning system
g	:	Gram
hrs.	:	Hours (s)
IAR	:	Intrinsic antibiotic resistance
i.e.	:	That is
<i>in vitro</i>	:	In Lab.
kb	:	Kilobase pair
LB broth	:	Luria bertani broth
mg.	:	Milligram
ml ⁻¹	:	Per milliliter
ml	:	Milli litre
mM	:	Millimolar
mm	:	Millimeter
µl	:	Micro liter

ng	:	Nanogram
µm	:	Micrometer
N lat.	:	North latitude
No.	:	Number (s)
PCR	:	Polymerase chain reaction
PDA	:	Potato dextrose agar
PDB	:	Potato dextrose broth
2D PCO	:	Two dimensional principal coordinate scatter plot analysis
3D PCO	:	Three dimensional principal coordinate scatter plot analysis
pH	:	Potential of hydrogen ion concentration
pp	:	Page (s)
Pa	:	<i>Pseudomonas aeruginosa</i>
Pf	:	<i>Pseudomonas fluorescens</i>
rpm	:	Revolution per minute
S	:	<i>Streptomyces</i>
S.E. ±	:	Standard error
spp.	:	Species
SOB	:	Sulphur oxidizing bacteria
UV	:	Ultra violet
viz.,	:	Videlicet (namely)
/	:	Per
%	:	Per cent
RAPD	:	Random amplified polymorphic DNA
RBA	:	Random bacterial primer

ABSTRACT

**“Variability mapping of actinomycetes, sulphur oxidiser
and plant growth promoting rhizobacteria from soils of
Maharashtra”**

by

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A candidate for the degree

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The studies on “Variability mapping of actinomycetes, sulphur oxidisers and plant growth promoting rhizobacteria from soils of Maharashtra” was conducted during 2009-2011 at Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri. In these studies the *Streptomyces* sp. (72), *Acidithiobacillus* sp. (8) and *Pseudomonas fluorescens* (34) were isolated from soils of the Maharashtra. These organisms were identified on the basis of morphological, cultural, biochemical characterization, functional

diversity and selected efficient strains were studied for their molecular diversity by using RAPD analysis.

There were 72 isolates have creamy colour, out of which 66 isolates having rectiflexibles (RF) in spore chain morphology and few of the isolates (6) showed retinaculiaperti (RA) in spore chain morphology. The *Streptomyces* sp. was found excellent on oat meal agar, good on starch casein agar and poor to moderate on nutrient agar medium.

The RHR-S-98 showed maximum percent inhibition 30.00% against *Alternaria alternata* while the RHR-S-23 showed maximum percent inhibition 28.80% against *Fusarium oxysporum* pv. *ciceri* *in vitro*.

The cellulose degradation ability of 72 *Streptomyces* sp. isolates was studied and observed that 56.94% have good cellulose degradation activity and 40.28% have moderate cellulose degradation activity.

Among the 72 *Streptomyces* sp., only 22.22% isolates showed the P solubilization ability in which less than 1cm P solubilization zone was observed and 77.78% isolates does not showed P solubilization activity.

The PCR amplification products of the 18 strains of *Streptomyces* sp. with respect to each of the 23 random primers were separated by agarose gel electrophoresis. A total of 1124 bands were produced by the 23 primers. The number of bands produced per primer varied from 36 to 72. The average number of bands per primer was 48.87. Polymorphic bands 88.52% and unique bands were observed 11.48% and no monomorphic band observed in all 23 primers. Most of the primers produced highly polymorphic bands varied from 2 to 24. The primers RBA 10 produced maximum polymorphic bands (55). The per cent polymorphism ranged from 73.33 to 97.43% and average polymorphism was observed 87.72%.

The genetic similarity matrix based on the pooled data of RAPD profiles from all the 23 primers revealed that highest genetic similarity

index of 0.65 between the isolates RHR S-40 and RHR S-41 and RHR S 41 and RHR S 48. The genetic similarity index was ranged from 0.13 to 0.65 among the all isolates. On the basis of RAPD analysis, the dendrogram constructed from the pooled data *Streptomyces* sp. had major two clusters. The first cluster consisted of five strains of *Streptomyces* (S-28, S-138, S-116 S-23 and S-21) and the second cluster consisted of 13 strains of *Streptomyces*.

Out of 150 locations, *Streptomyces* sp. were found in 48% of soils of Maharashtra. It was distributed in 72 location of Maharashtra soils. *Streptomyces* were prominently distributed maximum in Vidarbha (27.78%) followed by Western Maharashtra (19.44%), Western Konkan coast (15.28%), Marathwada region (13.89%) , Western Ghat (13.89%) and least was found in soils of North Maharashtra region (9.72%).

Sulphur oxidizer : All the *Acidithiobacillus* sp. have small yellow colour colonies grown in thiosulphate agar slants. They were found in short rods and size ranged from 0.3-0.7 x 0.9-1.6 μm . All the isolates were gram -ve in reaction. The pH was reduced in all the *Acidithiobacillus* sp. from 8.0 to ≤ 5.0 .

The PCR amplification products of the 9 isolates of *Acidithiobacillus* sp. with respect to each of the 15 random primers were separated by gel electrophoresis. A total of 484 bands were produced by the 15 random primers. The number of bands produced per primer varied from 18 to 48. On an average 32.2 bands were produced per primer. Polymorphic bands 58.68 % and unique bands were observed 41.32% and no monomorphic band observed in all 15 primers. The amplified DNA fragment ranged from 289 to 6021 bp. The number of polymorphic loci amplified varied with different primers and isolates.

The genetic similarity matrix based on the pooled data of RAPD profiles from all the 15 primers revealed that highest genetic similarity index of 0.65 between the isolates SOB-113 and SOB-115. The genetic

similarity index ranged from 0.13 to 0.65 among the all isolates. From the genetic similarity coefficient, it is observed that there was very high genetic diversity was observed in the SOB isolated from soils of Maharashtra on the basis of geographical region.

Acidithiobacillus sp. was occurred in 8 locations which contributes 5.33% of soils of Maharashtra. *Acidithiobacillus* sp. was distributed in Western Maharashtra (37.5%) and Vidarbha (37.5%) whereas less observed in Marathwada (12.5%) and Western Ghat (12.5%). The highly efficient *Acidithiobacillus* sp. was observed in Kolhapur, Solapur and Nagpur districts from Western Ghat, Western Maharashtra and Vidarbha regions, respectively.

Pseudomonas fluorescens : Out of the 65 isolates, 21 produced green colored colonies and 44 produced light green coloured colonies. Forty one isolates observed round and non-spreading type colonies whereas the remaining 24 isolates produced irregular shaped spreading type colonies.

All the 65 isolates were rod shaped bacteria and gram -ve in reaction. Among these fluorescent pseudomonads 65 isolates grouped on the basis of habitat, 40 isolates were rod shape living in clustering, 8 isolates were rod shape in isolate and 17 isolates were rod shape in pair and chain habitat. The size of these bacterial isolates are categorized into three on the basis of size. The results indicated that 29, 31 and 5 isolates were measured upto 1.0 μm , 1.01 to 2.00 μm and more than 2.01 μm length, respectively. The colonies showed wide variation in the intensity of fluorescence.

Based on the morphological and biochemical characteristics, 34 isolates were identified as *Pseudomonas fluorescens*, 31 as *Pseudomonas aeruginosa* from soils of Maharashtra. Out of 18 isolates from soils of Western Maharashtra, 8 were identified as *Pseudomonas fluorescens* and 10 as *Pseudomonas aeruginosa*. Out of 13 isolates from

Western Ghat, 10 isolates belonged to *Pseudomonas fluorescens* and three to *Pseudomonas aeruginosa*. Six out of 13 isolates from Vidarbha were identified as *P. fluorescens* and seven as *P. aeruginosa*. Among the 9 isolates of Marathwada, three isolates belonged to *Pseudomonas fluorescens* and six to *Pseudomonas aeruginosa*. Among the 4 isolates of Western Konkan coast region, two isolates belonged to *Pseudomonas fluorescens* and two *Pseudomonas aeruginosa*. In North Maharashtra, out of 8 isolates, 5 isolates were identified as *Pseudomonas fluorescens* and three belonged to *Pseudomonas aeruginosa*.

Among 34 *Pseudomonas fluorescens*, P-51 isolate was found more growth inhibition per cent (47.50%) followed by P-31 (45.62%), P-56 (45.62%) and P-121 (45.00%). Seven strains of *Pseudomonas fluorescens* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 were found more growth inhibition per cent than the commercial *Pseudomonas* against the *Macrophomina phaseolina*.

The *Pseudomonas fluorescens* isolates viz., P-121 (45.86%), P-51 (41.49%), P-134 (39.37%), P-136 (37.24%), P-31 (35.11%) and P-56 (34.04%) were found significantly superior in inhibition growth per cent over the commercial *Pseudomonas* (24.31%) against the *Fusarium oxysporium*.

The efficient *Pseudomonas fluorescens* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 isolated from soils from the locations Sawantwadi, Malegaon, Ahmedpur, Sevagram, Ramtek, and Kuhl, respectively were found superior to in inhibition growth percent of *Macrophomina phaseolina* and *Fusarium oxysporium*.

The PCR amplification products of the 14 isolates of *Pseudomonas fluorescens* with respect to each of 24 random bacterial primers were separated by gel electrophoresis. A total of 801 bands were produced by the 24 primers. The number of bands produced per primer varied from 12 to 50 and average was 33.33 bands per primer. Polymorphic bands

were observed 73.53 % and unique bands were observed 26.34% and only one monomorphic band observed in RBA-1 primer. Most of the primers produced highly polymorphic bands varied from 1 to 27. The amplified DNA fragment ranged from 199 to 3848 bp. The number of polymorphic loci amplified varied with different primers and isolates.

The genetic similarity matrix based on the pooled data of RAPD profiles from all the 24 primers revealed that highest genetic similarity index of 0.68 between the isolates P-40 and P-41 followed by 0.65 for the isolates P-91 and P-119. The genetic similarity index was ranged from 0.17 to 0.68 among the all isolates. From the genetic similarity coefficient, the results indicated that there was very distinct diversity (coefficient 0.0 to 0.35) observed in the *P. fluorescens* isolated from soils of Maharashtra on the basis of functional diversity and ecological regions.

On the basis of RAPD analysis, dendrogram constructed from the pooled data of *Pseudomonas fluorescens* isolates had two major clusters. The first cluster consist five strains of *Pseudomonas fluorscens* (P-31, P-134, CP, P-121 and P-136) and second cluster is sub-cluster into three groups.

Pseudomonas fluorescence strains P-31, P-51, P-56, P-121, P-134, P-136 and P-145 from different soils of Maharashtra were prominently observed from soils of Nagpur followed by Wardha district from Vidarbha followed by Sindudurg, Nasik and Latur from Western Ghat, North Maharashtra and Marathwada respectively. The occurrence of P-124, P-136 and P-145 isolate was observed from Ramtek, and Kuhl from Nagpur ditrict. The P-121 isolate was observed in the soils of Sewagram from Wardha district. The occurrence of *Pseudomonas fluorescence* strains P-31, P-51, P-56 was observed in soils of Sindudurg, Nasik and Latur from Western Ghat, North Maharashtra and Marathwada, respectively.

It was evident from the study that a great diversity exist among the *Pseudomonas fluorescens* in the different soils of Maharashtra with respect to colony morphology, biochemical characters, cultural and beneficial functions and genetic makeup.

Out of 150 soil samples from the Maharashtra, 72 isolates of *Streptomyces* sp., 8 isolates of *Acidithiobacillus* sp. and 34 isolates of *Pseudomonas fluorescens* were found in soil. Out of 72 isoaltes of *Streptomyces* sp., 8 isolates of *Acidithiobacillus* sp. and 34 isolates of *Pseudomonas fluorescens*, 40, 3 and 7 were found highly efficient isolates, respectively. The characteristics of *Streptomyces* sp. for cellulose degradation and antimicrobial activity, *Acidithiobacillus* sp. for reduction in pH and *Pseudomonas fluorescens* for biocontrol activity was considered for their efficiency.

From soils of Maharashtra, it was concluded that efficient *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* were found in Nagpur district of Maharashtra. While efficient isolates of *Streptomyces* sp., and *Pseudomonas fluorescens* were observed in Wardha, Latur, Nashik and Sindudurg districts of Maharashtra. The efficient strains of *Streptomyces* sp. and *Acidithiobacillus* sp. were observed in Kolhapur and Solapur district of Maharashtra

Only *Streptomyces* sp. was observed in Yavatmal, Gadchiroli, Washim, Akola, Nanded, Parbhani, Sangali, Satara, Ratnagiri, Raigad, Thane, Pune, Ahmednagar and Jalgaon districts of Maharashtra. While none of the efficient *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* were observed in Gondia, Chandrapur, Amaravati, Buldhana, Jalna, Beed, Osmanabad, Aurangabad and Nandurbar districts of Maharashtra. Such isolates could be useful in agriculture and industry. There is a need to conserve this treasure for the future.

1. INTRODUCTION

Streptomyces are gram positive, spore-forming bacteria found in soil. They are characterized by their tough, leathery, frequently pigmented colonies and their filamentous growth. They form discrete and lichenoid, leathery or butyrous colonies. Initially colonies are relatively smooth surfaced mycelium that may appear floccose granular, powdery, or velevety. Produce a wide variety of pigments responsible for the of the pigments responsible for the colour of the vegetative and aerial mycelia. Coloured diffusible pigments may also be formed. Many strains produce one or more antibiotics. Aerobes, Gram positive but non acid – alcohol fast. *Streptomyces* are chemoheteroorganotrophs, growing best at 25°C and pH 6.5-8.0. They are widely distributed and abundant in soil including compost. The mol% G+C of the DNA is 69-78. They are capable of using complex organic materials as carbon and energy sources and are involved in the breakdown of these products in the soil. This degradative ability makes these bacteria pivotal in the production of fertile soil for agriculture. They also give soil its characteristic smell by the production of a class of volatile low molecular weight compounds called geosmins. In the laboratory, different isolates were found to produce numerous compounds capable of inhibiting or killing other microorganisms.

Streptomycetes are known to be producers of many secondary metabolites, which have different biological activities, such as antibacterial, antifungal, antiparasitic, antitumor and immuno suppressive actions (Demain, 1999).

Streptomyces sp. 10 isolates obtained from different areas in Tamil Nadu studied for detection of antimicrobial substances it contained. The results revealed that out of 10 isolates of *Streptomyces* sp. three of the isolates were found to be of potential antagonistic against pathogenic organisms and thus providing production of secondary metabolites which has potential to control the soil borne pathogens (Kumari *et al.* 2006)

Streptomyces isolates (JS-1 to JS-20) were having strong antagonistic activity against various bacterial and fungal pathogens, but the activity was differing in all the 20 *Streptomyces*. Phylogenetic analysis revealed that the isolates are the divergent of *Streptomyces* which are associated in the rhizosphere soil of Mangrove (Kannan and Vincent 2011)

In Maharashtra, soils from nine districts viz., Aurangabad, Nanded, Osmanabad, Parabhani, Bhandara, Chandrapur, Kolhapur, Pune and Raigad have been reported to be of wide spread sulphur deficiency ranging from 5 to 20 per cent (Tandon, 1986).

Sulphur also affects the formation of flavour imparting compounds in crucifers and improve marketing quality of the agricultural produce. However, in view of the vital importance of sulphur in various biochemical transformations in plants and the alarming situation of sulphur deficiency in Indian soils, no adequate attention has been paid for its use in Indian agriculture.

Acidithiobacillus is obligately acidiphilic optimum ($\text{pH} < 4.0$) and aerobic, gram negative rods $0.4 \times 2.0 \mu\text{m}$ in size, motile by one or polar flagella, using reduced sulfur compounds to support autotrophic growth. *Thiobacillus* species exhibit almost as much diversity in DNA composition and physiology as is found collectively in all other proteobacterial groups. On the basis of physiological character and 16S rRNA gene sequence comparisons, eight of the existing *Thiobacillus* species are proposed for reassignment to three newly designated genera within gamma (r) sub class of the proteobacteria viz., *Acidithiobacillus*, *Halothiobacillus* and *Thermithiobacillus* (Kelly and Wood 2000).

The reports of the experiments conducted in India (Rupela and Tauro, 1973) showed that micro-organisms could be successfully exploited to reclaim the alkali soils since these soils have been formed to support very sparse population of sulphur oxidizing bacteria.

Due to adoption of intensive agriculture technology, some problems of soil fertility management are coming up in which the sulphur is an

important factor. The deficiency of sulphur nutrient results into poor growth and low yield with inferior quality produce. The role of S-oxidising microorganisms in the process of soil reclamation has been studied and it clearly showed that elemental S coupled with composite culture of bacteria and fungi could significantly decreases the soil pH and reflected in the reclamation of saline alkali soil.

Pseudomonas is a gram-negative rod shaped bacteria belonging to the family Pseudomonaceae. They are predominantly present in the soil and have versatile functional activities. They have the ability to colonize rhizosphere of a wide variety of crops including cereals, pulses, oilseeds and vegetables (Johri *et al.* 1997). The genus *Pseudomonas* includes both fluorescent and non-fluorescent species. The fluorescent species produce water soluble yellow green pigments and fluoresce under low wavelength UV radiation.

Pseudomonas fluorescens inhabit soil, plants, and water surfaces. The optimum growth temperature is between 25-30°C. The Pf-5 strain resides in the plant's rhizosphere and produces a variety of secondary metabolites including antibiotics against soil borne plant pathogens (Paulsen, *et al.* 2005). The G + C content ranges from 58% to 68%. The plant beneficial pseudomonads are heterogenous and they comprise of a collection of gram negative strains that are generally aerobic, non fermenting and motile (Holt *et al.* 1984) *Pseudomonas fluorescens* PFO-1 is well adapted to the soil where it was first isolated from agricultural land. *Pseudomonas fluorescens* strain SBW25 grow on plant leaves and roots where they can contribute to plant growth. Soluble, green fluorescent pigments are produced when the iron concentration is low. The significance of these organisms have increased because of their ability to degrade various pollutants and their use as bio-control against plant pathogens. *Pseudomonas fluorescens* is interesting and important to study because it produces a particular antibiotic (Mupirocin) (<http://www.thelabrat.com/restriction/sources/Pseudomonasfluorescens>).

shtml). The genus first described by Migula in 1984 has undergone repeated taxonomic revision (Palleroni, 1984)

Currently, two strains of *Pseudomonas fluorescens* have their genomes sequenced completely. *Pseudomonas fluorescens* Pf-5 genome contains one circular chromosome that has 7.1 Mbp and a GC content of 63.3%. It contains 87 RNAs and 6137 proteins and 5.7% of its genome contributes to secondary metabolism which is the largest of the pseudomonas (Paulsen, et al. 2005). The genome of *Pseudomonas fluorescens* PfO-1 has one chromosome with 6.4 Mbp and 60.5% GC content. There are 95 RNAs and 5736 proteins. The genome sequencing of *Pseudomonas fluorescens* SBW25 is still in progress.

Pseudomonas fluorescens produces the siderophore pyoverdine which is responsible for chelating iron. This siderophore is responsible for producing fluorescence of *Pseudomonas fluorescens*. This explains why the fluorescent pigment is only produced when iron concentrations are low. When iron concentrations are high, pyoverdine is not needed so colonies will not fluoresce under ultraviolet light. Strain Pf-5 possess many extracellular hydrolytic enzymes that degrade polymers found in soil as well as hydrolases used on plant-derived carbohydrates. They are also capable of degrading and using components of plant tissues such as hydrocarbon molecules, fatty acids and oils. *Pseudomonas fluorescens* are commensal species allowing plants to attain key nutrients, degrading pollutants, and suppressing pathogens via antibiotic productions. These microbes produce secondary metabolites that suppress plant disease and signal gene expression to neighboring cells inhabiting the rhizosphere. *Pseudomonas* also use siderophores from other microorganism to obtain iron which increases their survival in iron-limited environments. Plants provide these organisms with nutrients and shelter against stressful environments (Paulsen, et al. 2005).

Pseudomonas fluorescens are known to produce secondary metabolites like siderophores, antibiotics, HCN which have made them the most promising group of plant growth promoting rhizobacteria

involved in the biocontrol of plant diseases. *Pseudomonas fluorescens* is a antagonist micro-organisms play an important role in microbial equilibrium. *Pseudomonas fluorescens* produces hydrogen cyanide and the siderophores pyocheline and pyoverdine which compete with many pathogenic bacteria for iron availability for growth and suppress pathogens in the rhizosphere. They are ubiquitous soil micro organisms and common in habitats of rhizosphere. It plays an important role in biological control of plant pathogens (Kloepper et al. 1980b). Antibiotic compounds produced by fluorescent *Pseudomonas* strains play a key role in the suppression of various soil borne plant pathogens (Sharifi-Therani and Omati 1999). The rhizobacteria are known to produce siderophores. The strongest factor related to the mode of action of PGPR stimulated with response to the formation of a diffuse iron chelating siderophores (Kloepper and Schroth 1981).

A lot of diversity exists among this group of organisms and it can be studied by various methods like biochemical tests, multilocus enzyme electrophoresis, SDS-PAGE, RFLP and PCR based RAPD patterns (Jimenez-Salgado et al. 1997; Loganathan et al. 1999; Muthukumarswamy et al. 2000).

Genetic variability exists among the various isolates of *Pseudomonas* depending on the geographical locations from where they were collected, crops grown in a specific region and agricultural practices employed in the location (Picard and Bosco, 2008). The genotypic and phenotypic diversity of *Pseudomonas fluorescens* could be based on the geographic origin or functional differences in the genome of the strains (Naik et al. 2008a, Silby et al. 2009)

Biological diversity can be defined as “the variety of species in the ecosystems, as well as the genetic variability within each species”. Diversity is, therefore the range of significantly different kinds of organisms and their relative abundance in natural assemblage and habitat. Diversity can be regarded as the amount and distribution of genetic information in a natural community (Overeas and Torsvik, 1998).

However, the microorganisms that are isolated, characterized, preserved and sometimes exploited by humans constitute only a minute fraction of earth's microbial genetic diversity. Vast majority of microbial species are yet to be recognized and their genetic potential realized.

The microorganisms have successfully colonized every possible ecological niche on this planet and as such, the study of microbial diversity plays a key role in understanding microbial ecology of any ecosystem. Owing to their remarkable metabolic diversity and genetic adaptability, the microorganisms recycle important mineral elements and thereby improve the health and functioning of ecosystems and the whole environment. They play a vital role in maintaining soil fertility, pest and disease control and detoxification of natural and man-made pollutants. The variety of species in an ecosystem and the genetic variability within each species means biodiversity. Much better known and exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture (Johri *et al.* 2005).

Comparison of phenotypic and DNA based analysis of bacterial diversity contributes to a better understanding of microbial populations in soil. With the recent developments in the area of recombinant DNA technology and bioinformatics, it is evident that microbial resources will undoubtedly contribute increasingly to the development and improvement of agriculture, health and environment management.

Little research has been conducted to quantify the beneficial relationships between microbial diversity, soil quality and ecosystem sustainability. The ecosystem functioning is governed largely by soil microbial dynamics.

Keeping in view these facts, it is proposed to study the biodiversity, mapping and assessment of actinomycetes, sulphur oxidizer and plant growth promoting rhizobacteria (PGPR) from different soils of Western Konkan Coast, Western Ghats (Sahyadris) and North Deccan Plateau (Upper, Lower and Metamorphic) of Maharashtra with special interest on

beneficial activities of microorganisms for agriculture with the following objectives:

1. Isolation, enumeration, characterization and invitorization of actinomycetes, sulphur oxidiser and Plant Growth Promoting Rhizobacteria (PGPR) from soils of Maharashtra.
2. Assessing the geographical distribution and developing thematic maps for the above groups of Maharashtra.
3. Assessing the functional potentials of each group of organism for use in Agriculture.
4. Assessing the molecular diversity of key selected species.

2. REVIEW OF LITERATURE

The present investigation was carried out to isolate, characterize and identify *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* from 150 soils of the Maharashtra state and study their morphological, biochemical, cultural, functional properties with respect to beneficial functions for agriculture and also the molecular diversity of some selected isolates with multiple beneficial traits. The literature pertaining to these aspects is reviewed and presented below under the appropriate heads, which will provide an overview of the current status of the research work on the concerned aspects.

2.1. Actinomycetes micro-organisms :

2.1.1 Occurrence of streptomycetes in soil :

Rao and Subramanyan (1929) reported the presence in Indian soils (West Bengal) of 40,000 to 3,40,000 streptomycetes per gram.

Subramanyan and Noris (1929) reported that black Cotton soil had the highest streptomycete population while red sandy loam the least.

Singh (1937) observed a broad relation between numbers of these organisms and soil fertility ; the heavily manured soils gave the highest numbers and the unmanured the lowest.

Rangaswami and Venkatesan (1963) and Venkatesan (1964) reported that the streptomycetes population of Annamalai nagar paddy soil (alluvial) ranged between 1.14 to 5.40 millions per gram.

Rangaswami *et al.* (1967) reported that the percentage of streptomycetes to the total microbial population, varied from 5 to 41 and red soils had the maximum percentage followed by laterite, black and alluvial soils while Tandon (1966) reported that streptomycetes predominated in the lateritic soils of Maharashtra State.

Actinomycetes are most widely distributed groups of microorganisms in nature. They make up in many cases especially dry

alkaline conditions, a large part of the microbial population of soil (Athalye *et al.*, 1981 ; Williams *et al.*, 1983; Lacey, 1997).

Kumari *et al.* (2006) reported that actinomycetes population was found to be more in fertile areas particularly in rhizosphere and less in non rhizosphere region of the soil. Total number of actinomycetes colonies are found to be more in red soil and less in black soil which coincided with the soil pH, % of moisture content and total organic carbon content.

2.1.2 Isolation:

Studies of streptomycetes of soils are usually made with the dilution plate technique. The number of propgules of these organisms in most soils is intermediate between those of bacteria and fungi, so dilutions suitable for colony counting or isolation allow the development of large numbers of bacteria. At such dilutions, numbers of fungal colonies are usually low, but with their large radial spread they can still interfere with the estimation of streptomycetes on the plates. In acid soils, relative numbers of fungal colonies are often high enough to make estimation impossible. Many attempts have been made to overcome these drawbacks including use of selective nutrient media, pre treatment of soil samples or soil suspensions, and incorporation of selective inhibitors into the media.

Waksman (1950) used simple synthetic media such as nitrate-sucrose agar or egg-albumen agar and found that the number of streptomycetes in 25 soils varied from 7,500 per gram for an acid cranberry soil, to 2,400,000 for an Oregon adobe soil; these numbers represented 3.5 and 15.4 per cent respectively of the total microbial populations developing on the plate.

Tsao *et al.* (1960) reported increased development of streptomycetes when air dried soil was re-moistened, mixed with CaCO_3 and incubated at 28°C.

Kuster and Williams (1964) employed various media such as Glucose asparagines agar, Glucose nitrate agar, Glucose casein agar,

Glycerol asparagines agar, Glycerol arginine agar, Starch nitrate agar and Starch casein agar for isolation of streptomycetes but found low counts of streptomycetes in these media; they, therefore, recommended a streptomycetes medium containing starch, casein etc. which allows a large population of streptomycetes to develop.

Rangaswami *et al.* (1967) reported that the percentage of streptomycetes to the total microbial population, varied from 5 to 41 and that red soils have maximum percentage followed by laterite, black and alluvial soils while Tandon (1966) reported that streptomycetes predominated in the lateritic soils of Maharashtra State.

Konde (1974) reported that streptomycetes were found more in black cotton soils than red alluvial, lateritic and coastal saline soils of Maharashtra State.

Ghule (1968) used various media viz., Nutrient agar, Jensen's agar, Glycerol asparagine agar and M₂ agar for the isolation of streptomycetes and stated that the Glycerine asparagines agar was the best.

Rifaat and Kansoh (2005) revealed that different strains of *Streptomyces* spp. isolated from Egyptian soils in different localities at Sharkia Governorate were tested for antimicrobial activity.

You *et al.* (2005) revealed that 94 marine actinomycetes strains were isolated. Out of these isolates 87.5% actinomycetes were *Streptomyces* sp. and 12.5% were *Nocardia* sp. Busaya *et al.* 2005 isolated 32 strains of actinomycetes obtained from soil samples of Thailand.

Several research workers obtained the *Streptomyces* sp. from rhizosphere soils of different geographic regions. Busaya *et al.* (2005) isolated 32 strains of actinomycetes obtained from soil samples of Thailand. Taddei *et al.* (2006) isolated 71 *Streptomyces* sp. from soil samples collected at different places of Venezuela. Saadoun *et al.* (2008) isolated 110 isolates of Streptomycetes from soil samples collected from three regions of Ajlun forests in Northern Jordan.

Lakshmipathy and Kannabrian (2009) isolated actinomycetes colonies from the marine soil samples which were collected at Ennore Saltpan region of Bary of Bengal by using Starch Caesin Agar medium.

Dehnad *et al.* (2010) reported that 150 actinomycetes isolated from 100 soil samples collected from Northwest of Iran by using spreading technique on starch casein agar.

Mythili and Das (2011) isolated the four *Streptomyces* sp. from the rhizosphere soils of Ooty tea plantastion areas in Tamil Nadu.

Kannan and Vincent (2011) isolated 20 isolates of *Streptomyces* sp. from rhizosphere soil samples of Manakkudy mangroves, West Coast of India during 2007.

2.1.3 Morphological and cultural characters:

In Bergey's Manual is reported that vegetative hyphae (0.5 – 2.0 μm in diameter) produce an extensively branched mycelium that rarely fragments. The aerial mycelium at maturity forms chain of three or many spores. A few species bear short chains of spores on the substrate mycelium. Sclerotia, pycnidial-sporangia, and synnemata like structures may be formed by some species. Spores are non motile. Form discrete and lichenoid, leathery or butyrous colonies. Initially colonies are relatively smooth surfaced mycelium that may appear floccose granular, powdery , or velevety. Produce a wide variety of pigments responsible for the of the pigments responsible for the colour of the vegetative and aerial mycelia. Coloured diffusible pigments may also be formed. Many strains produce one or more antibiotics. Aerobes, Gram positive but non acid – alcohol fast. Catalase positive. Temperature grow optimum 25-35°C. Optimum pH range for growth 6.5-8.0. They are widely distributed and abundant in soil including compost (Williams *et al.* 1989).

Seung *et al.* (1998) reported that several phenotype properties, notably some morphological *viz.*, spore chain , spore surface and aerial spore masss colour and pigmentation features can be weighed to distinguish between carboxydotrophic strains AT50, AT51 and AT52 and

representatives of related validity described *Streptomyces* species composed of thermophilic strains. Spore chain morphology was observed rectiflexibles, retinaculiapertii and spirales. Melanin production was not observed in all the isolates except one isolate.

Stefka *et al.* (2004-2005) studied cultural of *Streptomyces* sp. strain 3B was good development. The colour of the aerial mycelium was white. It changed from white to gray-white or pale pink depending on the medium. The colouring of vegetative mycelium varied from pale yellow to yellow brown according to cultural age. The strain did not form melanoid pigment on any investigated for melanin media. The growth temperature range is 18-45°C.

Rifaat and Kansoh (2005) revealed that different strains of *Streptomyces* spp. isolated from Egyptian soils in different localities at Sharkia Governorate were test for antimicrobial activity. The microscopic examination of selected isolates (HM3) revealed that aerial mycelia were morphologically branched with compact spirals of sporophore. Each spore chain consisted of 10-20 turns with grayish white and smooth surface. The isolates grew well on most of the tested organic and synthetic media. Colonies were elevated, spread and covered with grey aerial mycelia and spores. Diffused melanoid pigments were sometimes observed. In general, microbial growth was monitored by naked eye and assigned as well or moderate.

Taddei *et al.* (2006) reported that the genus *Streptomyces* is represented in nature by the largest number of species and varieties among the family Actinomycetaceae. They differ greatly in their morphological, physiology and biochemical activities, producing the majority of known antibiotics. A comparative analysis using statistical software Minitab shows that 67 of these isolates are presumably new strains, since they possess a very low percentage of similarity with other reported species. Only four isolates shared 100% identity with one, two or three reported *Streptomyces* spp.

Lakshmipathy and Kannabrian (2009) isolated actinomycetes colonies from the marine soil samples which were collected at Ennore Saltpan region of Bay of Bengal by using Starch Casein Agar medium. The 3 isolates of *Streptomyces* sp. exhibited cultural and morphological characters. Actinomycetes isolates grew well at 28°C on starch casein agar medium producing pinpoint to medium sized, slow growing, powdery, irregular to regular, flat to raised colonies possessing an earthy odour characteristics of actinomycetes. The spore chain morphology and spore surface ornamentation of the isolates were evaluated by SEM analysis (S3400, 20 KV, 5.00 µm). The cultures were grown on starch casein agar for 7 days at 30°C. Degradation of starch, gelatin was determined by presence of zone of clearance around colonies after flooding plates with mercuric chloride and iodine, respectively.

Venkatachalam *et al.* (2010) reported that *Streptomyces* sp. isolates were Gram positive, filamentous and sensitive to lysozyme.

Umashankar *et al.* (2010) isolated 50 actinomycetes on the basis of colony morphology, gram staining and acid fast staining was performed to check morphology of cells and spore chain morphology. The 9 each isolates were identified as *Streptomyces* sp. (3), *Saccharopolyspora* sp. (3) and *Saccharomonospora* sp. (3) by using morphological, biochemical and Bergey's manual of determinative bacteriology studies.

2.1.4 Biochemical characterization:

Williams *et al.* (1989) reported that oat meal agar also used for spore chain morphology, colony reverse, diffusible pigmentation, antimicrobial activity, antibiotic resistance and degradation activity were also considered as special characters for morphological and biochemical studies of *Streptomyces* sp.

Kumari *et al.* (2006) reported that 10 isolates obtained from different areas in Tamil Nadu used for biochemical characterization of *Streptomyces* sp. and indicated that pigment production was very well observed in most of the *Streptomyces* spp. On the other hand, most of the

isolates were efficient in hydrolyzing starch and casein except *Streptomyces* spp. 9 and *Streptomyces* spp. 7. Indole production was strictly negative but catalase test was positive in all the isolates. Production of hydrogen sulfide, gelatin hydrolysis, casein and starch hydrolysis showed positive result in majority of the isolates.

Stefka *et al.* (2004-2005) studied cultural of *Streptomyces* sp. strain 3B was good development. The strain did not form melanoid pigment on any investigated for melanin media. The growth temperature range is 18-45°C. The strain liquefies gelatin and peptonises milk has not tirozinase activity and does not hydrolyze starch.

Venkatachalam *et al.* (2010) isolated and characterized the *Streptomyces* sp. and reported that the isolates were positive reaction in casein hydrolysis and starch hydrolysis. The negative reaction was observed in indole production, methyl red and Vogues Prouskauer and most of the *Streptomyces* sp. were catalase positive.

Umashankar *et al.* 2010. isolated 50 actinomycetes and biochemical tests were performed for 9 isolates of actinomycetes by using casein and starch hydrolysis, indole test, Methyl Red test and reported that all 9 actinomycetes were negative for casein, starch hydrolysis and indole production tests. The citrate test was observed positive for all 9 actinomycetes isolates. Whereas 3 *Streptomyces* sp. were negative reaction.

Dehnad *et al.* (2010) revealed that the actinomycetes were isolated considering morphological characters and examined microbial activity. Biochemical and molecular study also performed by them.

2.1.5 Pigmentation :

Among the most characteristic properties of streptomycetes is their ability to produce a great variety of pigments, both on organic and inorganic media. This pigment producing capacity is so characteristic as to serve for the naming of a large number of species, notably those belonging to the genus *Streptomyces*. Very few of these pigments are pure.

Von Plotho (1940) divided streptomycetes into four large groups according to the pigments produced.

1. Red yellow, 2 red blue 3. red brown 4 colourless.

Some of the pigments possess indicator properties, at an acid reaction, the red pigment predominates whereas at an alkaline reaction the second pigment prevails. The colourless groups have given the greatest number of antibiotics producing strains. Members of red blue group produce strong pigments but weak antibiotics. Members of red yellow group produce both strong pigments and highly active antibiotics. The members of red brown group produce neither true pigments nor active antibiotics.

Conn (1947) concluded that the two blue pigments produced by the two species of streptomycetes, *S. coelicolor* and *S. violaceus-ruber* are not identical. On the basis of this differentiation, Conn believed that the two organisms represented distinct species.

Benedict and Lindenfelser (1951) reported that a majority of Streptomycin producing strains of *S. grieseus* formed a green soluble pigment only in calcium malate medium and a yellow pigment in calcium succinate.

Waksman (1957, 1959) also considered the production of soluble pigments in both organic and inorganic media as an important characteristics of the streptomycetes.

Mencher and Hein (1962) reported that *S. lavendulae* synthesised brown pigment when tyrosine was added to the medium; without tyrosine only a trace of this pigment was detected. The data indicate that the brown black pigment synthesized by this organism is melanin.

Tresner and Hamlin (1964) observed that the chromogenic reactions of certain *Streptomyces* spp., in peptone iron agar (Difco) were the results of melanoid pigments formed and not due to production of hydrogen sulphide. According to them peptone iron agar (Difco) is an excellent substrate for demonstrating melanoid pigment formation by *Streptomyces*

species. Chromogenity of the aerial mycelium is also considered as an important character for the grouping and identification of streptomycetes by Waksman (1957, 1959) and Shinobu(1958).

Rangaswami *et al* (1967) found considerable variation in the chromogenity of aerial mycelium of the Streptomycetes from south Indian soils. Among the Streptomycetes from black soil, white forms were more and this was followed by grey, lavender, mauve, red and blue coloured groups. In alluvial soils they were in the following order : white, lavender, gray, red, blue and yellow. In red soils, they were in the order of mauve, white, gray, red, yellow, blue and black. In the lateritic soils the Streptomycetes were in the following order : mauve, gray, white, yellow red and black.

2.1.6 Functional Diversity of actinomycetes:

2.1.6.1 Antimicrobial activity :

Although the soil may be considered to be a source of antagonistic streptomycetes (Waksman and Schatz, 1946), the reason for this is that the growth inhibiting effect of streptomycetes upon bacteria and fungi is brought about largely through the production of toxic agents, which are known as antibiotics. The production of such substances can easily be demonstrated for some organisms by the agar cross streak method.

Nakhimovskaia (1937) studied on distribution of streptomycetes antagonistic first time surveyed was made on in nature, Out of 80 cultures isolated from a variety of soils, 47 possessed antagonistics properties however, only 27 of these were found capable of liberating antibiotic substances into the medium. These streptomycetes possessed the property of inhibiting the growth of gram positive bacteria but not of gram negative bacteria or of fungi. The antibacterial properties were manifested, not only in artificial culture media, but also in the soil.

Waksman *et al* (1942) came to the conclusion that streptomycetes possessing antibiotic properties against bacteria and fungi are widely distributed in nature, especially in soil and in composts. Two hundred

and forty cultures were isolated at random from different soils. Of these, 43.4 per cent possessed some antagonistic properties and 20 per cent were highly antagonistic. Similar relations were observed in examining a large series of well identified organism kept for a number of years in a Type Culture Collection (Welch, 1942). The antagonistic forms were most abundantly represented by the genus *Streptomyces* .

Meredith (1943, 1944) surveyed the distribution of organisms antagonistic to *Fusarium oxysporum f. cubense* in Jamaica soils. Most of these antagonists belonged to the streptomycetes. Out of 66 cultures, 10 cultures were the antagonistic organisms and not evenly distributed in the various soil samples.

Rehm and Rehm (1953) stated that 31 of the 100 strains of *Streptomyces* exerted an antibiotic effect on *Fusarium culmorum* (W.G. Smith) Sacc. and 43 on *Calonectria nivalis* without damaging the host. Inoculation of the seed with an active strain of *Streptomyces* reduced the incidence of 'Fusariosis' by about 1/3rd .

Benerjee *et al.* (1954) reported that Out of 196 *Streptomyces*, 128 were found to have antifungal activity of varying degrees against one or more of the fungi tested. Of the test fungi, *Aspergillus oryzae* (Ahlburg) Cohn, *Curvularia* sp. and *Helminthosporium oryzae* Breda de Haan were more susceptible to antagonism by *Streptomyces* spp. than *Verticillium* spp., *Fusarium* spp. or *Alternaria solani* (Ellis & Mart.) Jones and Grout.

Mukherjee and Nandi (1955) reported that of 170 *Streptomyces* spp. isolated from Indian soils, 68 were antagonistic towards gram negative bacteria and 54 to fungi including *Helminthosporium oryzae*, *Curvularia specifera*, *Fusarium* spp., *Alternaria solani* and *Rhizoctonia* spp. Different isolates of the same species of *Streptomyces* possessed different antagonistic properties. They tested 170 strains of soil streptomycetes for their antagonistic properties and found that 40 per cent were inhibitory to gram positive bacteria and 38 per cent were inhibitory to gram negative bacteria.

Pirdham *et al.* (1956) studied growth inhibiting effect of 200 streptomycetes, largely *Streptomyces* strains and found that only 52 of the 200 strains showed promise against *Helminthosporium* spp., *Fusarium oxysporum* and other fungi and bacteria .

Vitgeet (1960) studied the antagonistic effect of 2721 Streptomycetes cultures isolated from soils under cotton and Lucerne. 122 (10.4%) of 1171 were antagonistic to *Fusarium oxysporum* var. *Vasinfecum* and six hundred and eleven (22.5%) were antagonistic to *Verticillium dahliae*. Specially antagonists were *S. violaceus*, *S. globisporus*, *S. griseus* and *S. albus*.

Rangaswami and Vidyasekaran (1963) reported that *Streptomyces* spp. inhibited the spore germination, growth and lysed the germ tubes of *Helminthosporium sativum* Pammel, King and Bakke in the corn rhizosphere.

Basu Chaudhory (1964) stated that strains of *S. griseus* isolated from cultivated field soil, was shown to be antagonistic towards *Alternaria solani* from potato, *Fusarium oxysporum* var. *vasinfecum*, *Aspergillus niger* Tiegham, *Curvularia spicifera* (Bain) Boedijn, *Chaetomium indicum* Corda *Helminthosporium oryzae* and *H. sativum*

Whaley (1965) stated that 10 *Streptomyces* spp selected from 386 isolates of rhizosphere streptomycetes were antagonistic to *Fusarium oxysporum*, *Phymatotrichum omnivorum*, *Rhizoctonia solani* kuhn and *Verticillium albo-atrum*.

Ghule [1968] stated that of 16 isolates of *Streptomyces* 9 cultures inhibited the growth of *Helminthosporium tritici*, 7 cultures have inhibited the growth of *Curvularia lunata* and only 5 cultures have inhibited the growth of *Fusairum oxysporum*, *F. lycopersici*, *Alternaria tennissima*, *Sclerotium oryzae*, and *Malustella aeria*.

Goodfellow *et al.* (1987) revealed that 88 streptomycetes representating numerically circumscribed species groups were examined for their ability to use a range of 4-methyl-umbelliferone – linked

substrates. The test strains produced acid and alkaline phosphatases, a α -hydrolase (C₇) but the discontinuous distribution of the remaining enzymes provided information of taxonomic value.

Sardi *et al.* (1992) revealed that *Streptomyces* antimicrobial activity were screened against plant pathogen like *E. coli*, *Fusarium oxysporum* f. sp. *cyclaminis* and *Micrococcus luteus* and observed that some *Streptomyces* sp. showed the antimicrobial activity against the plant pathogens.

Busaya *et al.* (2005) reported that 32 strains of actinomycetes obtained from soil samples of Thailand were selected. Actinomycetes strain SU-1 is the most effective in terms of antagonism of *Fusarium moniliformae*. It produces antifungal substances on agar medium against *F. moniliformae*. On the basis of morphological and biochemical tests the strain was identified as *Streptomyces fradiae*.

You *et al.* (2005) revealed that all 94 marine actinomycetes strains were screened for their antagonism to seven species of vibrio. 23 actinomycete isolates were found antagonistic and to inhibit all 7 *Vibrio* sp. 57 strains could grow on CAS agar and 38 strains were considered as siderophore producers due to formation of orange haloes around the colonies. Most of them belongs to *Streptomyces* sp.

Kumari *et al.* (2006) reported that 10 isolates obtained from different areas in Tamil Nadu studied for detection of antimicrobial substances contained it. The results revealed that out of 10 isolates of *Streptomyces* sp. three of the isolates were found to be of potential antagonistic against pathogenic organisms and thus providing production of secondary metabolites which has potential to control the soil borne pathogens.

Rifaat and Kansoh (2005) revealed that different strains of *Streptomyces* spp. isolated from Egyptian soils in different localities at Sharkia Governorate were test for antimicrobial activity. The strain HM3 showed a broad antimicrobial spectrum against G +ve and G – ve

bacteria, fungi and yeast. The *Streptomyces* HM3 isolated showed antimicrobial activity against *Aspergillus niger*, *Aspergillus terreus*, *Fusarium oxysporum*, *Bacillus subtilis*, *E. coli* except *Pseudomonas aureoginosa* and *Candida albicans*.

Hassanin *et al.* (2007) studied that 43 isolates of actinomycetes and 8 isolates of bacteria were isolated from the rhizosphere associated soil of cotton plant. Four actinomycete isolates were found to have a potent antagonistic activities against *Rhizoctonia solani* and were identified as *Streptomyces erumpens*, *S. purpureus*, *S. aurantiacus* and *S. microflavus*. Four bacterial isolates were found to have strongest antagonistic activities and were identified as *Arthrobacter ramosus*, *Aureobacterium terregens*, *Curtobacterium pusillum* and *Pseudomonas putida*.

Jeffrey (2008) reported that 62 actinomycetes were isolated from Sarawak and studied for their morphological, enzymatic and antagonistic activities against selected phytopathogens and observed that 3, 25, 35 and 37 of isolates showed antagonistic reaction with *Fusarium palmivora*, *Bacillus subtilis*, *Pantoea dispersa* and *Ralstonia solanacearum*, respectively. The antagonistic actinomycetes should be used as biocontrol or bioremediation agents.

Lakshmipathy and Kannabrian (2009) revealed that the 3 isolates screened for antidermatophytic activity against *Trichophyton* and *Microsporon gypsum* and *Epidermatophyton floccosum* by well diffusion method.

Nonoh *et al.* (2010) studied that actinobacterial isolates from Kenya were screened for antagonism against *Fusarium oxysporum*, *Fusarium* spp and *Colletotrichum kahawae*. Twenty-three isolates showed antagonistic activity to one or all of the test fungi. Five isolates that were antagonistic against all test fungi were investigated further and were also found to have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The results suggest that protected areas may be ideal habitats for isolation of antagonistic actinobacterial species which may have the

potential for beneficial application in biological control of fungal pathogens.

Mythili and Ayyappa Das (2011) reported that actinomycetes are microscopic soil microorganisms known to play a very supporting role in the degradation of organic matter in coffee habitats. Antimicrobial activity of *Streptomyces* sp. collected from tea plantation soil sample was studied. The isolates were classified according to the color of its mature sporulated aerial mycelia. Isolates showed antimicrobial activity against some gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

2.1.6.2 Cellulose decomposition:

Kellerman and McBeth (1912) published a formula for preparing cellulose agar medium to test cellulose digestion. Since then this medium or a modification of it has been generally used in cellulose fermentation studies.

Konde (1974) who reported that out of 150 cultures tested, 106 cultures showed the cellulose degradation ability indicates that a large number of cellulose degrading streptomycetes were present in the soils of Maharashtra.

Segung *et al.* (1998) reported that starch degradation was observed in most of the isolates of *Streptomyces* sp.

Arora *et al.* (2005) isolated *Streptomyces griseus* B1 from soil and reported that when isolate B1 grown on cellulose powder as submerged culture, it produces high levels of all the three components *i.e.* filter paper lyase (FPase), CMCellulase and β -glucosidase of the cellulolytic enzyme system. When it grows on hardwood and softwood powder under solid state conditions, it depletes the cellulose (36% in softwood and 14.4% in hardwood).

Arunachalam *et al.* (2010) studied the cellulose producing actinomycetes from soil of southwest ghats, Tamil Nadu, India and

characterization was done by morphology, cultural and physiological and phylogenetic analysis of TBG-V20 and identified as *Streptomyces* sp. The Carboxy Methyl Cellulose (CMCase) activities 935 UL⁻¹ of glucose 1000 ml growth was recorded on 8th day and on 1st day strain TBG-V20 shows exocellulase activity and produced an amount of 500 µg/mL glucose.

Kishore (2010) reported that all the strains showed good cellulose degradation activity which is in conformity with the present results.

2.1.6.3 Antibiotic sensitivity study

Stefka Antonova- Nikolova *et al* (2004-2005) revealed that Strain 3B has tested for antiobiotic sensitivity to different antibiotics and recorded that strain 3B sensitive to the antibiotic gentamycin, erhytromycin, streptomycin and chloramphenicol and resistant to the group of the penicillin antibiotics.

Ibrahaim (2006) studied the antibiotic resistance of *Streptomyces* isolates and indicated that these *Streptomyces* isolates were sensitive to gentamycin, tobramycin, rifamycin and penicillin G, trtacycline but resistant against streptomycin, neomycin, vanomycin and lincomycine.

Lakshmipathy and Kannabrian (2009) studied that sensitivity to various antibiotics varied among the isolates and reported that *Strptomyces* sp DKDVIT1 was found to be sensitive to many antibiotics but resistant to chlorophenicol, penicillin, tetracycline and erythromycin.

2.1.7 Molecular diversity :

Seunga *et al.* (1998) reported that RAPD analysis of strains AT50, AT51 and AT52 and representatives of related validity described *Streptomyces* species composed of thermophillic strains. The number of DNA amplification products obtained for the test and marker strains varied from 5 to 12 with the lengths of the products lying between 0.2 and 2.2 kbp. Several strongly amplified fragments were detected in all preparations. Random DNA amplification profiles clearly distinguished strains AT 50, AT 51 and AT 52 from *Streptomyces thermodiastations* end from strain AT 37.

The polymerase chain reaction (PCR) is the *in-vitro* amplification of particular DNA sequences using random or specific primers and a thermostable DNA polymerase (Joshi *et al.* 1999). The diversity analysis as well as finger printing of individuals can be performed with the help of PCR based RAPD markers.

Malkawi *et al.* (1999) used for 10-ner primers to identify and assess genetic diversity among *Streptomyces* isolates and two reference strain. Cluster analysis revealed that incidence of polymorphisum among the isolates and none of them was identical to reference starin although there was some common amplification bands.

Martin *et al.* (2000) showed that RAPD analysis is suitable to reveal interspecific, intraspecific and intraclonal differences between *Streptomyces* sp. and strain. They also reported that RAPD analysis allows rapis, sensitive and specific detection of genetic diversity among species and strains of *Streptomyces*.

Roberts and Crawford (2000) reported that 36 *Streptomyces* species including 17 taxonomically undefined strains, 25 non streptomycetes actinomycetes, and 12 out group consisting of Gram positive and negative strains were analysed with 20 RAPD primers. Profile of most interest were those that produced clearly distinguishable major products of between 0.2 and 1.2 kDa, PCR- generated DNA fragments were subsequently labeled and tested for genus specificity. Most of the primers were useful in identifying unique DNA polymorphisms of all strains tested. A strain specific probe unique to *S. lydicus* WYEC108 genome was developed.

Throne and Cloete (2000) suggested the use of molecular techniques for determining microbial diversity and community structure in natural environments. They proposed the methods of recombinant DNA techniques, 16S rRNA sequence analysis and the use of fluorescently labelled population specific rRNA probes in the studies of diversity and phylogeny of microorganisms.

Gharaibeh *et al.* (2003) used RAPD analysis to determine the relatedness of 73 antibiotic producing soil streptomyces isolated from different habitats in Jordan. The obtained results cluster the tested isolates into two main clusters. The first cluster was more homogenous than the second one and contains all the reference strains. The second cluster contains unrelated isolates within small groups.

Besides remarkable inherent physiological and functional diversity, microbes have found application in agriculture, industry, medicine and environment. Much better known and exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture (Johri *et al.* 2005).

Rabbani *et al.* (2007) reported that the study of *Streptomyces griseus* by using morphological, biochemical and molecular techniques from the Isfahan soil. Distinguishable morphological factors such as the size of colony, pigmentation and colony status on the plate were used to separate *Streptomyces* sp. from others from the others in first step. They screened total of 50 colonies and 19 were found to be of *Streptomyces* origin. Large spores, mode of branching and non- segmented mycelia were among the most prominent factors for discriminating *Streptomyces* sp. from other genera within *Actinomycetes* family. PCR technique was used to further identification of colonies that were found to be a member of *S. anulatus* cluster group and colonies with positive antimicrobial activity.

Saadoun *et al.* (2007) reported that the soil streptomycetes were screened for hydrolytic enzymes and characterized them. A total of 110 isolates of streptomycetes were recovered from 15 soil samples collected from three regions of Ajiun forests in northern Jordan and screened for hydrolytic enzymes ability. The results indicated that 79% of the isolates were able to produce atleast one of the enzymes with cellulase being the highest (71.8%). Thirty five isolates (32%) were able to produce the three enzymes with 24 of them were characterized morphologically and physiologically as *Streptomyces*. RAPD DNA analysis has been used to

determine the relatedness of 11 active isolates based on their RAPD-PCR fingerprints. Genotypic polymorphisms between active isolates showed two common bands of 500 and 800 pb shared by approximately (90%) of them.

Munira *et al.* (2008) studied that the molecular fingerprints for fifteen *Streptomyces* isolates gathered from Riyadh city by using RAPD-PCR. Three 10-mer random primers (OPB-16, OPF-05 and OPK-10) out of eight has successfully generated reproducible polymorphic products. Each of the three primers displayed a strong amplification with distinct bands. Primer OPB-16 generates a total of 19 polymorphic bands. Two of these polymorphic bands were identified as unique ones. While the remaining ones were detected as 17 non unique bands. The generated RAPD profile with the primer OPF-05 shows a total of 23 polymorphic bands among the studied isolates. It comprises one unique band and 22 non unique polymorphic ones. Similarly, primer OPK-10 develops 14 polymorphic bands which scored as 4 unique and 10 non unique bands. The unique bands were used to discriminate between some of the studied *Streptomyces* isolates. The results generated from RAPD profiles were pooled for drawing the genetic relationships among the examined *Streptomyces* species. The constructed dendrogram tree divided the fifteen isolates into two groups. The highest similarity value (0.851) was observed between the two isolates no. 13 and 14. On the other hand, the lowest similarity value (0.000) was scored between the two isolates no. 5 and 6.

Kannan and Vincent (2011) revealed that actinomycetes were isolated, analyzed for the phylogenetic relationship from the rhizosphere soil of Manakkudy mangroves, West Coast of India and screened for its antimicrobial potential. The phylogenetic relationship among the 20 *Streptomyces* sp. were studied. Phylogeny analysis showed that all the 20 isolates were having strong similarity (98%) with *Streptomyces*. Eight different species of *Streptomyces* and 8 new strains (*Streptomyces* JS-1, *Streptomyces* JS-8, *Streptomyces* JS-10, *Streptomyces* JS-12,

Streptomyces JS-14, *Streptomyces* JS-17, *Streptomyces* JS-19 and *Streptomyces* JS-20) were identified among the 20 isolates. The *Streptomyces* isolates JS-1, JS-8, JS-10, JS-12, JS-14, JS-17, JS-19 and JS-20 were considered as the new lineages of *Streptomyces*. All the isolates (JS-1 to JS-20) were having strong antagonistic activity against various bacterial and fungal pathogens, but the activity was differing in all the 20 *Streptomyces*. Phylogenetic analysis revealed that the isolates are the divergent of *Streptomyces* which are associated in the rhizosphere soil of Mangrove.

2.2. Sulphur oxidizing micro-organisms: Sulphur is one of the inert elements found in nature and it must be oxidized before it is utilized by plants. Micro-organisms are mainly responsible for oxidation of elemental sulphur and reduced forms of sulphur eg. Thiosulphate salts and polysulphides.

The effective oxidation of elemental sulphur, a close contact between sulphur granules and sulphur bacteria is required (Starkey *et al.* 1956 and Schreffer and Umbreit, 1983).

There has been considerable amount of debate with regard to oxidation of sulphur to sulphate (Peck, 1962). However, recent evidence suggested that thiobacilli oxidized the sulphur to sulphite eventually to sulphate (Lyric and Suzuki, 1970).

The thiobacilli in general, the specialized chemolithotrophic sulphur oxidizers have long been viewed as the most important group of organisms contributing to the transformations of sulphur in soil and sediments, though only a few systemic studies on their occurrence and distribution of thiobacilli have been carried out. However, more and more evidence are available to indicate that heterotrophic bacteria can be atleast equally important as specialists in the overall conversion of inorganic sulphur compounds to oxidized compounds (Kelly, 1972 and Kuenen, 1975).

Vishniac and Santer (1957) studied the morphological and physiological characters of the genus *Thiobacillus* and reported *T. thioparum* as a type species. These bacteria have a limited capacity to assimilate organic carbon into cellular material, but energy generation is obligately dependent on the oxidation of organic sulphur compounds. It is differentiated from *T. neapolitanus* by its ability to grow anaerobically in the presence of nitrates, thiocynate and thiosulphate.

Frobisher (1961) reviewed that *T. thiooxidans*, *T. thioparus*, *T. denitrificans* and *T. ferrooxidans* oxidize the elemental sulphur and other reduced forms of sulphur into sulphate and sulphuric acid. *T. thiooxidans* is of interest in having great resistance to acid and grows even upto pH of 1 and also produce acid low pH i.e. 1 to 3. These bacteria are of great importance as agents in the acid disintegration of various rocks and minerals with the liberation of such valuable elements as phosphorus, magnesium and sodium.

In general, sulphur and iron oxidizers are ubiquitous in soil, fresh water and marine environments, as well as in sediments. The wide range of adaptation of thiobacilli to environmental conditions, including pH values between 1 and 9 and temperature optima up to 60°C found for some thermophillic isolates (Egorova and Deryugina, 1963, Williams and Hoare, 1972), make it understandable that it is relatively easy to find these organisms in nature.

Sokolova and Karavaika (1968) stated that the enrichment and isolation procedures, as well as enumeration techniques for sulphur-oxidizing bacteria, are almost invariably based on either thiosulphate or elemental sulphur as a substrate. In nature, the distribution of reduced inorganic sulphur compounds is only one of the factors governing the presence and activities of these organisms. In soil, water and sediments the distribution of the sulphur oxidizing bacteria is governed by a complex of chemical, physical and microbial interactions. A very important factor is that the sulphur oxidizers require the simultaneous presence of the reduced sulphur compound and oxygen (or sometime nitrate) in order to

be able to grow. Sulphide is one of the important natural substrates and many sulphur oxidizer often grow in the narrow zones and gradients of coexistence of sulphide and oxygen (or nitrate). Similarly, it can be seen that gradients for other critical factors influence the growth and selection of sulphur oxidizers. *Thiobacillus thiooxidans* were rod shaped cells of 0.5-0.5 x 1-1.2 μ , gram negative occurring singly and occasionally in pair and short rods. Thus, it has been shown that different species of *Thiobacillus* respond differently to varying redox potentials in their environment.

The fact that acidophilic thiobacilli commonly occur in apparently neutral environments which indicates the existence of pH gradients due to acidic microniches. Currently there seems to be contradictory evidence as to whether the heterotrophic sulphur oxidizers can actually derive energy from exothermic transformations of sulphur. However, it should be realized that there may be a spectrum of heterotrophic sulphur oxidizers with a varying potential to derive energy from these oxidations (Kuenen, 1975).

Mahoney and Edwards (1966) studied that the chemosynthetic autotroph *Thiobacillus thiooxidans* revealed structures comparable to gram-negative heterotrophic bacteria. Although this species is unique in that it oxidizes elemental sulfur for energy, uses carbondioxide as its solesource of carbon, and can withstand a pH of less than 1, thin sections revealed a profile of the cell envelope (cell wall and plasma lemma) similar together gram-negative species which have more common physiological traits. The cell wall is composed of five layers with an overall width of approximately 200A, and the plasma lemma appears as a conventional "unit membrane" with a width of about 85 A. Volutin granules and less-dense bodies of similar shape and size were frequently observed in close association with the nucleoplasm.

In soils, thiobacilli sometimes may account for the solubilization of sulphur compounds to make sulphur available (as sulphate) for assimilation by other micro organisms and plants. The sulphur budget

and the role of micro organisms in the sulphur cycle of cultivated soils is out of course, important to assess for fertilization. In Australia for example, *Thiobacilli* are scarce in areas of sulphur deficiency. Under suitable climatic conditions in tropics, rock phosphate pelleted with sulphur and seeded with *thiobacilli* has been shown to be useful, slow-releasing source of phosphate and sulphate for soil fertilization (Kelly, 1969).

One of the most common problems in obtaining pure cultures of chemolithotrophs is the extreme persistence with which heterotrophic contaminants may some times remain associated with the chemolithotrophs colonies on agar plates (Taylor and Hoare, 1971). This is partly due to the presence of trace amount of soluble organic material in virtually all agar brands and partly due to the fact that obligate chemolithotroph excrete organic compounds (Borichewski, 1967 and Chohen and Kuenen, 1976).

All *Thiobacilli* species can grow at the expenses of the oxidation of inorganic sulphur compounds but the conditions (e.g. pH) required for growth on a specific substrate may vary some what (Jackson *et al.* 1968).

Gupta and Agate (1986) revealed that cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are very difficult to preserve by conventional methods. The temperature of storage observed to have a definite effect on activity in that when the preserved cultures were stored at 8°C, the activity was retained whereas at 28-30°C (RT) storage, the activity of all the cultures preserved by various techniques, dropped significantly.

Chapman (1990) detected neutrophilic *Thiobacillus* in 84 per cent of the soils examined with a mode of 10^{-2} to 10^{-3} organisms per gram soil.

2.2.1 Isolation

For the isolation of sulphur oxidising micro organisms *viz.*, bacteria, the enrichment culture technique was used (Waksman, 1922). The medium consisted of sodium thiosulphate as sulphur source. The S –

oxidising bacteria are purified by streaking on thiosulphate of sulphur agar media.

Waksman and Joffe (1922) reported that *Thiobacillus thiooxidans* was isolated from compost of soil, sulfur and rock phosphate, by the use of inorganic media. It oxidizes elementary sulfur to sulphuric acid, derives the necessary carbon from CO₂ of the atmosphere and its nitrogen need from inorganic nitrogen salts. *Acidithiobacillus thiooxidans* grown in liquid medium on elemental sulphur thiosulphate or tetra thionate, short rod with round ends, 1 μ in length and 0.5 μ wide, gram negative.

Swaby and Fedel (1973) isolated five *Thiobacillus* sp. out of 56 Australian soil analysed.

Kapoor and Mishra (1985) studied the oxidation of sulphur and pyrite in sandy loam field soil of pH 8.0. They found that sulphur oxidized in soil under sterile and unsterile conditions though the oxidation was slow in the absence of micro-organisms. Thiobacilli were isolated from soil and pyrite by enrichment culture technique. The oxidation of sulphur in soil was enhanced by inoculation with Thiobacilli. Pyrite oxidation was also enhanced by inoculation with *T. fero-oxidans* and *T. thiooxidans*.

Shinde (1993) isolated sulphur oxidizer from soils, purification and biochemical analysis was also carried out.

Vidyalakshmi and Sridar (2006-2007) isolated sulphur oxidizing bacteria from different samples viz., paddy rhizosphere, pulse rhizosphere, sewage, biogas slurry, tannery effluent and mine soil.

Nareshkumar *et al.* (2007) isolated sulfur oxidizing bacteria, *Acidithiobacillus thiooxidans* using Starkey medium. The pH was dropped from neutral to below 1 over a period of 28 days; drop in pH was rapid when ratio was 0.57 and it took only 6 days for pH to drop from 7.12 to 0.94.

Yang Yu *et al.* (2008) revealed that an acidophilic, chemolithotrophic and ferrous oxidizing bacterium strain GF was isolated from the acid mine drainage of Gaofeng Mine, China using 9K enrichment medium.

Babana *et al.* (2011) isolated three *Thiobacillus* sp. strains from the 40 soil samples collected at Mali and Canada

2.2.2 Morphology and Cultural characters:

Micro-organisms that participate in the transformation of sulphur compounds are both diversified in physiological and morphological characters, and the best known group of sulphur oxidizing bacteria non-filamentous, aerobic autotrophs belonging to family thiobacteriaceae. *Acidithiobacillus thiooxidans* grown in liquid medium on elemental sulphur thiosulphate or tetra thionate, gram negative, short rod with round ends, 1 μ in length and 0.5 μ wide which have the capacity to oxidize the elemental sulphur to sulphuric acid utilize CO₂ as the source of cell carbon (Waksman, 1922 and Iwatsuka, 1962). When they are grown in presence of elemental sulphur, the thiobacilli produced considerable amount of acidity in their environments. *T. thiooxidans* could produce and tolerance extreme conditions of acidity (pH less than 1.5) while others varied in their degree of acid tolerance (Iwatsuka and Mori, 1960).

Kempner (1966) reported decrease in the pH of culture media by *T. thiooxidans* from 3 to 0.9 in about 2 to 3 weeks taxonomically all members of genus *Thiobacillus* are capable of oxidizing sulphur though the degree of oxidation varied from species to species (Jackson *et al.* 1988).

Sokolova and Karavaiko (1968) studied that *Thiobacillus thiooxidans* were rod shaped cells of 0.5-0.5 x 1-1.2 μ , gram negative occurring singly and occasionally in pair and short rods.

Guay and Silver (1975) characterised that *Thiobacillus acidophilus* as short rods, 0.5 – 0.8 μ m x 1-1.5 μ m with round ends, gram negative and motile non sporulating. The cells occurred in singly, mainly in pairs and rarely in chains.

Thiobacillus are Gram –ve, rod shaped cells (0.5 x 1.0 – 4.0 μ m) with some specific motile i.e. polar flagella. The genus includes obligate

aerobes and facultative denitrifying types. pH optimum 2 – 8 and optimum temp requires 20-43°C (Kelly and Harison, 1988).

Kelly and Wood (2000) reclassified some species of genus *Thiobacillus* fall into alfa, beta and gamma subclasses of the proteobacteria, the type species *Thiobacillus thioparus* being located in the B – sub class. *Acidithiobacillus* is obligately acidiphilic optimum (pH < 4.0) and aerobic, Gram negative rods 0.4 x 2.0 µm in size, motile by one or polar flagella, using reduced sulfur compounds to support autotrophic growth. *Thiobacillus* species exhibit almost as much diversity in DNA composition and physiology as is found collectively in all other proteobacterial groups. On the basis of physiological character and 16S rRNA gene sequence comparisions, eight of the exsisting *Thiobacillus* species are proposed for reassignment to three newly designated genera within gamma (γ) sub class of the proteobacteria viz., *Acidithiobacillus*, *Halothiobacillus* and *Thermithiobacillus*.

Lee *et al.* (2000) characterized that the Thiobacilli ASWW-2 was yellowish colonies 1-1.5 mm in diameter, short rods, size 0.5 x 1.5 µm and gram negative in reaction. During cultivation, the increase of sulfate concentration was accompanied by an increase in cell mass. The pH value of the culture gradually decreased according to the accumulation of sulfate. The final sulfate concentration was approximately 30 g/L, indicated that elemental sulfur (initial conc. 10 g/L) was stoichiometrically oxidized to sulfate by strain ASWW-2.

Vidyalakshmi and Sridar (2006-2007) reported that sulphur oxidizing bacteria were isolated from different samples viz., paddy rhizosphere, pulse rhizosphere, sewage, biogas slurry, tannery effluent and mine soil. The obtained isolates were inoculated in the growth media with initial pH adjusted to 8.0 and incubated at 32°C for 15 days. Out of the 28 isolates obtained, 14 were screened based on their efficacy to reduce the pH of the growth medium from 8.0 to ≤ 5.0 . The selected isolated were further studied for their morphology, gram reaction, colony characters and nutrition type. The selected isolates were characterized to

be short rods, Gram negative, utilizing biotin and one or more of the sulphur sources. The heterotrophic colonies were smooth, round and straw colour probably due to sulphur deposition.

Patel (2010) revealed that the *Thiobacillus thermosulfatus* isolated from fresh coal mine overburden spoil sample in medium – A enriched with elemental sulfur. The bacteria was gram negative, motile, rod shaped, facultatively autotrophic, strictly aerobic. The changes occurred in pH from 6.0 to 2.1 during the bacterial growth. Optimum growth of *Thiobacillus thermosulfatus* was obtained at pH 5.2 to 5.6 and temperature 50°C to 53°C, suggesting that the bacterium is acidophilic and moderately thermophilic.

Babana *et al.* (2011) studied that the *Thiobacillus* sp. were isolated from agricultural soils and morphologically characterized. The results showed that analyzed agricultural soils are poor in *Thiobacillus* sp. and they are gram negative, rod shaped and yellowish in colour. The colony diameter is ranged from 0.8 to 1.9 mm in size.

2.2.3 Molecular diversity :

Total genomic DNA was isolated using the Sambrook *et al.* (1989) protocol with slight modifications where needed. Forty seven primers were used for the PCR as reported by William *et al.* (1990) with minor modifications for isolation of good quality of DNA. The DNA denaturation was done at 94°C for 5 min followed by 40 cycles amplification (94°C for 1 min, 36°C for 1 min and 72°C for 2 min) and a final extension step at 72°C for 10 min. Amplification products were separated on 1.2% agarose gel.

Selenska – pobell and Kutschke (2002) studied that the taxonomic and discriminatory power of RAPD, ARDREA and rep-APD analytical approaches were compared. RAPD and Pre-APD methods provide taxonomic results which are in agreement with the classification on based on the of the highly conservative rRNA operon of the strains were studied. Moreover, both kind of genomic PCR fingerprints to the fact that they

derive information from different parts of the whole bacterial genome which may possess different variability among *Thiobacillus ferrooxidans*.

Karavaiko *et al.* (2003) revealed that phylophasic genotypic analysis of the *Acidithiobacillus ferrooxidans* strains isolated from ores and ore concentrates collected in different regions of the world showed considerable strain heterogeneity. The distribution of strains among the phylogenetic groups correlated with their distribution among the genomovars, except that the representatives of two different genomovars fell into one phylogenetic group. Thus, at least two levels of phylogenetic heterogeneity for *A. ferrooxidans* have been found. The phylogenetic heterogeneity of *Acidithiobacillus ferrooxidans* strains, which are phenotypically indistinguishable, suggests the occurrence of microevolutionary processes in different niches.

He *et al.* (2004) studied the molecular diversity and reported that Random amplified polymorphic DNA (RAPD) was used in analyzing the polymorphisms of *Thiobacillus ferrooxidans* from seven different places. Of the 120 primers, four could generate reproducible RAPD profiles, and each one produced approximately 9 bands. The similarity coefficients obtained from profiles generated by four primers among *Thiobacillus ferrooxidans* were about 44% to 83%.

Tanveer *et al.* (2005) studied that RAPD – a PCR based technique was applied to evaluate genomic diversity among three strains of *Acidithiobacillus thiooxidans* using 45 random primers of five different series. More than 220 bands were observed, with an average 45 bands per primer. Primer OPC-3 produced maximum number of fragments whereas minimum numbers of fragments were produced with primer OPA-5. The dendrogram showed three groups with similarity ranging from 29 to 85%. The maximum similarity (85%) was observed between the strains T.t₁ and T.t₂ of *Acidithiobacillus thiooxidans*.

Waltenbury *et al.* (2005) studied that twelve strains of *Acidithiobacillus ferrooxidans* were recovered from acid mine drainage (AMD) sites from three different geographical locations. The spread plate

and various culture media were used to isolate and purify all strains. DNA was extracted from each purified culture and amplified using PCR and twenty, 10-mer primers. Amplification products were separated by gel electrophoresis and photographed under UV light. The RAPD profiles were compared on the basis of the presence or absence of each DNA band and a data matrix was constructed. Strain diversity was calculated using the Jaccard's coefficient and UPGMA cluster analysis. The variations in banding patterns indicated genomic variability among the twelve *A. ferrooxidans* strains tested. The primer used in this study show that the grouped twelve strains in five major groups. Similarities between the strains ranged from 5.49% to 85.14%. These results showed that the strains have a high degree of genomic diversity that the RAPD procedure is a powerful technique to assess strain variability in this bacterium.

Yongqing *et al.* (2008) reported that 35 Chinese *Acidithiobacillus* isolates and three reference strains representing three validly described species and used them to construct phylogenetic trees. The two phylogenetic trees were roughly similar topologically, and *Acidithiobacillus* strains were assigned to eight phylogenetic groups. In addition, the results of phylogenetic analysis were consistent with those obtained by randomly amplified polymorphic DNA (RAPD) cluster analysis. The genomic DNA of each strain used in this work was amplified using 35 different random primers. Twenty-five of the primers tested produced a large number of specific and reproducible banding patterns revealing polymorphisms, whereas the other 10 primers (S-21, S-22, S-27, S-28, S-32, S-33, S-34, S-35, S-36, OPC-09) were excluded from the data analysis due to the production of non-reproducible banding pattern. The number of bands for each primer varied from 4 to 32 with an average of about 18 bands per primer. The size of the amplified product ranged from approximately 200 to 4000 bp. Coupling phylogenetic analysis and phenotypic characteristics, we concluded that at least each of the three *Acidithiobacillus ferrooxidans* phylogenetic groups should be considered a separate subspecies, and that five sulfur-oxidizing Chinese

Acidithiobacillus-like isolates represent one or two new species of the genus *Acidithiobacillus*.

Luciana *et al.* (1995) reported that 19 strains of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* isolated from similar sources in other countries and the type strains of the two species were characterized together with the type strain of *A. caldus* by using a combination of molecular systematic methods, viz., ribotyping, BOX- and ERIC-PCR assays. Data derived from the molecular fingerprinting analyses showed that the tested strains encompassed a high degree of genetic variability. Two of the Brazilian *A. ferrooxidans* organisms (strains SSP and PCE) isolated from acid coal mine waste and uranium mine effluent, respectively, and *A. thiooxidans* strain DAMS, isolated from uranium mine effluent, were the most genetically divergent organisms.

2.3. *Pseudomonas fluorescence* micro-organisms :

Microorganisms has small size and invisibility to the naked eye due to this ability the diversity has been least studied. Price (1988) stated that the existence of each living organism depends upon microorganisms since they are the decomposers in the food chain. The realization of various potentials of microorganisms viz., production of plant growth promoting substances, biological control, ability of nitrogen fixation, solubilization of inorganic insoluble phosphate and they were exploited for today's agriculture. The knowledge accumulated on the importance of microorganisms has prompted the scientists to think about the microbial diversity. Thus, the study of microbial diversity is gaining increasing interest.

Sorheim *et al.* (1989) reported that the study of phenotypic divergence of soil bacteria isolated by using three different media. The bacterial populations obtained through planting are mainly dependent on the isolated media used. Later, the strains were phenotypically characterized and cluster analysis revealed that the isolates belonged to biotypes that were common to all the three populations.

The activity of microorganisms and its presence is essential for the health and proper functioning of the ecosystems and the whole environment through mineralization and recycling of important elements, photosynthesis, proper conditioning and fertility of the soil, pest and vector control as well as detoxification of the naturally occurring and man-made pollutants (Olembo, 1991).

Deleij *et al.* (1993) reported that the characterization of bacterial communities on roots and in soil by considering the use of colony development and suggested that simple agar plating can be used to quantify microbial communities in different habitats.

Nei and Li (1979) revealed that they developed a mathematical theory for studying genetic variation in terms of restriction enzymes. Biological diversity defines the total diversity of all the living organisms. Biodiversity may represent a form of biological insurance, against the loss or poor performance of selected species. It also includes the diversity found at the genetic, species and ecosystem levels.

Hugenholtz *et al.* (1998) studied the phylogenetic trees based on rRNA sequences show that bacterial diversity is represented by natural relatedness groups, phylogenetic divisions. Comparative analyses of small subunit rRNA (16S or 18S rRNA) and other gene sequences show that life falls into three primary domains *viz.*, bacteria, eukarya and archaea. Based on studies using rRNA trees, the main extent of earth's biodiversity was found to be amongst microbes.

Kari and James (2001) characterized the rhizosphere and root interior microbial communities through fatty acid methyl ester (FAME) analysis and community level physiological profiles and reported that the plant variety has notable influence on microbial composition of rhizosphere. Results of this analysis indicated that genetically modified canola varieties was different in composition of microbial community as compared to the of traditional canola varieties.

The main diversity of life is microbial, distributed among three primary relatedness groups or domains: Archaea, Bacteria, and Eucarya. The application of molecular-phylogenetic methods to study natural microbial ecosystems without the traditional requirement for cultivation has resulted in the discovery of many unexpected evolutionary lineages; members of some of these lineages are only distantly related to known organisms but are sufficiently abundant that they are likely to have impact on the chemistry of the biosphere. The use of molecular tools and perspectives based on gene sequences were applied into microbial diversity studies since the morphological and nutritional criteria used to describe microbes failed to provide a natural taxonomy ordered according to evolutionary relationships (Pace, 1997).

Borneman *et al.* (1996) studied the rapid and efficient method for extraction of DNA from soils which resulted in highly purified DNA with minimal shearing was developed. Universal small-subunit-rRNA primers were used to amplify DNA extracted from the pasture soil. A culture independent survey of the microbial diversity in clover grass pasture conducted by using 16S rRNA analysis which found that three kingdoms viz., the Proteobacteria (16.1%), the Cytophaga-Flexibacter-Bacteroides group (21.8%), and the low G+C- content gram-positive group (21.8%) were dominant.

Palleroni (1977) was of opinion that the modern approaches based on use of molecular techniques fail to provide sufficient and reliable information for estimation of prokaryotic diversity. However, Throne and Cloete (2000) suggested the simple morphology of most microbes provides few clues for their identification and physiological traits are often ambiguous. Molecular approaches based on 16S ribosomal RNA (rRNA) sequence analysis allow direct investigation of the community structure, diversity, and phylogeny of microorganisms in almost any environment, while quantification of the individual types of microorganisms or entire microbial communities may be addressed by nucleic acid hybridization techniques. They proposed the methods of recombinant DNA techniques,

16S rRNA sequence analysis and use of fluorescently labeled population of specific rRNA probes for studying diversity and phylogeny of microorganisms.

Zhou *et al.* (2002) examined the four geographically different soil samples from 29 locations for defining the determinants that drive microbial community structure in soil using rRNA based cloning approach and reported that spatial and resource factors influence microbial diversity in soil.

The molecular techniques used for studying microbial diversity have also helped to identify the factors influencing microbial activity. Bowman and McCuaig (2003) used 16S rDNA clone library analysis to assess the prokaryotic diversity and changes within the community structure of Mertz glacier, Polynya region of the Antarctic continental shelf and stated that the species richness decreases with increase in depth of sampling. The results indicated that at the oxic sediment surface (depth, 0 to 0.4 cm) the microbial community appeared to be dominated by a small subset of potentially *r*-strategist (fast-growing, opportunistic) species, resulting in a lower-than-expected species richness of 442 operational taxonomic units. At a depth of 1.5 to 2.5 cm, the species richness was much higher, with the community dominated by numerous gamma and delta proteobacterial phylotypes. At a depth of 20 to 21 cm, a clear decline in species richness occurred, accompanied by a larger number of more phylogenetically divergent phylotypes and a decline in the predominance of *Proteobacteria*.

Girvan *et al.* (2003) studied the soil bacterial communities in three separate arable farms in eastern England using a polyphasic approach combining traditional soil analysis, analysis of the physiological properties of the isolates as well as nucleic acid profiling and reported that soil factor was the key determinant of the bacterial diversity in arable soils. Leguminous crops on particular soil types had a positive soil types has a positive effect upon organic matter and resulted in small changes in

the active bacterial population. The active population was therefore more indicative of short-term management changes.

Okonski *et al.* (2003) studied the influence of soil amendments on microbial properties of the acidic, heavy metal contaminated soil from Cu-Ni smelter-damaged landscape of Canada and reported that the abundance of bacteria, actinomycetes and microfungi was found to be extremely low in these soils. They counted and identified representatives of 14 species of soil micro-fungi. The highest biodiversity of microfungal community and actinomycetes was recorded in soil treated with zeolite whereas limed soil had the least diversity of both the groups.

Microorganisms have come to stay as a cosmopolitan conglomerate of highly compatible organisms. They are found in all the extreme habitats of pH, temperature, water and salt stress. The 'deep hot biosphere' has been recognized due to unique microbial-animal assemblages and nutrient dynamics. It points out the versatility and importance of microbes in sustaining the life. The microbes play important role in agriculture, environment, medicine, industry and because of their remarkable inherent physiological and functional diversity. Very well recognized, efficient and commercially exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, which play a vital role in sustainable agriculture (Johri *et al.* 2005).

2.3.1 Isolation and microbial population :

Jayaswal *et al.* (1990) isolated and characterised the antagonistic bacteria from unshed caryopses of *T. dactyloides*. The colonies grown on King's B medium or LB agar found yellow pigmented, circular smooth and pigmented. They were confirmed as *Pseudomonas fluorescens* on the basis of morphological and physiological characterization.

Picard *et al.* (2000) studied the biodiversity of 188 isolates of 2,4-diacetylphloroglucinol (DAPG) producing pseudomonads isolated from maize rhizosphere at different stages of plant growth by using 16S rDNA

analysis which indicated increase in the frequency of DAPG producers in rhizosphere over time.

Kumar *et al.* (2002) *Pseudomonas* sp. were isolated using King's B medium from 31 soil samples collected from rhizosphere of rice and sugarcane around Madurai which were confirmed such as colonies are fluoresced under UV light ($\lambda=356$ nm) and further purified on same medium.

Spiers *et al.* (2005) observed that the pseudomonads are found in large numbers in terrestrial, freshwater and marine environments and also form intimate associations with plants and animals. Their universal distribution indicates a remarkable degree of physiological and genetic adaptability.

Ramette *et al.* (2006) studied *Pseudomonas* populations in the tobacco rhizosphere showing antagonistic properties towards *Thielaviopsis basicola*, the incitant of black root rot of tobacco.

Botelho and Mendonça-Hagler (2006) stated that one of the most abundant microorganisms in the rhizosphere is fluorescent *Pseudomonas* spp. They have been considered as an alternative to agrochemicals for controlling plant diseases.

Joseph *et al.* (2007) isolated the PGPR bacteria from soil samples collected from west of Allahabad Agriculture Institute, India by using King's B medium. Megha *et al.* 2006 isolated 52 isolates of fluorescent pseudomonads from forest soils of Western Ghat, Uttara Kannada district from Karnataka.

Das *et al.* (2008) isolated 126 fluorescent *Pseudomonas* spp. from different sorghum growing regions of India and evaluated for *in-vitro* antifungal activities.

Rekha *et al.* (2010) isolated *Pseudomonas fluorescens* from the rhizosphere soil samples obtained around Chennai and confirmed by morphological, biochemical test. They reported that 1.3×10^7 CFU/ml bacteria isolated from the soil samples. 54 strains were isolated from soil

samples and 5 were selected and among these strains, *Pseudomonas fluorescens* potential strain was observed.

2.3.2 Morphological and cultural characterization:

Brown and Lobury (1968) suggested that direct detection of fluorescence around the colonies could be used for the identification of fluorescent pseudomonads.

While some species of *Pseudomonas* are known to produce various kinds of pigments, the allocation of these pigments in the genus is uncertain (Palleroni *et al.* 1970).

Pseudomonas are typically Gram-negative, chemoheterotrophic and motile rods with polar flagella. The genus *Pseudomonas* includes the most diverse and ecologically important bacteria. (Palleroni *et al.* 1973). Some of them produce water-soluble yellow-green pigments and fluoresce under low wavelength UV radiation. Most *Pseudomonas* species can be assigned to one of the five natural clusters called RNA similarity groups. Group one is the largest among the five groups, which includes, fluorescent and non- fluorescent species.

The fluorescent pseudomonads are gram-negative, rod shaped and aerobic bacteria associated with rhizosphere soil and plants. They include *Pseudomonas fluorescens*, *P. aeruginosa*, *P. putida* and *P. aureofaciens*. A number of phenotypic and biochemical methods have been developed and used for characterization of the pseudomonad isolates. The tests conducted for characterization and identification of fluorescent pseudomonads are primarily based on physiological and nutritional studies (Holt *et al.* 1984).

Johnson and Palleroni (1989) reported that the *Pseudomonas* species have ability to produce water-soluble yellow green pigments and make up a diverse group of bacteria that can be visually distinguished from the other bacteria in soil due to its pigmentation ability. Due to the simple nutritional requirements, they are relatively abundance of these organisms in soil. The important fluorescent *Pseudomonas* species are *P.*

fluorescens, *P. aeruginosa*, *P. putida*, *P. cichorii*, *P. syringae*, *P. viridiflava* and *P. chlororaphis*.

Jayaswal *et al.* (1990) reported that all the antifungal bacteria isolated were short rods (approximately 1 x 1.6 μm) that mainly grew in pairs or in clusters, and they were gram negative, lophotrichous and motile. Strain RJ2 was used for further characterization. This strain grew at 20 to 40°C but not at 41°C. Colonies grown on LB agar or King B medium at 30°C for 16h were yellow pigmented, non fluorescent, raised, circular, smooth and metallic. The strain was aerobic, failed to grow under acidic conditions was oxidase and catalase positive but indole negative and did not produce gas from sugars. Because these characteristics are main features of genus *Pseudomonas*, the strain RJ2 belongs to this genus.

Cartwright *et al.* (1995) also reported that *Pseudomonas* were aerobic, G-ve, strictly aerobic, rod shape bacteria.

Dwivedi and Johri (2003) reported that Fluorescent pseudomonads are ubiquitous bacteria that are common inhabitants of the rhizosphere and are the most studied group within the genus *Pseudomonas*. They can be visually distinguished from the other *Pseudomonas* species of soil by their ability to produce water-soluble yellow green pigments. They comprise of *Pseudomonas aeruginosa*, the type species of the genus, *P. aureofaciens*, *P. chlororaphis*, *P. fluorescens*, *P. putida* and the plant pathogenic species *P. cichorii* and *P. syringae*. Most plant beneficial pseudomonads are quite heterogenous and comprise a collection of non-enteric, gram-negative strains that are generally aerobic, non-fermenting and motile.

Siddiqui *et al.* (2005) reported that 20 isolates of fluorescent pseudomonads and *Bacillus* were isolated from pathogen suppressive soils of pigeon pea fields from Uttar Pradesh, India. These isolates were characterized for the Gram reaction, Growth at 4°C and 41°C and fluorescens on the Kings B medium and the isolates showing fluorescens

under the UV are selected which are grow at 4°C and not grow at 41°C are confirmed as *P. fluorescens*.

Da Silva and Almeida (2006) revealed that the medium was prepared from brewery waste yeast with and without mineral salts to study growth and yellow green fluorescent pigment production (YGFP) by *Pseudomonas fluorescens*. The King's medium used for detection of siderophore production were expressively weaker inducers of YGFP formation when compared to FYE medium. Joseph *et al.* 2007 reported that *Pseudomonas* were rod shape, gram negative in reaction and producing pigments on King's B medium. The fluorescent was observed on King's B medium. King *et al.* (1954) studied that they prepared two different media containing as common ground potassium and magnesium salts and glycerol. The medium with porease peptone was denominated as medium B. The other one with peptone was called medium A.

Megha *et al.* (2006) studied that all 52 fluorescent *pseudomonas* isolates from forest soils of Western Ghat of Uttara Kannada District, Karanataka. Out of the 52 isolates, 16 produced green colored colonies, 34 produced light green coloured colonies and two isolates produced light orange coloured colonies. Twenty four isolates formed round and non-spreading type colonies whereas the remaining 28 isolates produced irregular shaped spreading type colonies. The colonies showed wide variation in the intensity of fluorescence. These isolates were tested for biochemical characters *viz.*, starch hydrolysis, lipid hydrolysis, casein hydrolysis, arginine hydrolysis, H₂S hydrolysis, acid and gas production, indole production, catalase test, oxidase test, urase test, denitrification and gelatin liquaefication as per the procedures by Cappuccino and Sherman. On the basis of morphological and biochemical characteristics, 37 isolates were tentatively identified as *Pseudomonas fluorescens*, 13 as *P. aeruginosa* and two as *P. aureofaciens*.

Yusran *et al.* (2008) revealed that *Pseudomonas* isolated from Indonesian soils have been investigated with respect to biochemical and physiological characteristics and their effectiveness against *Fusarium*

oxysporium f.sp. radicis-lycopersici Jarvis and Shomaker (FORL 11r). Four soil samples collected from the rhizosphere soil land 28 pseudomonads strains were isolated and used for the biochemical and physiological study. Distinct colonies showing fluorescence under UV light at 360 nm were picked and streaked on KB agar medium.

Battu *et al.* (2009) studied that plant growth promoting rhizobacterial strains belonging to fluorescent pseudomonads were isolated from the rhizosphere of rice. Among 30 strains that were confirmed as *Pseudomonas fluorescens*, these, *P. fluorescens* strains were characterized by PCR-RAPD analysis and biochemical methods. Ten exhibited strong antifungal activity against *P. oryzae* and *R. solani* mainly through the production of antifungal metabolites.

Siddiqui and Shakeel (2009) revealed that two hundred soil and root samples were collected from different localities of Aligarh. Twenty one isolates of rhizobacteria belonging to *Pseudomonas* were isolated from *M. icognita* suppressive soils of pigeon pea fields. After gram staining *Pseudomonas* identification was confirmed by growing on King's B medium. Green fluorescence of *pseudomonas* spp. was very clear on King's B medium. Characterization test for the identification of *Pseudomonas* showed that out of twenty one isolates, only six isolates were positive for levan production, gelatin liquefaction, growth at 41°C and catalase test while negative for starch hydrolysis and growth at 41°C.

Rekha *et al.* (2010) reported that *Pseudomonas fluorescens* are gram negative, cell size small, rod shape, yellow green in colour, large colony size and irregular was observed. Whereas, the biochemical tests citrate, oxidase, catalase nitrate reduction and gelatin liquefaction were found to be positive reaction and Indole production, methyl red, VP and starch hydrolysis was found negative in reaction.

2.3.3 Biochemical characterization:

On the basis of similarity observed biochemical tests, the species of fluorescent pseudomonads are again grouped into different biovars and

subgroups (Barett *et al.* 1986). Thus, rapid identification of economically viable and potentially bioagents is possible through various biochemical characterization methods (Weller *et al.* 2002; Onczena *et al.* 1999 and Singh *et al.* 2000).

Jayaswal *et al.* (1990) reported that all the antifungal bacteria isolated were aerobic, failed to grow under acidic conditions was oxidase and catalase positive but indole negative and did not produce gas from sugars. Because these characteristics are main features of genus *Pseudomonas*, the strain RJ2 belongs to this genus.

Pseudomonas species associated with most plants belong to *Pseudomonas fluorescens* and *Pseudomonas putida* complex. There was no clear distinction between *Pseudomonas fluorescens* and *Pseudomonas putida* (Sheath *et al.* 1981). However, these two species are identified based on gelatin liquefaction and trehalose utilization. In this, *Pseudomonas fluorescens* exhibits a positive reaction for both the tests whereas *Pseudomonas putida* shows negative reaction to these tests (Hildebrand *et al.* 1992).

Rangarajan *et al.* (2001) reported that all 124 *Pseudomonas* isolates from three rhizosphere were positive for oxidase, indole formation and Simmons citrate but negative for lysine decarboxylase, and cellulobiose.

Joseph *et al.* (2007) characterised the selected isolates of *Pseudomonas* (35), *Bacillus* (40), *Azotobacter* (40) and *Rhizobium* (35) for oxidase test, H₂S production, IMViC tests, NO₂ reduction, starch and gelation hydrolysis and observed that *Pseudomonas* was positive in oxidase, H₂S production, starch hydrolysis and negative for indol, gelation hydrolysis and Methyl red test.

Megha *et al.* (2007a) tested fluorescent pseudomonads for biochemical characters viz., starch hydrolysis, lipid hydrolysis, casein hydrolysis, arginine hydrolysis, H₂S hydrolysis, acid and gas production, indole production, catalase test, oxidase test, urase test, denitrification and gelatin liquaefication as per the procedures by Cappuccino and

Sherman(1992). All the 52 isolates of fluorescent pseudomonads isolates from forest soils of Western Ghat of Uttara Kannada District, Karanataka were positive for indole production, gelatin liquefaction and arginine hydrolysis. 48 isolates were positive for lipid and casein hydrolysis while most of the isolates produce H₂S and were positive for oxidase and catalase test and none was positive for starch hydrolysis, urease activity and acid and gas production. 13 isolates of *Pseudomonas aeruginosa*, 2 isolates of *Pseudomonas aureofaciens* tentitatively identified based on the morphological, biochemical and physiological characters. Similarly, the remaining 37 isolates were tentatively identified as *Pseudomonas fluorescens*.

Rekha *et al.* (2010) tested *Pseudomonas fluorescens* for biochemical characters viz., indole production, catalase test, oxidase test, nitrate reduction and gelatin liquaefication and results showed that and positive in catalase test, oxidase test, nitrate reduction and gelatin liquaefication and negative in indole production reaction.

Maleki *et al.* (2010) reported that the *Pseudomonas fluorescens* CV6 was physiologically and biochemically characterised by oxidase, gelatin liquefaction and Aginine dihydrolase and growth at 4⁰C and positive in reaction wheras no growth was observed at 41⁰C.

2.3.4 Functional Diversity:

2.3. 4.1 Antagonistic property:

The most widely studied group of rhizosphere bacteria with respect to the biocontrol is the fluorescent. The worldwide interest in the fluorescent *Pseudomonas* sp. as biocontrol agents gained momentum after the initial studies conducted at the University of California, Berkeley, during the late 1970's by Weller *et al.* (1988).

Howell and Stipanovic (1979) reported that a strain of *Pseudomonas fluorescens* antagonistic against *R. solani* was isolated from rhizosphere of cotton seedlings. An antagonistic strongly inhibitory to *R. solani* was isolated from *P. fluorescens* cultures and identified as pyrrolnitrin. The

antibiotic also inhibited growth of other fungi associated with the cotton seedling disease complex.

Howell and Stipanovic (1980) found pyoluteorin producing fluorescent pseudomonad to be effective against *Pythium*-induced damping-off in cotton seedlings. The strain Pf-5 of *Pseudomonas fluorescens* was antagonistic in vitro to *Pythium ultimum*. An antibiotic, pyoluteorin was isolated from cultures of the Pf-5 strain and found to be inhibitory to *P. ultimum*, but not to *R. solani*. Treatment of cottonseed with pyoluteorin or with *P. fluorescens* at the time of planting in *P. ultimum*-infested soil increased seedling survival from 33 to 65% and from 28 to 71%, respectively.

In vitro antagonistic potential of *P. fluorescens* against *F. solani* was assessed by dual culture technique (Upadhyay and Rai, 1987) by measuring radial growth of the pathogen as well as that of *P. fluorescens*. 3 mm diameter mycelium disc of 4 day old culture of *F. solani* and disc of *P. fluorescens* were placed in PDA medium in petriplate at the opposite sides keeping at equal distance from the centre of the petriplate. The plates were incubated at 28 ± 2 °C for 3 days, five replications were maintained. The petriplate incubated only with pathogen served as control.

According to Pal and Jalali (1998), the major factor in suppression of root pathogens by antagonistic bacteria is the production and release of certain inhibitory metabolites which mainly include the antibiotics, bacteriocins, hydrocyanic acid (HCN) and several other extracellular metabolites.

The fluorescent pseudomonads are classified into three categories viz., pathogens, biodegraders and root colonizers/biocontrol agents. The root colonizers/ biocontrol agents exert a protective effect on the roots through antagonism against phytopathogenic fungi and bacteria (Dwivedi and Johri, 2003). O'Sullivan and O'Gara (1992) explained siderophore/antibiotic mediated suppression, HCN production, competition and successful root colonization as major mechanisms with

which the fluorescent pseudomonads suppress plant root pathogens. Different mechanisms involved in biocontrol activity of fluorescent pseudomonads are reviewed hereunder.

The presence of *Pseudomonas fluorescens* in the rhizosphere of wheat plants was reported. Migration was significantly stimulated by various wheat genotypes and by synthetic attractants, though they did not in the intensity of their effect. (Bashan, 1986). The fluorescent pseudomonads are now well recognized as plant growth promoting rhizobacteria and as biocontrol agents against fungal and bacterial plant pathogens.

The rhizosphere bacteria are ideal bioagents as they are able to provide frontline defense to plant roots against attack of plant pathogens. The bacterial antagonism of plant pathogens leads to sustainable disease control (Pal and Jalali, 1998).

Rao *et al.* (1999) studied the influence of five fluorescent pseudomonad strains (GRP₃, GRP₆, PRS₉, RBP₂ and PEn₄) on growth and nodulation of lentil in a *Fusarium* infested soil. PRS₉ and GRP₃ reduced the population of pathogen by 25-50 and 50-75 per cent in rhizosphere and rhizoplane respectively besides improved nodulation and growth.

In the microbial society the antagonist bacterium like *Bacillus thermophilic* (Borkar *et al.*, 1999), *P. fluorescence* and fungi trichoderma plays important role to maintain the social colonization of microbes. Among the naturally occurring population of fluorescent pseudomonas and *Trichoderma harzianum* in rhizosphere has high antagonistic potential against several plant pathogens.

Varshney and Chaube (1999) studied fluorescent pseudomonads and *Trichoderma spp.* (isolated from rhizosphere of tomato) showing plant growth promoting activity and high biocontrol potential for rhizosphere competence. The bioagents colonized root segments up to length of 1 cm but with extension of roots, the population counts decreased sharply and significantly. *Trichoderma spp.* could not be recovered from segments measuring 4-5 cms. The decline in bacterial counts was over 90 per cent.

However, co-inoculation of *Trichoderma harzianum* and *Fluorescens pseudomonas* bacterial strain were highly inhibitory to *T. harzianum* (Varsheny *et al.* 2000)

Isaac *et al* (2000) reported that *Pseudomonas fluorescence* isolates with confirmed antagonistic activity against *Aspergillus flavus*. There was reduction of infection produced by *A. flavus* from 35.8 % to 3.2% of maize ear bioassays.

Sutra *et al.* (2000) studied total aerobic bacteria and fluorescent pseudomonads were counted in bulk and from rhizospheric soils of banana plants of 14 plantations in Martinique. Fluorescent *Pseudomonas* isolates were then identified and investigated for *in vitro* antagonism towards *Cylindrocladium* sp., a fungal pathogen. They reported that the total aerobic bacteria and fluorescent pseudomonads were significantly more abundant in banana rhizosphere soils than in bulk soils. Among 58 fluorescent *Pseudomonas* isolates, 41 were identified as *Pseudomonas fluorescens* biovar V and 17 as *P. putida* biovar A. Six strains exhibited an antagonism towards *Cylindrocladium* which causing banana root infections. Among them, *P. putida* strain 93.1 totally blocked fungal growth. No relationship was established between the antifungal effect and enzyme or hydrogen cyanide production by bacteria, suggesting that siderophores and other compounds were involved in fungal inhibition. Antagonistic *fluorescens* pseudomonads represent a potential for the biological control of banana root infections by *Cylindrocladium* sp.

Kumar *et al.* (2002) reported that Plant growth promoting rhizobacterial strains belonging to fluorescent pseudomonads were isolated from the rhizosphere of rice and sugarcane. Among 40 strains that were confirmed as *Pseudomonas fluorescens*, 18 exhibited strong antifungal activity against *Rhizoctonia bataticola* and *Fusarium oxysporum*, mainly through the production of antifungal metabolites.

Tripathi and Johri (2002) isolated fluorescent pseudomonads from rhizoplane (PEn-4) and rhizosphere of pea (PRS-1) and wheat (WRS-24) and studied for *in-vitro* inhibition of *Colletotrichum dematium*, *Rhizoctonia*

solani and *Sclerotium rolfsii*. They were more effective against *C. dematium* and least effective against *S. rolfsii*. The isolates, however, delayed the sclerotial germination of *R. solani* and *S. rolfsii*.

Cornellis and Matthijs (2002) reported that the siderophores, like pyoverdines or pseudobactins (which are efficient iron scavengers) produced by the majority of fluorescent pseudomonads, could be used as an interesting tool to study the diversity and taxonomy of fluorescent pseudomonads as each species produce a different pyoverdine.

Kurek and Jaroszuk-Scise (2003) conducted a study to determine effect of different forms of N-fertilization (NO_3 , urea, $\text{NO}_3 + \text{NH}_4^+$ (1:1)) and enrichment of the soil with clay on the efficiency of *P. fluorescens* strains as PGPR and as antagonists to *Fusarium culmorum* in rye rhizosphere. Two streptomycin and kanamycin-resistant *P. fluorescens* strains produced Fe(III)-chelating compounds (including siderophores) and could inhibit *in-vitro* growth of *F. culmorum* (cycloheximide resistant) strain by competition for Fe(III). They also observed that a mixture of NO_3 and NH_4^+ was the best form of fertilization for enhancing the capacity of the *P. fluorescens* strains to promote plant growth and inhibit *Fusarium* growth.

Mercado-Blanco *et al.* (2004) noted effective control of *Verticillium* wilt of olive plants under controlled conditions by all the eight isolates of *P. fluorescens* and *P. putida*. All isolates produced the green fluorescent siderophore, pseudobactin, under *in-vitro* conditions while some *P. fluorescens* isolates produced either salicylic acid in succinate medium or HCN in *in-vitro* assays. The antagonistic activity of *P. fluorescens* and *P. putida* isolates from olive against defoliating (D) and nondefoliating (ND) *V. dahliae* pathotypes varied with culture media. On PDA, isolates of *P. putida* were more inhibitory to the pathogen than those of *P. fluorescens*. Results indicated that root treatment with some of *P. fluorescens* isolates significantly delayed the onset of symptoms, and reduced the final disease incidence and severity by 31–82% and 73–96%, respectively, compared with the nontreated controls, under conditions of severe *Verticillium* wilt.

Suryakala *et al.* (2004) reported that *P. fluorescens* isolated from different crop rhizosphere were antagonistic to fungal pathogens like *F. oxysporum*, *Alternaria sp.* and *Colletotrichum capsicii*. They reported that siderophores exerted maximum impact on *F. oxysporum* than on *Alternaria sp.* and *Colletotrichum capsicii*

Bhatia *et al.* (2005) reported that seeds of sunflower treated with fluorescent pseudomonads PS I and PS II reduced the collar rot incidence by 56.9 to 69.8 per cent in *S. rolfsii* infested soil.

Sottero *et al.* (2006) studied the *in-vitro* antagonism between rhizobacteria and *Fusarium sp.* to verify if growth promotion ability was determined by biocontrol. Sixty-four fluorescent pseudomonads from the rhizosphere of different lettuce varieties were tested out of which 12 isolates showed antagonistic activity against *Fusarium sp.* However, only three of them enhanced growth in lettuce and colonized the root collar.

Ahmadzadeh *et al.* (2006) reported that collection of 47 fluorescent *Pseudomonas spp.*, some with known *in-vitro* biocontrol activity against soil-borne phytopathogenic fungi and the potential to produce siderophore, HCN and protease. *Pseudomonas fluorescence* strains showed antagonistic activity against soil borne plant pathogens viz., *Macrophomina phaseolina*, *Rhizoctoniasolani*, *Phytophthora nicotianae* var. *parasitica*, *Pythium sp.* and *Fusarium sp.* The results indicated that 66.0, 40.4, 63.8, 48.9 and 27.6 per cent of the strains were antagonistic to *R. solani*, *M. phaseolina*, *Pythium sp.*, *P. nicotianae* and *Fusarium sp.*, respectively. Among the 47 strains, 76.59%, 97.87% and 17% produced protease, siderophore and HCN, respectively.

Megha *et al.* (2007b) Fluorescent pseudomonads are well recognized as plant growth promoting rhizobacteria with nutrient mobilizing ability and as bio control agents against plant pathogens. 52 Fluorescent pseudomonas from Uttara Kanada district from Karnataaka were tested for their biocontrol potential against two bacterial and two fungal pathogens, 44 were inhibitory against one or the other test pathogen. Among the isolates tested, 14 were inhibitory to *Ralstonia solanacearum*,

10 were inhibitory to *Xanthomonas campestris*, 32 to *Fusarium oxysporum* and 24 to *Rhizoctonia bataticola*. Many of them showed biocontrol potential against more than one pathogen.

Siddiqui (2007) evaluated the 18 isolates of fluorescent pseudomonads and *Bacillus* spp. in laboratory and greenhouse for biocontrol of *A. triticina* which were obtained from *Alternaria triticina* suppressive soils of wheat fields. The six isolates (Pa22, Pf27, Pa28, B25, B28, and B30) were considered potential biocontrol agents on the basis of fluorescence, inhibitory effect on *A. triticina* and colonization of wheat roots.

Rini and Sulochana (2007) studied the antagonism property 56 fluorescent pseudomonad isolates *in-vitro* against *R. solani* and *F. oxysporum* infecting tomato. *P. fluorescens* isolates P51 and P28 showed the greatest inhibition of *R. solani* whereas the isolates P20 and P28 were most effective against *F. oxysporum*.

Vinod Kumar *et al.* (2007) revealed that the effectiveness of *Pseudomonas fluorescens* isolates were tested against charcoal rot of chickpea. The results revealed that isolate Pf4-99 is quite effective to reduce the charcoal rot disease both in field and greenhouse, and also increases seed yields significantly. Therefore, this isolates used as an efficient biocontrol agent against charcoal rot disease.

Weller, (2007) revealed that the *Pseudomonas* spp. are ubiquitous bacteria in agricultural soils and findings that have had many traits that make them well suited as biocontrol agents of soilborne significant impact on shaping our current understanding of biological pathogens. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents. These include the ability to grow rapidly *in vitro* and to be mass-produced; rapidly utilize seed and root exudates; colonize and multiply in the rhizosphere and spermosphere environments. They produce an antibiotics, siderophores, volatiles, and growth-promoting substances, compete aggressively with other microorganisms, and adapt to environmental stresses.

Malathi *et al.* (2008) tested 12 isolates of *Pseudomonas* spp. against 13 pathotypes of *Colletotrichum falcatum*. The antagonistic strains exhibited greater variation in their activity depending on virulence of the pathotype. Among the four efficient *P. fluorescens* strains selected based on inhibition of various pathotypes, ARR1G and VPT4 were effective against tropical pathotypes and FP7 showed moderate effect against all the pathotypes. The strain KKM2 was effective against sub-tropical and weaker tropical pathotypes.

Hultberg *et al.* (2008) studied the presence of antibiotic and biosurfactant producing strains of fluorescent pseudomonads in a closed hydroponic system equipped with a slow filter. A total of 271 strains of Pseudomonads were isolated and their ability to inhibit the growth of plant pathogens, *Pythium ultimum*, *Phytophthora cryptogea* and *F. oxysporum* was determined using dual culture plating. Production of antibiotics or biosurfactants was observed to be a common trait among the fluorescent pseudomonads. Production of antibiotics or biosurfactants was observed to be a common trait among the fluorescent pseudomonads within the closed hydroponic system and it was affected by the filter. *P. ultimum* was the pathogen that was most sensitive to antibiotics produced by the fluorescent pseudomonads.

Naik *et al.* (2008b) reported that the fluorescent pseudomonads from banana rhizosphere isolates were screened for antifungal activity towards phytopathogenic fungi. Gene-specific primers identified the putative antibiotic producing isolates. The production of antibiotics was confirmed by TLC and HPLC. Genotypic analysis by BOX PCR resulted into 3 distinct genomic clusters at a 50% similarity level and 62 distinct BOX profiles. The isolates were designated as *Pseudomonas monteilii*, *P. plecoglossicida*, *P. fluorescens*, *P. fulva*, *P. mosselii*, *P. aeruginosa*, *P. alcaligenes* and *P. pseudoalcaligenes* based on the sequence similarity of 16S rRNA and construction of subsequent phylogenetic tree analysis.

Das *et al.* (2008) revealed that 126 fluorescent *Pseudomonas* spp. from different sorghum growing regions of India were isolated, selected

and evaluated for *in-vitro* antifungal activities and tested three selected strains for management of charcoal rot under field conditions. The isolates, SRB127, SRB129 and SRB288 inhibited mycelial growth of *M. phaseolina* ranging from 30.5 to 76.5% in dual culture assay. The cell-free culture filtrates of these strains at 20 per cent (v/v) concentration significantly reduced the formation and germination of microsclerotia of *M. phaseolina*. In the field, *P. chlororaphis* SRB127 emerged as effective biocontrol agent of charcoal rot of sorghum. The bacterium, when applied as seed treatment, reduced the charcoal rot incidence by >40 % and crop-lodging by >20 % coupled with the increased grain mass. The results indicates that the use of a selection of effective strains of bacteria can be used as useful component of integrated management of charcoal rot of sorghum.

Ahmadzadeh and Sharifi-Tehrani (2009) studied 103 fluorescent pseudomonads from wheat and common bean rhizosphere out of which 52 were antagonistic to *R. solani*.

Elmer (2009) conducted greenhouse studies to determine if soils infested with soil-borne pathogens and augmented with earthworms could reduce disease of asparagus, eggplant and tomato. Soils planted with asparagus were infested with *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*, eggplant with *Verticillium dahliae*, and tomato with *F. oxysporum* f. sp. *lycopersici*. In each host-disease system, earthworm activity showed increase in plant growth and decrease in disease. Plant weights were increased by 60 to 80 % and disease incidence was reduced to 50-70 %.

Singh and Sinha (2009) screened 5 fluorescent pseudomonads against *R. solani* causing rice sheath blight. Pfr1 and Pfr 5 grew very fast and attained a radial growth of 23.7 mm after 48 h. In dual culture test, 71.2 % inhibition of mycelial growth of *R. solani* was observed with Pfr 1 followed by Pfr 5 (66.4%). Viability of sclerotia after emergence in bacterial suspension declined with prolonged incubation and at the end of 5 weeks, most of the isolates caused 7-100 % loss in sclerotial germination.

Choudhary *et al.* (2009) stated that new biotechnological products are currently being developed based on stimulation of the plant defence response and on the use of plant-beneficial bacteria for biological control of plant diseases (biopesticides) and for plant growth promotion (biofertilizers). PGPR are employed as inoculants for biofertilization, phytostimulation and biocontrol. Various bacterial strains, in particular the fluorescent *Pseudomonas* spp., play an important role in the prevention of plant infectious diseases. They promote plant growth by suppressing pathogens, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance.

Prasanna Reddy and Rao (2009) reported that out of 30 fluorescent pseudomonad isolates from rice rhizosphere confirmed as *P. fluorescens*, 10 were highly antagonistic to *P. oryzae* and *R. solani* mainly through the production of antifungal metabolites.

Mehnaz *et al.* (2009) studied that they isolated, identified and characterized seven fluorescent pseudomonads from roots, shoots and rhizosphere soil of sugarcane and studied their impact on growth of sugarcane plantlets. The 16S rRNA gene sequence of five isolates showed close homology with *P. putida*, one with *P. graminis* and one with *P. fluorescens*. Physiological and biochemical characterization were also studied. Biocontrol potential was determined from agar diffusion assays of HCN production and production of antifungal compounds against local isolates of *Colletotrichum falcatum* inciting red rot disease. All the seven isolates showed significant increase in fresh and dry masses but only five strains increased the shoot height.

Siddiqui and Shakeel (2009) revealed that at the 21 isolates of *Pseudomonas* were tested for their antifungal activity against *F. udum* in dual inoculation test. Only 8 isolates of *Pseudomonas* (P704, P710, Pf718, Pf719, P725, Pf738, Pa737 and Pf 740) showed antifungal activity, while the remaining 13 isolates had no antifungal effect against *F. udum*.

Srivastava and Shalini (2009) studied the antifungal activity of different strains of *Pseudomonas fluorescens* were tested against some

plant pathogens such as *Alternaria cajani*, *Curvularia lunata*, *Fusarium* sp., *Bipolaris* sp. and *Helminthosporium* sp. in *in vitro*. Different concentrations (1000, 2000, 3000, 4000 and 5000 µg/mL) of *Pseudomonas fluorescens* were used and maximum spore germination of fungus was inhibited at 4000 and 5000 µg/mL. The result indicated that all the strains of *Pseudomonas fluorescens* presented a most significant value against *Alternaria cajani* and *Curvularia lunata*. Out of the five strains studied, the best result was shown by A-5, which showed almost complete inhibition against pathogenic fungi such as *Curvularialunata* and *Fusarium* sp. at 4000 and 5000 µg/mL while strain L-5 was resistant against *Fusarium* sp. and *Helminthosporium* sp. at 5000 µg/mL.

Couillerot *et al.* (2009) reported that many *Pseudomonas fluorescens* strains show potential to control plant pathogens. In taxonomic terms, several of them are indeed *P. fluorescens sensu stricto*, while others belong in fact to neighbouring species of the *Pseudomonas fluorescens* complex or to ill-defined related species within the fluorescent *Pseudomonas* spp. These bacteria have been prominent models for rhizosphere ecological studies and analysis of bacterial secondary metabolism, and in recent years knowledge on their plant-beneficial traits has been considerably enhanced by widening the focus beyond the case of phytopathogen-directed antagonism. *Pseudomonas fluorescens* biocontrol agents and a better exploitation of their plant-beneficial properties for sustainable agriculture.

Rekha *et al.* (2010) studied the antibacterial activity of *Pseudomonas fluorescens* against pathogenic bacteria. The *Pseudomonas fluorescens* strain isolated from the study seemed to be highly potential in controlling pathogens of health significance. The results indicated that strains of *Pseudomonas fluorescens* presented a significant value against *Salmonella typhi*, *Streptococcus mutans*, *Bacillus subtilis*, *Shigella sonnei* and no activity against *Vibrio cholerae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens*.

Maleki *et al.* (2010) studied that antagonistic rhizobacteria, more especially fluorescent pseudomonads and certain species of *Bacillus*, are known to control of fungal root diseases of agronomic crops. 144 bacteria were isolated from cucumber rhizosphere and screened as potential biological control agents against *Phytophthora drechsleri* in vitro and glass house condition. They studied then broad spectrum antifungal activity of *Pseudomonas fluorescens* CV6 against phytopathogenic fungi like *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporium* f.sp. *vasinfectum*, *Fusarium oxysporium* f.sp. *lycopersici* etc. The % mycelium inhibition was ranged from 45.32 to 85.82% in most of the pathogen except no inhibition in *Alternaria solani* and *F. oxysporium* f.sp. *lycopersici* (10.02 – 15.32 % inhibition) It was able to produce considerable amount of siderophore and acid indole-3-acetic acid (IAA). The bacterium showed positive reactions for HCN, catalase, protease and phosphatase and negative for production of pectinase, lipase and cellulose.

2.3.4.2 Siderophore and Hydrocyanic acid production:

A great diversity among rhizosphere microorganisms have been described, the microorganisms have been characterized and in many cases tested for their beneficial activity of biocontrol against soil-borne plant pathogens. They can produce antibiotics, HCN, siderophores, and a variety of enzymes or induce systemic resistance in host plants that may limit the damage caused by phytopathogens. In addition, they can also compete with the pathogens for colonization sites and nutrients.

Kloepper *et al.* (1980a) reported the concept of siderophores which helps disease suppression for the first time. The siderophore production has been correlated with disease suppression potential of certain fluorescent *Pseudomonas* species. Competition for iron between pathogens and siderophores of fluorescent pseudomonads has been implicated in the biocontrol of wilts caused by *F. oxysporum* Similarly, Kloepper *et al.* (1980b) Fluorescent pseudomonad particularly *Pseudomonas putida* and *Pseudomonas fluorescens* which are commonly isolated from plant rhizosphere; have been shown to protect plants from

fungal infection. Two factors have been cited as essential for biocontrol : colonization of the rhizosphere and production of antibiotics. The antagonistic activities are presumably due to the production of siderophores.

Siderophores are the low-molecular-weight, extracellular protein synthesized by many microbes under iron limiting conditions to obtain iron essential for the growth of the organism. Siderophores have a high specific affinity for ferric iron which enters the into microbial cells as a ferric siderophore complex through outer-membrane receptors specific for the siderophore (Neilands, 1981).

Leong (1986) reported that siderophores are iron- chelating compounds produced by many micro organisms growing under iron-limiting conditions. It has been hypothesized that siderophores function as biostatic compounds by drastically reducing the amount of ferric ions available to certain rhizosphere microflora.

The production of siderophores have been linked to the disease suppression potential of certain fluorescent pseudomonads. A membrane receptor that specifically recognizes ferric-siderophore complex mediates the transport of iron into the cells. The ferric iron, whose concentration is very low in well-aerated soils, is sequestered in a form that cannot be utilized by the pathogen. Fluorescent siderophores that have very strong affinity for ferric ions are secreted during growth under low iron conditions. The resulting ferric siderophore complex is unavailable to other organisms but producing strain can utilize this complex via very specific receptor in the outer cell membrane (Hemming, 1986).

Buyer and Leong (1986) reported that *Pseudomonas* B10 produces yellow-green, pseudobactin and they secrete siderophores who have very strong affinity for ferric ion when growing under Fe-deficient conditions. The growth inhibition of fluorescent pseudomonads by specific bean-beneficial fluorescent pseudomonads is due in part to the inability of susceptible strains to utilize siderophores from beneficial strains to transport iron(III). The ability of a given pseudomonad to utilize another

pseudomonad's siderophore may depend upon its possessing a specific outer membrane receptor protein for that pseudomonad's ferric siderophore.

Fluorescent pseudomonads produce yellow green fluorescent siderophores of the pyoverdine type when grown under low iron. They are generally produced fluorescent, yellow-green, water-soluble siderophores, either pyoverdines or pseudobactins (Demange *et al.* 1987).

Later, the siderophores produced by the fluorescent pseudomonads were reported to be implicated in the suppression of plant pathogens (Bakker *et al.* 1986; Becker and Cook, 1988).

Dileep Kumar and Dubey (1993) reported siderophore production by fluorescent *Pseudomonas* sp. effective against several fungal and bacterial pathogens.

Rao *et al.* (1999) reported that siderophore production was an important mechanism in growth promotion of lentil and biocontrol of wilt caused by *Fusarium oxysporum* f. sp. *lini* with five strains of fluorescent pseudomonads

Manwar *et al.* (2000) isolated siderophore producing pseudomonads from soil and the amount of siderophores produced were calculated to be 57 and 50 per cent siderophore units respectively which exerted killing effect on plant deleterious fungi while promoting the growth of wheat plants.

Tripathi and Johri (2002) reported production of siderophores as one of the mechanisms for *in vitro* inhibition of *Colletotrichum dematium*, *Rhizoctonia solani* and *Sclerotium rolfsii* by fluorescent pseudomonads.

Suryakala *et al.* (2004) reported that the PGPR belonging to fluorescent pseudomonads produce extracellular siderophores of trihydroxamate type pyoverdines forming hexadentate ligands with Fe^{3+} ions when grown in casamino acid medium under iron deficiency. These siderophores were antagonistic to fungal pathogens like *F. oxysporum*, *Alternaria* and *Colletotrichum*. They reported that siderophores exerted

maximum impact on *Fusarium oxysporum* than on *Alternaria sp.* and *Colletotrichum capsicii*

Rachid and Ahmed (2005) reported that the four basal media, supplemented with different concentrations of iron were employed to study the effect of iron and different organic carbon sources on siderophore production in *Pseudomonas fluorescens*. The highest siderophore concentration was obtained in succinate medium. Ferric iron increased the growth yield and completely repressed siderophores production above 200 µg/l.

Dhanya and Potty (2007) revealed that *P. fluorescens* colonies were isolated from the rhizosphere soil of *Solenostemon rotundifolius* with serial dilution technique. Spectrophotometric analysis of the culture in standard succinate medium showed a peak between 420 to 450nm and indicated that the prescence of siderophores of ferrate hydroxamate nature. SDS-PAGE analysis of the extracted sample revealed the prescence of bands having molecular weight similar to siderophores and were determined based on the protein markers. *P. fluorescens* produced siderophores with molecular weight of 8.3, 9.5 and 12.5 KD and the average molecular weight of siderophore was 10 KD.

Schalk (2008) revealed that the fluorescent pseudomonads secrete siderophores called pyoverdines (Pvd), which form complexes with iron that are then taken up by the bacteria under iron-limiting conditions. Pyoverdines consist of a fluorescent chromophore derived from 2,3-diamino-6,7-dihydroxyquinoline and containing one of the bidentate groups involved in iron chelation, linked to a peptide moiety containing the other two bidentate groups required for binding to Fe³⁺. More than 100 different pyoverdines have been identified forming a wide family of siderophores. In the human opportunistic pathogen *Pseudomonas aeruginosa*, pyoverdine is necessary for infection and is considered a virulence factor.

Ahmadzadeh and Sharifi-Tehrani (2009) reported that the isolate UTPF16 to produce prolific amounts of siderophores, creating a large zone

of orange up to 160 mm². In all, 52 bacterial isolates with antifungal activity against *Rhizoctonia solani* were found. Of the 52 isolates, 41 were selected according to their high efficiency in *in vitro* antagonism, which was shown as inhibition zones in the dual-culture assay. Six of the 41 rhizobacteria, including isolates UTPF7, UTPF13, UTPF18, UTPF22, UTPF27 and strain CHA0 produced HCN.

2.3.4.3 Antibiotic sensitivity :

Somasegaran and Hoban (1994) have demonstrated genetic diversity of soil bacteria using the pattern of antibiotic resistance. Dobereiner and Baldani (1979) also reported diversity among *Azospirillum* strains with respect to their resistance to various antibiotics.

Deepa (2000) has reported that her isolates of fluorescent pseudomonads were sensitive to streptomycin, spectinomycin, kanamycin and tetracycline.

Suneesh (2004) also reported that most of the fluorescent pseudomonads of moist deciduous forests of Western Ghats were resistant to spectinomycin, ampicillin, nalidixic acid, chloramphenicol and kanamycin.

Jayaswal *et al.* (1990) reported that *Pseudomonas* strain RJ2 was showed the antifungal activities against the various corn pathogens viz., *Aspergillus niger*, *Fusarium moniliformae*, *Helminthosporium sp.*, *Macrophomona phaseolina*, *Trichoderma viride* etc. *Pseudomonas* strain RJ2 was tested for the antibiotic sensitivity activities against the chloramphenicol, tetracycline, ampicillin, gentamycin, kanamycin, penicillin, neomycin and streptomycin. The *Pseudomonas* strain RJ2 was found to be sensitive to chloramphenicol, tetracycline and resistant to antibiotics like ampicillin, gentamycin, kanamycin, penicillin, neomycin and streptomycin.

Siddiqui *et al.* (2005) reported that 20 isolates of fluorescent pseudomonads and *Bacillus* were isolated from pathogen suppressive soils of pigeon pea fields from Uttar Pradesh, India. The antibiotic sensitivity test was determined by standard disc diffusion method of Baur

et al. 1966. Sensitivity of these 20 isolated was checked against antibiotics ampicillin, penicillin, rifampicin, ciprofloxacin, co-trimoxazole and nalidixic acid. *Pseudomonas* isolates were found resistant to the antibiotic ampicillin and co-trimoxazole and nalidixic acid whereas most of the *Pseudomonas* strains are also resistant except Pa12, Pa20 and Pa310. The antibiotic rifampicin was observed sensitive to all *Pseudomonas* isolates except Pa20 and Pa116.

Megha *et al* (2006) studied that all the 52 isolates were resistant to ampicillin (100 µg/ml), chloramphenicol (5 µg/ml), nalidixic acid (10 µg/ml), rifampicin (25 µg/ml) and spectinomycin (20 mg/ml). While 39 isolates were resistant to kanamycin (50 µg/ml), 28 were resistant to tetracycline (20 µg/ml) and 30 were resistant to streptomycin (100 µg/ml).

2.3.4.4 Molecular diversity :

Microbial diversity studies are important in order to understand the microbial ecology in soil and other ecosystems. Several methods have been developed for analyzing the structure and diversity of bacterial communities. A lot of diversity exists among the fluorescent pseudomonads which can be studied by various methods. Study of molecular diversity by PCR based RAPD analysis allows identification and placement of the strains in genetically distinct and related groups.

Welsh and McClelland, (1990) revealed that the simple and reproducible fingerprints of complex genomes can be generated using single arbitrarily chosen primers and the polymerase chain reaction (PCR). No prior sequence information is required. The method, arbitrarily primed PCR (AP-PCR), involves two cycles of low stringency amplification followed by PCR at higher stringency. The strains can be distinguished by comparing polymorphisms in genomic fingerprints. The diversity analysis as well as finger printing of individuals can be performed with the help of PCR based RAPD markers. PCR based methodologies have been used in the past to study variation among closely related individuals and in phenotypically less polymorphic species.

Welsh *et al.* (1992) revealed that the large number of isolates have been collected, and studied the relationships among the various strains. Using genomic fingerprinting by an arbitrarily primed polymerase chain reaction, we resolved into three groups a collection of Eurasian and North American isolates of spirochetes that are generally categorized as *B. burgdorferi*. Group I strains have been identified in both North America and Eurasia, while strains belonging to *Borrelia* groups II and III have been found only in Eurasia. In this method, strain specific arrays of DNA fragments are generated by PCR amplification of arbitrary oligonucleotides.

The random primers are used in PCR reactions for randomly amplified polymorphic DNA studies. Molecular genetic maps are commonly constructed by analyzing the segregation of restriction fragment length polymorphisms (RFLPs) among the progeny of a sexual cross. A new DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence. These polymorphisms, simply detected as DNA segments which amplify from one parent but not the other, are inherited in a Mendelian fashion and can be used to construct genetic maps in a variety of species (Williams *et al.* 1990).

The RAPD has been widely used for differentiating the closely related organisms (McClelland and Welsh, 1994, Bassam *et al.* 1992) based on polymorphisms in the RAPD product patterns. As no information concerning specific sequence is needed because of use of the random primers, the RAPD is a very useful, fast, informative and unique technique. Further, the technique gives the opportunity to get information about the biodiversity in a group of isolates.

Hadrys *et al.* (1992) reported that molecular genetic markers have been developed into powerful tools to analyse genetic relationships and genetic diversity. As an extension to the variety of existing techniques using polymorphic DNA markers, the Random Amplified Polymorphic DNA (RAPD) technique may be used in molecular ecology to determine

taxonomic identity, assess kinship relationships, analyse mixed genome samples, and create specific probes.

Wiedmann *et al.* (2000) reported that 70 *Pseudomonas* sp. isolated from milk studied for phenotypic and molecular characterization and grouped them into five clusters using biolog system and enzyme profile analysis. These 70 isolates were further grouped into 38 ribogroups indicating that the ribotyping is highly discriminatory for *Pseudomonas* isolates.

Picard *et al.* (2000) analysed the diversity of a 2,4-diacetylphloroglucinol (DAPG) producing *Pseudomonas* population occurring naturally on the roots, in rhizosphere soil of maize and in the non-rhizosphere soil to assess the microbial diversity at five stages of plant growth. The total of 188 DAPG producing isolates were further classified into four amplified DNA restriction analysis groups. The main group comprised 89.8 per cent of the isolates. They were analyzed for the genetic diversity using RAPD PCR, which showed a high genetic diversity among those isolates.

Campbell *et al.* (2000) analyzed the diversity of *P. aeruginosa* strains by RAPD and also studied the reproducibility and discriminating power. A total of 200 isolates were evaluated by RAPD. All 600 samples were typeable, 197 of 200 isolates gave identical results. The technique was found to be highly reproducible and they were able to identify 131 distinct RAPD types.

Rangarajan *et al.* (2001) studied the *Pseudomonas* populations and analysed for biochemical characters and genetic diversity using molecular tools like RAPD and PCR-RFLP and found that increased salinity helped selection of *P. pseudoalcaligens* and *P. alcaligens* irrespective of the host rhizosphere. RAPD has been used as a tool to study the diversity of microorganisms.

Moenne-Loccoz *et al.* (2001) studied that fluorescent pseudomonads biocontrol strains affect interspecific diversity of resident culturable

fluorescent pseudomonads. The impact of 2, 4-DAPG producing biocontrol agent (*Pseudomonas fluorescens* F113 R.f.) on the diversity of the resident community of the culturable fluorescent pseudomonads at the rhizoplane of sugar beet seedlings grown in field was studied phenotypic as well as molecular tools like RAPD and rDNA restriction analysis. They found an increase of about 30 per cent in the culturable fluorescent pseudomonas community capable of growing on three carbon substrates viz., erythritol, adonitol and L-tryptophan.

Koh and Nou (2002) reported that screening a specific DNA fragment of *P. syringae* pv. *actinidiae* as well as similar strains that were isolated from kiwi fruits by using RAPD analysis and reported high diversity among the strains.

Ramesh Kumar *et al.* (2002) reported that Plant growth promoting rhizobacterial strains belonging to fluorescent pseudomonads were isolated from the rhizosphere and molecular diversity analysed by RAPD of rice and sugarcane. PCR-RAPD analysis was repeated at least three times and fingerprints were compared. The RAPD bands which appeared consistently were evaluated. RAPD analysis revealed that strains JAI2, JAI5 and JAI6 formed distinct groups compared to other strains and showed maximum similarity with strains PNC1, PAON3 and MKU3 respectively, which were isolated from different locations. Genotyping of these *P. fluorescens* strain was made by PCR-RAPD analysis, since differentiation by biochemical methods was limited.

Kwon *et al.* (2005) analyzed 160 isolates from seven soil samples using RAPD and revealed 53 genotypes, which were subsequently identified by their 16S ribosomal DNA sequences. Phylogenetic analyses of the 53 genotypes along with 43 fluorescent pseudomonad type strains separated the genotypes into 10 distinct clusters that included two phylogenetic groups that were not represented by previously described type strains. They concluded that the diversity of genotypes that was obtained from the soil samples was highly variable among the different

soils and appeared to be associated with different soil management practices also influence plant yields.

Megha *et al.* (2007a) subjected 15 functionally beneficial isolates out of 52 fluorescent pseudomonads from soils of different forests in Western Ghats of Uttara Kannada district to RAPD-PCR with eight random primers and reported that the PCR amplicons of fluorescent pseudomonads (15 selected isolates and 5 reference strains) obtained from eight random primers produced 127 polymorphic bands across the 20 strains. While the primers OPD-02 and OPO-09 produced minimum number of bands (9), maximum number of bands (25) was produced by OPO-16. All the primers showed 100% polymorphism. The dendrogram constructed from the pooled data had six clusters with maximum genetic similarity of 0.73 and minimum genetic similarity of 0.00. The dendrogram for the pooled data had six clusters which clearly grouped the biochemically identified species of pseudomonads together and high level genetic diversity.

Shilpi Mital and Bhavdish Johri (2007) revealed that the species variation was determined based on classical biochemical and physiological tests. Numerical analysis of these characteristics showed that 36% isolates from mandua were 100% similar whereas 18% strains from wheat showed 100% similarity. Based on numerical analysis of phenotypic characters, the Shannon diversity index was 0.64. Functional characterization based on plant growth promoting properties clearly showed greater diversity in wheat rhizosphere than mandua.

Picard and Bosco (2008) reported considerable genetic variation among plant-probiotic fluorescent pseudomonads (PFP) which plays an important role in rhizosphere competence and biocontrol ability of PFP strains. Understanding the mechanisms by which genotypic and phenotypic diversity occurs is helpful to choose agricultural practices that best exploit the indigenous PFP populations or to isolate new PFP strains for using them as inoculants. Genetic variability exists among the various isolates of pseudomonads depending on the geographical locations from

where these were collected, crops grown in a specific region and the agricultural practices employed in the location.

Tran *et al.* (2008) reported that fluorescent pseudomonads that produce biosurfactants against *Phytophthora capsici* causing root rot and studied their genotypic diversity as well as potential to control the disease and concluded that these strains indigenous to black pepper rhizosphere are genotypically diverse and provide a novel resource for the control of *P. capsici* root rot and growth promotion of black pepper stem cuttings. The results indicated that it is strong basis for further development of supplementary strategies with antagonistic bacteria to control foot and root rot of black pepper and to promote plant growth.

Moura *et al.* (2008) analyzed 15 non-fluorescent pseudomonad strains by RAPD using 19 primers. Numerical analysis of the RAPD band patterns allowed the differentiation of two major groups corresponding to *P. corrugata* and *P. mediterranea* isolates sharing approximately 50% similarity. At 80% similarity level, 4 clusters were distinguished. Cluster II, the largest one, had 6 strains closely related to *P. corrugata* type strain. Cluster III, included 5 strains closely related to *P. mediterranea* type strain. Cluster I was represented by 2 strains that shared 65% similarity with cluster II, which contained the *P. corrugata* type strain. One unique strain that showed a slightly different banding pattern from the rest of the *Pseudomonas* strains was alone in Cluster IV. The 19 primers used in this RAPD study provided evidence for the great genetic heterogeneity within *P. corrugata* and *P. mediterranea* species with the presence of divergent strains.

Lawongsa *et al.* (2008) isolated *Pseudomonas* species from rice (cv. Pathum Thani 1) and maize (cv. DK888) rhizosphere. The genotypic diversity of isolates was determined by amplified rDNA restriction analysis (ARDRA). This analysis showed that both plant varieties selected for two distinct populations of *Pseudomonas*. The actual biocontrol and plant promotion abilities of these strains was confirmed by bioassays on fungal viz., *Verticillium* sp., *Rhizoctonia solani* and *Fusarium* sp. and bacterial viz.,

Ralstonia solanacearum and *Bacillus subtilis* plant pathogens. There was a significant difference between isolates from rice and maize rhizosphere in terms of biological control against *R. solanacearum* and *B. subtilis*. Interestingly, none of the pseudomonads isolated from maize rhizosphere showed antagonistic activity against *R. solanacearum*. The results indicated that the percentage of pseudomonad isolates obtained from rice rhizosphere which showed the ability to produce fluorescent pigments was almost threefold higher than pseudomonad isolates obtained from maize rhizosphere.

Prasanna Reddy and Rao (2009) isolated PGPR strains belonging to fluorescent pseudomonads from the rhizosphere of rice. Among thirty isolates that were confirmed as *P. fluorescens*, were characterized by PCR-RAPD analysis and biochemical methods. Ten exhibited strong antifungal activity against *P. Oryzae* and *R. solani* mainly through the production of antifungal metabolites.

Battu *et al.* (2009) reported that the *Pseudomonas fluorescens* strains were isolated from the rhizosphere of rice and characterized by PCR-RAPD analysis and biochemical methods. The *P. fluorescens* showed strong antifungal activity against *P. Oryzae* and *R. solani*.

Silby *et al.* (2009) reported that the genotypic and phenotypic diversity of *P. fluorescens* could be based on the geographic origin or functional differences in the genome of the strains.

Charan *et al.*, (2010) revealed that the genetic diversity of *P. fluorescens* strains was assessed by two PCR based molecular techniques RAPD and Rep-PCR. 40 primers used in RAPD and 26 were found to be polymorphic. The number of DNA bands amplified varied from 2-10 with each primer and level of polymorphism was mostly 100% in all cases. The amplified DNA fragments ranged from 300 – 6500 bp. The number of polymorphic loci amplified varied with the different primers and isolates. All bands were scored for their presence and absence in the 15 bacterial isolates and similarity matrix was constructed using the UPGMA program. Clusted analysis was carried out. The similarity among these standard

isolates ranged from 0.42 to 0.95. Both methods effectively assessed the diversity of the 15 *P. fluorescens* isolates. The uniqueness of one of the isolates, pf-6 was demonstrated by the formation of a separate group in both methods.

3. MATERIALS AND METHODS

The present investigation entitled “Variability mapping of actinomycetes, sulphur oxidiser and plant growth promoting rhizobacteria from soils of Maharashtra” was undertaken at the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri during the period from June, 2009 to March, 2011. The *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* isolated from soils of the Maharashtra and characterized for morphological, cultural and biochemical tests. Attempts were also made to assess the molecular diversity of those isolates with multiple beneficial properties like biocontrol potential against plant pathogens, phosphate solubilization and siderophore production, antibiotic sensitivity. The materials used and methods employed in the investigation are outlined below.

3.1. Collection of soil samples

A total of 150 rhizosphere soil samples (0-15 cm depth) were collected by using GPS technique as per the soil series prescribed by Challa *et al.* (1999) from different physiographic regions of Maharashtra viz., Western Konkan Coast, Western Ghats (Sahyadris) and North Deccan Plateau (Upper, Lower and Metamorphic) so as to study the variability of *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens*, to ascertain most efficient strains with special interest on beneficial activities for agriculture. The abstract of soil samples collected from various physiographic regions of Maharashtra for studying the microbial diversity is presented in Table-1.

3.2 Population dynamics study :

The population of actinomycetes and fluorescent pseudomonads was estimated by counting the number of colonies of the respective organisms per plate by a colony counter and computing the average

Table 1. Abstract of soil samples collected from different physiographic regions of Maharashtra for studying the microbial diversity

Sr. No.	Name of the District	*No. of soil samples to be collected for studying the microbial diversity					Total number of soil samples
		Western Konkan Coast	Western Ghat	North Deccan Maharashtra Plateau			
				Upper	Lower	Lower (M)	
1.	Sindhudurg	7	5	-	-	-	12
2.	Ratnagiri	7	2	-	-	-	9
3.	Raigad	4	-	-	-	-	4
4.	Thane	4	4	-	-	-	8
5.	Kolhapur	-	6	5	-	-	11
6.	Satara	-	1	5	-	-	6
7.	Sangli	-	-	5	2	-	7
8.	Pune	-	2	6	-	-	8
9.	Solapur	-	-	3	5	-	8
10.	Ahmednagar	-	-	8	1	-	9
11.	Nasik	1	4	4	6	-	15
12.	NandurBAR	-	2	-	-	-	2
13.	Jalgaon	-	-	-	2	-	2
14.	Dhule	-	1	1	2	-	4
15.	Aurangabad	-	-	-	1	-	1
16.	Beed	-	-	1	2	-	3
17.	Latur	-	-	3	-	-	3
18.	Osmanabad	-	-	1	-	-	1
19.	Parbhani	-	-	-	1	-	1
20.	Hingoli	-	-	-	1	-	1
21.	Akola	-	-	-	1	-	1
22.	Nanded	-	-	-	1	-	1
23.	Jalana	-	-	-	1	-	1
24.	Buldhana	-	-	-	1	-	1
25.	Washim	-	-	-	1	-	1
26.	Amaravati	-	-	-	1	-	1
27.	Yeotmal	-	-	-	1	-	1
28.	Wardha	-	-	-	4	-	4
29.	Nagpur	-	-	-	2	11	13
30.	Chandrapur	-	-	-	1	3	4
31.	Bhandara	-	-	-	-	1	1
32.	Gondia	-	-	-	-	1	1
33.	Gadchiroli	-	-	-	-	5	5
Total soil samples		23	27	42	37	21	150

* As per the soil series prescribed by Challa *et al.*, (1999)

number of particular groups per gram of soil by using the following formula adopted by James (1978) :

$$\text{Viable cell count (CFU/g soil)} : \frac{\text{Average plate count}}{\text{Dry weight of soil}} \times \text{X dilution factor}$$

3.3 Isolation Method of Actinomycetes:

The streptomycetes were isolated by the soil dilution plate technique which is briefly described here.

A 10 g of representative soil sample was transferred to an Erlenmeyer flask containing 100 ml sterile distilled water and mixed thoroughly by shaking for 5 to 10 minutes. 10 ml samples were immediately drawn from the suspension in motion and transferred to 90 ml sterile water blank and shaken for one minute. From each suspension serial dilutions were prepared to obtain the desired dilution. One ml of the final dilution was transferred aseptically to a Petri dish and 15-18 ml of Knight agar medium was added to the Petri dish. The dish was rotated by hand in different directions so as to disperse it uniformly in the agar medium. When the medium was solidified, the petri dishes were incubated at 28 ± 2 °C) for 7 days, before they were taken for counting the streptomycete population.

Single colonies of streptomycetes were picked up, numbered and maintained on starch casein agar medium for further studies after ascertaining their purity.

3.4 Morphological characters of actinomycetes isolates

i) Shape and Size : The isolates grown in starch casein agar slants were prepared and examined by microscope under 40x.

All the actinomycetes isolates were examined for their spore chain cell morphology (cell shape, cell size and cell arrangement) using the trinocular microscope along with image analysis software DigiPro™ version 4.0.

ii) Gram reaction: All the cultures were stained by using Burke and Kopeloff Beerman's modified method (Anonymous, 1957) of staining. All the isolates were Gram positive in reaction.

iii) Acid fast stain:

Prepare smear of culture on clean slide, dry it and fix smear by heating gently. Cover with carbon fuschsion. Heat the smears from lower side of slide until steam rises of about 8 to 10 minutes. Do not allow to stain boil or slide become dry. Under heating the slide will fail to stain acid-fast cells. Drain the slide and decolorize with acid-alcohol for 2 min. Rinse, drain and add counterstain with methylene blue for 30 seconds. Wash with water, dry and then examine mixed smear. Acid-fast organisms will appear red and non-acid-fast organisms will be blue.

3.5 Cultural characters:

To observe the growth characteristics such as colour of aerial mycelium and production of soluble pigments, the streptomycetes were grown on Nutrient Agar, Starch Caseion Agar and Oat Meal Agar medium as per Manual of Microbiological Methods (Anonymous, 1957).

3.6 Biochemical properties:

The oxidase, catalase, hydrolysis of starch, hydrolysis of casein (These tests were performed according to the procedures given in " The Actinomycetes" Vol. II (Waksman, 1961 and Lechevalier, 1989).

3.7 Functional diversity of *Streptomyces* sp. :

3.7.1. Antimicrobial activity of *Streptomyces* by dual plate method :

The antibiotics producing capacity of each of the isolates was determined on agar plates by the following method :

An antagonist and pathogen was inoculated at 3 cm distance between them and incubated for seven days at 30°C. The zones of inhibition were measured after 7 days' of incubation at 30°C.

These test fungi viz., *Aternaria alternata* and *Fusarium oxysporum* were obtained from Culture Collection Centre of Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri.

3.7.2. Decomposition of cellulose:

The capacity of the isolates to decompose cellulose was studied by cultivating each isolate in broth containing 3 g KH_2PO_4 , 2 g K_2HPO_4 , 0.5 g MgSO_4 , 2 g asparagines, 10 g filter paper strips, and 1000 ml distilled water. 10 g filter paper strips were boiled, crushed and added in the medium. The medium was neutralized with bromothymol blue indicator. Tubes were incubated at 30 °C for 15 days. The decomposition of cellulose was evidenced by the production of acid in the broth, which was tested by using litmus paper strips.

3.7.3. Solubilization of phosphate:

Cultures were grown on modified Pikovskaya's medium (Pikovskaya, 1948) in plates at 30°C for 6 days. Only those cultures which showed clear zones of phosphate solubilization around their colonies were further studied for phosphate estimation in liquid culture. The average diameter of clear zone was recorded in mm. Composition of the medium was :

Tri calcium phosphate	5.0 g
Glucose	10.0 g
Ammonium phosphate	0.5 g
Sodium chloride	0.2 g
Potassium chloride	0.2 g
Magnesium sulphate	0.1 g
Mn & Fe sulphates	Traces
Yeast extract	0.5 g
Agar	15 g
Distilled water	1000 ml
pH	6.5

3.8. Molecular Diversity of selected *Streptomyces* spp.

3.8.1. DNA isolation procedure :

The genomic DNA of the 18 *Streptomyces* spp. was isolated by following the modified methodology of Stahl and Flesher (1987). The chemicals and reagents used for DNA isolation are listed in Appendix III.

Grow the pure culture of *Streptomyces* spp. in LB broth for late exponential phase and culture was taken in centrifuge tube and centrifuged at 12000 rpm at 4 °C for 2 min. The supernatant was discarded and the cell pellets redissolved in 500 µl of extraction buffer (T₅₀ mM E₁₀ mM) containing 2% sarkosyl, add 10 µl of RNase and 5 µl of Protease K per sample and incubated at 50 °C for 60 min. Each sample was vortex for 1-2 min. The DNA lysate was then extracted using phenol.

The lysate was treated with equal volume of 500 µl Tris saturated phenol (pH 8.0) and mixed well to form milky emulsion. It was centrifuged at 15,000 rpm for 10 minutes at 4°C to separate organic and aqueous phases. The upper aqueous phase was collected in fresh new tubes and add equal volume 500 µl of chloroform. Centrifuged at 14000 rpm for 10 min. and upper face was collected. To the upper face 0.7 volume of chilled isopropanol was added and mix thoroughly and keep in -20°C for over night. Centrifuged at 14000 rpm at 4°C for 15 min. The supernatant was discarded and 250 µl 70 % chilled ethanol was added. Thick DNA pellet was washed with 70% chilled ethanol and centrifuged at 14000 rpm for 5 min. at 4°C. The supernatant was discarded and dried DNA pellet and resuspend in 100 ul of T₁₀mM E₁mM and store at 4°C for further study.

3.8.2 Quantification of genomic DNA

The quantification of genomic DNA was done using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). The blank was run using 1 µl miliQ water. Then 1 µl of DNA sample was used for quantification of genomic DNA. The concentration of DNA was expressed as ng/µL. The Optical density

(O.D.) of DNA sample having the ratio (260/280 nm) near 1.8 showed good quality DNA.

3.8.3 Electrophoresis

The genomic DNA of each of the *Streptomyces* sp. was isolated and purified following the methodology of Stahal and Flesher. Purified genomic DNA extracts from the individual isolates were used as template DNA. The genomic DNA samples were electrophoresed on 0.8 per cent agarose gel along with molecular weight standard marker DNA. The gel was observed under gel documentation system by using image analyzer.

3.8.4 RAPD-PCR

3.8.4.1 Requirements

3.8.4.1.1 Template DNA

The purified genomic DNA extracts from the individual isolates were diluted to working of concentration and used as template DNA.

3.8.4.1.2 Random primers

Commercial Kit of RBA random decamer DNA primers were obtained from M/s Bangalore Genie Pvt. Ltd., Bangalore, India. A total of 24 random decamer primers, viz., RBA-1, RBA-2, RBA-3, RBA-4 RBA-5, RBA-6, RBA-7, RBA-8, RBA-9, RBA-10 RBA-11, RBA-12, RBA-13, RBA-14 RBA-15, RBA-16, RBA-17, RBA-18, RBA-19, RBA-20, RBA-21 RBA-22 RBA-23 and RBA-24 were used for RAPD analysis. Each of these primers (3 O.D. = 99 µg = 30000 picomoles) were dissolved in 1500 µl sterilized milli Q water to get final concentration of 20 picomoles/µl and incubated at room temperature for 3-4 hrs. with vortexing frequently in between.

Bacterial RAPD Primer Set : The DNA sequences (5"-3") of all primers used for DNA amplification are given in Table 2.

3.8.4.1.3 Taq DNA polymerase

Taq DNA polymerase and 10 X Taq buffer A and buffer B obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore, were used.

Table 2. Bacterial RAPD primers with their sequences

Sr. No.	Primer Name	Primer Sequence
1	RBA 1	5' AAA ACC GGG C 3'
2	RBA 2	5' ACA GGG CTC T 3'
3	RBA 3	5' ACA GGG GTG T 3'
4	RBA 4	5' ACC GGG TTT C 3'
5	RBA 5	5' AGG GGC GGC A 3'
6	RBA 6	5' ATC CTG CCT G 3'
7	RBA 7	5' ATC GGG TCC T 3'
8	RBA 8	5' ATC GGG TCG A 3'
9	RBA 9	5' ATC TGC GAG C 3'
10	RBA 10	5' CCC GCC TTC C 3'
11	RBA 11	5' CCG GCC CCA A 3'
12	RBA 12	5' CCG GCC TTA A 3'
13	RBA 13	5' CCG GCC ATA C 3'
14	RBA 14	5' CCG GCC TTC C 3'
15	RBA 15	5' CCG GCT GGA A 3'
16	RBA 16	5' CCG GGG AAA C 3'
17	RBA 17	5' CCG GGG TTA T 3'
18	RBA 18	5' CCG GGG TTT G 3'
19	RBA 19	5' CCT GCG CTT A 3'
20	RBA 20	5' CCT GGC GGT G 3'
21	RBA 21	5' CCT GGG CCA G 3'
22	RBA 22	5' CCT GGG CTG G 3'
23	RBA 23	5' CCT GGG CTT G 3'
24	RBA 24	5' CCT GGG CTT A 3'

3.8.4.1.4 dNTP's

dNTP's mix. obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore, were used.

3.8.4.1.5 Thermocycler

Palm Cycler, Germany was used for the cyclic amplification of DNA.

3.8.4.2 Reaction mixture:

One primer at a time was used to study polymorphism between the 18 strains by RAPD-PCR assay with genomic DNA extracts from all strains as template DNA. The RAPD - PCR reaction was carried out in 20µL volume. Master mix was prepared afresh using 10x assay buffer with MgCl₂ (2.5µl)/ Taq buffer A 2.0 µl and 25 mM MgCl₂ 0.4 µl , dNTPs 2.5 mM (1.6 µl), primer (1.0 µl), Taq DNA polymerase 30 U/ µl (0.33 µl) and sterile deionized water (13.67 µl) for all the strains to avoid handling errors. The dNTPs and Taq DNA polymerase were obtained from M/s Bangalore Genie Pvt. Ltd., Bangalore. The master mixture was distributed to 15 microfuge (PCR) tubes of 0.5 ml capacity @ 19 µl per tube and 1 µl of template DNA from the respective isolates was added to make total reaction volume in each tube equal to 20 µl.

3.8.4.3 PCR amplification

The PCR amplification was done by following the method proposed by Castrillo and Brooks (1998) with certain modifications as shown below:

Stage	Stage	Temperature (°C)	Duration (min.)	No. of cycles
I	Denaturation	94	10	1
II	Denaturation	94	1	40
	Annealing	37.1	1	
	Extension	72	2	
III	Extension	72	10	1
	Hold	4	-	-

3.8.4.4 Separation of amplified products by agarose gel electrophoresis

The reagents required for agarose gel electrophoresis are listed in Appendix III. Twenty μl of the amplified products from each tube along with 4 μl of loading dye (Appendix III) were separated on 1.2 per cent agarose gel (Appendix III) using 1x TBE buffer of pH 8.0 (Appendix III) along with 100 bp DNA ladder as DNA molecular weight marker.

Gel loading buffer comprised of Bromophenol blue, Xylene cyanol FF (0.5 %), Glycerol (30%), the 20 μL of RAPD Product was loaded on 1.2 % agarose gel using TBE buffer. Electrophoresis was performed for 3 hr. at 80 volts in an submarine electrophoresis apparatus (Bio Rad No.96). The gel was stained with Ethidium bromide (0.1%) and photographed using gel documentation system (using FluorChem software).

3.8.4.5 Analysis of RAPD profiles

The amplification profiles for all the primers were compared with each other and the bands of DNA fragments were scored as present (1) or absent (0) generating the binary matrices.

The binary data were analyzed under the SIMQUAL module of NTSYS PC-2.0 software programme (Rohlf, 1998) using DICE coefficient (Nei and Lei, 1979). A dendrogram based on the UPGMA clustering method (SAHN) (Sneath and Sokal, 1973) was used to generate a tree (dendrogram)

Principle coordinate analysis (PCO) was performed to estimate the denetic distance between each group of the isolates by using NTSYS software. It involves first transforming similarity value to scalar products by DCENTER. Then analyzing the product matrix by EIGEN to get Eigenvectors (which is PCO) and Eigenvalues, and finally getting its 2D / 3D scatter plot graph.

The per cent polymorphism was computed as,

$$\text{Per cent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

3.9. Isolation, purification and maintenance of sulphur oxidizing Bacteria :

All the 150 soil samples previously subjected to chemical analysis were used for isolations of sulphur oxidizing bacteria. For isolation purpose an enrichment technique was used (Waksman, 1922 and Kuenen and Tuovien, 1981). The medium used contained powdered elemental sulphur and without any carbon source. The composition of the medium is given in Appendix-II .

The pH of the medium was adjusted to 5. Five ml of bromocresol purple (0.25%) indicator was added to per litre medium, which was then distributed in each of the 250 ml. Erlenmeyer flasks.

The flasks were, then autoclaved at 1 kg/cm² pressure for 20 minutes. The sulphur used in the medium was sterilized separately in an autoclave on three successive days, and added aseptically to the previously sterilized medium. The flasks were then inoculated with one gram of soil and incubated on to and fro shaker (150 to and fro actions) at room temperature for eight days. The flasks showing the fall in the pH of the medium as evidenced by the colour change of the bromocresol purple indicator (from purple to colourless) were presumed to contain the growth of sulphur oxidizing bacteria. Therefore, transfers (1 ml quantity) were made from these flasks to the fresh medium of the same composition.

For culture purification thiosulphate medium (Waksman, 1922) containing sodium thiosulphate was employed. Since sodium thiosulphate was soluble in water, it provided a medium in which the sulphur source was uniformly distributed. The composition of the medium is given in Appendix II.

Five ml of bromocresol purple (0.25%) indicator was added to a litre of medium before sterilization. This indicator helped to recognize the colonies which produced sulphuric acid from thiosulphate oxidation by its nature to change the colour of the medium with simultaneous change in the pH of the medium.

A loopful of the inoculum from the enrichment broth showing positive indication for the presence of the sulphur oxidizing bacteria was streaked on the duplicate agar plates of the above medium. The medium of the different pH values were tried to make out the isolates with different pH optima. The plates were then incubated at 30°C for 5 days. The colonies producing the acid as indicated by the change in the indicator from purple to colourless, were selected and streaked on to the slants of the fresh agar medium of the same composition and pH. By this procedure, 8 bacterial isolates were obtained and purified. They were maintained on the same medium by keeping the appropriate pH values and transferred on to the fresh medium, fortnightly.

3.10. Screening of the isolates for sulphur oxidizing efficacy:

The bacteria isolates were inoculated in the sulphur liquid medium (Waksman, 1922) and incubated for 20 days. The sulphur oxidizing efficacy of the different isolates was judged by the reduction of pH of liquid medium from the oxidation of element sulphur. The pH of the medium was measured by Digital pH meter was determined.

3.11. Morphological characters of bacterial isolates The identification of bacterial isolates was done by studying morphological and staining reaction by using standard methods (Frobisher, 1961; Kuenen and Thovinen, 1981; Buchanan and Gibbons, 1974).

i) Shape : Smear of 72 hours old growth of all isolates grown in thiosulphate agar slants were prepared and stained with Ziehl's carboc fuchsin and examined under microscope

ii) Size : All the bacterial isolates were examined for their cell morphology (cell shape, and cell size) using the trinocular microscope along with image analysis software DigiPro™ version 4.0.

iii) Gram reaction: The bacterial isolates were stained as per Burke and Kopeloff Beerman's modification (Anonymous, 1957).

3.12. Cultural characters On thiosulphate medium slants:

All the bacterial isolates were streaked with a straight needle and examined for intensity of growth and surface.

3.13 Molecular Diversity of *Acidithiobacillus* sp. :

The genomic DNA of the 9 *Acidithiobacillus* spp. was isolated by following the modified methodology of Stahl and Flesher (1987). The chemicals and reagents used for DNA isolation are listed in Appendix III.

3.13.1 Procedure for DNA isolation of *Acidithiobacillus* sp.

1. The pure culture of *Acidithiobacillus* spp. were grown in Basal salt medium broth for late exponential phase.
2. Bacterial culture was taken in centrifuge tube and centrifuged at 12500 rpm for 2 min. (4°C).
3. Three washing with Sterilized deionized water with 2.5 pH and the cell pellets dissolved in 500 µl of extraction buffer (2% sarkosyl) , add 20 µl of RNase and 10 µl of Protease K per sample.
4. The samples were incubated in water bath at 50 °C for 60 min.
5. Each sample was vortexed for 1-2 min.
6. After that equal volume of Tris saturated phenol (pH 8.0) was added and centrifuged at 13500 rpm for 10 min. (10°C).
7. The upper surface was collected in new micro centrifuge tubes and to that tube equal volume of chloroform 500 µl was added and centrifuged at 13500 rpm for 10 min. and the upper face was collected.
8. To the upper face 0.7 volume of isopropanol was added and mix thoroughly and keep in -20°C for over night.
9. Again centrifuged at 13500 rpm for 15 min. at 4°C.
10. The supernatant was discarded and 250 µl 70 % chilled ethanol was added and centrifuged at 13500 rpm for 5 min. at 4°C.
11. The supernatant was discarded.

12. The DNA pellet was dried and resuspend in 100 µl of T₁₀E₁ buffer.

Thus, the genomic DNA of each of the *Thiobacillus spp.* was isolated and purified following the methodology of Stahal and Flesher (1987) and Tanveer *et al.* (2005). Purified genomic DNA extracts from the individual isolates were used as template DNA. Commercial Kit of RBA random decamer DNA primers were obtained from M/s Bangalore Genie Pvt. Ltd., Bangalore, India. A total of 21 random decamer primers were used for RAPD analysis. Out of 21 primers, 15 primers were amplified.

3.13.2. Reaction mixture : One primer at a time was used to study polymorphism between the 9 strains by RAPD-PCR assay with genomic DNA extracts from all strains as template DNA. The RAPD - PCR reaction was carried out in 20µL volume. Master mix was prepared afresh using 10x assay buffer with MgCl₂ (2.5ul)/ Taq buffer A 2.0 ul and 25 mM MgCl₂ 0.4 ul, dNTPs 2.5 mM (1.6 ul), primer (1.0 ul), Taq DNA polymerase 30 U/ul (0.33 ul) and deionized water (13.67 ul) for all the strains to avoid handling errors. The dNTPs and Taq DNA polymerase were obtained from M/s Bangalore Genie Pvt. Ltd., Bangalore, India. The master mixture was distributed to 15 microfuge (PCR) tubes of 0.5 ml capacity @ 19 µl per tube and 1 µl of template DNA from the respective isolates was added to make total reaction volume in each tube equal to 20 µl. PCR tubes were placed in Thermocycler (Eppendropp, Germany) for DNA amplification.

3.13.3 PCR amplification was done following the method of Castrillo and Brooks (1998) with slight modification (I stage : one cycle of denaturation at 94°C for 10 min; II stage : 40 cycles (consisting of denaturation at 94°C for 1 min; annealing at 36°C for 1 min and extension at 72°C for 2 min) and III stage : one cycle of extension at 72°C for 10 min).

After the completion of the required cycles of amplification, the content were loaded onto 1.2% agarose gel for electrophoretic analysis.

3.13.4 Analysis of RAPD profiles: Molecular data was scored as binary data (0 band absent and 1 band present) used for diversity analysis using suitable software (NTSYS-PC) (Dice, 1945; Nei and Li, 1979).

3.14 Characterization and identification of the bacterial isolates

Sixty five isolates of fluorescent pseudomonads isolated from the soils of Maharashtra and out of this after confirmation 34 isolates of *Pseudomonas fluorescens* maintained as culture collection centre in the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, were used in the present investigations.

3.15. Morphological characterization

All the 65 bacterial isolates were examined for their colony morphology, pigmentation, epifluorescence by streak plate method using King's B agar plates incubating at $28 \pm 2^{\circ}\text{C}$ for 24 hrs. All the isolates were examined for their cell morphology (cell shape, cell size and cell arrangement) using the trinocular microscope along with image analysis software DigiPro™ version 4.0. The colony morphology and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewer (1950) and Anonymous (1957).

3.16 Cultural characterization

All the 65 isolates were grown on different media viz., King's B, Nutrient Agar and Pikovaskayas' medium are examined for their growth pigmentation, colony morphology as per the standard procedures given by Barthalomew and Mittewer (1950) and Anonymous (1957).

3.17 Biochemical characterization

Biochemical characterizations of all the isolates were done as per the procedures outlined by Cappuccino and Sherman (1992). The tests performed and protocols used are briefly outlined below:

3.17.1 Catalase test

Nutrient agar (Appendix II) plates were inoculated with 10 μl overnight cultures of test organisms and were incubated at 30°C for 24 hours. After incubation, the tubes were flooded with 5-10 drops of three per cent hydrogen peroxide and observed for gas bubbles. **The occurrence of gas bubbles was scored positive for catalase.**

3.17.2 Hydrogen sulfide test

The sterilized Hydrogen sulfide Indole Motility agar (SIM agar Appendix II) stabs were inoculated along the inner side wall with the 24 hrs cultures of isolates. The inoculated tubes were incubated at $28 \pm 2^{\circ}\text{C}$ for 48 hours. After incubation, **the development of black colour along the line of the stab was noted as positive reaction for the test.**

3.17.3 Indole production

The test cultures isolates were grown for 24 hours were inoculated in the pre-sterilized SIM agar (Appendix II) slants. The tubes were incubated at $28 \pm 2^{\circ}\text{C}$ for 48 hours. After incubation, each tube was added with 10 drops of Kovac's reagent (Appendix II). **The isolates showing production of red colour were recorded as positive reaction for the indole production.**

3.17.4 Gelatin liquefaction

The overnight cultures of test isolates were inoculated to the pre-sterilized nutrient gelatin (Appendix II) deep tubes and incubated at $28 \pm 2^{\circ}\text{C}$ for 24 hours. Then the tubes were kept in a refrigerator at 4°C for 30 minutes. **The test isolates that remained liquified were taken as positive for the test** and the tubes with cultures that solidified on refrigeration were taken as negative for the test.

3.17.5 Oxidase test

The test isolates were inoculated and cultures were incubated at $28 \pm 2^{\circ}\text{C}$ for 24 hours. After incubation, the filter paper is soaked into tetramethyl-phenylenediamine dihydrochloride and dried the filter paper. Then the overnight growth of each test organism was inoculated on the surface of dried filter paper which is soaked into tetramethyl-phenylenediamine dihydrochloride. **The isolates showing colour change to maroon was reported as oxidase positive.**

3.17.6 Urease test

The overnight cultures were inoculated to the test tubes containing sterilized urea broth (Appendix II) and incubated at $28 \pm 2^{\circ}\text{C}$ for 48 hours. **The pink colour discoloration was taken as positive for the test and colour remain unchange reported as negative test.**

3.17.7 Starch hydrolysis

The test cultures were spotted with 10 μl overnight culture on the starch agar (Appendix II) plates and incubated at $28 \pm 2^{\circ}\text{C}$ for 48 hours. After incubation, the plates were flooded with iodine solution. **The formation of blue colour i.e. transparent zone around the colony was taken as positive reaction for the test.**

3.17.8 Casein hydrolysis

Overnight cultures of the test isolates were spotted with 10 μl overnight culture on skimmed milk agar (Appendix II) plates and incubated at $28 \pm 2^{\circ}\text{C}$ for 48 hours. **The isolate showing halo zone around the colony was taken as positive reaction for the test.**

3.17.9 Denitrification

Nitrate broth tubes (Appendix II) with inverted Durham's tube without air bubbles inside were inoculated with overnight grown culture of the test organism and were incubated at 25°C for 15 days. After incubation, **the inverted Durham's tubes were observed for the accumulation of gas were recorded as positive.**

3.18 Physiological characterization

3.18.1 Growth at 4°C

Twenty four hours old cultures of the test organisms in nutrient broth were spotted on the Trypticase soy agar (Appendix I) plates and incubated at 4°C for 48 hours and the observation on growth was recorded at the end of incubation period.

3.18.2 Growth at 41°C

Twenty-four hours old cultures of the test organisms in nutrient broth were spotted on the Trypticase soy agar (Appendix II) plates and incubated at 41°C for 48 hours and the observation on growth was recorded at the end of the incubation period.

3.19 Functional diversity of the isolates

3.19.1 Antagonistic property :

A) Dual plate method : The 34 *Pseudomonas fluorescens* isolates were tested for antagonistic property against *Macrophomina phaseolina* and *Fusarium oxysporum* by dual plate method. These pathogen were procured from Culture collection centre of Department of Plant Pathology and Agril. Microbiology, MPKV., Rahuri. Fungal culture was grown on potato dextrose agar (PDA) at 28°C for 3 days. A fungal disc (5 mm dia.) was removed from PDA and placed centrally on prepoured PDA : nutrient agar (1:11, v/v) plate. Bacterial cultures were spot inoculated towards the edge of the plates, which were then incubated at 28°C for 2-4 days. Inhibition of fungal mycelium around the bacterial colony was scored after 4 days and calculated by using the following formula (Shilpi Mital and Johri 2007, Jayaswal *et al.* 1990).

$$\% \text{ Growth Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 = radial dist. of control

R_2 = radial dist. in dual culture

B) Dual inoculation method : 5 ml sterilized potato dextrose broth in test tubes was used for interaction studies. A loopful of each isolated bacteria was added in the broth in separate test tube. Then loopfull of *Macrophomina phaseolina*/*Fusarium oxysporum* culture was added in each tube. These tubes were incubated at 27°C $\pm$ 1°C for 48 hours.

After incubation period a small aliquot from each tube was plated on the PDA medium and spread with the help of curve glass rod. These

plates were incubated at $28 \pm 1^{\circ}\text{C}$ and observed after every 12 hours to record the effect of interaction i.e. growth of *Macrophomina phaseolina*/*Fusarium oxysporum* or bacterium or both (Joshi, 2010).

3.19.2 HCN production

Whatman No. 1 filter paper pads were placed inside the lids of petri plates and the plates were sterilized. Then the plates (bottom) were poured with sterilized trypticase soy agar medium added with glycine @ 4.4 g/lit. and allowed to solidify. The antagonist isolates were streaked on the medium and the filter paper padding in the lids was soaked with 2 ml sterile picric acid solution (picric acid 2.5 g and Na_2CO_3 12.5 g/lit). Then the plates were sealed with parafilm in order to retain the gaseous metabolites produced by the antagonists and to allow for chemical reaction with picric acid present in the filter paper padding (Wei *et al.* 1991). After incubation at 30°C for a week, the colour change of the filter paper was noted and the HCN production potential of the antagonists was assessed as shown below:

No colour change	-	No HCN production
Brownish colouration	-	Weak HCN production
Brownish to orange	-	Moderate HCN production
Complete orange	-	Strong HCN production

3.19.3 Siderophore production

For the assay of siderophore production, all the glassware were first soaked in 2N HCl solution for 24 hrs. to avoid contamination of iron from the glassware. The chrome azurol S (CAS) solution was prepared by dissolving 60.5 mg dehydrated chrome azurol S in 50 ml double distilled water and further mixing with 10 ml of iron solution (1 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 10 mM HCl). This was then slowly added with 40 ml aqueous solution containing 72.9 mg cetyl trimethyl ammonium bromide with continuous stirring and the final solution was autoclaved.

The King's B agar was prepared using PIPES buffer (30.2 g) and Difco agar (18.0 g) and the pH was adjusted to 6.8 by addition of 0.1 N NaOH before autoclaving (Schwyn and Neilands, 1987). After cooling, the CAS solution (100 ml) was added along the wall of flask with gentle agitation to mix without formation of foam. The CAS agar thus prepared was poured in to the plates. After solidification, the plates were kept in the refrigerator (4° C) for 24 hrs. The overnight cultures of fluorescent pseudomonads (10 µl each) were spotted on these CAS agar plates and incubated at $28 \pm 2^\circ \text{C}$ for 48 hrs. **Formation of orange coloured zone around the colony was taken as positive for the siderophore production** (Mital and Johri 2007).

3.19.4 Antibiotic sensitivity test:

Antibiotic sensitivity of fluorescent pseudomonads were determined by the standard disc diffusion method of Baur *et al* (1966) and as adopted by Antoun *et al* (1982). The antibiotic discs were obtained from the Hi-media laboratories Pvt. Ltd., Mumbai, India. Antibiotics discs used were streptocyclin, tetracycline rifampicin, and nystin (Siddiqui *et al* 2005).

3.20 Molecular Diversity of selected *Pseudomonas fluorescens*

Thirteen isolates of *Pseudomonas fluorescens* showing multiple functional properties and one commercial reference strain were subjected to diversity analysis through RAPD-PCR as detailed below:

3.20.1 Isolation of genomic DNA

The genomic DNA of the 14 *Pseudomonas fluorescens* was isolated by following the modified methodology of Stahl and Flesher (1987). The chemicals and reagents used for DNA isolation are listed in Appendix III.

Grow the pure culture of *Pseudomonas fluorescens* in LB broth at $28 \pm 2^\circ \text{C}$ for 36 hrs bacterial culture was taken in centrifuge tube and centrifuged at 12000 rpm at 4°C for 2 min. The supernatant was discarded and the cell pellets redissolved in 500 µl of extraction buffer (T₅₀mM E₁₀mM) containing 2% sarkosyl, after that add 10 µl of RNase A

and 5 µl of Protease K per sample and incubated at 50 °C for 60 min. Each sample was vortex for 1-2 min. The DNA lysate was then extracted using phenol.

The lysate was treated with equal volume of 500 µl Tris saturated phenol (pH 8.0), mixed well to form milky emulsion and centrifuged at 13,000 rpm for 10 minutes at 4°C to separate organic and aqueous phases. The upper aqueous phase was collected in fresh new tubes and add equal volume of chloroform. Centrifuged at 13000 rpm for 10 min. and upper aqueous face was collected. To the upperface 0.7 volume of chilled isopropanol was added and mix thoroughly and keep in -20 °C for overnight. Centrifuged at 13000 rpm for 15 min at 4°C. The supernatant was discarded and 250 µl 70 % chilled ethanol was added. Thick DNA pellet was washed with 70% chilled ethanol and centrifuged at 13000 rpm for 5 min. at 4°C. The supernatant was discarded and dried DNA pellet and resuspend in 100 ul of T₁₀mM E₁mM and store at 4°C for further study.

3.20.2 Reaction mixture:

The RAPD - PCR reaction was carried out in 20µL reaction volume. One primer at a time was used to study polymorphism between the 20 strains by RAPD-PCR assay with genomic DNA extracts from all strains as template DNA. Master mix was prepared afresh using 10x assay buffer with MgCl₂ (2.5µl), dNTPs 2.5 mM (1.0 µl), primer (1.0 µl), *Taq* DNA polymerase 30 U/µl (0.33 µl) and deionized water (19.17 µl) for all the strains to avoid handling errors. The dNTPs and *Taq* DNA polymerase were obtained from M/s Bangalore Genie Pvt. Ltd., Bangalore. The master mixture was distributed to 15 microfuge (PCR) tubes of 0.5 ml capacity @ 19 µl per tube and 1 µl of template DNA from the respective isolates was added to make total reaction volume in each tube equal to 20 µl.

3.20.3 PCR amplification : PCR amplification was done following the method of Castrillo and Brooks with slight modification (I stage : one cycle of denaturation at 94°C for 10 min; II stage : 40 cycles (consisting of denaturation at 94°C for 1 min; annealing at 35.4°C for 1 min and

extension at 72°C for 2 min) and III stage : one cycle of final extension at 72°C for 10 min).

After the completion of the required cycles of amplification, the content were loaded onto 1.2% agarose gel for electrophoretic analysis.

3.20.4 Analysis of RAPD profiles: Recorded molecular data was used for diversity analysis using suitable software (NTSYS-PC) (Dice, 1945; Nei and Li, 1979).

3.21 Statistical analyses

The statistical analyses of the data were carried out by employing completely randomized design (CRD). The critical differences were calculated at $P = 0.05$ for the *in-vitro* and interpretation of the results was carried out in accordance with Panse and Sukhatme (1985).

4. RESULTS

The present investigation entitled “Variability mapping of actinomycetes, sulphur oxidiser and plant growth promoting rhizobacteria from soils of Maharashtra” was undertaken at the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri during the period from June, 2009 to March, 2011. The *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* isolated from soils of the Maharashtra and characterized for morphological, cultural and biochemical tests. Attempts were also made to assess the molecular diversity of those isolates with multiple beneficial properties like biocontrol potential against plant pathogens, phosphate solubilization and siderophore production, antibiotic sensitivity and molecular diversity of each selected organisms is studied. The results of this investigation are outlined below.

4.1 Isolation of actinomycetes, sulphur oxidisers and fluorescent pseudomonads

A total 150 soil samples collected which represent physiographically different soil types of Maharashtra. viz., Western Konkan Coast, Western Ghats (Sahyadris) and North Deccan Plateau (Upper, Lower and Metamorphic) used for isolation. Out of that 131 actinomycetes, 22 sulphur oxidizer microorganisms and 65 fluorescent pseudomonads were isolated (Table 3, Plate 1). Among these isolates 72 *Streptomyces* sp., 8 *Acidithiobacillus* and 34 *Pseudomonas fluorescens* were characterized on morphological, biochemical basis and functional activities to ascertain most efficient strains and to study the variability of microorganisms with special interest on beneficial activities for agriculture.

Table3. Abstract of fluorescent pseudomonads, actinomycetes and sulphur oxidizing microorganisms isolated from different physiographic regions of Maharashtra.

physiographic regions	Fluorescent pseudomonads	Actinomycetes	Sulphur oxidizing microorganisms	Total isolates
Konkan coast region	4	23	2	29
Western Ghat	13	24	3	40
Western Maharashtra	18	31	7	56
North Maharashtra	8	13	-	21
Marathwada	9	11	2	22
Vidarbha	13	29	8	50
Total isolates	65	131	22	218

4.2. Actinomycetes

4.2.1 Microbial Population of Actinomycetes :

In the present studies, the microbial population of actinomycetes was found 87.33% of the soils of Maharashtra. The microbial population of actinomycetes varied from 1×10^{-4} to 18×10^{-5} cfu/g in different soils of Maharashtra (Table 4). They were observed maximum (23.66%) in Western Maharashtra followed by Vidarbha (22.14%), Western Ghat (18.32%), Western Konkan coast (17.56), North Maharashtra (9.92%) and Marathwada (8.40%) from soils of Maharashtra. The microbial population of actinomycetes A-56 was found 18×10^{-5} cfu/g maximum in Latur district from Marathwada followed by Sangali isolate A-63 (16×10^{-5} cfu/g), Latur isolate A-55 (15×10^{-5} cfu/g), Nashik isolate A-52 (15×10^{-5} cfu/g) from Western Maharashtra, Marathwada and North Maharashtra, respectively.

Table 4 : Microbial population of Actinomycetes from soils of Maharashtra.

Sr..No.	Isolate	Microbial population	Sr.No.	Isolate	Microbial population
1.	A-1	1×10^{-5}	34.	A-35	1×10^{-5}
2.	A-2	2×10^{-4}	35.	A-36	5×10^{-5}
3.	A-3	4×10^{-5}	36.	A-37	2×10^{-5}
4.	A-4	1×10^{-5}	37.	A-39	6×10^{-5}
5.	A-5	5×10^{-4}	38.	A-40	13×10^{-5}
6.	A-6	3×10^{-4}	39.	A-41	3×10^{-5}
7.	A-7	1×10^{-5}	40.	A-42	14×10^{-5}
8.	A-8	2×10^{-4}	41.	A-43	1×10^{-4}
9.	A-9	6×10^{-5}	42.	A-44	3×10^{-5}
10.	A-10	3×10^{-5}	43.	A-45	2×10^{-5}
11.	A-11	1×10^{-5}	44.	A-46	3×10^{-5}
12.	A-12	1×10^{-5}	45.	A-48	10×10^{-5}
13.	A-13	5×10^{-4}	46.	A-49	3×10^{-5}
14.	A-14	5×10^{-4}	47.	A-50	4×10^{-5}
15.	A-15	3×10^{-5}	48.	A-51	1×10^{-5}
16.	A-16	3×10^{-5}	49.	A-52	15×10^{-5}
17.	A-17	12×10^{-5}	50.	A-53	8×10^{-5}
18.	A-18	1×10^{-4}	51.	A-55	15×10^{-5}
19.	A-19	2×10^{-5}	52.	A-56	18×10^{-5}
20.	A-20	13×10^{-5}	53.	A-57	1×10^{-5}
21.	A-21	4×10^{-5}	54.	A-58	1×10^{-5}
22.	A-22	10×10^{-5}	55.	A-59	8×10^{-5}
23.	A-23	5×10^{-5}	56.	A-60	4×10^{-5}
24.	A-24	1×10^{-5}	57.	A-62	3×10^{-5}
25.	A-25	1×10^{-5}	58.	A-63	16×10^{-5}
26.	A-26	5×10^{-5}	59.	A-64	6×10^{-5}
27.	A-28	4×10^{-5}	60.	A-65	7×10^{-5}
28.	A-29	9×10^{-5}	61.	A-67	5×10^{-5}
29.	A-30	10×10^{-5}	62.	A-71	1×10^{-5}
30.	A-31	11×10^{-5}	63.	A-73	3×10^{-5}
31.	A-32	11×10^{-5}	64.	A-74	2×10^{-5}
32.	A-33	3×10^{-5}	65.	A-75	3×10^{-4}
33.	A-34	4×10^{-5}	66.	A-76	1×10^{-5}

Contd

Table 4 contd

Sr..No.	Isolate	Microbial population	Sr.No.	Isolate	Microbial population
67.	A-78	7×10^{-5}	100	A-115	2×10^{-5}
68.	A-79	3×10^{-5}	101	A-116	5×10^{-5}
69.	A-80	2×10^{-5}	102	A-119	2×10^{-5}
70.	A-81	1×10^{-5}	103	A-120	7×10^{-5}
71.	A-82	2×10^{-5}	104	A-121	2×10^{-5}
72.	A-83	1×10^{-5}	105	A-122	6×10^{-5}
73.	A-85	2×10^{-5}	106	A-123	4×10^{-5}
74	A-87	1×10^{-4}	107	A-124	1×10^{-5}
75	A-88	3×10^{-5}	108	A-125	3×10^{-5}
76	A-89	2×10^{-5}	109	A-126	3×10^{-5}
77	A-90	3×10^{-5}	110	A-127	1×10^{-5}
78	A-91	1×10^{-5}	111	A-128	5×10^{-5}
79	A-92	2×10^{-5}	112	A-129	5×10^{-5}
80	A-93	2×10^{-5}	113	A-130	7×10^{-5}
81	A-94	3×10^{-5}	114	A-132	3×10^{-5}
82	A-95	8×10^{-5}	115	A-133	3×10^{-4}
83	A-97	3×10^{-5}	116	A-134	3×10^{-5}
84	A-98	2×10^{-5}	117	A-135	1×10^{-4}
85	A-99	7×10^{-5}	118	A-136	12×10^{-4}
86	A-100	2×10^{-5}	119	A-137	1×10^{-5}
87	A-101	2×10^{-5}	120	A-138	4×10^{-5}
88	A-102	7×10^{-5}	121	A-139	5×10^{-5}
89	A-104	5×10^{-5}	122	A-140	2×10^{-4}
90	A-105	6×10^{-5}	123	A-141	5×10^{-5}
91	A-106	3×10^{-5}	124	A-142	2×10^{-5}
92	A-107	2×10^{-5}	125	A-143	4×10^{-5}
93	A-108	1×10^{-4}	126	A-144	5×10^{-5}
94	A-109	3×10^{-5}	127	A-145	9×10^{-5}
95	A-110	9×10^{-4}	128	A-146	4×10^{-5}
96	A-111	11×10^{-5}	129	A-147	6×10^{-5}
97	A-112	18×10^{-4}	130	A-148	2×10^{-4}
98	A-113	9×10^{-5}	131	A-150	3×10^{-5}
99	A-114	2×10^{-5}			

4.2.2 Morphological and Cultural characters :

4.2.2.1 Morphological characters, gram staining and acid fast staining :

In the present studies, the morphological studies of the actinomycetes isolates were studied regarding gram reaction, acid fast staining, colour, spore chain morphology and pigmentation. All the 131 isolates were gram positive and non acid fast in reaction. There were 72 isolates have creamy colour, 55 isolates were grey in colour and 4 isolates were white to milky in colour. In all 72 isolates spores of aerial mycelium and substrate mycelium were found in chain (Plate 2) The most of isolates (66) having rectiflexibles (RF) in spore chain morphology and few of the isolates (6) showed retinaculiaperti (RA) in spore chain morphology is the main features of spore chain morphology of *Streptomyces* genus (Table 5, Plate 3). The spores are observed rectiflexibles in straight to flexuous spores in chain. While in retinaculiaperti open loops, hooks of diameter, coils or spirales. In some *Streptomyces* sp. the sclerotial like structures are also observed. The aerial mycelium and substrate mycelium is also recorded on different media. The five isolates showed different pigmentation colour in the medium. It is observed that the 72 isolates spores of aerial mycelium and substrate mycelium were found in chain and they were identified as *Streptomyces* sp., whereas 4 isolates are cottony growth and 55 isolates grey or powdery growth and their aerial mycelium found in chain or most of spores are not in chain. The substrate mycelium of cottony growth isolates and powdery growths spores were not the chain. These isolates were other than *Streptomyces* sp. were probably *Chania* sp. and cottony growth was *Streptoverticillium* sp. The 72 isolates of *Streptomyces* sp. were used for further studies.

4.2.2.2 Cultural characters:

The *Streptomyces* sp. were grown on the nutrient agar medium, starch casein agar and oat meal agar medium for the cultural studies. All the 72 isolates were grown excellent on oat meal agar medium followed by starch casein agar and nutrient agar media. The growth of all the

Table 5: Morphology of Actinomycetes isolated from soils of Maharashtra

S. N.	Isolates No.	Gram reaction	Acid fast stain	Colour	Spore chain morphology	en-tation
1	A-1	Positive	Negative	Grey	Spores in chain	-
2	A-2	Positive	Negative	Blackish grey	Spores not in chain	-
3	A-3	Positive	Negative	Creamy	Rectifexibles, spores in chain	-
4	A-4	Positive	Negative	Whitish Grey	spores in cluster	-
5	A-5	Positive	Negative	Creamy	Rectifexibles,	-
6	A-6	Positive	Negative	Dark Creamy	Rectifexibles,	-
7	A-7	Positive	Negative	Creamy	Rectifexibles, Cluster, thread like hypae	-
8	A-8	Positive	Negative	Grey	spores in cluster	-
9	A-9	Positive	Negative	Grey	spores in cluster	-
10	A-10	Positive	Negative	Whitish Creamy	Rectifexibles, spores in chain	-
11	A-11	Positive	Negative	Grey	spores in cluster	-
12	A-12	Positive	Negative	Grey	spores in isolate, cluster	-
13	A-13	Positive	Negative	Creamy	Rectifexibles, spores in long chain	-
14	A-14	Positive	Negative	Whitish Grey	spores in isolate, cluster	-
15	A-15	Positive	Negative	Creamy	spores in isolate, cluster	-
16	A-16	Positive	Negative	Creamy	Rectifexibles, Cluster	-
17	A-17	Positive	Negative	Creamy	Rectifexibles, Cluster	-
18	A-18	Positive	Negative	Grey	spores in isolate	-
19	A-19	Positive	Negative	Grey	spores in isolate	-
20	A-20	Positive	Negative	Milky white	Spores in chain	-
21	A-21	Positive	Negative	Creamy	Individual spore	-
22	A-22	Positive	Negative	Whitish Grey	Individual spore	-
23	A-23	Positive	Negative	Creamy	Rectifexibles, spore in long chain	-
24	A-24	Positive	Negative	Creamy	Rectifexibles, spore in cluster, long chain	-

S. N.	Isolates No.	Gram reaction	Acid fast stain	Colour	Spore chain morphology	Pigmentation
25	A-25	Positive	Negative	Creamy	Rectifexibles, spore in long chain	-
26	A-26	Positive	Negative	Creamy	Rectifexibles, spore in chain, structure	-
27	A-28	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
28	A-29	Positive	Negative	Creamy	Rectifexibles, spore in long chain	-
29	A-30	Positive	Negative	Grey	Spores in chain	-
30	A-31	Positive	Negative	Whitish Creamy	Rectifexibles, spore in long chain	-
31	A-32	Positive	Negative	Grey	Spore isolates	-
32	A-33	Positive	Negative	White Grey	Spores in cluster	-
33	A-34	Positive	Negative	Grey	Spore isolates	-
34	A-35	Positive	Negative	Grey	Spore isolates	-
35	A-36	Positive	Negative	Whitish Grey	Spores in cluster	-
36	A-37	Positive	Negative	Whitish Grey	Spores in cluster	-
37	A-39	Positive	Negative	Whitish Creamy	Rectifexibles, spore in long chain	-
38	A-40	Positive	Negative	Creamy	Rectifexibles, spore in cluster and structure	-
39	A-41	Positive	Negative	Creamy	Spore in cluster and structure	-
40	A-42	Positive	Negative	Whitish Grey	Rectifexibles, spore in chain	-
41	A-43	Positive	Negative	Grey	Spore isolates	-
42	A-44	Positive	Negative	Milky white	Rectifexibles, chain, hair like	-
43	A-45	Positive	Negative	White	spore in chain	-
44	A-46	Positive	Negative	White	spore in chain	-
45	A-48	Positive	Negative	Creamy	Rectifexibles, spore in long chain	Pinkish
46	A-49	Positive	Negative	Grey	Spore in cluster	-
47	A-50	Positive	Negative	Whitish Grey	Spore in chain	-
48	A-51	Positive	Negative	Blackish Grey	Spore in cluster	-
49	A-52	Positive	Negative	Creamy	Rectifexibles, spore in long chain, structure	-

S. N.	Isolates No.	Gram reaction	Acid fast stain	Colour	Spore chain morphology	Pigmentation
50	A-53	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
51	A-55	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
52	A-56	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
53	A-57	Positive	Negative	Grey	Spore in chain	-
54	A-58	Positive	Negative	Creamy	Rectifexibles, spore in chain	Yellowish
55	A-59	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
56	A-60	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
57	A-62	Positive	Negative	Creamy	Rectifexibles,	-
58	A-63	Positive	Negative	Grey	Spores isolated	-
59	A-64	Positive	Negative	Creamy	Rectinaculiapetri, Spore in long chain, structure	-
60	A-65	Positive	Negative	Creamy	Rectifexibles,Spore in chain	-
61	A-67	Positive	Negative	Creamy	Rectifexibles,Spore in long chain	-
62	A-71	Positive	Negative	Grey	Spore in chain	-
63	A-73	Positive	Negative	Creamy	Rectinaculiapetri, Spore in cluster, structure	-
64	A-74	Positive	Negative	Whitish Grey	Rectifexibles,Spore in short chain , structure	-
65	A-75	Positive	Negative	Grey	Spore in chain	-
66	A-76	Positive	Negative	Creamy	Rectinaculiapetri, Root hair like structure	-
67	A-78	Positive	Negative	Grey	Rectifexibles,Spore in cluster	Yellowish
68	A-79	Positive	Negative	Grey	Rectifexibles, Individual spore	-
69	A-80	Positive	Negative	Grey	Rectifexibles, spore in chain	-
70	A-81	Positive	Negative	Whitish Grey	Rectifexibles, spore in cluster	-
71	A-82	Positive	Negative	Creamy	Rectifexibles, spore in chain	-

S. N.	Isolates No.	Gram reaction	Acid fast stain	Colour	Spore chain morphology	Pigmentation
72	A-83	Positive	Negative	Whitish Grey	Rectifexibles, spore in cluster	-
73	A-85	Positive	Negative	Creamy	Rectifexibles, spore in chain , cluster	-
74	A-87	Positive	Negative	Grey	Rectifexibles, spore cluster, structure	-
75	A-88	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
76	A-89	Positive	Negative	Grey	Rectifexibles, spore in cluster	-
77	A-90	Positive	Negative	Grey	Spore in chain	-
78	A-91	Positive	Negative	Whitish Grey	Spore in chain	-
79	A-92	Positive	Negative	Grey	Spore in cluster	-
80	A-93	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
81	A-94	Positive	Negative	Grey	Spore in cluster	-
82	A-95	Positive	Negative	Creamy	Rectifexibles	-
83	A-97	Positive	Negative	Creamy	Rectifexibles, spore in long chain	-
84	A-98	Positive	Negative	Blackish Creamy	Rectifexibles, spore cluster structure	-
85	A-99	Positive	Negative	Whitish Grey	Spore in cluster	-
86	A-100	Positive	Negative	Blackish Grey	Spores in isolates	-
87	A-101	Positive	Negative	Creamy	Rectifexibles, spore in very long chain	-
88	A-102	Positive	Negative	Creamy	Rectifexibles,	-
89	A-104	Positive	Negative	Creamy	Rectifexibles, spore cluster structure	-
90	A-105	Positive	Negative	Creamy	Rectifexibles, spore in very long chain	-
91	A-106	Positive	Negative	Whitish Grey	Rectifexibles,	-
92	A-107	Positive	Negative	Grey	Spore in cluster	-
93	A-108	Positive	Negative	Creamy	Rectifexibles,	-
94	A-109	Positive	Negative	Creamy	Rectifexibles, spore in cluster	-
95	A-110	Positive	Negative	Creamy	Rectifexibles, spore in short chain, cluster	-

S. N.	Isolates No.	Gram reaction	Acid fast stain	Colour	Spore chain morphology	Pigmentation
96	A-111	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
97	A-112	Positive	Negative	Creamy	Spore in long chain	-
98	A-113	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
99	A-114	Positive	Negative	Whitish Grey	Spore in chain	-
100	A-115	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
101	A-116	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
102	A-119	Positive	Negative	Blackish Grey	Spore in chain	-
103	A-120	Positive	Negative	Creamy	Rectifexibles, Root hair structure	Bluish
104	A-121	Positive	Negative	Whitish Creamy	Rectifexibles, Short chain structure	-
105	A-122	Positive	Negative	Blackish Grey	Spore in chain	-
106	A-123	Positive	Negative	Creamy	Rectifexibles, Short chain cluster structure	-
107	A-124	Positive	Negative	Creamy	Rectifexibles, spore in long chain with structure	-
108	A-125	Positive	Negative	Blackish Grey	spore in chain , individual	-
109	A-126	Positive	Negative	Blackish Grey	spore in chain , individual	-
110	A-127	Positive	Negative	Creamy	Rectifexibles	-
111	A-128	Positive	Negative	Whitish Grey	spore in chain , individual	-
112	A-129	Positive	Negative	Creamy	Rectifexibles, spore in very long chain	-
113	A-130	Positive	Negative	Creamy	Rectifexibles, spore in chain	-
114	A-132	Positive	Negative	Creamy	Rectifexibles, spore in long chain structure	-
115	A-133	Positive	Negative	Whitish Creamy	Rectifexibles,	-
116	A-134	Positive	Negative	Creamy	Rectifexibles, Root hair structure	-

S. N.	Isolates No.	Gram reaction	Acid fast stain	Colour	Spore chain morphology	Pigmentation
117	A-135	Positive	Negative	Whitish Creamy	Rectifexibles, hair like structure	-
118	A-136	Positive	Negative	Creamy	Rectinaculiapetri	-
119	A-137	Positive	Negative	Grey	spore in individual	-
120	A-138	Positive	Negative	Creamy	Rectinaculiapetri, spore in cluster	-
121	A-139	Positive	Negative	Creamy	Rectifexibles, Spore in cluster groups	-
122	A-140	Positive	Negative	Grey	Spore in individual	-
123	A-141	Positive	Negative	Grey	Spore in individual	-
124	A-142	Positive	Negative	Grey	spore in individual	-
125	A-143	Positive	Negative	Creamy	Rectifexibles, spore in chain , individual	-
126	A-144	Positive	Negative	Creamy	Rectifexibles, spore in long chain, structure	-
127	A-145	Positive	Negative	Blackish Grey	spore in individual	-
128	A-146	Positive	Negative	Creamy	Rectifexibles, spore in cluster	-
129	A-147	Positive	Negative	Creamy	Rectifexibles, spore in cluster	-
130	A-148	Positive	Negative	Creamy	Rectifexibles, spore in cluster, chain,	Dark pinkish
131	A-150	Positive	Negative	Creamy	Rectifexibles, spore in chain	-

Streptomyces sp. was found good on starch casein agar and poor to moderate on nutrient agar medium. (Table 6, Plate 4) The growth of aerial and substrate mycelium was observed on these media. It was found that there was no variation observed among the aerial mycelium on the different growth media whereas there was slight change observed in substrate mycelium.

4.2.3 Biochemical characterization :

All the 72 *Streptomyces* sp. isolates were tested for their biochemical characterization viz., gelatin liquefaction, H₂S production, catalase test and oxidase test (Table 7, Plate 5). All the isolates were positive in catalase test and oxidase test. The H₂S production was observed positive reaction in 60 cultures. It was observed that 68 isolates does not liquefy showed negative gelatin liquefaction ability.

4.2.4 Functional diversity of *Streptomyces* sp. :

Among the actinomycetes isolates from soils of Maharashtra, on the basis of morphological and cultural studies 72 isolates were identified as *Streptomyces* sp and studied for functional diversity like cellulose degradation, antibiotic sensitivity, antimicrobial activity and P solubilization ability.

4.2.4.1. Antimicrobial activity:

The *Streptomyces* sp. were tested antimicrobial activity against the pathogen viz., *Fusarium oxysporum* pv. *ciceri* and *Alternaria alternata* . The antibiotic activity of *Streptomyces* sp. were summarised in Table 8, Plate 6 .

The RHR-S-98 showed maximum percent inhibition was 30.00% against *A. alternata in vitro*. Among the 72 *Streptomyces* sp., 64 isolates showed antimicrobial activity against *Alternaria alternata* was ranged from 7.45% to 19.90% and 8 isolates showed more than 20% antimicrobial activity against *Alternaria alternata*.

Table 6 : Cultural characteristics of *Streptomyces* spp. isolated from soils of Maharashtra.

Sr. No.	Isolate No.	Nutrient Agar			Starch Casein Agar			Oat Meal Agar		
		Growth	Aerial mycelium	Vegetative mycelium	Growth	Aerial mycelium	Vegetative mycelium	Growth	Aerial mycelium	Vegetative mycelium
1.	RHR S -3	Moderate	Creamy	Creamy	Good	Creamy	Brown	Excellent	Creamy	Brown
2	RHR S -5	Poor	Creamy	Light Creamy	Moderate	Creamy	Light Creamy	Good	Light Creamy	Colourless
3	RHR S-6	Moderate	Creamy	Medium Creamy	Good	Creamy	Brown	Good	Creamy	Light Cream
4	RHR S-7	Moderate	Creamy	Light Creamy	Good	Creamy	Light Creamy	Good	Light Creamy	Yellow Brown
5	RHR S-10	Moderate	Creamy	Light Brown	Good	Creamy	Pale Yellow	Good	Creamy	Light Cream
6	RHR S-13	Moderate	Light Creamy	Light Brown	Moderate	Medium Cream	Colourless	Good	Med. Creamy	Brown
7	RHR S-15	Moderate	Creamy	Light Yellow	Good	Creamy	Light Creamy	Good	Light Creamy	Yellow Brown
8	RHR S-16	Moderate	Creamy	Cream	Good	Creamy	Light Creamy	Good	Creamy	Light Cream
9	RHR S-17	Poor	Creamy	Med. Cream	Good	Creamy	Pale Yellow	Good	Creamy	Light Cream
10	RHR S-21	Poor	Creamy	Light Cream	Moderate	Creamy	Light Creamy	Good	Med. Creamy	Yellow Brown
11	RHR S-23	Good	Creamy	Light Brown	Good	Creamy	Light Yellow	Good	Creamy	Pale Yellow
12	RHR S-24	Poor	Creamy	Light Yellow	Excellent	Creamy	Brown	Excellent	Creamy	Yellow Brown
13	RHR S-25	Moderate	Creamy	Cream	Moderate	Creamy	Light Creamy	Good	Creamy	Cream
14	RHR S-26	Moderate	Creamy	Cream	Moderate	Dark Creamy	Pale Yellow	Excellent	Creamy	Brown
15	RHR S-28	Poor	Creamy	Brown	Moderate	Moderate Cream	Colourless	Excellent	Creamy	Light Cream
16	RHR S-29	Poor	Creamy	Light Yellow	Excellent	Creamy	Brown	Excellent	Creamy	Yellow Brown
17	RHR S-31	Moderate	Creamy	Cream	Moderate	Creamy	Light Creamy	Good	Creamy	Cream

Sr. No.	Isolate No.	Nutrient Agar			Starch Casein Agar			Oat Meal Agar		
		Growth	Aerial mycelium	Vegetative mycelium	Growth	Aerial mycelium	Vegetative mycelium	Growth	Aerial mycelium	Vegetative mycelium
18	RHR S-39	Moderate	Creamy	Greenish yellow	Good	Creamy	Creamy	Good	Creamy	Creamy
19	RHR S-40	Moderate	Creamy	Pale Yellow	Good	Creamy	Pale Yellow	Excellent	Creamy	Pale Yellow
20	RHR S-41	Moderate	Creamy	Pale Yellow	Good	Creamy	Light Brown	Excellent	Creamy	Colourless
21	RHR S-48	Moderate	Creamy	Creamy	Good	Creamy	Colourless	Excellent	Creamy	Light Cream
22	RHR S-52	Moderate	Creamy	Med. Creamy	Good	Creamy	Light Creamy	Good	Creamy	Light Cream
23	RHR S-53	Moderate	Creamy	Med. Creamy	Good	Creamy	Light Creamy	Excellent	Creamy	Light Cream
24	RHR S-55	Moderate	Creamy	Brown	Good	Creamy	Yellow Brown	Excellent	Creamy	Yellow Brown
25	RHR S-56	Moderate	Creamy	Brown	Good	Creamy	Light Creamy	Excellent	Creamy	Yellow Brown
26	RHR S-58	Moderate	Creamy	Pale Yellow	Good	Creamy	Yellow Brown	Excellent	Creamy	Colourless
27	RHR S-60	Moderate	Creamy	Creamy	Good	Creamy	Yellowish	Excellent	Creamy	Colourless
28	RHR S-62	Moderate	Creamy	Brown	Good	Creamy	Light Creamy	Excellent	Creamy	Yellow Brown
29	RHR S-64	Good	Dark Creamy	Brown	Good	Creamy	Dark Creamy	Excellent	Creamy	Light Cream
30	RHR S-65	Moderate	Creamy	Brown	Good	Creamy	Yellow	Excellent	Creamy	Colourless
31	RHR S-67	Moderate	Creamy	Brown	Good	Creamy	Yellow	Excellent	Creamy	Colourless
32	RHR S-73	Moderate	Creamy	Brown	Good	Creamy	Brown	Excellent	Creamy	Brown
33	RHR S-76	Good	Creamy	Pale Yellow	Good	Creamy	Yellow	Excellent	Creamy	Colourless
34	RHR S-82	Moderate	Creamy	Brown	Good	Creamy	Creamy	Good	Creamy	Brown
35	RHR S-85	Good	Creamy	Pale Yellow	Good	Creamy	Yellow	Excellent	Creamy	Colourless
36	RHR S-88	Good	Creamy	Yellow Brown	Good	Creamy	Yellow Green	Good	Creamy	Pale Yellow

Sr. No.	Isolate No.	Nutrient Agar			Starch Casein Agar			Oat Meal Agar		
		Growth	Aerial mycelium	Vegetative mycelium	Growth	Aerial mycelium	Vegetative mycelium	Growth	Aerial mycelium	Vegetative mycelium
37	RHR S-93	Poor	Creamy	Yellow Green	Poor	Creamy	Pale Yellow	Good	Creamy	Pale Yellow
38	RHR S-95	Moderate	Creamy	Pale Yellow	Good	Creamy	Yellow Green	Good	Yellow Creamy	Dark Brown
39	RHR S-97	Poor	Creamy	Pale Yellow	Good	Creamy	Pale Yellow	Good	Creamy	Colourless
40	RHR S-98	Moderate	Creamy	Brown	Good	Creamy	Light Cream	Excellent	Creamy	Yellow Brown
41	RHR S-101	Good	Creamy	Yellow Green	Good	Creamy	Pale Yellow	Excellent	Creamy	Yellow Brown
42	RHR S-102	Moderate	Creamy	Brown	Good	Creamy	Light Cream	Good	Creamy	Pale Yellow
43	RHR S-104	Moderate	Creamy	Pale Yellow	Good	Creamy	Yellow Green	Good	Creamy	Pale Yellow
44	RHR S-105	Moderate	Creamy	Yellow Green	Good	Creamy	Pale Yellow	Good	Creamy	Yellowish
45	RHR S-108	Moderate	Creamy	Yellow Green	Good	Creamy	Yellow Green	Good	Creamy	Yellow Brown
46	RHR S-109	Moderate	Creamy	Yellow Green	Good	Creamy	Pale Yellow	Good	Creamy	Brown
47	RHR S-110	Moderate	Creamy	Brown	Good	Creamy	Light Cream	Excellent	Creamy	Yellow Brown
48	RHR S-111	Moderate	Creamy	Pale Yellow	Good	Creamy	Pale Yellow	Good	Creamy	Yellow Brown
49	RHR S-112	Moderate	Creamy	Yellow Green	Good	Creamy	Light Cream	Good	Creamy	Brown
50	RHR S-113	Moderate	Creamy	Brown	Good	Creamy	Pale Yellow	Excellent	Creamy	Yellow Brown
51	RHR S-115	Moderate	Creamy	Pale Yellow	Good	Creamy	Light Creamy	Excellent	Creamy	Yellow Brown
52	RHR S-116	Good	Creamy	Pale Yellow	Good	Creamy	Pale Yellow	Good	Yellow Creamy	Dark Brown
53	RHR S-120	Moderate	Creamy	Brown	Good	Creamy	Light Creamy	Excellent	Creamy	Yellow Brown
54	RHR S-121	Moderate	Creamy	Pale Yellow	Good	Creamy	Light Creamy	Good	Yellow Creamy	Dark Brown
55	RHR S-123	Moderate	Creamy	Light Yellow	Good	Creamy	Mod. Cream	Good	Creamy	Light Cream

Sr. No.	Isolate No.	Nutrient Agar			Starch Casein Agar			Oat Meal Agar		
		Growth	Aerial mycelium	Vegetative mycelium	Growth	Aerial mycelium	Vegetative mycelium	Growth	Aerial mycelium	Vegetative mycelium
56	RHR S-124	Moderate	Creamy	Light Yellow	Good	Creamy	Light Brown	Excellent	Mod. Creamy	Light Brown
57	RHR S-127	Moderate	Creamy	Light Yellow	Good	Creamy	Mod. Cream	Good	Creamy	Light Cream
58	RHR S-129	Moderate	Creamy	Light Yellow	Good	Creamy	Light Brown	Excellent	Mod. Creamy	Light Brown
59	RHR S-130	Moderate	Creamy	Light Yellow	Good	Creamy	Mod. Cream	Good	Creamy	Light Cream
60	RHR S-132	Moderate	Creamy	Light Yellow	Good	Creamy	Light Brown	Excellent	Mod. Creamy	Light Brown
61	RHR S-133	Poor	Creamy	Light Yellow	Good	Creamy	Light Yellow	Good	Mod. Creamy	Brown
62	RHR S-134	Moderate	Creamy	Light Yellow	Good	Creamy	Light Brown	Excellent	Creamy	Light Brown
63	RHR S-136	Moderate	Creamy	Greenish Yellow	Good	Creamy	Light Yellow	Good	Mod. Creamy	Yellow Cream
64	RHR S-138	Moderate	Creamy	Yellow Green	Good	Creamy	Light Cream	Good	Creamy	Dark Brown
65	RHR S-139	Moderate	Creamy	Light Yellow	Good	Creamy	Mod. Cream	Good	Creamy	Light Cream
66	RHR S-141	Poor	Creamy	Dark Brown	Good	Creamy	Light Creamy	Excellent	Creamy	Dark Brown
67	RHR S-143	Moderate	Creamy	Yellow Brown	Good	Creamy	Light yellow	Excellent	Creamy	Brown
68	RHR S-144	Poor	Creamy	Dark Brown	Good	Creamy	Light Creamy	Excellent	Creamy	Dark Brown
69	RHR S-146	Moderate	Creamy	Light Yellow	Good	Creamy	Mod. Cream	Good	Creamy	Light Cream
70	RHR S-147	Moderate	Creamy	Light Yellow	Moderate	Creamy	Light Brown	Excellent	Mod. Creamy	Light Brown
71	RHR S-148	Poor	Creamy	Dark Brown	Good	Creamy	Light Cream	Excellent	Creamy	Dark Brown
72	RHR S-150	Moderate	Creamy	Pale Yellow	Good	Creamy	Pale Yellow	Good	Creamy	Yellow Brown
73	RHR S	Moderate	Creamy	Light Cream	Good	Creamy	Light Cream	Good	Light Creamy	Colourless

Table 7 : Biochemical characteristics of *Streptomyces* spp. isolated from soils of Maharashtra.

Sr.No.	Isolate No.	Catalase	Oxidase	Gelatin Liquefaction	H₂S production
1.	RHR S -3	+	+	+	+
2	RHR S -5	+	+	+	+
3	RHR S-6	+	+	+	+
4	RHR S-7	+	+	+	+
5	RHR S-10	+	+	+	+
6	RHR S-13	+	+	+	-
7	RHR S-15	+	+	+	+
8	RHR S-16	+	+	+	-
9	RHR S-17	+	+	+	+
10	RHR S-21	+	+	+	+
11	RHR S-23	+	+	+	+
12	RHR S-24	+	+	+	+
13	RHR S-25	+	+	+	+
14	RHR S-26	+	+	+	+
15	RHR S-28	+	+	+	+
16	RHR S-29	+	+	-	-
17	RHR S-31	+	+	+	+
18	RHR S-39	+	+	+	+
19	RHR S-40	+	+	+	+
20	RHR S-41	+	+	+	+
21	RHR S-48	+	+	+	+
22	RHR S-52	+	+	+	+
23	RHR S-53	+	+	+	+
24	RHR S-55	+	+	+	+

Sr.No.	Isolate No.	Catalase	Oxidase	Gelatin Liquefaction	H₂S production
25	RHR S-56	+	+	+	-
26	RHR S-58	+	+	+	+
27	RHR S-60	+	+	+	+
28	RHR S-62	+	+	+	-
29	RHR S-64	+	+	+	+
30	RHR S-65	+	+	+	+
31	RHR S-67	+	+	-	+
32	RHR S-73	+	+	+	+
33	RHR S-76	+	+	+	-
34	RHR S-82	+	+	+	+
35	RHR S-85	+	+	+	+
36	RHR S-88	+	+	+	+
37	RHR S-93	+	+	+	+
38	RHR S-95	+	+	+	+
39	RHR S-97	+	+	+	-
40	RHR S-98	+	+	+	+
41	RHR S-101	+	+	+	+
42	RHR S-102	+	+	+	+
43	RHR S-104	+	+	+	+
44	RHR S-105	+	+	+	-
45	RHR S-108	+	+	-	+
46	RHR S-109	+	+	+	+
47	RHR S-110	+	+	+	+
48	RHR S-111	+	+	+	+

Sr.No.	Isolate No.	Catalase	Oxidase	Gelatin Liquefaction	H₂S production
49	RHR S-112	+	+	+	+
50	RHR S-113	+	+	+	+
51	RHR S-115	+	+	+	+
52	RHR S-116	+	+	+	+
53	RHR S-120	+	+	+	+
54	RHR S-121	+	+	+	+
55	RHR S-123	+	+	+	-
56	RHR S-124	+	+	+	+
57	RHR S-127	+	+	+	+
58	RHR S-129	+	+	+	+
59	RHR S-130	+	+	+	+
60	RHR S-132	+	+	+	+
61	RHR S-133	+	+	+	-
62	RHR S-134	+	+	+	+
63	RHR S-136	+	+	+	+
64	RHR S-138	+	+	+	+
65	RHR S-139	+	+	+	+
66	RHR S-141	+	+	+	+
67	RHR S-143	+	+	+	+
68	RHR S-144	+	+	+	-
69	RHR S-146	+	+	-	+
70	RHR S-147	+	+	+	+
71	RHR S-148	+	+	+	+
72	RHR S-150	+	+	+	-
73	RHR S	+	+	+	+

+ = Positive

- = Negative

Table 8 : Biocontrol activity of *Streptomyces* sp. isolated from soils of Maharashtra against *Alternaria alternata* and *Fusarium oxysporium*

Sr. No.	Isolate No	Dual plate method		Dual inoculation method	
		% GI of <i>A. alternata</i>	% GI of <i>F. oxysporium</i>	<i>A. alternata</i>	<i>F. oxysporium</i>
1	RHR S -3	16.65	10.52	+	+
2	RHR S -5	9.75	10.44	+	+
3	RHR S-6	10.80	11.85	+	+
4	RHR S-7	13.55	15.52	+	+
5	RHR S-10	13.25	14.95	+	+
6	RHR S-13	17.20	17.93	+	+
7	RHR S-15	14.15	15.31	+	+
8	RHR S-16	9.99	20.32	+	+
9	RHR S-17	15.55	15.83	+	+
10	RHR S-21	15.60	15.65	+	+
11	RHR S-23	19.50	28.80	+	++
12	RHR S-24	24.80	24.80	++	++
13	RHR S-25	25.00	25.40	++	++
14	RHR S-26	14.40	20.31	+	++
15	RHR S-28	19.02	20.95	+	++
16	RHR S-29	13.20	14.95	+	+
17	RHR S-31	14.20	15.32	+	+
18	RHR S-39	9.95	10.85	+	+
19	RHR S-40	16.40	14.83	+	+
20	RHR S-41	16.33	17.98	+	+
21	RHR S-48	13.55	14.50	+	+
22	RHR S-52	16.84	11.18	+	+
23	RHR S-53	9.83	10.84	+	+
24	RHR S -55	10.65	11.84	+	+
				Contd.....	

Sr. No.	Isolate No	Dual plate method		Dual inoculation method	
		% GI of <i>A. alternata</i>	% GI of <i>F. oxysporium</i>	<i>A. alternata</i>	<i>F. oxysporium</i>
25	RHR S-56	14.75	15.53	+	+
26	RHR S-58	14.35	14.93	+	+
27	RHR S-60	16.18	17.78	+	+
28	RHR S-62	14.15	15.31	+	+
29	RHR S-64	14.53	18.24	+	+
30	RHR S-65	12.80	14.83	+	+
31	RHR S-67	17.00	18.05	+	+
32	RHR S-73	7.45	20.05	+	++
33	RHR S-76	9.85	10.60	+	+
34	RHR S-82	15.55	14.55	+	+
35	RHR S-85	11.30	11.81	+	+
36	RHR S-88	21.51	15.53	++	+
37	RHR S-93	8.73	15.63	+	+
38	RHR S-95	8.83	20.35	+	++
39	RHR S-97	17.05	18.05	+	+
40	RHR S-98	30.00	21.89	+++	++
41	RHR S-101	20.63	18.65	++	+
42	RHR S-102	18.00	18.05	+	+
43	RHR S-104	14.25	14.55	+	+
44	RHR S-105	24.65	18.05	++	+
45	RHR S-108	16.10	14.90	+	+
46	RHR S-109	11.35	12.45	+	+
47	RHR S-110	11.80	10.90	+	+
48	RHR S-111	13.25	11.45	+	+
49	RHR S-112	14.65	15.74	+	+
				Contd.....	

Sr. No.	Isolate No	Dual plate method		Dual inoculation method	
		% GI of <i>A. alternata</i>	% GI of <i>F. oxysporium</i>	<i>A. alternata</i>	<i>F. oxysporium</i>
50	RHR S-113	10.98	18.25	+	+
51	RHR S-115	11.45	14.65	+	+
52	RHR S-116	13.00	17.85	+	+
53	RHR S-120	17.25	14.55	+	+
54	RHR S-121	13.05	16.45	+	+
55	RHR S-123	10.25	12.25	+	+
56	RHR S-124	15.00	12.85	+	+
57	RHR S-127	9.65	15.60	+	+
58	RHR S-129	15.95	20.15	+	++
59	RHR S-130	21.90	20.75	++	++
60	RHR S-132	19.25	19.50	+	+
61	RHR S-133	17.75	19.25	+	+
62	RHR S-134	15.85	10.75	+	+
63	RHR S-136	13.80	15.25	+	+
64	RHR S-138	25.20	19.65	++	+
65	RHR S-139	14.40	15.63	+	+
66	RHR S-141	16.35	15.90	+	+
67	RHR S-143	13.55	16.75	+	+
68	RHR S-144	15.25	15.50	+	+
69	RHR S-146	19.60	21.35	+	+
70	RHR S-147	13.65	17.50	+	+
71	RHR S-148	19.90	19.90	+	+
72	RHR S-150	14.85	17.90	+	+
73	RHR S	20.05	20.00	++	++
	+ = poor inhibition ++ = Moderate inhibition +++ = Good inhibition				

The RHR-S-23 showed maximum percent inhibition was 28.80% against *Fusarium oxysporum* pv. *ciceri* *in vitro*. Out of 72 *Streptomyces* sp., 12 *Streptomyces* sp. were showed more than 20% antimicrobial activity against *Fusarium oxysporum* pv. *ciceri* and 60 isolates showed less than 20% antimicrobial activity against *Fusarium oxysporum* pv. *ciceri*. The *Streptomyces* sp. showed percent inhibition was ranged from 10.44% to 28.80%

4.2.4.2. Cellulose degradation :

The ability to cellulose degradation of the 72 *Streptomyces* sp. isolates was studied by cultivating each isolate in dubos broth and incubated at 30 °C for 15 days. *Streptomyces* sp. isolated from soils of Maharashtra were tested for cellulose degradation activity. The ability to cellulose decomposition of *Streptomyces* sp. were summarized in Table 9. and Plate 7. It was observed that 56.94% *Streptomyces* sp. have good cellulose degradation activity and 40.28% *Streptomyces* sp. have moderate cellulose degradation activity and 2.78% have not cellulose degradation activity.

4.2.4.3 Antibiotic sensitivity :

The antibiotic sensitivity of *Streptomyces* sp. were tested by using the standard disc diffusion method of Baur *et al* (1966) and as adopted by Antoun *et al* (1982). Antibiotics discs R₅, S₁₀ and T₃₀ and NS₁₀₀ were used. The antibiotic sensitivity of *Streptomyces* sp. were summerised in Table 10. and Plate 8.

Among the 72 *Streptomyces* sp., it was observed that 21 isolates does not form the zone around the antibiotic disc which indicates that these 21 isolates were resistant and 51 were sensitive to antibiotics R₅, S₁₀ and T₃₀. All the 72 *Streptomyces* sp. isolated from soils of Maharashtra were found resistant to NS₁₀₀.

4.2.4.4 P solubilization ability:

The P solubilizing ability of *Streptomyces* sp. were summerised in Table 11. Among the 72 *Streptomyces* sp., only 22.22% isolates showed

Table 9 . Cellulose degradation of *Streptomyces* isolated from soils of Maharashtra

Sr. No.	Isolate No	Cellulose Degradation	Sr. No.	Isolate No	Cellulose Degradation	Sr. No.	Isolate No	Cellulose Degradation
1	RHR S-3	+	25	RHR S-56	++	49	RHR S-112	+
2	RHR S-5	+	26	RHR S-58	+	50	RHR S-113	+
3	RHR S-6	+	27	RHR S-60	++	51	RHR S-115	+
4	RHR S-7	++	28	RHR S-62	++	52	RHR S-116	+
5	RHR S-10	-	29	RHR S-64	+	53	RHR S-120	++
6	RHR S-13	++	30	RHR S-65	+	54	RHR S-121	++
7	RHR S-15	++	31	RHR S-67	++	55	RHR S-123	++
8	RHR S-16	+	32	RHR S-73	+	56	RHR S-124	++
9	RHR S-17	+	33	RHR S-76	++	57	RHR S-127	++
10	RHR S-21	++	34	RHR S-82	+	58	RHR S-129	+
11	RHR S-23	+	35	RHR S-85	++	59	RHR S-130	++
12	RHR S-24	++	36	RHR S-88	+	60	RHR S-132	+
13	RHR S-25	+	37	RHR S-93	++	61	RHR S-133	++
14	RHR S-26	++	38	RHR S-95	+	62	RHR S-134	+
15	RHR S-28	++	39	RHR S-97	++	63	RHR S-136	++
16	RHR S-29	++	40	RHR S-98	++	64	RHR S-138	++
17	RHR S-31	++	41	RHR S-101	++	65	RHR S-139	+
18	RHR S-39	++	42	RHR S-102	++	66	RHR S-141	-
19	RHR S-40	++	43	RHR S-104	++	67	RHR S-143	++
20	RHR S-41	++	44	RHR S-105	++	68	RHR S-144	+
21	RHR S-48	++	45	RHR S-108	+	69	RHR S-146	++
22	RHR S-52	++	46	RHR S-109	+	70	RHR S-147	+
23	RHR S-53	+	47	RHR S-110	+	71	RHR S-148	++
24	RHR S-55	+	48	RHR S-111	++	72	RHR S-150	++
						73	RHR-S	++

+ = moderate cellulose degradation

++ = Good cellulose degradation

- = No cellulose degradation

Table 10. Sensitivity of *Streptomyces* sp. isolated from soils of Maharashtra against different antibiotics

Sr. No.	Isolate No	R ₅				S ₁₀				T ₃₀				NS ₁₀₀			
		1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7
1	3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
2	5	R	R	R	R	10	10	10	10	16	16	16	16	R	R	R	R
3	6	13	13	13	13	R	R	R	R	R	R	R	R	R	R	R	R
4	7	9	9	9	9	R	15	15	15	9	13	13	13	R	R	R	R
5	10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
6	13	9	9	9	9	14	16	16	16	R	30	30	30	R	R	R	R
7	15	13	13	13	13	15	15	15	15	10	10	10	10	R	R	R	R
8	16	R	R	R	R	R	R	R	R	8	8	8	8	R	R	R	R
9	17	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
10	19	11	11	11	11	9	9	9	9	11	11	11	11	R	R	R	R
11	21	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
12	23	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
13	24	R	10	10	10	R	R	R	R	R	R	R	R	R	R	R	R
14	25	R	R	R	R	10	10	10	10	R	R	R	R	R	R	R	R
15	26	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
16	28	R	R	R	R	R	R	R	R	10	10	10	10	R	R	R	R
17	29	20	20	21	21	15	15	15	15	12	12	12	12	R	R	R	R
18	31	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
19	39	13	13	13	17	14	14	14	14	14	14	14	18	R	R	R	R
20	40	10	10	10	10	14	14	14	14	15	15	16	16	R	R	R	R
21	41	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
22	48	9	9	9	9	13	13	13	13	14	14	14	14	R	R	R	R
23	52	R	R	11	11	R	R	30	R	8	8	8	8	R	R	R	R
24	53	8	8	8	8	10	10	10	10	R	15	15	15	R	R	R	R

Contd ...

Sr. No.	Isolate No	R ₅				S ₁₀				T ₃₀				NS ₁₀₀			
		1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7
25	55	R	R	R	R	R	R	R	R	8	8	8	8	R	R	R	R
26	56	R	16	16	16	10	11	11	11	R	R	R	R	R	R	R	R
27	58	9	9	9	9	9	9	27	27	13	15	15	15	R	R	R	R
28	59	19	19	19	19	16	16	16	16	13	13	13	13	R	R	R	R
29	60	R	R	R	R	8	8	8	8	R	R	R	R	R	R	R	R
30	62	13	13	13	13	11	11	11	11	15	26	26	26	R	R	R	R
31	64	9	9	9	9	18	18	18	18	9	9	32	32	R	R	R	R
32	65	12	12	12	12	9	9	9	9	15	20	20	20	R	R	R	R
33	67	R	12	12	12	R	R	R	R	8	8	8	8	R	R	R	R
34	73	R	12	12	12	R	R	R	R	R	R	R	R	R	R	R	R
35	76	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
36	82	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
37	85	R	R	R	R	R	R	R	R	R	21	24	24	R	R	R	R
38	88	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
39	93	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
40	95	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
41	98	10	10	10	11	13	14	14	14	R	19	23	23	R	R	R	R
42	101	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
43	102	11	11	11	11	11	11	11	11	R	20	20	20	R	R	R	R
44	104	9	9	9	9	9	9	9	9	R	13	13	13	R	R	R	R
45	105	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
46	109	13	13	13	13	R	R	R	R	R	21	21	21	R	R	R	R
47	110	12	13	15	15	13	13	13	13	R	31	31	31	R	R	R	R
48	111	20	20	20	20	11	11	11	11	R	R	R	R	R	R	R	R
49	112	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
50	113	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
51	115	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
52	116	8	R	R	R	8	R	R	R	8	R	R	R	R	R	R	R

Contd

Sr. No.	Isolate No	R ₅				S ₁₀				T ₃₀				NS ₁₀₀			
		1	3	5	7	1	3	5	7	1	3	5	7	1	3	5	7
53	120	10	10	10	10	R	12	12	12	R	R	R	R	R	R	R	R
54	121	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
55	123	R	12	12	12	9	9	9	9	R	R	R	R	R	R	R	R
56	124	10	17	18	18	9	9	9	9	R	R	31	31	R	R	R	R
57	127	R	R	R	R	R	R	R	R	R	18	18	18	R	R	R	R
58	129	15	15	15	15	R	R	R	R	10	10	10	10	R	R	R	R
59	130	10	10	10	10	R	R	9	9	21	21	21	21	R	R	R	R
60	132	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
61	133	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
62	134	11	11	11	11	11	12	12	12	R	26	26	26	R	R	R	R
63	136	15	15	15	15	12	12	12	12	R	25	25	25	R	R	R	R
64	138	17	17	17	17	15	23	30	38	R	22	23	23	R	R	R	R
65	139	14	14	14	14	9	9	9	9	16	23	23	23	R	R	R	R
66	141	13	13	13	13	11	11	11	12	17	21	21	21	R	R	R	R
67	143	9	9	9	9	14	14	14	14	R	23	23	23	R	R	R	R
68	144	14	14	14	14	15	15	15	15	12	12	16	18	R	R	R	R
69	146	12	12	12	12	7	7	7	7	15	20	24	28	R	R	R	R
70	147	R	R	R	R	14	14	14	14	10	10	10	10	R	R	R	R
71	148	10	10	10	10	9	29	30	32	R	30	31	31	R	R	R	R
72	150	13	15	15	15	R	R	20	22	13	13	15	15	R	R	R	R

R : Resistance Fig. : Zone in mm

Note: Observations were recorded on 1,3,5 and 7 days

Table 11. P solubilization of *Streptomyces* sp. isolated from soils of Maharashtra

Sr. No.	Isolate No	P solubili zation	Sr. No.	Isolate No	P solubili zation	Sr. No.	Isolate No	P solubili zation
1	RHR S 3	-	25	RHR S 56	+	49	RHR S 112	-
2	RHR S 5	+	26	RHR S 58	-	50	RHR S 113	-
3	RHR S 6	-	27	RHR S 60	-	51	RHR S 115	-
4	RHR S 7	-	28	RHR S 62	-	52	RHR S 116	+
5	RHR S 10	-	29	RHR S 64	+	53	RHR S 120	-
6	RHR S 13	-	30	RHR S 65	-	54	RHR S 121	-
7	RHR S 15	-	31	RHR S 67	-	55	RHR S 123	-
8	RHR S 16	-	32	RHR S 73	-	56	RHR S 124	+
9	RHR S 17	-	33	RHR S 76	-	57	RHR S 127	-
10	RHR S 21	-	34	RHR S 82	-	58	RHR S 129	-
11	RHR S 23	-	35	RHR S 85	-	59	RHR S 130	+
12	RHR S 24	+	36	RHR S 88	-	60	RHR S 132	-
13	RHR S 25	-	37	RHR S 93	-	61	RHR S 133	-
14	RHR S 26	-	38	RHR S 95	-	62	RHR S 134	-
15	RHR S 28	+	39	RHR S 97	+	63	RHR S 136	-
16	RHR S 29	-	40	RHR S 98	+	64	RHR S 138	+
17	RHR S 31	-	41	RHR S 101	-	65	RHR S 139	-
18	RHR S 39	-	42	RHR S 102	-	66	RHR S 141	-
19	RHR S 40	+	43	RHR S 104	-	67	RHR S 143	-
20	RHR S 41	+	44	RHR S 105	-	68	RHR S 144	-
21	RHR S 48	+	45	RHR S 108	-	69	RHR S 146	+
22	RHR S 52	-	46	RHR S 109	-	70	RHR S 147	-
23	RHR S 53	-	47	RHR S 110	-	71	RHR S 148	+
24	RHR S 55	-	48	RHR S 111	-	72	RHR S 150	-

+ = Slight P solubilization

- = No P solubilization

the P solubilization ability in which less than 1cm P solubilization zone was observed and 77.78% isolates does not showed P solubilization activity.

4.2. 5 Molecular Characterization:

The PCR amplification products of the 18 strains of *Streptomyces* spp. were subjected to PCR amplification using 23 random primers Plate 9 . The amplification of *Streptomyces* sp. of 23 random primers were summerised in Table 12. The RAPD phenotypes were assessed from the photographs. It was observed that 1124 bands were produced by the 23 primers (Table 13, Plate 10 to Plate 16). The number of bands produced per primer varied from 36 to 72. The average number of bands per primer was 48.87. Out of 1124 bands, 986 polymorphic bands were observed (88.52%) and 138 unique bands were observed (11.48%) and no monomorphic band observed in all 23 primers. Most of the primers produced highly polymorphic bands varied from 2 to 24. Highly polymorphic bands observed maximum (24) in primer RBA-14 followed by RBA-16 (18) and minimum highly polymorphic bands observed in RBA-7. Less polymorphic bands were also recorded in all primers ranged from 18 to 46 in which <20% polymorphic band observed and most of the primers do not produce >80% ie less polymorphic bands. The amplified DNA fragment ranged from 195 to 4234 bp. The number of polymorphic loci amplified varied with different primers and isolates. The primers RBA 10 produced maxium polymorphic bands (55). The per cent polymorphism was ranged from 73.33 to 97.43% and average polymorphism was observed 87.72%. The fingerprints of 23 random primers were converted into a binary matrix based on the prescence of band was marked as “1” and absence of band was marked as “0”.

However, RBA-14 was found to be the best primer for determination of variability among the *Streptomyces* sp. isolates. This primer generated 50 bands of different molecular weight summerised in Table 14. Though some of the isolates share common bands most of the isolates differed in their banding pattern. Band 6 was present in isolates of *Streptomyces* sp.

Table 12 : Summery of RAPD analysis of *Streptomyces* spp. isolates from soils of Maharashtra

Sr. No.	Primer Name	Total No. of bands	No. of unique bands	No. of mono-morphic bands	Highly polymorphic	Less polymorphic bands (<20 & >80%)		% polymorphism
						<20%	>80%	
1	RBA 1	36	5	0	3	28	0	86.11
2	RBA 2	46	5	0	6	34	1	89.13
3	RBA 3	51	2	0	12	37	0	96.08
4	RBA 4	55	2	0	7	46	0	96.36
5	RBA 5	55	8	0	15	32	0	85.45
6	RBA 6	46	9	0	4	33	0	80.43
7	RBA 7	56	3	0	2	51	0	94.64
8	RBA 8	72	17	0	11	44	0	76.39
9	RBA 9	60	16	0	11	33	0	73.33
10	RBA 10	61	6	0	9	46	0	90.16
11	RBA 11	44	4	0	13	27	0	90.91
12	RBA12	44	6	0	8	30	0	86.36
13	RBA 13	48	5	0	16	27	0	89.58
14	RBA 14	50	7	0	24	19	0	86.00
15	RBA 15	41	4	0	14	23	0	90.24
16	RBA 16	39	1	0	18	20	0	97.43
17	RBA 17	44	5	0	11	28	0	88.64
18	RBA 18	42	2	0	17	22	1	95.24
19	RBA 19	37	6	0	7	24	0	83.78
20	RBA 20	36	5	0	12	18	1	86.11
21	RBA 21	55	9	0	9	37	0	83.64
22	RBA 22	50	5	0	11	34	0	90.00
23	RBA 23	55	6	0	10	39	0	89.09

Table 14 : Determination of genetic variability in different isolates of *Streptomyces isp.* by DNA amplification profiles by RBA- 14 primer

Sr. No.	Mole. weight (bp)	RHR S	RHR S-17	RHR S-21	RHR S-23	RHR S-24	RHR S-25	RHR S-28	RHR S-40	RHR S-41	RHR S-48	RHR S-52	RHR S-88	RHR S-98	RHR S-116	RHR S-130	RHR S-138	RHR S-147	RHR S-148
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	5202	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2	4767	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
3	4820	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
4	4664	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
5	4321	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
6	4137	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
7	3917	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	3709	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0
9	3512	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	3399	0	0	0	1	0	0	0	1	1	1	0	0	0	1	1	0	0	0
11	2982	0	0	0	1	0	0	0	1	1	1	0	0	1	0	0	0	1	0
12	2854	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
13	2732	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
14	2673	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
15	2521	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
16	2397	0	1	0	0	0	1	0	0	0	1	1	1	1	0	1	0	0	0
17	2345	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0
18	2149	1	0	0	0	0	1	0	1	1	1	1	1	0	0	0	0	1	1
19	1885	0	1	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0
20	2035	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
21	1785	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	1727	0	0	0	0	1	1	0	0	0	0	1	1	1	0	0	0	0	1
23	1654	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0
24	1618	0	0	1	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0
25	1549	0	0	0	1	0	1	1	1	1	1	1	1	0	0	0	1	0	1
26	1435	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
27	1344	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0
28	1286	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1
29	1245	0	1	1	1	0	0	1	0	0	0	0	0	0	0	1	1	0	0
30	1128	1	0	1	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0
31	1057	0	1	0	0	1	1	1	1	1	1	0	1	1	0	0	1	0	1
32	990	0	0	1	1	1	0	0	0	0	1	0	0	1	1	1	0	0	0
33	878	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1

Contd

Table 13. Summery of RAPD analysis of genomic DNA of eighteen different isolates of *Streptomyces* sp.

Sr.No.	Details	
1.	Total number of primers used	23
2.	Total number of polymorphic primers	23
3.	Total number of bands	1124
4.	Total number of unique bands	138
5.	Total number of polymorphic bands	986
6.	Percentage Polymorphic	87.72

RHRS-21 and RHRS-98, band 2 only present in RHRS-116, band 3 in RHRS-130, band 7 only in RHRS-23, band 14 present in RHRS and RHRS-24, band 34 present in RHRS-88 and RHRS-98 isolates, thereby differentiating these are the genetically different isolates. None of the *Streptomyces* sp. isolate share all the bands and are therefore genetically variable. Though all the isolates of *Streptomyces* sp. were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Genetic similarity index was computed based on pooled data of RAPD profiles for *Streptomyces* as DICE coefficient (Dice, 1945; Nei and Li, 1979) using the similarity routine of NTSYS PC 2.0 software. The genetic similarity matrix based on the pooled data of RAPD profiles from all the 23 primers revealed that highest genetic similarity index of 0.65 between the isolates RHR S-40 and RHR S-41 and RHR S 41 and RHR S 48. The minimum genetic similarity index was observed in the isolates RHR S-5 and RHR S-130 (0.13) The genetic similarity index ranged from 0.13 to 0.65 among the all isolates (Table 15). From the genetic similarity coefficient (0.21), the results indicated that there was very distinct diversity (coefficient 0.0 to 0.35) observed in *Streptomyces* sp. isolates from soils of Maharashtra.

Table 15. Genetic similarity as DICE coefficient based on pooled data of RAPD profiles for *Streptomyces* spp.

	RHR S	RHR S17	RHR S21	RHR S23	RHR S24	RHR S25	RHR S28	RHR S40	RHR S41	RHR S48	RHR S52	RHR S88	RHR S98	RHR S116	RHR S130	RHR S138	RHR S147	RHR S148
RHR S	1.00																	
RHR S17	0.26	1.00																
RHR S21	0.18	0.19	1.00															
RHR S23	0.20	0.17	0.36	1.00														
RHR S24	0.18	0.22	0.25	0.24	1.00													
RHR S25	0.22	0.21	0.26	0.22	0.24	1.00												
RHR S28	0.16	0.19	0.23	0.21	0.20	0.25	1.00											
RHR S40	0.27	0.23	0.16	0.17	0.17	0.26	0.25	1.00										
RHR S41	0.32	0.19	0.19	0.22	0.19	0.27	0.23	0.65	1.00									
RHR S48	0.26	0.18	0.14	0.19	0.21	0.25	0.19	0.61	0.65	1.00								
RHR S52	0.19	0.22	0.21	0.20	0.19	0.30	0.18	0.25	0.25	0.25	1.00							
RHR S88	0.23	0.24	0.22	0.23	0.215	0.30	0.20	0.24	0.27	0.24	0.48	1.00						
RHR S98	0.17	0.24	0.14	0.17	0.19	0.22	0.20	0.21	0.22	0.23	0.28	0.30	1.00					
RHR S116	0.17	0.19	0.31	0.32	0.18	0.23	0.20	0.19	0.20	0.18	0.21	0.28	0.25	1.00				
RHR S130	0.13	0.25	0.22	0.16	0.24	0.24	0.20	0.23	0.20	0.24	0.22	0.24	0.21	0.21	1.00			
RHR S138	0.20	0.20	0.24	0.30	0.15	0.21	0.22	0.16	0.19	0.21	0.23	0.25	0.17	0.36	0.23	1.00		
RHR S147	0.21	0.18	0.18	0.17	0.21	0.21	0.19	0.23	0.25	0.21	0.22	0.20	0.19	0.22	0.21	0.27	1.00	
RHR S148	0.22	0.16	0.24	0.21	0.22	0.27	0.22	0.21	0.17	0.18	0.22	0.22	0.23	0.19	0.18	0.20	0.22	1.00

The dendrogram representing the genetic relationship among isolates based on RAPD analysis was developed and presented in Fig. 1. On the basis of RAPD analysis, the dendrogram constructed from the pooled data of *Streptomyces* sp. had major two clusters. The first cluster consisted of five strains of *Streptomyces* (S-28, S-138, S-116 S-23 and S-21) The first cluster consisted of RHR S-28 is distinct and other 4 isolates correlated in two sub cluster S-138 and S-116 with a genetic similarity value of 0.36 and S-23 and S-21 with a genetic similarity value of 0.36. The second cluster consisted of 13 strains of *Streptomyces*. The second cluster is sub-cluster into three groups. The second cluster's sub-cluster is among the RHR S, RHR S-40, RHR S-41 and RHR S-48 and RHR S-147 is grouped in which RHR S-147 is distinct and RHR S, RHR S-40, RHR S-41 and RHR S-48 again grouped into two groups RHR S is distinct and isolates RHR S-40 is distinct and RHR S-41 and RHR S-48 with a genetic similarity value of 0.65. The second sub-cluster of second cluster is among five isolates RHR S-148, RHR S-98 , RHR S-88, RHR S-52 and RHR S25. The second sub-cluster is grouped into three groups in which RHR S-148 and RHR S-98 are distinct and RHR S25 is also correlated between S-52 and S-88 isolates with a genetic similarity value of 0.48. The third sub-cluster is between S-17 and S-130 with the genetic similarity value of 0.025 and the RHR S-24 is distinct. But the second sub-cluster of second cluster is among *Streptomyces* spp. five isolates RHR S-148, RHR S-98 , RHR S-88, RHR S-52 and RHR S-25. are genetically correlated with S-17, S-130 and the RHR S-24.

2D scatter plot PCO scatter plot (Fig.2) were also helpful to make up the group of isolates on the basis of prescence of points on particular axis. From the 2D scatter plot graph was observed that the isolates are grouped into four groups. The isolates RHRS-21, RHRS-23, RHRS-116 and RHRS-138 were genetically closely related in dendrogram and also represented in 2D scatter plot graph. The isolates RHRS-40, RHRS-41 and RHRS-48 were also graphically closely represented in 2D scatter plot graph. The isolates P-52 and P-88 were also closely grouped in 2D scater plot and rest of the isolates

other than above groups were grouped into fourth group which were closely related and represented in 2D scatter plot

Similarly 3D scatter plot PCO scatter plot (Fig.3) clearly represents the phylogenetic relationship among different isolates of *Streptomyces* sp.. It differentiates the distinctness or genetic similarity among the isolates. From 3D scatter plot graph, the isolates were grouped in four groups as per 2D plot was observed and represented in 3D scatter plot but does not add much more information. It is observed that the isolates RHR-S-41, RHR-S-41 and RHR-S-48 showed same place represents that they were genetically closely related. The isolates RHR-S-52 and RHR-S-88 were genetically closely related already observed in the dendrogram.

4.2.6 Geographical distribution of *Streptomyces* sp. from soils of Maharashtra:

Out of 150 locations, *Streptomyces* sp. were found in 48% of soils of Maharashtra and was distributed in 72 location of Maharashtra soils.

From soils of Maharashtra, it was observed that *Streptomyces* were prominently distributed maximum in Vidarbha (27.78%) followed by Western Maharashtra (19.44%), Western Konkan coast (15.28), Marathwada region (13.89%) , Western Ghat (13.89%) and least was found in soils of North Maharashtra region (9.72) The results are summarised in Table 16.

In Vidarbha region, Nagpur Wardha, Yavatmal, Akola, Washim and Gadchiroli districts having highly efficient *Streptomyces* spp. in soil. In Western Maharashtra, Solapur, Satara, Sangli, Pune and Ahmednagar district having efficient *Streptomyces* spp. in soil. In Western Konkan coast region, Ratnagiri and Raigad district having efficient *Streptomyces* sp. Whereas, Ratnagiri, Sindhudurg , Satara, Pune and Kolhapur district have efficient *Streptomyces* sp. in Western Ghat region soil. In Marathwada region, Latur, Nanded and Parbhani districts having efficient *Streptomyces*. In North Maharashtra, Jalgaon and Nashik district having

Table 16. Distribution of efficient *Streptomyces* sp. across the phylographic regions of Maharashtra.

Sr. No.	Physiographic region	District	Distribution of efficient <i>Streptomyces</i> sp.
I.	Western Konkan coast	Sindudurg	
		Ratnagiri	I,II
		Thane	II
		Raigad	I,II
II.	Western Ghats	Sindudurg	I
		Ratnagiri	I,II
		Thane	I
		Kolhapur	I
		Satara	I
		Pune	I
		Nasik	I
		Dhule	I
		Nandurbar	
III.	Western Maharashtra	Pune	I,II
		Kolhapur	
		Satara	I
		Sangali	I
		Solapur	I,II
		Ahmednagar	I,II
IV.	North Maharashtra	Jalgaon	I
		Dhule	
		Nandurbar	
		Nasik	I,II
V.	Marathwada	Latur	I,II
		Osmanabad	
		Beed	II
		Nanded	I
		Parbhani	I
		Hingoli	
		Jalna	
		Aurangabad	I
		Buldhana	
VI.	Vidarbha	Washim	I
		Akola	I
		Amaravati	
		Yawatmal	I,II
		Wardha	I
		Nagpur	I,II
		Bhandara	I
		Chandrapur	II
		Gondia	
		Gadchiroli	I,II

I – Highly efficient

II- Moderately efficient

efficient *Streptomyces* spp. On the basis of functional diversity, the map of *Streptomyces* sp. of the soil of Maharashtra State has been prepared and representing the district wise distribution of efficient *Streptomyces* presented in Fig.4.

4.3. Sulphur oxidizer :

All the *Acidithiobacillus* sp. isolated from soils of Maharashtra were studied for their isolation, morphology and molecular studies.

4.3. 1. Occurrence of sulphur oxidizer microorganisms:

In the present studies, the occurrence of sulphur oxidizer microorganisms was found 14.67% of the soils of Maharashtra (Table 17). Out of 22 locations, maximum found in Vidarbha (9 location) followed by Western Maharashtra (7), Western ghat (3) and Western konkan coast (2) and least was observed in Marathwada region (1 location) from soils of Maharashtra.

4.3.2. Morphology and Cultural characters of *Acidithiobacillus* sp.

All the *Acidithiobacillus* sp. isolated from different soils of Maharashtra were studied for their morphology and it was observed that all the *Acidithiobacillus* sp. have small yellow colour colonies and grown in thiosulphate agar slants. They were found in short rods and size ranged from 0.3-0.7 x 0.9-1.6 μm (Table 18, Plate 17). All the isolates were gram -ve in reaction. The pH reduction was observed in all the *Acidithiobacillus* sp. from 8.0 pH and it ranged in between 4.0 to 5.0.

4.3.3 Molecular Characterization:

The PCR amplification products of the 9 isolates of *Acidithiobacillus* sp. (8 isolates alongwith RHR-At.) were subjected to PCR amplification using 15 random primers (Table 19). The amplification of 9 isolates of 15 random primers were summerised in Table 20 and Plate 18 to Plate 20. The RAPD phenotypes were assessed from the photographs. It was observed that 484 bands were produced by the 15 random primers. The number of bands produced per primer varied from 18 to 48. On an

Table 17 : Occurrence of Sulphur oxidizer from soils of Maharashtra.

Sr. No.	Location	Isolate	Sr.No.	Location	Isolate
1.	Rajur babla, Nasik	SO 15	12.	Niphad, Nasik	SO 110
2.	Mahad, Raigad	SO 20	13.	Malshiras, Solapur	SO 111
3.	Murbad, Thane	SO 27	14.	North Solapur, Solapur	SO 113
4.	Mhakoshi, Jawahar, Thane	SO 41	15.	Kalwan, Nasik	SO 115
5.	Chandgad, Kolhapur	SO 44	16.	Umrer, Nagpur	SO 130
6.	Ambegaon, Pune	SO 68	17.	Sindewahi Chandrapur	SO 132
7.	Gargoti, Kolhapur	SO 70	18.	Umrer, Nagpur	SO 133
8.	Bamani, Latur	SO72	19.	Umrer, Nagpur	SO 134
9.	Rahuri	SO 80	20.	Kuhi, Nagpur	SO 135
10.	Biloli, Nanded	SO 93	21.	Tekala, Etapalli, Gadchoroli	SO 141
11.	Gondpimpri, Chandrapur	SO 103	22.	Palora, Ramtek,	SO 146

Table 18. Morphology of Sulphur oxidizing bacteria isolated from soils of Maharashtra

S. N.	Isolates No.	Gram reaction	Shape	Size (µm)	Colony character	pH reduction
1	SOB 44	- ve	Short rods	0.4 x 1.1	Very small yellow colony	4.4
2	SOB 72	- ve	Short rods	0.7 x 1.6	Small yellow colony	5.0
3	SOB 80	- ve	Short rods	0.6 x 1.5	Very small yellow colony	4.7
4	SOB 113	- ve	Short rods	0.3 x 0.9	Very small close yellow colony	4.5
5	SOB 115	- ve	Short rods	0.4 x 1.0	Very small close yellow colony	4.7
6	SOB 130	- ve	Short rods	0.6 x 1.2	Medium scatter yellow colony	4.8
7	SOB 135	- ve	Short rods	0.6 x 1.1	Very small scatter yellow colony	4.3
8	SOB 146	- ve	Short rods	0.6 x 1.6	Large, yellow colony	4.9
9	SOB – R (std.)	- ve	Short rods	0.7 x 1.5	Yellow	4.6

Table 19: RAPD analysis of *Acidithiobacillus* spp. isolates from soils of Maharashtra

Sr. No.	Primer Name	Total No. of bands	No. of unique bands	No. of monomorphic bands	Highly polymorphic	Less polymorphic bands (<20% >80%)		% poly - morphism
						<20%	>80%	
1	RBA 1	28	10	0	10	8	0	64.28
2	RBA 2	28	10	0	9	9	0	64.28
3	RBA 3	40	16	0	12	12	0	60.00
4	RBA 4	26	10	0	5	11	0	61.54
5	RBA 5	46	17	0	14	15	0	63.04
6	RBA 6	31	9	0	9	13	0	70.97
7	RBA 7	22	16	0	3	3	0	27.27
8	RBA 8	26	16	1	5	4	0	34.62
9	RBA 9	27	13	0	8	6	0	51.85
10	RBA 10	46	12	0	17	17	0	73.91
11	RBA 11	40	12	0	18	10	0	70.00
12	RBA 12	18	12	0	2	4	0	33.33
13	RBA 13	48	21	0	15	12	0	56.25
14	RBA 14	33	12	0	9	12	0	63.64
15	RBA 21	24	14	0	2	7	1	41.67

average 32.2 bands were produced per primer. Out of 484 bands, 284 polymorphic bands were observed (58.68 %) and 200 unique bands were observed (41.32%) and no monomorphic band observed in all 15 primers. Most of the primers produced highly polymorphic bands varied from 2 to 18. Highly polymorphic bands observed maximum (18) in primer RBA-11 followed by RBA-10 (17) and minimum highly polymorphic bands observed in RBA-13. Less polymorphic bands were also recorded in all primers ranged from 2 to 17 in which <20% polymorphic band observed and most of the primers do not produce >80% ie less polymorphic bands. The amplified DNA fragment ranged from 289 to 6021 bp. The number of polymorphic loci amplified varied with different primers and isolates. The primer RBA 10 showed maximum per cent polymorphisum 73.91%. The average polymorphisum was observed 58.68%. The fingerdata of 15 random primers were converted into a binary matrix based on the prescence of band was marked as “1” and absence of band was marked as “0”.

Table 20 . Summery of RAPD analysis of genomic DNA of thirteen different isolates of *Acidithiobacillus* sp.

Sr.No.	Details	
1.	Total number of primers used	15
2.	Total number of polymorphic primers	15
3.	Total number of bands	484
4.	Total number of unique bands	200
5.	Total number of polymorphic bands	284
6.	Percentage Polymorphic	58.68

However, RBA-11 was found to be the best primer for determination of variability among the *Acidithiobacillus* sp. isolates. This primer generated 40 bands of different molecular weight and is summerised in Table 21. Though some of the isolates share common bands most of the isolates differed in their banding pattern.

Table 21 : Determination of genetic variability in different isolates of *Acidithiobacillus* by DNA amplification profiles by RBA-11 primer

Sr. No.	Molecular weight (bp)	SOB44	SOB72	SOB80	SOB113	SOB115	SOB130	SOB135	SOB146	SOB
		1	2	3	4	5	6	7	8	9
1	5284	0	0	0	1	1	0	1	0	1
2	3215	0	0	1	0	0	0	0	0	1
3	2973	0	0	1	0	0	0	0	0	0
4	2859	0	0	0	0	0	0	0	1	0
5	2609	0	0	1	1	1	0	1	0	0
6	2508	0	0	0	0	0	1	0	0	0
7	2350	1	0	1	0	0	0	0	0	1
8	2264	0	0	0	0	1	0	1	0	0
9	2009	0	0	0	1	1	1	0	1	0
10	1944	1	0	1	0	0	0	0	0	0
11	1763	0	1	1	0	0	0	0	1	0
12	1833	0	0	0	0	0	0	0	0	1
13	1630	1	0	0	0	0	1	1	0	1
14	1526	0	0	0	1	1	1	0	1	0
15	1393	0	0	1	0	0	0	1	1	1
16	1206	1	0	0	1	0	0	1	1	0
17	1175	0	0	1	0	1	0	0	0	0
18	1130	0	0	0	0	0	0	0	0	1
19	1087	0	1	0	0	0	0	0	0	0
20	1045	1	0	0	0	0	1	1	1	0
21	1018	0	0	0	1	1	0	0	0	1
22	979	0	0	1	0	0	0	0	0	0
23	929	1	0	0	0	0	0	0	0	0
24	848	0	1	0	0	0	1	0	0	0
25	784	1	0	1	0	1	1	0	0	1
26	753	0	0	0	1	0	0	1	1	1
27	631	0	1	0	0	0	1	0	0	1
28	558	0	0	0	0	1	0	1	1	0
29	519	1	0	0	0	0	0	0	1	1
30	477	0	0	0	1	0	0	0	0	0
31	447	1	0	1	0	1	1	1	0	1
32	387	0	1	0	0	0	0	0	1	0
33	357	1	0	0	0	0	1	0	0	0
34	331	0	0	0	1	1	0	0	1	0
35	295	0	0	1	0	0	0	1	0	0
36	245	0	0	0	0	0	1	1	0	0
37	235	0	1	0	0	0	0	0	0	0
38	229	0	0	0	0	0	0	0	1	0
39	206	0	0	0	0	0	1	1	0	0
40	157	1	0	0	0	0	0	0	0	0

Band 23 and 40 were only present in SOB-44, band 19 and 37 in SOB-72, band 9 and 14 were present in SOB-113, SOB-115, SOB-130 and SOB-146, bands 36 and 39 present in SOB-130 and SOB-135, thereby differentiating that these are the genetically different isolates. None of the *Acidithiobacillus* sp. isolate share all the bands and are therefore genetically variable. Though all the isolates of *Acidithiobacillus* sp. were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Genetic similarity index was computed based on pooled data of RAPD profiles for SOB as DICE coefficient (Dice, 1945; Nei and Li, 1979) using the similarity routine of NTSYS PC 2.0 software. The genetic similarity matrix based on the pooled data of RAPD profiles from all the 15 primers revealed that highest genetic similarity index of 0.65 between the isolates SOB-113 and SOB-115. The minimum genetic similarity index was observed in the isolates SOB-44 and SOB-115 (0.13). The genetic similarity index ranged from 0.13 to 0.65 among the all isolates (Table 22). From the genetic similarity coefficient, the results indicated that there was very distinct diversity (coefficient 0.20) observed in the *Acidithiobacillus* isolated from soils of Maharashtra on the basis of geographical region.

The dendrogram representing the genetic relationship among isolates based on RAPD analysis was developed and presented in Fig. 5. On the basis of RAPD analysis, the dendrogram constructed from the pooled data had four clusters. The first cluster consisted of four strains of SOB (SOB 135, SOB-130, SOB 115 and SOB 113) The first cluster consisted of two sub cluster SOB 135 and S 115 with a genetic similarity value of 0.26 and S 115 and S113 with a highest genetic similarity value of 0.65. The second cluster consisted of 3 strains of SOB. The second cluster is sub-clustered into two groups. The second cluster's sub-cluster is among the SOB-146, SOB and SOB-80 is grouped in which SOB-146 is distinct and SOB and SOB -80 with a genetic similarity value of 0.31

Table 22. Genetic similarity as DICE coefficient based on pooled data of RAPD profiles for *Acidithiobacillus* sp.

	SOB 44	SOB 72	SOB 80	SOB 113	SOB 115	SOB 130	SOB 135	SOB 146	SOB
SOB 44	1.00								
SOB 72	0.18	1.00							
SOB 80	0.21	0.19	1.00						
SOB 113	0.15	0.17	0.26	1.00					
SOB 115	0.13	0.17	0.23	0.65	1.00				
SOB 130	0.28	0.20	0.19	0.25	0.27	1.00			
SOB 135	0.19	0.20	0.19	0.24	0.26	0.30	1.00		
SOB 146	0.14	0.19	0.21	0.16	0.16	0.28	0.21	1.00	
SOB	0.17	0.23	0.31	0.20	0.22	0.24	0.29	0.30	1.00

The third (SOB 72) and fourth cluster (SOB 44) are distinct which are correlated with other SOB.

2D scatter plot PCO scatter plot (Fig.6) was also helpful to make up the group of isolates on the basis of presence of points on particular axis. In 2D scatter plot, *Acidithiobacillus sp.* are grouped into four groups. SOB-113 and SOB-115 were genetically closely related and mostly same than the other isolates. The isolates SOB-130, SOB-135, SOB-72, SOB-44 and SOB-146 were closely related and grouped in one group. However, SOB and SOB-80 were separately plotted in 2D plot.

Similarly 3D scatter plot PCO scatter plot (Fig. 7) clearly represents the phylogenetic relationship among different isolates of *Acidithiobacillus sp.* It differentiates the distinctness or genetic similarity among the isolates. From the 3D scatter plot graph, it is observed that the isolate SOB113 and isolates SOB 115 were genetically closely related and also represented in 3D scatter plot graph. The isolates SOB-130, SOB135, SOB-72, SOB-44 and SOB-146 were closely related and plotted in 3D scatter plot. Whereas, SOB and SOB-80 were separately plotted in 3D scatter plot.

From the genetic similarity coefficient, it is observed that there was very high genetic diversity in the *Acidithiobacillus sp.* isolated from soils of Maharashtra.

4.3.4 Geographical distribution of *Acidithiobacillus* from soils of Maharashtra:

Acidithiobacillus sp. occurred in 8 locations which contributes 5.33% of soils of Maharashtra (Table 23).

The results indicated that *Acidithiobacillus* was distributed in Western Maharashtra (37.5%) and Vidarbha (37.5%) whereas less observed in Marathwada (12.5%) and Western Ghat (12.5%). *Acidithiobacillus sp.* occurred 3 locations viz., Rahuri, Kalwan and North Solapur of Western Maharashtra and Vidarbha (3 location) viz,

Table 23. Distribution of efficient *Acidithiobacillus* sp. across the phylographic regions of Maharashtra.

Sr. No.	Physiographic region	District	Distribution of efficient <i>Acidithiobacillus</i> sp.
I.	Western Konkan coast	Sindudurg	
		Ratnagiri	
		Thane	
		Raigad	
II.	Western Ghats	Sindudurg	
		Ratnagiri	
		Thane	
		Kolhapur	I
		Satara	
		Pune	
		Nasik	
		Dhule	
		Nandurbar	
III.	Western Maharashtra	Pune	
		Kolhapur	
		Satara	
		Sangali	
		Solapur	I
		Ahmednagar	II
IV.	North Maharashtra	Jalgaon	
		Dhule	
		Nandurbar	
		Nasik	II
V.	Marathwada	Latur	II
		Osmanabad	
		Beed	
		Nanded	
		Parbhani	
		Hingoli	
		Jalna	
		Aurangabad	
		Buldhana	
VI.	Vidarbha	Washim	
		Akola	
		Amaravati	
		Yawatmal	
		Wardha	
		Nagpur	I,II
		Bhandara	
		Chandrapur	II
		Gondia	
		Gadchiroli	

I – Highly efficient

II- Moderately efficient

Umred, Kuhi in Nagpur, Chandrapur and least was observed in Latur of Marathwada region and Kolhapur in Western ghat region.

The highly efficient *Acidithiobacillus* sp. was observed in Kolhapur, Solapur and Nagpur districts from Western Ghat, Western Maharashtra and Vidarbha regions, respectively.

On the basis of efficiency, the map of *Acidithiobacillus* sp. of the soil of Maharashtra State has been prepared and representing the district wise distribution of *Acidithiobacillus* presented in Fig. 8.

4.4 *Pseudomonas fluorescens*

4.4.1 Microbial Population of fluorescent pseudomonads :

The microbial population of fluorescent pseudomonads was found 43% of the soils of Maharashtra. The microbial population of fluorescent pseudomonads varied from 9×10^{-3} to 6×10^{-6} cfu/g in soils of Maharashtra (Table 24). Microbial population were observed maximum in Western Maharashtra (18 locations) followed by Western ghats (13) and Vidarbha region (13) soil, respectively from soils of Maharashtra. The less fluorescent pseudomonads were observed in the soils of Konkan coast region.

A wide variation in microbial population among the fluorescent pseudomonads isolates was observed in the soils of Maharashtra. The maximum population of fluorescent pseudomonads FP-57 (6×10^{-6} cfu/g of soil) was recorded in Kolhapur district of Western Maharashtra followed by FP-52 and FP-43 isolates (4×10^{-6} cfu/g of soil) from Nasik and Sindudurg districts of North Maharashtra and Western Ghat region. The minimum fluorescent pseudomonads population was FP-146 isolates (9×10^{-3} cfu/g soil) in Ramtek from Nagpur district of Vidarbha region.

4.4.2 Morphological, Biochemical and Cultural characters:

Sixty five isolates of fluorescent pseudomonads from soils of all district from Maharashtra were characterized and studied for their morphological, biochemical studies and identified *Pseudomonas*

Table 24 : Microbial population of Fluorescent pseudomonads from soils of Maharashtra.

Sr. No	Isolate	Microbial population	Sr. No	Isolate	Microbial population
1.	FP 15	1×10^{-6}	34.	FP 90	3×10^{-4}
2.	FP 16	1×10^{-5}	35.	FP 91	2×10^{-4}
3.	FP 19	3×10^{-5}	36.	FP 92	12×10^{-4}
4.	FP 22	6×10^{-5}	37.	FP 93	4×10^{-4}
5.	FP 25	4×10^{-6}	38.	FP 94	2×10^{-4}
6.	FP 31	1×10^{-5}	39.	FP 95	8×10^{-4}
7.	FP 33	5×10^{-4}	40.	FP 96	6×10^{-4}
8.	FP 34	3×10^{-6}	41.	FP 97	8×10^{-5}
9.	FP 35	2×10^{-6}	42.	FP 100	3×10^{-5}
10.	FP 39	2×10^{-6}	43.	FP 101	1×10^{-5}
11.	FP 40	6×10^{-4}	44.	FP 102	2×10^{-4}
12.	FP 41	2×10^{-4}	45.	FP 104	7×10^{-5}
13.	FP 42	2×10^{-5}	46.	FP 105	6×10^{-4}
14.	FP 43	4×10^{-6}	47.	FP 106	1×10^{-4}
15.	FP 44	1×10^{-6}	48.	FP 109	4×10^{-4}
16.	FP 49	3×10^{-5}	49.	FP 110	1×10^{-6}
17.	FP 50	1×10^{-5}	50.	FP 111	2×10^{-5}
18.	FP 51	9×10^{-5}	51.	FP 113	7×10^{-4}
19.	FP 52	4×10^{-6}	52.	FP 118	6×10^{-4}
20.	FP 54	3×10^{-5}	53.	FP 119	9×10^{-5}
21.	FP 55	3×10^{-6}	54.	FP 120	2×10^{-5}
22.	FP 56	1×10^{-5}	55.	FP 121	2×10^{-5}
23.	FP 57	6×10^{-6}	56.	FP 129	7×10^{-4}
24.	FP 58	2×10^{-5}	57.	FP 131	3×10^{-5}
25.	FP 63	1×10^{-4}	58.	FP 134	1×10^{-5}
26.	FP 65	6×10^{-5}	59.	FP 136	4×10^{-5}
27.	FP 67	2×10^{-5}	60.	FP 139	2×10^{-5}
28.	FP 68	17×10^{-4}	61.	FP 145	15×10^{-4}
29.	FP 75	1×10^{-5}	62.	FP 146	9×10^{-3}
30.	FP 77	13×10^{-5}	63.	FP 148	5×10^{-5}
31.	FP 80	2×10^{-5}	64.	FP 149	2×10^{-5}
32.	FP 82	27×10^{-4}	65.	FP 150	6×10^{-5}
33.	FP 85	7×10^{-4}			

fluorescens were used for further cultural, functional and molecular diversity. The cultures were maintained at culture collection centre in the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV., Rahuri.

4.4.2.1 Morphological characters:

The isolates were examined for their colony and cell morphology, cell size and gram reaction of the cells. Wide variations were observed among the isolates in their colony morphology. Out of the 65 isolates, 21 produced green colored colonies and 44 produced light green coloured colonies. Forty one isolates observed round and non-spreading type colonies whereas the remaining 24 isolates produced irregular shaped spreading type colonies (Table 25, Plate 21).

All the 65 isolates were rod shaped bacteria and gram negative in reaction. Among these fluorescent pseudomonads 65 isolates grouped on the basis of habitat, 40 isolates were rod shape living in grouping/clustering, 8 isolates were rod shape in isolated habitat and 17 isolates were rod shape in pair and chain habitat. The size of these bacterial isolates are categorized into three on the basis of size viz., upto 1.0 μm , 1.01 to 2.00 μm and more than 2.01 μm length. The results indicated that 29, 31 and 5 isolates were measured upto 1.0 μm , 1.01 to 2.00 μm and more than 2.01 μm length, respectively. The colonies showed wide variation in the intensity of fluorescence (Plate 22). Out of 65 isolates, 11 showed high fluorescence, 33 showed medium fluorescence and the remaining 21 isolates showed weak fluorescence.

4.4.2.2 Biochemical characterization :

All the 65 Fluorescent pseudomonads isolates were tested for their biochemical characterization viz., starch hydrolysis, H_2S production, indole production, catalase test, oxidase test, urease test, denitrification and gelatin liquefaction (Table 26, Plate 23).

All the 65 Fluorescent pseudomonads isolates were positive for indole production and gelatin liquefaction tests and were negative for

Table 25 : Morphological characteristics of the Fluorescent pseudomonas isolated from different soils of Maharashtra

Sr. No.	Isolate No	Colony morphology			Gram reaction	Cell shape	Size	Fluorescence
		Colour	Shape	Nature				
1	P 15	Light Green	Irregular	Spreading	Negative	Rod group	0.1-0.2 x 0.2-0.6	++
2	P 16	Green	Round	Non spreading	Negative	Rod group	0.2-0.8 x 0.4 – 1.8	+++
3	P 19	Light Green	Round	Non spreading	Negative	Rod group	0.2-0.4 x 0.4-1.2	++
4	P 22	Light Green	Round	Non spreading	Negative	Rod chain & pair	0.1- 0.2 x 0.3-1.7	++
5	P 25	Light Green	Round	Non spreading	Negative	Rod group	0.1-0.2 x 0.3-1.2	++
6	P 31	Light Green	Round	Non Spreading	Negative	Rod group	0.1-0.2 x 0.2-0.3	++
7	P 33	Green	Irregular	Spreading	Negative	Rod group	0.1-0.1 x 0.2-1.1	++
8	P 34	Green	Irregular	Spreading	Negative	Rod pair	0.1-0.1 x 0.1-0.9	+
9	P 35	Light Green	Round	Non spreading	Negative	Rod group	0.2-0.2 x 0.3 -0.6	++
10	P 39	Light Green	Round	Non spreading	Negative	Rod group	0.1-0.1 x 0.7-1.2	+++
11	P 40	Light Green	Irregular	Spreading	Negative	Rod in group	0.2-0.7 x 0.4-1.7	+
12	P 41	Green	Irregular	Spreading	Negative	Rod group	0.2-0.4 x 0.6-1.9	+++
13	P 42	Green	Irregular	Spreading	Negative	Rod isolate	0.2-0.3 x 0.2-0.9	+
14	P 43	Light Green	Irregular	Spreading	Negative	Rod chain	0.1-0.2 x 1.1 -2.3	++
15	P 44	Light Green	Irregular	Spreading	Negative	Rod group	0.1- 0.2 x 0.2-1.1	+++
16	P 49	Light Green	Round	Non spreading	Negative	Rod group	0.1-0.2 x 0.3-0.9	++
17	P 50	Green	Irregular	Spreading	Negative	Rod pair	0.1-0.3 x 0.4-1.7	+
18	P 51	Green	Round	Non spreading	Negative	Rod in group	0.1-0.2 x 0.1-1.2	++
19	P 52	Light Green	Irregular	Spreading	Negative	Rod group	0.2-0.3 x 0.2- 1.04	+++
20	P 54	Light Green	Round	Non spreading	Negative	Rod group	0.2-0.4 x 0.4– 1.7	+
21	P 55	Green	Irregular	Spreading	Negative	Rod in group	0.1-0.1 x 0.1-1.1	++
22	P 56	Light Green	Irregular	Spreading	Negative	Rod in group	0.1-0.3 x 0.6 -1.3	+++

Contd...

Sr. No.	Isolate No	Colony morphology			Gram reaction	Cell shape	Size	Fluorescence
		Colour	Shape	Nature				
23	P 57	Light Green	Irregular	Spreading	Negative	Rod in pair	0.1-0.6 x 0.4-2.0	+++
24	P 58	Green	Irregular	Spreading	Negative	Rod in pair	0.1-0.2 x 0.7-2.3	+
25	P 63	Light Green	Round	Non spreading	Negative	Rod group	0.1-0.2 x 0.2-1.4	++
26	P 65	Light Green	Round	Non spreading	Negative	Rod isolate	0.1-0.1 x 0.4-0.8	++
27	P 67	Light Green	Round	Non spreading	Negative	Rod in pair	0.1-0.1 x 0.1-0.6	+
28	P 68	Light Green	Round	Non spreading	Negative	Rod group	0.1-0.1 x 0.1-0.9	+
29	P 75	Light Green	Round	Non spreading	Negative	Rod isolate	0.1-0.1 x 0.1-0.4	+
30	P 77	Light Green	Round	Non spreading	Negative	Rod group	0.1-0.3 x 0.8-2.5	++
31	P 80	Light Green	Round	Non spreading	Negative	Rod long chain	0.1-0.1 x 1.0 – 3.5	++
32	P 82	Green	Round	Non spreading	Negative	Rod in pair	0.1-0.2 x 0.3-0.8	+
33	P 85	Green	Round	Non spreading	Negative	Rod group	0.1-0.1 x 0.1-1.2	++
34	P 90	Light Green	Round	Non spreading	Negative	Rod in pair	0.1-0.3 x 0.1-1.6	++
35	P 91	Light Green	Irregular	Spreading	Negative	Rod in group	0.1-0.6 x 0.1-1.2	+++
36	P 92	Light Green	Round	Non spreading	Negative	Rod in pair	0.1-0.1 x 0.3 – 0.8	++
37	P 93	Green	Round	Non spreading	Negative	Rod in pair	0.1-0.2 x 0.4-1.1	+
38	P 94	Green	Round	Non spreading	Negative	Rod group	0.1-0.2 x 0.3-1.0	++
39	P 95	Light Green	Irregular	Spreading	Negative	Rod group	0.1 x 0.2 - 0.6	+
40	P 96	Light Green	Round	Non spreading	Negative	Rod group	0.1-0.4 x 0.6 – 1.0	++
41	P 97	Green	Round	Non spreading	Negative	Rod pair	0.1-0.2 x 0.2 -0.8	+
42	P 100	Light Green	Round	Non spreading	Negative	Rod isolate	0.1-0.3 x 0.2 -0.8	++
43	P 101	Light Green	Round	Non spreading	Negative	Rod in pair	0.1-0.1 x 0.1 -0.4	++
44	P 102	Light Green	Irregular	Spreading	Negative	Rod group	0.1-0.2 x 0.4-1.2	++

Sr. No.	Isolate No	Colony morphology			Gram reaction	Cell shape	Size	Fluorescence
		Colour	Shape	Nature				
45	P 104	Light Green	Irregular	Spreading	Negative	Rod in pair	0.1-0.2 x 0.4-1.6	+
46	P 105	Light Green	Round	Non spreading	Negative	Rod in group	0.1-0.2 x 0.2-0.8	++
47	P 106	Light Green	Round	Non spreading	Negative	Rod in group	0.1-0.1 x 0.4 – 1.0	+
48	P 109	Light Green	Round	Non spreading	Negative	Rod in group	0.1-0.2 x 0.3-0.8	+
49	P 110	Light Green	Round	Non spreading	Negative	Rod in group	0.1-0.2 x 0.4 – 1.0	++
50	P 111	Light Green	Round	Non spreading	Negative	Rod in group	0.1-0.3 x 0.2-1.0	++
51	P 113	Light Green	Irregular	Spreading	Negative	Rod in group	0.2 x 0.3-1.4	++
52	P 118	Light Green	Round	Non spreading	Negative	Rod in group	0.1-0.2 x 0.2-1.3	+
53	P 119	Green	Irregular	Spreading	Negative	Rod in groups	0.1-0.2 x 0.3 -1.7	+
54	P 120	Green	Irregular	Spreading	Negative	Rod in group	0.1-0.2 x 0.4- 0.6	+++
55	P 121	Light Green	Round	Non spreading	Negative	Rod isolate	0.1-0.2 x 0.6-0.9	++
56	P 129	Green	Round	Non spreading	Negative	Rod in pair	0.1-0.2 x 0.2-0.8	+
57	P 131	Light Green	Irregular	Spreading	Negative	Rod in group	0.2-0.3 x 0.3-1.8	++
58	P 134	Green	Round	Non spreading	Negative	Rod isolate	0.2-0.2 x 0.3-1.4	++
59	P 136	Green	Round	Non spreading	Negative	Rod isolate	0.1-0.4 x 0.3 – 0.7	++
60	P 139	Green	Irregular	Spreading	Negative	Rod isolate	0.1-0.4 x 0.3-1.7	+
61	P 145	Light Green	Round	Non spreading	Negative	Rod in groups	0.1-0.3 x 0.2 – 1.8	++
62	P 146	Light Green	Round	Non spreading	Negative	Rod in group	0.1-0.2 x 0.2-1.4	++
63	P 148	Light Green	Round	Non spreading	Negative	Rod in group	0.2-0.3 x 0.1-1.8	+++
64	P 149	Light Green	Round	Non spreading	Negative	Rod in group	0.1-0.2 x 0.2-1.0	+
65	P 150	Green	Round	Non spreading	Negative	Rod in pair	0.1-0.3 x 0.2 -0.8	++

Table 26: Biochemical and physiological characteristics of the Fluorescent pseudomonas isolated from different soils of Maharashtra

Sr. No.	Isolates	Starch hydrolysis	H ₂ S production	Indole production	Catalase	Oxidase	Gelatin	Urease Test	Denitrification	Growth at 4°C	Growth at 41°C	Tentative Identification
1	FP 15	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
2	FP 16	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
3	FP 19	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
4	FP 22	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
5	FP 25	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
6	FP 31	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
7	FP 33	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
8	FP 34	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
9	FP 35	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
10	FP 39	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
11	FP 40	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
12	FP 41	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
13	FP 42	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
14	FP 43	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
15	FP 44	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
16	FP 49	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
17	FP 50	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
18	FP 51	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
19	FP 52	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
20	FP 54	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
21	FP 55	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
22	FP 56	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>

Contd.....

Sr. No.	Isolates	Starch hydrolysis	H ₂ S production	Indole production	Catalase	Oxidase	Gelatin	Urease Test	Denitrification	Growth at 4°C	Growth at 41°C	Tentative Identification
23	FP 57	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
24	FP 58	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
25	FP 63	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
26	FP 65	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
27	FP 67	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
28	FP 68	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
29	FP 75	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
30	FP 77	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
31	FP 80	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
32	FP 82	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
33	FP 85	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
34	FP 90	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
35	FP 91	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
36	FP 92	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
37	FP 93	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
38	FP 94	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
39	FP 95	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
40	FP 96	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
41	FP 97	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
42	FP 100	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
43	FP 101	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
44	FP 102	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
45	FP 104	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>

Contd.....

Sr. No.	Isolates	Starch hydrolysis	H ₂ S production	Indole production	Catalase	Oxidase	Gelatin	Urease Test	Denitrification	Growth at 4°C	Growth at 41°C	Tentative Identification
46	FP 105	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
47	FP 106	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
48	FP 109	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
49	FP 110	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
50	FP 111	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
51	FP 113	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
52	FP 118	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
53	FP 119	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
54	FP 120	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
55	FP 121	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
56	FP 129	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
57	FP 131	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
58	FP 134	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
59	FP 136	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
60	FP 139	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
61	FP 145	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
62	FP 146	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
63	FP 148	-	+	+	+	+	+	-	+	-	+	<i>P. aeruginosa</i>
64	FP 149	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>
65	FP 150	-	+	+	+	+	+	-	-	+	-	<i>P. fluorescens</i>

starch hydrolysis and urease activity. Similarly, all the 65 isolates showed positive H₂S production and oxidase activity, 61 positive for catalase test and 4 were negative. 34 Fluorescent pseudomonads isolates showed negative for denitrification test and 31 were positive. Thirty-four isolates showed growth at 4°C and 31 isolates were not able to grow at 4°C. where as 31 isolates were able to grow at 41°C and 34 isolates were not able to grow at 41°C .

Based on the morphological and biochemical characteristics, 34 isolates were identified as *Pseudomonas fluorescens*, 31 as *Pseudomonas aeruginosa* from soils of Maharashtra. Out of 18 isolates from soils of Western Maharashtra, 8 were identified as *Pseudomonas fluorescens* and 10 as *Pseudomonas aeruginosa*. Out of 13 isolates from Western Ghat, 10 were identified as *Pseudomonas fluorescens* and 3 to *Pseudomonas aeruginosa*. Six out of 13 isolates from Vidarbha were identified as *P. fluorescens* and seven as *P. aeruginosa*. Among the 9 isolates of Marathwada, three isolates belonged to *Pseudomonas fluorescens* and six to *Pseudomonas aeruginosa*. Among the 4 isolates of Western Konkan coast region, two isolates belonged to *Pseudomonas fluorescens* and two to *Pseudomonas aeruginosa* . In North maharashtra, out of 8 isolates, 5 isolates were identified as *Pseudomonas fluorescens* and three isolates were *Pseudomonas aeruginosa*.

4.4.2.3 Cultural Characters :

All the 34 isolates were identified as *Pseudomonas fluorescens* on their morphological, physiological and biochemical characters which were studied for cultural characterization. All the 34 *Pseudomonas fluorescens* were grown on King's B medium, Nutrient Agar and Pikovaskay's medium (Table 27, Plate 24).

All the 34 *Pseudomonas fluorescens* were grown on King's B medium were showed the water soluble yellow colonies and produced pigmentation on media. Whereas, The colonies of 34 *Pseudomonas fluorescens* isolates showed whitish colour and does not produce

Table 27 : Cultural characters of *Pseudomonas fluorescens* on isolated from soils of Maharashtra

Sr. No.	Isolate No	Colony growth on King'B		Colony growth on Nutrient Agar		Colony growth on Pikovaskiyas medium	
		Colony colour	Pigmen-tation	Colony colour	Pigmen-tation	Colony colour	Pigmen-tation
1	P 16	Green	+	White	-	White	-
2	P 22	Light Green	+	White	-	White	-
3	P 31	Light Green	+	White	-	White	-
4	P 33	Green	+	White	-	White	-
5	P 35	Light Green	+	White	-	White	-
6	P 39	Light Green	+	White	-	White	-
7	P 40	Light Green	+	White	-	White	-
8	P 41	Green	+	White	-	White	-
9	P 42	Green	+	White	-	White	-
10	P 43	Light Green	+	White	-	White	-
11	P 44	Light Green	+	White	-	White	-
12	P 49	Light Green	+	White	-	White	-
13	P 51	Green	+	White	-	White	-
14	P 52	Light Green	+	White	-	White	-
15	P 55	Green	+	White	-	White	-

Contd

Sr. No.	Isolate No	Colony growth on King'B		Colony growth on Nutrient Agar		Colony growth on Pikovaskiyas medium	
		Colony colour	Pigmentation	Colony colour	Pigmentation	Colony colour	Pigmentation
16	P 56	Light Green	+	White	-	White	-
17	P 57	Light Green	+	White	-	White	-
18	P 77	Green	+	White	-	White	-
19	P 80	Light Green	+	White	-	White	-
20	P 85	Green	+	White	-	White	-
21	P 91	Light Green	+	White	-	White	-
22	P 92	Light Green	+	White	-	White	-
24	P 105	Light Green	+	White	-	White	-
25	P 106	Light Green	+	White	-	White	-
26	P 110	Light Green	+	White	-	White	-
27	P 119	Green	+	White	-	White	-
28	P 120	Green	+	White	-	White	-
29	P 121	Light Green	+	White	-	White	-
30	P 134	Green	+	White	-	White	-
31	P 136	Green	+	White	-	White	-
32	P 145	Light Green	+	White	-	White	-
33	P 149	Light Green	+	White	-	White	-
34	P 150	Green	+	White	-	White	-

pigmentation on Nutrient Agar and Pikovaskaya's medium. The colony growth of *Pseudomonas fluorescens* on King's B medium was yellow in colour whereas colony was white in colour on Nutrient Agar and Pikovaskaya's medium

4.4.3 Functional diversity:

4.4.3.1 Biocontrol ability:

From the soils of Maharashtra, 34 *Pseudomonas fluorescens* were isolated, and tested for their biocontrol ability against soil borne pathogens. The per cent growth of inhibition by *Pseudomonas fluorescens* against *Macrophomina phaseolina* ranged from 11.25% to 47.50% (Plate 25). The *Pseudomonas fluorescens* isolates P-51 was found more growth inhibition per cent (47.50%) followed by P-31 (45.62%), P-56 (45.62%) and P-121 (45.00%) (Table 28). Seven strains of *Pseudomonas fluorescens* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 were found more growth inhibition per cent but the commercial *Pseudomonas* is also at par with these isolates in dual plate method assay and the same trend of growth of inhibition was observed against *Macrophomina phaseolina* in dual inoculation method.

The *Pseudomonas fluorescens* isolates viz., P-121 (45.86%), P-51 (41.49%), P-134 (39.37%), P-136 (37.24%), P-31 (35.11%) and P-56 (34.04%) were found significantly superior in inhibition growth per cent over the commercial *Pseudomonas* (24.31%) against the *Fusarium oxysporium* in dual plate assay whereas in dual inoculation the *Pseudomonas fluorescens* isolates P-31, P-56, P-121 and P-136 were observed good in growth inhibition against *Fusarium oxysporium* (Plate 26).

By dual plate and dual inoculation method, 7 strains of *Pseudomonas fluorescens* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 showed antagonistic activity superior than commercial *Pseudomonas* against soil borne plant pathogens viz., *Macrophomina phaseolina* and *Fusarium* sp. The efficient *Pseudomonas fluorescens* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 isolated from soils from

Table 28 : Biocontrol activity of *Pseudomonas fluorescens* isolated from soils of Maharashtra against *Macrophomina phaseolina* and *Fusarium oxysporium*

Sr. No.	Isolate No	Dual plate method		Dual inoculation method	
		% GI of <i>M. phaseolina</i>	% GI of <i>F. oxysporium</i>	<i>M. phaseolina</i>	<i>F. oxysporium</i>
1	P 16	19.38	11.70	+	+
2	P 22	15.00	14.13	+	+
3	P 31	45.62	35.11	+++	+++
4	P 33	35.63	13.83	++	+
5	P 35	19.37	12.67	+	+
6	P 39	35.63	11.71	+++	+
7	P 40	25.00	12.77	+	+
8	P 41	36.88	27.66	++	++
9	P 42	33.75	11.71	++	+
10	P 43	31.88	9.58	++	+
11	P 44	20.00	14.90	+	+
12	P 49	25.63	11.71	+	+
13	P 51	47.50	41.49	+++	++
14	P 52	38.75	20.22	++	++
15	P 55	38.12	17.02	++	+
16	P 56	45.62	34.04	+++	+++
17	P 57	18.13	13.83	+	+
18	P 77	40.88	15.96	++	+
19	P 80	21.88	14.90	+	+

Contd.....

Sr. No.	Isolate No	Dual plate method		Dual inoculation method	
		% GI of <i>M. phaseolina</i>	% GI of <i>F. oxysporium</i>	<i>M. phaseolina</i>	<i>F. oxysporium</i>
20	P 85	33.13	15.96	++	+
21	P 91	39.38	27.66	++	++
22	P 92	11.25	14.90	+	+
23	P 96	14.98	11.71	+	+
24	P 105	25.00	9.58	+	+
25	P 106	26.88	10.64	+	+
26	P 110	21.25	11.71	+	+
27	P 119	28.13	10.64	+	+
28	P 120	30.63	13.83	++	+
29	P 121	45.00	45.86	+++	+++
30	P 134	43.88	39.37	+++	++
31	P 136	43.38	37.24	+++	+++
32	P 145	43.50	19.68	+++	++
33	P 149	22.63	8.87	+	+
34	P 150	39.38	13.83	++	+
35	CP	41.25	24.37	+++	+++
	S.E.±	2.49	2.45	-	-
	C.D. at 5%	7.16	7.03	-	-

GI = Growth inhibition

+ = Poor inhibition

++ = Moderate inhibition

+++ = Good inhibition

the locations Sawantwadi, Malegaon, Ahmedpur, Sevagram, Ramtek, and Kuhi, respectively.

4.4.3.2 Antibiotic sensitivity :

Intrinsic antibiotic resistance of all the 35 *Pseudomonas fluorescens* isolates including 1 commercial *Pseudomonas fluorescens* strain was tested against four antibiotic discs viz., NS₁₀₀, S₁₀, R₅ and T₃₀. These antibiotic discs were obtained from the Hi-Media Lab. Pvt. Ltd., Mumbai, India (Table 29, Plate 27). All the *Pseudomonas fluorescens* isolates were resistant to NS₁₀₀. While 28 isolates resistant and 7 isolates were sensitive to S₁₀, whereas 34 *Pseudomonas fluorescens* were observed sensitive (range from 11 to 31 mm) and P 40 was found resistant to R₅. The minimum zone 8mm and maximum zone 31 mm was found in P-120 and P-134, respectively. Similarly, all the *Pseudomonas fluorescens* isolates were found sensitive to T₃₀ and the zone ranged from 8 to 55mm. Minimum in P-96 and maximum was observed in P-134. Thus, the results indicated that the *Pseudomonas fluorescens* were found to be resistant to NS₁₀₀ and S₁₀ whereas *Pseudomonas fluorescens* was found sensitive to the R₅ and T₃₀.

4.4.3.3 HCN and siderophore production :

The antagonistic strains were tested *in vitro* HCN production by the picric acid assay method and all the 35 isolates (34 *P. fluorescens* and one commercial production) were found to produce HCN (Table 30, Plate 28). However, 7 of them and CP were high HCN producers which turned the orange colour of the filter paper. Sixteen *Pseudomonas fluorescens* isolates were moderate HCN producers and eleven were weak HCN producers. All the *Pseudomonas fluorescens* showed the siderophore ability.

It is observed that all the 35 isolates were found to produce siderophore production and the effective isolates showed larger zone than the other isolates (Table 30).

Table 29 : Sensitivity of *Pseudomonas fluorescens* isolated from soils of Maharashtra against antibiotics

Sr. No.	Isolate No	S ₁₀	NS ₁₀₀	R ₅	T ₃₀
1	P 16	S(13 mm)	R	S(13 mm)	S(14 mm)
2	P 22	R	R	S(12 mm)	S(10 mm)
3	P 31	S(11 mm)	R	S(11 mm)	S(10 mm)
4	P 33	R	R	S(12 mm)	S(14 mm)
5	P 35	R	R	S(14 mm)	S(15 mm)
6	P 39	R	R	S(13 mm)	S(12 mm)
7	P 40	R	R	R	S(12 mm)
8	P 41	R	R	S(13 mm)	S(12 mm)
9	P 42	HS(20 mm)	R	S(13 mm)	S(9 mm)
10	P 43	R	R	S(13 mm)	S(10 mm)
11	P 44	R	R	S(12 mm)	S(10 mm)
12	P 49	R	R	S(15 mm)	S(10 mm)
13	P 51	HS(29 mm)	R	S(15 mm)	S(10 mm)
14	P 52	R	R	S(12 mm)	HS(20mm)
15	P 55	R	R	S(13 mm)	S(10 mm)
16	P 56	S(13 mm)	R	S(14 mm)	HS(35mm)
17	P 57	S(8 mm)	R	S(11 mm)	S(11 mm)
18	P 77	R	R	S(14 mm)	S(13 mm)
19	P 80	R	R	S(13 mm)	HS(21mm)
20	P 85	R	R	S(15 mm)	S(17 mm)
21	P 91	R	R	S(13 mm)	S(13 mm)
22	P 92	R	R	HS(26mm)	HS(21mm)
23	P 96	R	R	S(18 mm)	S(8 mm)
24	P 105	HS(135 mm)	R	S(15 mm)	HS(29mm)
25	P 106	R	R	S(18 mm)	S(12 mm)
26	P 110	R	R	S(18 mm)	HS(20mm)
27	P 119	R	R	S(17 mm)	HS(20mm)
28	P 120	R	R	S(8 mm)	S(9 mm)
29	P 121	R	R	S(17 mm)	HS(23mm)
30	P 134	R	R	HS(31mm)	HS(55mm)
31	P 136	R	R	HS(25 mm)	S(19 mm)
32	P 145	R	R	S(14 mm)	S(17 mm)
33	P 149	R	R	S(15 mm)	S(18 mm)
34	P 150	R	R	S(9 mm)	S(12 mm)
35	CP	R	R	S(15 mm)	S(10 mm)

S₁₀ = Streptocyclin NS₁₀₀ = Nystin R₅ = Rifamycin T₃₀ = Tetracycline

R = Resistant

S= Less than 20 mm formation of zone

HS= 20 mm and above formation of zone

Table 30 : Production of siderophore and HCN by *Pseudomonas fluorescens* from soils of Maharashtra.

Sr.No.	Isolate No	Siderophore production	HCN producion
1.	P-16	+	++
2.	P-22	+	++
3.	P-31	+	+++
4.	P-33	+	+
5.	P-35	+	++
6.	P-39	+	+
7.	P-40	+	++
8.	P-41	+	++
9.	P-42	+	+
10.	P-43	+	++
11.	P-44	+	++
12.	P-49	+	+
13.	P-51	+	+++
14.	P-52	+	++
15.	P-55	+	++
16.	P-56	+	+++
17.	P-57	+	+
18.	P-77	+	+
19.	P-80	+	++
20.	P-85	+	+
21.	P-91	+	++
22.	P-92	+	++
23.	P-96	+	++
24.	P-105	+	+
25.	P-106	+	++
26.	P-110	+	+
27.	P-119	+	++
28.	P-120	+	+
29.	P-121	+	+++
30.	P-134	+	+++
31.	P-136	+	+++
32.	P-145	+	+++
33.	P-149	+	++
34.	P-150	+	+
35.	CP	+	+++

+ = Weak HCN producer ++ = moderate HCN producer +++ =High HCN producer
+ = siderophore producer

4.4.4 Molecular characterization

The PCR amplification products of the 14 isolates of *Pseudomonas fluorescens* (13 selected isolates alongwith one commercial *Pseudomonas* strain) were subjected to PCR amplification using 24 random bacterial primers (Table 31, Plate 29 and Plate 30). The amplification profile of 14 different *Pseudomonas fluorescens* isolates with 24 random bacterial primers were summerised in Table 32, Plate 31 to Plate 33. The RAPD phenotypes were assessed from the photographs. It was observed that 801 bands were produced by the 24 primers. The number of bands produced per primer varied from 12 to 50. The average number of bands per primer was 33.33. Out of 801 bands, 589 polymorphic bands were observed (73.53 %) and 211 unique bands were observed (26.34%) and only one monomorphic band observed in RBA-1 primer. Most of the primers produced highly polymorphic bands varied from 1 to 27. Highly polymorphic bands observed maximum (27) in primer RBA-20 followed by RBA-14 (19) and minimum highly polymorphic bands observed in RBA-19 and no highly polymorphic bands observed in all RBA-17 and RBA-24 primers. Less polymorphic bands were also recorded in all primers and ranged from 4 to 27 in which <20% polymorphic band observed and most of the primers do not produce >80% ie less polymorphic bands. The amplified DNA fragment ranged from 199 to 3848 bp. The number of polymorphic loci amplified varied with different primers and isolates. The primer RBA 20 showed maximum per cent polymorphisum 97.30% and average polymorphisum was observed 73.53%. The fingerdata of 24 random primers were converted into a binary matrix based on the prescence of band was marked as “1” and absence was marked as “0”.

However, RBA-14 was found to be the best primer for determination of variability among the *Pseudomonas fluorescens* isolates. This primer generated 37 bands of different molecular weight summerised

Table 32. Summary of RAPD analysis of genomic DNA of fourteen different isolates of *Pseudomonas fluorescens*

Sr.No.	Details	
1.	Total number of primers used	24
2.	Total number of polymorphic primers	24
3.	Total number of bands	801
4.	Total number of unique bands	211
5.	Total number of polymorphic bands	589
6.	Total number of monomorphic bands	1
7.	Percentage Polymorphic	73.53%

in Table 33. Though some of the isolates share common bands most of the isolates differed in their banding pattern. Band 3 was only present in P-52, band 6 and 10 were present in P-51 and P-52, band 9 was present in P-40, P-121 and P-145, band 12 present in P-51, P-121 and P-136, band 22 present in P-121 and CP, band 36 in P-41 and P-51, thereby differentiating these are the genetically different isolates. None of the *Pseudomonas fluorescens* isolate share all the bands and are therefore genetically variable. Though all the isolates of *Pseudomonas fluorescens* were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Genetic similarity was computed based on pooled data of RAPD profile for *Pseudomonas fluorescens* as DICE coefficient (Dice, 1945; Nei and Li, 1979) using the similarity routine of NTSYS PC 2.02i software. The genetic similarity matrix (Table 34) based on the pooled data of RAPD profiles from all the 24 primers revealed that highest genetic similarity index of 0.68 between the isolates P-40 and P-41 followed by 0.65 for the isolates P-91 and P-119. The minimum genetic similarity index was observed in the isolates P-31 and P-52 (0.17) and P-121 and CP (0.18) The genetic similarity index was ranged from 0.17 to 0.68 among the all isolates. From the genetic similarity coefficient, the results indicated that there was very distinct diversity (coefficient 0.0 to 0.35) observed in the

Table 31 : RAPD analysis of *Pseudomonas fluorescens* isolates from soils of Maharashtra

Sr. No.	Primer Name	Total No. of bands	No. of unique bands	No. of monomorphic bands	Highly polymorphic	Less polymorphic bands (<20 & >80%)		% polymorphism
						<20%	>80%	
1	RBA 1	38	11	1	11	15	0	71.05
2	RBA 2	46	14	0	11	21	0	71.74
3	RBA 3	45	23	0	8	14	0	48.89
4	RBA 4	35	14	0	9	12	0	60.00
5	RBA 5	46	14	0	8	24	0	69.57
6	RBA 6	32	5	0	7	20	0	84.38
7	RBA 7	34	8	0	13	13	0	76.47
8	RBA8	28	7	0	10	10	1	75.00
9	RBA 9	33	14	0	10	9	0	57.58
10	RBA 10	46	7	0	12	27	0	84.78
11	RBA 11	35	1	0	14	19	1	97.14
12	RBA 12	20	8	0	1	11	0	60.00
13	RBA 13	42	9	0	7	26	0	78.57
14	RBA 14	37	1	0	19	17	0	97.30
15	RBA 15	50	22	0	14	14	0	56.00
16	RBA 16	28	9	0	9	8	2	67.86
17	RBA 17	12	8	0	0	4	0	33.33
18	RBA 18	22	5	0	4	13	0	77.27
19	RBA 19	13	4	0	1	8	0	69.23
20	RBA 20	43	3	0	27	13	0	93.02
21	RBA 21	29	2	0	16	9	2	93.10
22	RBA 22	33	3	0	18	12	0	90.91
23	RBA 23	38	10	0	8	20	0	73.68
24	RBA 24	15	9	0	0	6	0	40.00

Table 33 : Determination of genetic variability in different isolates of *P. fluorescens* by DNA amplification profiles by RBA-14 primer

Sr. No.	Primer and Molecular weight (bp)	<i>Pseudomonas fluorescens</i> isolates													
		P-31	P-40	P-41	P-51	P-52	P-55	P-56	P-91	P-119	P-121	P-134	P-136	P-145	CP
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	3965	1	0	0	0	0	0	0	0	0	0	1	0	0	0
2	3236	0	0	0	1	0	0	0	0	1	0	0	0	1	1
3	3134	0	0	0	0	1	0	0	0	0	0	0	0	0	0
4	2970	0	0	0	0	0	1	1	0	1	0	0	0	0	1
5	2877	0	1	1	0	0	0	1	0	0	0	0	0	1	1
6	2540	0	0	0	1	1	0	0	0	0	0	0	0	0	0
7	2450	1	0	0	0	0	0	0	1	0	0	0	0	0	0
8	2373	0	0	1	0	0	1	1	0	1	0	0	0	0	0
9	2323	0	1	0	0	0	0	0	0	0	1	0	0	1	0
10	2075	0	0	0	1	1	0	0	0	0	0	0	0	0	0
11	2000	0	0	0	0	0	0	1	0	0	0	0	0	0	1
12	1957	0	0	0	1	0	0	0	0	0	1	0	1	0	0
13	1816	1	0	0	0	1	0	0	0	0	0	1	0	0	1
14	1759	0	0	0	0	0	1	0	1	1	0	0	0	0	0
15	1722	0	1	1	0	0	0	0	0	0	1	0	0	0	0
16	1667	0	1	0	0	0	0	1	0	0	0	0	0	1	1
17	1530	0	0	0	1	0	0	0	1	0	0	0	0	0	0
18	1466	0	0	0	0	1	1	0	0	1	0	1	0	0	0
19	1420	0	1	1	0	0	0	1	0	0	0	0	0	0	0
20	1361	1	0	0	1	1	0	0	1	0	0	0	1	1	1
21	1276	0	0	0	0	0	1	1	0	1	0	1	0	0	0
22	1151	0	0	0	0	0	0	0	0	0	1	0	0	0	1
23	1052	1	0	1	1	1	1	1	1	1	0	1	1	0	0
24	998	0	1	0	0	0	0	0	0	0	0	0	0	0	1
25	936	0	0	1	0	1	1	0	1	1	1	1	0	1	0
26	887	1	1	0	1	0	0	1	0	0	0	0	1	1	1
27	780	0	1	1	0	1	0	1	1	1	0	0	0	0	0
28	708	0	0	0	0	0	0	0	0	0	1	1	0	1	0
29	623	0	1	1	1	0	1	1	1	1	0	0	0	0	0
30	560	1	1	1	1	1	1	1	1	1	0	0	0	1	1
31	492	0	0	0	0	0	1	0	0	0	0	0	1	1	0
32	452	0	1	0	0	0	0	1	1	0	0	0	0	0	0
33	369	0	1	1	1	1	1	1	1	1	0	0	1	1	1
34	314	0	1	0	1	1	1	1	1	1	1	1	0	0	0
35	256	0	1	1	0	0	1	0	1	1	0	1	0	1	0
36	210	0	0	1	0	1	0	0	0	0	0	0	0	0	0
37	165	0	0	0	1	1	0	0	1	1	0	0	0	0	0

Table 34. Genetic similarity as DICE coefficient based on pooled data of RAPD profiles for *Pseudomonas fluorescens*.

	P 31	P 40	P 41	P 51	P 52	P 55	P 56	P 91	P 119	P 121	P 134	P 136	P 145	CP
P 31	1.00													
P 40	0.26	1.00												
P 41	0.24	0.68	1.00											
P 51	0.20	0.30	0.31	1.00										
P 52	0.17	0.33	0.39	0.44	1.00									
P 55	0.23	0.42	0.49	0.34	0.49	1.00								
P 56	0.27	0.48	0.52	0.30	0.39	0.60	1.00							
P 91	0.25	0.31	0.35	0.42	0.51	0.47	0.49	1.00						
P 119	0.26	0.36	0.43	0.31	0.44	0.56	0.570	0.65	1.00					
P 121	0.24	0.20	0.23	0.22	0.22	0.28	0.20	0.19	0.22	1.00				
P 134	0.32	0.25	0.28	0.21	0.21	0.26	0.29	0.23	0.27	0.31	1.00			
P 136	0.26	0.21	0.22	0.26	0.21	0.26	0.22	0.20	0.24	0.37	0.29	1.00		
P 145	0.28	0.34	0.34	0.25	0.33	0.41	0.43	0.36	0.41	0.22	0.31	0.30	1.00	
CP	0.30	0.26	0.23	0.28	0.24	0.24	0.26	0.24	0.32	0.18	0.25	0.29	0.34	1.00

P. fluorescens isolated from soils of Maharashtra on the basis of functional diversity and ecological regions.

The dendrogram representing the genetic relationship among isolates based on RAPD analysis was developed and presented in Fig. 9. On the basis of RAPD analysis, dendrogram constructed from the pooled data of *Pseudomonas fluorescens* isolates had major two clusters. The first cluster consisted of five strains of *Pseudomonas fluorescens* (P-31, P-134, CP, P-121 and P-136). The first cluster consisted of P-31 and P134 having a genetic similarity coefficient 0.31. The genetic similarity coefficient between CP , P-31 and P-134 varied 0.25 to 0.30 and between P121 and P-136 having a genetic similarity coefficient 0.37. The second cluster consisted of 9 strains of *P. fluorescens* . The second cluster is sub-clustered into three groups. P-40 and P-41 is grouped in first sub-cluster having a genetic similarity coefficient 0.68. The second sub-cluster of P-52, P-55, P-56 , P-91 and P-119 isolates in which P-55 and P-56 *P. fluorescens* isolates having a genetic similarity coefficient 0.60. and isolates P-91 and P-119 having a genetic similarity coefficient 0.65 and P-52 is correlated to these four isolates. The third sub-cluster is of P-145 and P-51 of *P. fluorescens* isolates having a genetic similarity with other related isolates varied from 0.33 to 0.45 and 0.30 to 0.44, respectively.

2D scatter plot PCO scatter plot (Fig.10) were also helpful to make up the group of isolates on the basis of prescence of points on particular axis. In 2D scatter plot graph, the isolates P-40 and P-41 were also graphed in one group whereas isolates of *Pseudomonas fluorescens viz.*, P-31, P-134, CP, P-136 and P-121 were observed genetically closely related and graphed in one group. The isolates P-51 and P-145 were also observed genetically closely related and grouped in one group whereas, *Pseudomonas* isolates P-56, P-55, P-52, P-119 and P91 were also observed closely related in clustering.

Similarly 3D scatter plot PCO scatter plot (Fig.11) clearly represents the phylogenetic relationship among different isolates of *P. fluorescens*. It differentiates the distinctness or genetic similarity among the isolates. For 3D scatter plot graph, it was observed that P-51 and P-145 isolates were close in dendrogram and 2D scatter plot graph. But in 3D scatter plot P-51 and P-145

were different from rest of the isolates from the same group. The isolates P-31, P-134, CP, P-136 and P-121 were represented closely in 3D scatter plot graph. The isolates P-31 and P-134 were different from rest of the isolates from the same group by three dimensions. Whereas the isolates P-40 and P-41 were also same pick represents that are closely genetically grouped in dendrogram and 3D scatter plot graph.

4.4.5 Geographical distribution of *Pseudomonas fluorescens* from soils of Maharashtra:

Out of 150 locations, *Pseudomonas fluorescens* was distributed in 34 location of Maharashtra which contributes 22.67% of soils of Maharashtra. *Pseudomonas fluorescens* was found maximum in soils of Western Ghats (29.41%) followed by Western Maharashtra (23.53%), Vidarbha(17.65%), North Maharashtra (14.71%), Marahwada region (8.82%) and least was found in Western Konkan region (5.88%) (Table 35).

Pseudomonas fluorescence strains P-31, P-51, P-56, P-121, P-134, P-136 and P-145 showed antagonistic activity superior than commercial *Pseudomonas fluorescens* from different soils of Maharashtra. The *P. fluorescens* was prominently observed from soils of Nagpur followed by Wardha district from Vidarbh followed by Sindudurg, Nasik and Latur from Western Ghat, North Maharashtra and Marathwada respectively. The occurrence of P-124, P-136 and P-145 isolate was observed from Ramtek, and Kuhl from Nagpur ditrict. The P-121 isolate was observed in the soils of Sewagram from Wardha district. The occurrence of *Pseudomonas fluorescence* strains P-31, P-51, P-56 was observed in soils of Sindudurg, Nasik and Latur from Western Ghat, North Maharashtra and Marathwada, respectively. On the basis of functional diversity, the map of *Pseudomonas fluorescens* of the soils of Maharashtra State has been prepared and representing the district wise distribution of efficient *Pseudomonas fluorescens* presented in Fig. 12.

Table 35. Distribution of efficient *Pseudomonas fluorescens* across the phylographic regions of Maharashtra.

Sr. No.	Physiographic region	District	Distribution of efficient <i>Pseudomonas fluorescens</i>
I.	Western Konkan coast	Sindudurg	
		Ratnagiri	III
		Thane	III
		Raigad	
II.	Western Ghats	Sindudurg	I,II
		Ratnagiri	
		Thane	II
		Kolhapur	II,III
		Satara	III
		Pune	
		Nasik	II
		Dhule	
		Nandurbar	III
III.	Western Maharashtra	Pune	
		Kolhapur	III
		Satara	II
		Sangali	III
		Solapur	
		Ahmednagar	II,III
IV.	North Maharashtra	Jalgaon	II,III
		Dhule	
		Nandurbar	
		Nasik	I,II,III
V.	Marathwada	Latur	I,II
		Osmanabad	
		Beed	
		Nanded	
		Parbhani	III
		Hingoli	
		Jalna	
		Aurangabad	
		Buldhana	
VI.	Vidarbha	Washim	
		Akola	
		Amaravati	
		Yawatmal	
		Wardha	I
		Nagpur	I,II,III
		Bhandara	
		Chandrapur	
		Gondia	
		Gadchiroli	

I – Highly efficient (more than CP)

II- Moderately efficient

III – Less efficient

4.4.6 Geographical distribution of *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* from soils of Maharashtra:

Out of 150 soil samples from the Maharashtra, 72 isolates of *Streptomyces* sp., 8 isolates of *Acidithiobacillus* sp. and 34 isolates of *Pseudomonas fluorescens* were found. Out of 72 isolates of *Streptomyces* sp., 8 isolates of *Acidithiobacillus* sp. and 34 isolates of *Pseudomonas fluorescens*, 40, 3 and 7 were found highly efficient isolates, respectively. The efficiency of *Streptomyces* sp. for cellulose degradation and antimicrobial activity, *Acidithiobacillus* sp. for reduction in pH and *Pseudomonas fluorescens* for biocontrol activity was considered.

From soils of Maharashtra, it was concluded that efficient *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* were found in Nagpur district of Maharashtra. While efficient isolates of *Streptomyces* sp., and *Pseudomonas fluorescens* were observed in Wardha, Latur, Nashik and Sindudurg districts of Maharashtra. The efficient strains of *Streptomyces* sp. and *Acidithiobacillus* sp. were observed in Kolhapur and Solapur district of Maharashtra.

Only *Streptomyces* sp. was observed in Yavatmal, Gadchiroli, Washim, Akola, Nanded, Parbhani, Sangali, Satara, Ratnagiri, Raigad, Thane, Pune, Ahmednagar and Jalgaon districts of Maharashtra. While none of the efficient *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* were observed in Gondia, Chandrapur, Amaravati, Buldhana, Jalna, Beed, Osmanabad, Aurangabad and Nandurbar districts of Maharashtra. Such isolates could be useful in agriculture and industry. There is a need to conserve this treasure for the future.

5. DISCUSSION

Soil is considered a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than five per cent of the total space. It is a crucial resource not only for agricultural production and food security but also towards maintenance of most life processes. The functions of soil microorganisms are central to the decomposition processes and nutrient cycling. They play an important role in soil processes that determine plant productivity. For successful functioning of introduced microbial bioinoculants and their influence on soil health, efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behaviour in soil habitats (Hill, 2000).

The study of microbial diversity plays a key role in understanding microbial ecology of any ecosystem. Owing to their remarkable metabolic diversity and genetic adaptability, the microorganisms recycle important mineral elements and thereby improve the health and functioning of ecosystems and the whole environment. They play a vital role in maintaining soil fertility, pest and disease control and detoxification of natural and man-made pollutants. The variety of species in an ecosystem and the genetic variability within each species means biodiversity. Much better known and exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture (Johri *et al.* 2005).

Beneficial free living soil bacteria isolated from rhizosphere of plants, which have been shown to improve plant health or increase yield are usually referred to as plant growth promoting rhizobacteria i.e. PGPR (Kloepper *et al.* 1980b). *Pseudomonas fluorescens* is a antagonist micro-organisms plays an important role in microbial equilibrium. They are ubiquitous soil microorganisms and common in habitats of rhizosphere. It plays an important role in biological control of plant pathogens.

Pseudomonas fluorescens are commensal species with plants, allowing plants to attain key nutrients, degrading pollutants, and suppressing pathogens via antibiotic productions. These microbes produce secondary metabolites that suppress plant disease and signal gene expression to neighboring cells inhabiting the rhizosphere. *Pseudomonas* also use siderophores from other microorganism to obtain iron which increases their survival in iron-limited environments. Plants provides nutrients and shelter against stressful environments (Paulsen, *et al.* 2005).

Streptomyces isolates (JS-1 to JS-20) were having strong antagonistic activity against various bacterial and fungal pathogens, but the activity was differing in all the 20 *Streptomyces*. Phylogenetic analysis revealed that the isolates are the divergent of *Streptomyces* which are associated in the rhizosphere soil of Mangrove (Kannan and Vincent, 2011)

The reports of the experiments conducted in India Rupela and Tauro, (1973) showed that micro-organisms could be successfully exploited to reclaim the alkali soils since these soils have been formed to support very sparse population of sulphur oxidizing bacteria.

The study of molecular diversity by PCR based RAPD analysis facilitates identification and placement of the strains in genetically distinct and related group (Lee and Henry, 2001).

The very little study has been undertaken for region wise diversity of microorganisms in the Maharashtra State. So, there is a need to formulate meaningful programmes for its conservation and sustainable utilization. It was in this background that the efforts were made to study the variability mapping of *Pseudomonas fluorescens*, *Streptomyces* sp. and *Acidithiobacillus* sp. isolated from soils of Maharashtra regarding morphological, biochemical and cultural characterization as well as beneficial activities and genetic variation. The results obtained in the investigation are discussed in this chapter.

5.1 Isolation of actinomycetes, sulphur oxidizer and Fluorescent pseudomonads:

A total 150 soil samples collected which represent physiographically different soil types of Maharashtra. viz., Western Konkan Coast, Western Ghats (Sahyadris) and North Deccan Plateau (Upper, Lower and Metamorphic) were used for isolation. Out of that 131 actinomycetes, 22 sulphur oxidizing microorganisms and 65 fluorescent pseudomonads were isolated. Among these isolates 34 *Pseudomonas fluorescens*, 72 *Streptomyces* sp. and 8 *Acidithiobacillus* were characterized on morphological, biochemical basis and functional activities to ascertain most efficient strains and to study the variability of microorganisms with special interest on beneficial activities for agriculture.

Several research workers isolated the *Streptomyces* sp. from rhizospheric soils of different geographic regions. Busaya *et al.* (2005) isolated 32 strains of actinomycetes from soil samples of Thailand. Rifaat and Kansoh (2005) isolated *Streptomyces* spp. from Egyptian soils Taddei *et al.* (2006) isolated 71 *Streptomyces* sp. from different places of Venezuela. Saadoun *et al.* (2008) isolated 110 isolates of Streptomyces from rhizosphere soils of Ajlun forests in Northern Jordan. Dehnad *et al.* (2010) isolated 150 actinomycetes from 100 soil samples collected from Northwest of Iran. Lakshmipathy and Kannabrian (2009); Mythili and Das (2011) and Kannan and Vincent (2011) who isolated *Streptomyces* sp. from the rhizosphere soils from different geographical regions of India. The results regarding isolation of *Streptomyces* sp. are in conformity to the work done by various research workers from different geographical regions in India and other countries.

Several research workers isolated Sulphur oxidizer from rhizospheric soils of different geographic regions. Swaby and Fedel (1973) isolated five *Thiobacillus* sp. out of 56 Australian soil analysed. Shinde (1993); Vidyalakshmi and Sridar (2006-2007) and Nareshkumar *et al.* (2007) isolated sulphur oxidizing bacteria *Acidithiobacillus thiooxidans* using Starkey medium from different samples in India. Yang Yu *et al.* (2008) and Babana *et al.* (2011)

isolated *Thiobacillus* sp. strains from the soil samples collected from China and Canada, respectively. The results regarding isolation of *Acidithiobacillus* sp. are in conformity with the work done by various research workers from different geographical regions.

Similar results were obtained by various research workers regarding isolation of *Pseudomonas fluorescens* from rhizosphere soils of different geographic regions. Jayaswal *et al.* (1990) isolated circular smooth and yellow pigmented colonies and confirmed as *Pseudomonas fluorescens* on the basis of morphological and physiological characterization. Kumar *et al.* (2002) isolated *Pseudomonas* sp. from rhizosphere of rice and sugarcane around Madurai. Joseph *et al.* (2007) isolated the PGPR bacteria from soil samples collected from Allahabad Agriculture Institute, India by using King's B medium. Megha *et al.* (2006) isolated 52 isolates of fluorescent pseudomonads from forest soils of Western Ghat, Uttara Kannada district from Karnataka. Das *et al.* (2008) and Rekha *et al.* (2010) isolated *Pseudomonas fluorescens* from the rhizosphere soil samples. The results regarding isolation of *Pseudomonas fluorescens* are in conformity with the work done by earlier research workers at different geographic regions of India.

5.2. Actinomycetes :

5.2.1 Microbial Population of Actinomycetes :

In the present studies, the microbial population of actinomycetes was found 87.33% of the different soils of Maharashtra. The microbial population of actinomycetes varied from 1×10^{-4} to 18×10^{-5} cfu/g in different soils of Maharashtra. They were observed maximum (23.66%) in Western Maharashtra followed by Vidarbha, Western Ghat, Western Konkan coast, North Maharashtra and Marathwada 22.14%, 18.32%, 17.56, 9.92% and 8.40%, respectively from different soils of Maharashtra. The microbial population of actinomycetes A-56 was found 18×10^{-5} cfu/g maximum in Latur district from Marathwada followed by Sangali isolate A-63 (16×10^{-5} cfu/g), Latur isolate A-55 (15×10^{-5} cfu/g), Nashik isolate A-52 (15×10^{-5} cfu/g) from Western Maharashtra, Marathwada and North Maharashtra, respectively.

The present results are comparable with the results of Rao and Subramanyan (1929) who reported the presence in Indian soils (West Bengal) of streptomycetes which ranged from 4×10^4 to 3.4×10^5 CFU per gram of soil. Rangaswami and Venkatesan (1963) and Venkatesan (1964) also reported that the streptomycetes population of Annamalainagar paddy soil (alluvial) ranged between 1.14 to 5.40 millions per gram. Rangaswami *et al.* (1967) reported that the percentage of streptomycetes to the total microbial population, varied from 5 to 41%.

The similar results were obtained by Konde (1974) who reported that *Streptomyces* population varied from 5.11×10^6 to 7.67×10^6 CFU/g of soil from soils of Maharashtra. Kumari *et al.* 2006 isolated the actinomycetes from soils collected at different areas in Tamil Nadu and microbial population ranged from 4×10^3 to 12×10^3 CFU/g of soil. Jeffrey 2008 isolated 62 actinomycetes from soil samples collected from Sarawak and estimated microbial population ranged from 9.8×10^6 to 8.0×10^7 CFU/g of soil. The present results are in confirmity with the work done by earlier research workers at different geographic regions of India and other countries.

5.2.2 Morphological and Cultural characters :

5.2.2.1 Morphological characters and staining reaction :

In the present studies, the morphological studies of the actinomycetes isolates is studied regarding gram reaction, colour, spore chain morphology and pigmentation. All the 131 isolates are gram positive in reaction but non acid fast in reaction. There are 72 isolates having creamy colour, 55 isolates are grey in colour and 4 isolates are white to milky in colour. The most of isolates (66) having rectiflexibles (RF) in spore chain morphology and few of the isolates (6) showed retinaculiaperti (RA) in spore chain morphology is the main features of spore chain morphology of *Streptomyces* genus. The spores are observed

rectiflexibles in straight to flexuous spores in chain. While in retinaculiaperti open loops, hooks, coils or spirales. In some *Streptomyces* sp. the sclerotial like structures are also observed. The aerial mycelium and substrate mycelium is also recorded on different media. Only five isolates showed different pigmentation colour in the medium. It is observed that in 72 isolates spores of aerial mycelium and substrate mycelium were found in chain and they were identified as *Streptomyces* sp., whereas 4 isolates had cottony growth and 55 isolates grey or powdery growth and their aerial mycelium found in chain or most of spores are not in chain. The substrate mycelium of cottony growth isolates and powdery growths spores were not in the chain. These isolates were other than *Streptomyces* sp. probably *Chania* sp. and cottony growth was *Streptoverticillium* sp. The present results were obtained in conformity with those of Rangaswamy *et al.* (1967); Konde, 1974; Locci, 1989; Williams *et al.* 1989; Goodfellow, 1989; Oskay *et al.* 2004; Srivasatava *et al.* 2010, Reddy *et al.* 2011 who reported that filamentous rod shaped bacteria, gram positive, violet in colour but non acid fast in reaction. They reported that rectiflexibles per cent was more than retinaculiaperti and others and no biverticillium spira observed in these soil samples.

The results regarding morphology, spore chain morphology and melanin pigmentation are in conformity with the results of Seung *et al.* 1998; Taddei *et al.* 2006; Rabbani *et al.* 2007; Nonoh *et al.* 2010 and Venkatachalam *et al.* 2010; Kishore 2010 and Mythili and Das 2011 who reported that the isolates of *Streptomyces* sp. was gram positive in reaction, pale brown, white and gray colour in aerial mycelia and growth was moderate to abundant and leathery, rectiflexibles per cent was more than retinaculiaperti and melanin pigment was not observed in the most of the *Streptomyces* sp.

5.2.2.2 Cultural characters:

The *Streptomyces* sp. were grown on the nutrient agar medium, starch casein agar and oat meal agar medium for the cultural studies. All the 72 isolates were grown excellently on oat meal agar medium followed by starch casein agar and nutrient agar media. The growth of all the *Streptomyces* sp.

was found good on starch casein agar and poor to moderate on nutrient agar medium. The growth of aerial and substrate mycelium was observed on these media. It was found that there was no variation observed among the aerial mycelium on the different growth media whereas there was slight change observed in substrate mycelium. These findings are in agreement with those of Rangaswami *et al.* 1967; Konde 1974 and Rifaat and Kanosh, 2005 who reported that Oat meal agar medium gave better sporulation than other media. There was no variation observed among the aerial mycelium on the different growth media whereas there was slight change observed in substrate mycelium. Jain and Jain 2007 and Lakshmipathy and Kannabiran 2010 who also reported that there was no change in aerial mycelium but variation observed in substrate mycelium and growth pattern was varied from poor to good as per the media used for its growth. Thus, the present results are in conformity with the workdone by earlier research workers.

5.2.3 Biochemical characterization :

All the 72 isolates of *Streptomyces* sp. were biochemically characterized viz., H₂S production, catalase test, oxidase test and gelatin liquefaction. The H₂S production was observed positive reaction in 60 cultures. It was observed that 68 isolates showed gelatin liquefaction ability. Similar results were observed and are in conformity with those of Konde 1974 who reported that H₂S production was observed 71% positive reaction and 90% reaction positive in gelatin liquefaction. All the isolates were found in positive reaction in oxidase and catalase test. The present results are in conformity with the workdone by earlier research worker for biochemical tests positive in H₂S production (Kannan and Vincent, 2011; Rifaat and Kanosh 2005); positive gelatin liquefaction (Jain and Jain 2007; Mythili and Das 2011); positive in oxidase and catalase reaction (Srivastava *et al.* 2010) and positive reaction in catalase (Kumari *et al.* 2006; Nonoh *et al.* 2010; Umashankar,

et al. 2010) and Venkatachalam *et al.* 2010 reported that most of the *Streptomyces* sp. positive in catalase reaction.

5.2.4 Functional diversity of *Streptomyces* sp. :

Among the actinomycetes isolates from different soils of Maharashtra, on the basis of morphological and cultural studies 72 isolates were tentatively identified as *Streptomyces* sp and studied for functional diversity like cellulose degradation, antibiotic sensitivity, antimicrobial activity and P solubilization ability.

5.2.4.1. Cellulose degradation :

The capacity for cellulose decomposition of the 72 *Streptomyces* sp. isolates was studied and after 15 days, it was observed that 56.94% *Streptomyces* sp. have good cellulose degradation ability and 40.28% *Streptomyces* sp. isolates have moderate cellulose degradation activity and 2.78% have not cellulose degradation activity. Similarly, these finding are also inconfirmity with those of Konde (1974) who reported that out of 150 cultures tested, 106 cultures showed the cellulose degradation ability indicates that a large number of cellulose degrading streptomycetes were present in the soils of Maharashtra. Waksman (1931) reported that streptomycetes especially *Streptomyces* sp. were able to grow on cellulose as only source of carbon. Arora *et al.* (2005) reported that *Streptomyces griseus* B1 from soil produces high levels of all the three components *i.e.* filter paqper lyase (FPase), CMCellulase abd bglucosidase of the cellulolytic enzyme system and it depletes the cellulose (36% in softwood and 14.4% in hardwood). Saadoun *et al.* 2007 reported that 79% isolates of *Streptomyces* were able to produce atleast one of the enzymes with celluase being the highest (71.8%). Arunachalam *et al.* (2010) and Kishore 2010 reported that all the strains showed good cellulose degradation activity which are inconfirmity with the present results.

5.2.4.2 Antibiotic sensitivity :

Among the 72 *Streptomyces* sp., it was observed that 21 isolates do not form the zone around the antibiotic disc which indicates that these 21 isolates

were resistant and 51 were sensitive to antibiotics R₅, S₁₀ and T₃₀. All the 72 *Streptomyces* sp. isolated from soils of Maharashtra were found resistant to NS₁₀₀. Our investigation also resulted in a similar types of finding which are comparable to that of Oskay *et.al.* (2004); Ibrahim (2006) reported that *Streptomyces* isolates were sensitive to gentamycin, tobramycin, rifamycin and penicillin G, tetracycline but resistant against streptomycin, neomycin, vanomycin and lincomycine. Lakshmipathy and Kannabrian (2009) reported that *Strptomyces* sp DKDVIT1 sensitive to many antibiotics but resistant to chlorophenicol, penicillin, tetracycline and erythromycin.

5.2.4.3. Antimicrobial activity:

The *Streptomyces* sp. were tested for antimicrobial activity against the pathogen *viz.*, *Fusarium oxysporum pv. ciceri* and *Alternaria alternata* . The antibiotic activity of *Streptomyces* sp. were summerised in Table 20.

The RHR-S-98 showed maximum percent inhibition and was 30.00% against *A. alternata in vitro*. Among the 72 *Strptomyces* sp., 64 isolates showed antimicrobial activity against *Alternaria alternata* which ranged from 7.45% to 19.90% and 8 isolates showed more than 20% antimicrobial activity against *Alternaria alternata*.

The RHR-S-23 showed maximum percent inhibition and was 28.80% against *Fusarium oxysporum pv. ciceri in vitro*. Out of 72 *Strptomyces* sp., 12 *Streptomyces* sp. showed more than 20% antimicrobial activity against *Fusarium oxysporum pv. ciceri* and 60 isolates showed less than 20% antimicrobial activity against *Fusarium oxysporum pv. ciceri*. The *Streptomyces* sp. percent inhibition ranged from 10.44% to 28.80%. The results are in confirmity with the results of Konde, 1974.

The present results are in conformity with the results of research workers who showed antibacterial activity against the *Fusarium oxysporium*, *Aspergillus niger*, *Aspergillus terreus* (Rifaat and Kanosh 2005, *Fusarium palmivora* (Jeffrey *et al.* 2007); *Fusarium oxysporum* (Nonoh *et al.* 2010) and actinobacterial

isolates from Kenya showed the antibacterial activity against the plant pathogens like *Aspergillus niger*, *Aspergillus flavour*, *Pseudomonas aureoginosa*, *Bacillus subtilis* (Kannan and Vicent 2011).

5.2.4.4 P solubilization ability:

Among the 72 *Streptomyces* sp., only 22.22% isolates showed the P solubilization ability in which less than 1cm P solubilization zone was observed and 77.78% isolates does not showed P solubilization activity. The results are in confirmity with the results of Konde, 1974 who also reported that out of 150 cultres, only 9% showed P solubilization in liquid cultre.

5.2. 5 Molecular Characterization:

The present results of PCR amplification products of the 18 strains of *Streptomyces* spp. for RAPD analysis by using 23 random primers. It was observed that 1124 bands were produced by the 23 primers. The number of bands produced per primer varied from 36 to 72. The average number of bands per primer was 48.87. Out of 1124 bands, 986 polymorphic bands were observed (88.52%) and 138 unique bands were observed (11.48%) and no monomorphic band observed in all 23 primers. Most of the primers produced highly polymorphic bands varied from 2 to 24. Highly polymorphic bands observed were maximum (24) in primer RBA-14 followed by RBA-16 (18) and minimum highly polymorphic bands observed in RBA-7. The amplified DNA fragment ranged from 195 to 4234 bp. The per cent polymorphisum ranged from 73.33 to 97.43% and average polymorphism was observed 87.72%. Similar results are in confirmity with those of Munira *et al.* (2008) studied that the molecular fingerprints for fifteen *Streptomyces* isolates gathered from Riyadh city by using RAPD-PCR. The generated RAPD profile with the primer OPF-05 shows a total of 23 polymorphic bands among the studied isolates. It comprises one unique band and 22 non unique polymorphic ones. Similarly, primer OPK-10 develops 14 polymorphic bands which scored as 4 unique and 10 non unique bands.

The present results of genetic similarity matrix based on the pooled data of RAPD profiles from all the 23 primers revealed that highest genetic similarity of 0.65 between the isolates RHR S-40 and RHR S-41 and RHR S 41 and RHR S 48. The minimum genetic similarity was observed in the isolates RHR S-5 and RHR S-130 (0.13). The genetic similarity ranged from 0.13 to 0.65 among the all isolates. From the genetic similarity coefficient (0.21), the results indicated that there was very distinct diversity observed in *Streptomyces* sp. isolates from soils of Maharashtra. The results are comparable with the results of Munira *et al.* (2008) who studied RAPD profiles for drawing the genetic relationships among the examined *Streptomyces* species. The constructed dendrogram tree divided the fifteen isolates into two groups. The highest similarity value (0.851) was observed between the two isolates No. 13 and 14.

The present results of representing the genetic relationship among isolates based on RAPD analysis and dendrogram was developed and on the basis of RAPD analysis, the dendrogram constructed from the pooled data *Streptomyces* sp. had two main clusters. The first cluster consisted of five strains of *Streptomyces* (RHR S-28, RHR S-138, RHR S-116, RHR S-23 and RHR S-21) and had good cellulose degradation activity, poor biocontrol ability and substrate mycelium was brown to dark brown varied from rest of the other isolates. The first cluster consisted of RHR S-28 is distinct and other 4 isolates correlated in two sub cluster RHR S-138 and RHR S-116 with a genetic similarity value of 0.36 and RHR S-23 and RHR S-21 with a genetic similarity value of 0.36. The second cluster consisted of 13 strains of *Streptomyces*. The second cluster is sub-clustered into three groups. The second cluster's sub-cluster is among the RHR S, RHR S-40, RHR S-41 and RHR S-48 and RHR S-147 is grouped in which substrate mycelium was pale yellowish colour, good cellulose activity, poor biocontrol ability and RHR S-40, RHR S-41 and RHR S-48 isolated from ghat region except RHR S-147 which isolated from Vidarbha region soils. The second sub-cluster of second cluster is among five isolates RHR S-25, RHR S-52, RHR S-88, RHR S-98 and RHR S-148. In these isolates

substrate mycelium was light cream in colour, poor biocontrol ability except RHR S-98 whose spore morphology *rectinaculiaperti* (RA) and good biocontrol ability vary from other isolates (RF) within the same group. The second sub-cluster is grouped into three groups in which RHR S-148 and RHR S-98 are distinct and RHR S-25 is also correlated between RHR S-52 and RHR S-88 isolates with a genetic similarity value of 0.48. The third sub-cluster is between RHR S-17 and RHR S-130 with the genetic similarity value of 0.025 and the RHR S-24 is distinct. These isolates were poor to moderate cellulose and biocontrol ability. But the second sub-cluster of second cluster is among *Streptomyces* spp. five isolates RHR S-148, RHR S-98, RHR S-88, RHR S-52 and RHR S-25 are genetically correlated with S-17, S-130 and RHR S-24. The present results are in conformity with the results of Gharaibeh *et al.* (2003). They used RAPD analysis of 73 antibiotic producing soil *streptomyces* and reported that *Streptomyces* cluster resulted into two main clusters. The first cluster was more homogenous than the second one and contains all the reference strains. The second cluster contains unrelated isolates within small groups.

The present results are in conformity with the earlier work for studying genetic diversity of *Streptomyces* by Seung *et al.* 1998; Malkawi *et al.* (1999); Martin *et al.* (2000); Roberts and Crawford (2000) and (Saadoun *et al.* 2007), they analyzed genetic diversity by RAPD analysis using RAPD primers for clearly distinguishable among the species. Kannan and Vincent (2011) reported that *Streptomyces* analysed for phylogenetic analysis and among 20 *Streptomyces* sp. JS-20 showed similarity among them. The isolates observed divergent of *Streptomyces* which are associated with rhizosphere soil of Mangroove.

5.2.6 Geographical distribution of *Streptomyces* sp. from soils of Maharashtra:

Out of 150 locations, *Streptomyces* sp. were found in 48% soils of Maharashtra. It was distributed in 72 location of Maharashtra soils.

From soils of Maharashtra, it was observed that *Streptomyces* were prominently distributed maximum in Vidarbha (27.78%) followed by Western Maharashtra (19.44%), Western Konkan coast (15.28), Marathwada region (13.89%) , Western Ghat (13.89%) and least was found in soils of North Maharashtra region (9.72).

In Vidarbha region, Nagpur Wardha, Yawatmal, Akola, Washim and Gadchiroli districts having highly efficient *Streptomyces* spp. in soil. In Western Maharashtra, Solapur, Satara, Sangli, Pune and Ahmednagar district having efficient *Streptomyces* spp. in soil. In Western Konkan coast region, Ratanagiri and Raigad district having efficient *Streptomyces* sp. Whereas, Ratnagiri, Sindudurg , Satara, Pune and Kolhapur district have efficient *Streptomyces* sp. in Western Ghat region soil. In Marathwada region, Latur, Nanded and Parbhani districts having efficient *Streptomyces*. In North Maharashtra, Jalgaon and Nashik district having efficient *Streptomyces* spp. The present finding are in confirmity with the results of Rangaswany *et. al.* (1967); Konde, (1974).

5.3. Sulphur oxidizer :

All the *Acidithiobacillus* sp. isolated from different soils of Maharashtra were studied for their cultural, morphology, biochemical and molecular studies.

5.3. 1. Occurrence of sulphur oxidizer microorganisms:

In the present studies, the occurrence of sulphur oxidizer microorganisms was found 14.67% of the soils of Maharashtra. Out of 22 locations, maximum found in Vidarbha (9 location) followed by Western Maharashtra (7), Western ghat (3) and Western konkan coast (2) and least was observed in Marathwada region (1 location) from soils of Maharashtra. Similarly the results are comparable to the earlier work by Swaby and Fedel, (1973); Lawrence and Germida, (1985); Shinde *et al.*(1996) and Babana *et al.* (2011) that isolated *Thiobacillus* from various soil samples and observed that the soils are poor in sulphur oxidizer microorganisms.

5.3.2. Morphology and Cultural characters of *Acidithiobacillus* sp.

All the *Acidithiobacillus* sp. isolated from different soils of Maharashtra were studied for their morphology and it was observed that all the

Acidithiobacillus sp. have small yellow colour colonies and grow in thiosulphate agar slants. They were found in short rods and size ranged from 0.3-0.7 x 0.9-1.6 μm . All the isolates were gram -ve in reaction. The pH reduction was observed in all the *Acidithiobacillus* sp. from 8.0 to ≤ 5.0 . These present results are in confirmity with the earlier research work by Waksman and Joffe (1922) who reported that *Acidithiobacillus thiooxidans* grown in liquid medium on elemental sulphur thiosulphate or tetra thionate, short rod with round ends, 1 μ in length and 0.5 μ wide, gram negative. Sokolova and Karavaiko (1968) studied that *Thiobacillus thiooxidans* were rod shaped cells of 0.5-0.5 x 1-1.2 μ , gram negative occurring singly and occasionally in pair and short rods. Guay and Silver (1975) characterised that *Thiobacillus acidophilus* as short rods, 0.5 – 0.8 μm x 1-1.5 μm with round ends, gram negative and motile non sporulating. The cells occurred in singly, mainly in pairs and rarely in chains. The present results are inconfirmitly with the results of Kelly and Wood (2000); Lee *et al.* (2000).

The present results regarding colony size and pH reduction are in conformity with the results of Vidyalakshmi and Sridar (2006-2007) and Babana *et al.* (2011), they reported that *Thiobacillus* sp. efficacy screened based on to reduce the pH of the growth medium from 8.0 to ≤ 5.0 , isolates short rods, Gram negative, smooth, round and straw colour, yellowish colony diameter ranged from 0.8 to 1.9 mm in size.

5.3.3 Molecular Characterization:

The PCR amplification products of the 9 isolates of *Acidithiobacillus* sp. (8 isolates alongwith At.-RHR) were subjected to PCR amplification using 15 random primers. The RAPD phenotypes were assessed from the photographs. It was observed that 484 bands were produced by the 15 random primers. The number of bands produced per primer varied from 18 to 48. The average number of bands produced per primer was 32.2. Out of 484 bands, 284 polymorphic bands were observed (58.68 %) and 200 unique bands were observed (41.32%) and no monomorphic band observed in all 15 primers.

Most of the primers produced highly polymorphic bands varied from 2 to 18. Highly polymorphic bands observed maximum (18) in primer RBA-11 followed by RBA-10 (17) and minimum highly polymorphic bands observed in RBA-13. The amplified DNA fragment ranged from 289 to 6021 bp. The number of polymorphic loci amplified varied with different primers and isolates.

The genetic similarity matrix based on the pooled data of RAPD profiles from all the 15 primers revealed that highest genetic similarity of 0.65 between the isolates SOB-113 and SOB-115. The minimum genetic similarity was observed in the isolates SOB-44 and SOB-115 (0.13). The genetic similarity ranged from 0.13 to 0.65 among the all isolates. From the genetic similarity coefficient, the results indicated that there was very high diversity was observed in the *Acidithiobacillus* isolated from soils of Maharashtra on the basis of geographical region.

On the basis of RAPD analysis, the dendrogram constructed from the pooled data had four clusters. The first cluster consisted of four isolates of *Acidithiobacillus* sp. (SOB 135, SOB-130, SOB-115 and SOB-113). The first cluster consisted into two sub cluster SOB-135 and SOB-130 whose yellow colonies were round and scattered and size range 0.6 x 1.1-1.2 μ m from Vidarbha region soils with a genetic similarity value of 0.26 and SOB-115 and SOB-113 whose yellow colonies were very small, close, size vary 0.3-0.4 x 0.9-1.0 μ m and from irrigated soils of western and north Maharashtra with a highest genetic similarity value of 0.65. The second cluster consisted of 3 isolates of *Acidithiobacillus* sp. The second cluster is sub-clustered into two groups. The second cluster's sub-cluster among the SOB-146, SOB and SOB-80 whose colonies were medium to large, size vary 0.6 x 1.5 to 1.6 μ m grouped in which SOB-146 is distinct from Vidarbha soils and SOB and SOB-80 were from Western Maharashtra with a genetic similarity value of 0.31. The third (SOB 72) and fourth cluster (SOB 44) are distinct and small colony size and size vary 0.4-0.7 x 1.1 to 1.6 μ m which are correlated with other *Acidithiobacillus* sp.

The present results are comparable with the workdone by earlier research workers (Selenska – Pobell, Otto and Kutschke, 2002; Karavaiko *et al.* 2003; He *et al.* (2004) studied the molecular diversity and reported that random amplified polymorphic DNA (RAPD) was used in analyzing the polymorphisms of *Thiobacillus ferrooxidans*. Tanveer *et al.* (2005) studied that RAPD – a PCR based technique applied to evaluate genomic diversity among three strains of *Acidithiobacillus thiooxidans* using 45 random primers of five different series. More than 220 bands were observed, with an average 45 bands per primer. Primer OPC-3 produced maximum number of fragments whereas minimum numbers of fragments were produced with primer OPA-5. The dendrogram showed three groups with similarity ranging from 29 to 85%. The maximum similarity (85%) was observed between the strains T.t₁ and T.t₂ of *Acidithiobacillus thiooxidans*. Waltenbury *et al.* (2005) reported that the variations in banding patterns indicated genomic variability among the twelve *Acidithiobacillus ferrooxidans* strains tested and grouped twelve strains in five major groups. Similarities between the strains ranged from 5.49% to 85.14%. Yongqing Ni *et al.* (2008) reported that three *Acidithiobacillus ferrooxidans* phylogenetic groups should be considered a separate subspecies, and that five sulfur-oxidizing Chinese *Acidithiobacillus*-like isolates represent one or two new species of the genus *Acidithiobacillus*. Luciana *et al.* 1995 reported that *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* analysed for molecular fingerprinting showed that the tested strains encompassed a high degree of genetic variability. Two *A. ferrooxidans* isolates from Brazil were the most genetically divergent organisms. Thus, the present results are in conformity with research workdone by earlier research workers.

5.3.4 Geographical distribution of *Acidithiobacillus* from soils of Maharashtra:

Acidithiobacillus sp. occurred in 8 locations which contributes 5.33% of different soils of Maharashtra.

The results indicated that *Acidithiobacillus* was distributed in W. Maharashtra (37.5%) and Vidarbha (37.5%) whereas less observed in Marathwada (12.5%) soil and Western Ghat (12.5%). *Acidithiobacillus* sp. occurred in 3 locations viz., Rahuri, Kalwan and North Solapur of soils of Western Maharashtra and Vidarbha (3 location) viz, Umred, Kuhi in Nagpur, Chandrapr and least was observed in Latur from soils of Marathwada region and Kolhapur in Western ghat region soils. From the genetic similarity coefficient, it is observed that there was very high diversity in the SOB isolated from soils of Maharashtra. The present results are comparable to the earlier work done by Swaby and Fedel, (1973); Lawrence and Germida, (1985); Shinde *et al.*(1996) and Babana *et al.* (2011), they isolated *Thiobacillus* from various soil samples and observed that the soils are poor in sulphur oxidizer microorganisms.

5.4 *Pseudomonas fluorescens*

5.4.1 Microbial Population of fluorescent pseudomonads :

The microbial population of fluorescent pseudomonads was found 43% of the soils of Maharashtra. The microbial population of fluorescent pseudomonads varied from 9×10^{-3} to 6×10^{-6} cfu/g in different soils of Maharashtra. A wide variation in microbial population among the Fluorescent pseudomonads isolates was observed in the soils of Maharashtra. The maximum population of fluorescent pseudomonads FP-57 (6×10^{-6} cfu/g of soil) was recorded in Kolhapur district of Western Maharashtra followed by FP-52 and FP-43 isolates (4×10^{-6} cfu/g of soil) from Nasik and Sindudrurg districts of North Maharashtra and Western Ghat region. The minimum fluorescent pseudomonads population was FP-146 isolates (9×10^{-3} cfu/g soil) in Ramtek from Nagpur district of Vidarbha region. The similar results was obtained by Rekha *et al.* 2010 they reported that 1.3×10^7 CFU/ml bacteria were isolated from the soil samples of Chennai region.

5.4.2 Morphology of fluorescent pseudomonads :

The isolates were examined for their colony and cell morphology, cell size and gram reaction of the cells. Wide variations were observed among the isolates in their colony morphology. Out of the 65 isolates, 21 produced green colored colonies and 44 produced light green coloured colonies. Forty one isolates observed round and non-spreading type colonies whereas the remaining 24 isolates produced irregular shaped spreading type colonies.

All the 65 isolates were rod shaped bacteria and gram negative in reaction. Among these fluorescent pseudomonads 65 isolates were grouped on the basis of habitat, 40 isolates were rod shape living in clustering, 8 isolates were rod shape in isolate and 17 isolates were rod shape in pair and chain habitat. The size of these bacterial isolates are categorized into three on the basis of size *viz.*, upto 1.0 μm , 1.01 to 2.00 μm and more than 2.01 μm length. The results indicated that 29, 31 and 5 isolates were measured upto 1.0 μm , 1.01 to 2.00 μm and more than 2.01 μm length, respectively. The colonies showed wide variation in the intensity of fluorescence. Out of 65 isolates, 11 showed high fluorescence, 33 showed medium fluorescence and the remaining 21 isolates showed weak fluorescence.

Similar type of results were reported by Jayaswal *et al.* (1990) reported that colonies grown on King's B medium were circular, smooth and metallic, yellow pigment, aerobic, gram negative short rods approximately 1 x 1.6 μm in size, rods mainly grew in pairs or in clusters. Cartwright *et al.* (1995) also reported that *Pseudomonas* were aerobic, G-ve, strictly aerobic, rod shape bacteria. Siddiqui *et al.* 2005 also reported that all the *Pseudomonas* sp. were G-ve in reaction, Fluorescence was observed on King's B medium. Joseph *et al.* (2007) reported that *Pseudomonas* were rod shape, G-ve in reaction and producing pigments on King's B medium. Siddiqui and Shakeel (2009) reported that *Pseudomonas* were confirmed by green fluorescence of *Pseudomonas* sp. observed very clear on King's B medium. Megha (2006) and Rekha *et al.* (2010) reported that *Pseudomonas* sp. are G-ve, small rod shape bacteria, colonies are yellow green in colour, round and non spreading and

larger colony size and irregular surface. The present results are in conformity with these results.

5.4.3 Biochemical characterization of fluorescent pseudomonads :

The results on biochemical tests indicated that all the 65 fluorescent pseudomonads isolates were positive for indole production and gelatin liquefaction tests and were negative for starch hydrolysis and urease activity. Similarly, all the 65 isolates showed positive H₂S production and oxidase activity, 61 positive for catalase test and 4 were negative. 34 fluorescent pseudomonads isolates showed negative for denitrification test and 31 were positive. Thirty-four isolates showed growth at 4°C and 31 isolates were not able to grow at 4°C. whereas 31 isolates were able to grow at 41°C and 34 isolates were not able to grow at 41°C.

Based on the morphological and biochemical characteristics, 34 isolates were identified as *Pseudomonas fluorescens*, 31 as *Pseudomonas aeruginosa* from soils of Maharashtra. Out of 18 isolates from soils of Western Maharashtra, 8 were identified as *Pseudomonas fluorescens* and 10 as *Pseudomonas aeruginosa*. Out of 13 isolates from Western Ghat, 10 were identified as *P. fluorescens* and three were identified as *P. aeruginosa*. Six out of 13 isolates from Vidarbha were identified as *P. fluorescens* and seven as *P. aeruginosa*. Among the 9 isolates of Marathwada, three isolates belonged to *Pseudomonas fluorescens* and six to *Pseudomonas aeruginosa*. Among the 4 isolates of Western Konkan coast region, two isolates belonged to *Pseudomonas fluorescens* and two to *Pseudomonas aeruginosa*. In North Maharashtra, out of 8 isolates, 5 isolates were identified as *Pseudomonas fluorescens* and 3 were *Pseudomonas aeruginosa*.

The similar results were obtained by Megha *et al.* 2007 who reported that all the 52 isolates of fluorescent pseudomonads were positive for indole production and gelatin liquefaction. 48 isolates were positive for casein hydrolysis while most of the isolates produce H₂S and were positive for oxidase and catalase test and none was positive for starch hydrolysis, urease activity and acid and gas production. 13 isolates of *Pseudomonas aeruginosa*, 2 isolates

of *Pseudomonas aureofaciens* identified based on the morphological, biochemical and physiological characters. Similarly, the remaining 37 isolates were identified as *Pseudomonas fluorescens*. The results obtained in the present investigation are comparable to those of earlier workers (Jayaswal *et al.* 1990; Rangarajan *et al.* 2001; Joseph *et al.* 2007; Rekha *et al.* 2010; Maleki *et al.* 2010).

5.4.4 Cultural characterization of *Pseudomonas fluorescens* :

For cultural studies, all the 34 *Pseudomonas fluorescens* were grown on King's B medium, Nutrient Agar and Pikovaskay's medium. It is observed that all 34 *Pseudomonas fluorescens* grown on King's B medium showed the water soluble yellow colonies and produced pigmentation on media. Whereas, colonies of 34 *Pseudomonas fluorescens* isolates showed whitish colour and does not produce pigmentation on Nutrient Agar and Pikovaskaya's medium. Similar results were reported by King *et al.* (1954) who prepared two different media containing as common ground potassium and magnesium salts and glycerol. The medium with protease peptone was denominated as medium B. The other one with peptone was called medium A. Joseph *et al.* (2007) observed pigments on King's B medium. The fluorescent was observed on King's B medium. The results are incofirmity with the work done by earlier research workers.

5.4.5 Biocontrol activity of *Pseudomonas fluorescens* :

All the 34 isolates were examined for their biocontrol potential against two plant pathogenic fungi (*Macrophomina phaseolina* and *Fusarium oxysporum*). The *Pseudomonas fluorescens* isolate P-51 was found more growth inhibitor (47.50%) followed by P-31 (45.62%), P-56 (45.62%) and P-121 (45.00%) (Table 9). Seven strains of *Pseudomonas fluorescence viz.*, P-31, P-51, P-56, P-121, P-134, P-136 and P-145 were

found more growth inhibition per cent than the commercial *Pseudomonas* against *Macrophomina phaseolina* in dual inoculation method.

The *Pseudomonas fluorescens* isolates viz., P-121 (45.86%), P-51 (41.49%), P-134 (39.37%), P-136 (37.24%), P-31(35.11%) and P-56 (34.04%) were found significantly superior in inhibition growth per cent over the commercial *Pseudomonas* (24.31%) against the *Fusarium oxysporium*.

Seven strains of *Pseudomonas fluorescence* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 showed antagonistic activity superior than commercial *Pseudomonas* against soil borne plant pathogens viz., *Macrophomina phaseolina* and *Fusarium* sp. The efficient *Pseudomonas fluorescens* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 isolated from soils from the locations Sawantwadi, Malegaon, Ahmedpur, Sevagram, Ramtek, and Kuhl, respectively.

The similar results were obtained by the earlier research workers on the effectiveness of fluorescent pseudomonads against plant pathogenic fungi like *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Pythium* (Ganeshan and Ganamanickam, 1987; Rao et al. 1999; Siddiqui et al. 2005;); *Pseudomonas fluorescens* against *Macrophomina phaseolina*, *Fusarium*, *Trichoderma viride*, *Helminthosporium*, *Aspergillus flavus*, *Penicillium* (Jayaswal et al. 1990); *Pseudomonas fluorescens* against *Fusarium*, *Rhizoctonia* (Kumar et al. 2002; Maleki et al. 2010); *Macrophomina phaseolina* (Vinod Kumar et al. 2007; *Fusarium* (Negi et al. 2005; Yusran et al. 2008; Siddiqui and Shakeel, 2009); *Fusarium*, *Rhizoctonia solani*, *Pythium ultimum*, *Drechslera poae*, (Couillerot et al. 2009). Thus the results are in conformity with the research work carried out by earlier research workers.

5.4. 6 Intrinsic antibiotic resistance of *Pseudomonas fluorescens* :

Intrinsic antibiotic resistance of all the 35 *Pseudomonas fluorescens* isolates was tested against four antibiotic discs viz., NS₁₀₀, S₁₀, R₅ and T₃₀. All the *Pseudomonas fluorescens* isolates were resistant to NS₁₀₀. While 28 isolates resistant and 7 isolates were sensitive to S₁₀, whereas 34 *Pseudomonas fluorescens* observed sensitive (range from 11 to 31 mm) and P 40 was found resistant to R₅. The minimum zone 8 mm and maximum zone 31 mm was found in P-120 and P-134, respectively. Similarly, all the *Pseudomonas fluorescens* isolates were found sensitive to T₃₀ and the zone ranged from 8 to

55 mm. Minimum in P-96 and maximum was observed in P-134. Thus, the results indicated that the *Pseudomonas fluorescens* were found to be resistant to NS₁₀₀ and S₁₀ whereas R₅ and T₃₀ were found sensitive to the *Pseudomonas fluorescens*.

Somasegaran and Hoban (1994) have demonstrated genetic diversity of soil bacteria using the pattern of antibiotic resistance. The results obtained in the present investigation are comparable to those of earlier workers (Jayaswal *et al.* 1990; Suneesh, 2004); sensitive to streptomycin, and tetracycline (Deepa, 2000); sensitive to rifamycin (Siddiqui *et al.* 2005).

5.4.7 HCN and siderophore production by *Pseudomonas fluorescens* :

All the 35 isolates (34 *P. fluorescens* and one commercial production) were found to produce HCN. However, 7 of them and CP were high HCN producers which turned the orange colour of the filter paper. Sixteen *Pseudomonas fluorescens* isolates were moderate HCN producers and eleven were weak HCN producers. All the *Pseudomonas fluorescens* showed the siderophore ability. It is observed that all the 35 isolates were found to produce siderophore production and the effective isolates showed larger zone than the other isolates.

Tripathi and Johri (2002) have reported that fluorescent pseudomonads have ability to secrete antifungal metabolites, siderophore production and HCN production which is the mechanism of biocontrol of pathogens. Bakker and Schippers (1987); Voisard *et al.* (1989); Weller and Thomashow (1993) and Megha, 2004; Siddiqui and Shakeel, 2007 have reported HCN production as a mechanism of biocontrol of pathogens. The siderophore production was produced by *Pseudomonas fluorescens* (Sayyed *et al.* 2005; Mittal and Johri, 2007; Joseph *et al.* 2007; Siddiqui and Shakeel, 2009; Maleki *et al.* 2010) The results regarding HCN and siderophore production are in conformity to the work done by various research workers.

5.4.8 Molecular characterization of *Pseudomonas fluorescens* :

Diversity in a group of isolates can be studied by molecular techniques also. The PCR based RAPD markers help in diversity analysis as well as fingerprinting of an individual. On the basis of this information, 14 isolates of *Pseudomonas fluorescens* (13 selected isolates alongwith one commercial *Pseudomonas* strain) were analysed to PCR amplification using 24 random bacterial primers and 589 polymorphic and 211 unique bands were observed. The number of bands produced per primer varied from 12 to 50. Most of the primers produced highly polymorphic bands varied from 1 to 27. Highly polymorphic bands were observed maximum (27) in primer RBA-20 followed by RBA-14 (19). The amplified DNA fragment ranged from 199 to 3848 bp.

Genetic similarity was computed based on pooled data of RAPD profile for *Pseudomonas fluorescens* and results indicated that highest genetic similarity of 0.68 between the isolates P-40 and P-41 followed by 0.65 for the isolates P-91 and P-119. The minimum genetic similarity was observed in the isolates P31 and P 52 (0.17) and P121 and CP (0.18) The genetic similarity ranged from 0.17 to 0.68 among the all isolates.

On the basis of RAPD analysis, dendrogram constructed from the pooled data of *Pseudomonas fluorescens* isolates had major two clusters. The first cluster consisted of five strains of *Pseudomonas fluorscens* (P-31, P-134, CP, P-121 and P-136) were rod shape bacteria living in isolate, colonies round and non spreading having size less than 1.0 μm and highly efficient biocontrol ability clustered in one group due to genetic close relationship among them. The first cluster consisted P-31 and P134 having a genetic similarity coefficient 0.31. The genetic similarity coefficient between CP , P-31 and P-134 varied 0.25 to 0.30 and between P121 and P-136 having a genetic similarity coefficient 0.37. The second cluster consisted of 9 isolates of *P. fluorescens* . The second main cluster is sub-clustered into three groups. P-40 and P-41 is grouped in

first sub-cluster having a genetic similarity coefficient 0.68. The P-40 and P-41 isolates characters were irregular and spreading types, rod shape and living in groups and size more than 1 μm and less fluorescens and poor to moderate biocontrol ability. The second sub-cluster of P-52, P-55, P-56 , P-91 and P-119 isolates in which colonies were irregular and spreading type, fluorescence moderate to high and rod shape living in groups , size range from 0.1-0.6 x 1.04 to 1.7 and poor biocontrol ability except P-56. the P-56 isolate variable in colonies shape and nature than rest of the efficient biocontrol isolates. The third sub-cluster was of P-145 and P-51 of *P. fluorescens* isolates having a genetic similarity with other related isolates and varied from 0.33 to 0.43 and 0.30 to 0.44, respectively. The isolates P-145 and P-51 of *P. fluorescens* were round and spreading types and size more than 1 μm and rods were observed in grouping which varies from other efficient isolates vary with from size of rods, living habitat.

Using RAPD as a genetic tool, Rangarajan *et al.* 2001 analysed molecular diversity of 31 *Pseudomonas* sp. including one type strain isolated from coastline of Southern India and observed at 60% dissimilarity level a grouping of 30 strains into four clusters was observed. The size of amplified fragments ranged from 0.1 to 3.5 kb, and number of fragments varied from 6 to 14 bands per primer. Raaijmakers and Weller (2001) also analyzed the genotypic diversity of 2, 4-Diacetylphoroglucinol producing *Pseudomonas fluorescens*, from the roots of wheat using RAPD and identified 16 groups among 160 isolates.

Kumar *et al.* (2002) analysed the 18 *Pseudomonas fluorescens* for PCR-RAPD analysis and observed that strain ASOCO- and SOCO 12 isoalted from same area were placed in separate subgroups. Strain SOCO6 shared greater similarity with strain PAON2, thus confirming that bacteria isolated from entirely different geographical areas can also share some genetic relatedness. Many molecular methods are used to detect the prescence of soil borne pathogens and assess the genetic variability among the different isolates. Suneesh (2004) analyzed molecular diversity of ten fluorescent pseudomonads

from moist deciduous forest soils of Western Ghats and observed them to be distributed in five clusters. Megha *et al.* (2007) also analysed the 15 fluorescent pseudomonads along with five reference strains obtained from eight random primers were analysed for RAPD analysis and observed that eight random primers produced 127 polymorphic bands across 20 fluorescent pseudomonads and they were grouped into six clusters. The results are in conformity with the results of RAPD analysis by Battu *et al.* 2009; Prasanna Reddy and Rao (2009) *P. fluorescens* from rich rhizosphere, through RAPD used for molecular diversity analysis. Charan *et al.* 2010 studied the genetic diversity of *Pseudomonas fluorescens* by PCR based molecular techniques and observed that similarity among these standard isolates ranged from 0.42 to 0.95 and three groups were seen among 15 isolates of *Pseudomonas fluorescens*. Thus, the present results are in conformity with the results of earlier research workers.

5.4.9 Geographical distribution of *Pseudomonas fluorescens* from soils of Maharashtra:

Out of 150 locations, *Pseudomonas fluorescens* was distributed in 34 locations of Maharashtra which contributes 22.67% of soils of Maharashtra. *Pseudomonas fluorescens* was found maximum in soils of Western Ghats (29.41%) followed by Western Maharashtra (23.53%), Vidarbha (17.65%), North Maharashtra (14.71%), Marathwada region (8.82%) and least was found in Western Konkan region (5.88%).

Pseudomonas fluorescens strains P-31, P-51, P-56, P-121, P-134, P-136 and P-145 showed antagonistic activity superior than commercial *Pseudomonas fluorescens* from different soils of Maharashtra. The *P. fluorescens* was prominently observed from soils of Nagpur followed by Wardha district from Vidarbha followed by Sindudurg, Nasik and Latur from Western Ghats, North Maharashtra and Marathwada respectively. The occurrence of P-124, P-136 and P-145 isolate was observed from Ramtek, and Kuhl from Nagpur district. The P-121 isolate was observed in the soils of Sewagram from Wardha district. The occurrence of *Pseudomonas fluorescens* strains P-31, P-51, P-56 was

observed in soils of Sindudurg, Nasik and Latur from Western Ghat, North Maharashtra and Marathwada, respectively.

The results are in confirmity with the research work carried out by Megha *et. al.* 2007. The highly efficient *Pseudomonas fluorescens* isolates compared to commercial pseudomonas needs to be exposed to field conditions to study their bio-control performance against soil borne pathogens. Therefore, there is a need to conserve this treasure of diversity for the future exploitation.

6. SUMMARY AND CONCLUSION

The studies on “Variability mapping of actinomycetes, sulphur oxidiser and plant growth promoting rhizobacteria from soils of Maharashtra” was conducted during 2009-2011 at Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri. In these studies, the *Streptomyces* sp. (72), *Acidithiobacillus* sp. (8) and *Pseudomonas fluorescens* (34) were isolated from soils of the Maharashtra. These organisms were identified on the basis of morphological, cultural, biochemical characterization, functional diversity and selected efficient strains were studied for their molecular diversity by using RAPD analysis. The summary of this investigation are given as below.

In the present studies, the microbial population of actinomycetes was found 87.33% of the soils of Maharashtra. The microbial population of actinomycetes varied from 1×10^{-4} to 18×10^{-5} cfu/g in soils of Maharashtra. They were observed maximum (23.66%) in Western Maharashtra followed by Vidarbha (22.14%), Western Ghat (18.32%), Western Konkan coast (17.56), North Maharashtra (9.92%) and Marathwada (8.40%) from different soils of Maharashtra. The microbial population of actinomycetes isolate A-56 was found maximum 18×10^{-5} cfu/g in Latur district of Marathwada. There were 72 isolates having creamy colour, out of which 66 isolates having rectiflexibles (RF) in spore chain morphology and few of the isolates (6) showed retinaculiaperti (RA) in spore chain morphology. The *Streptomyces* sp. was found excellent on oat meal agar, good on starch casein agar and poor to moderate on nutrient agar medium.

The RHR-S-98 showed maximum percent inhibition 30.00% against *A. alternata* *in vitro*. Among the 72 *Strptomyces* sp., 64 isolates showed antimicrobial activity and it ranged from 7.45% to 19.90% and 8 isolates showed more than 20% antimicrobial activity against *Alternaria alternata*. *In vitro*, the RHR-S-23 showed maximum percent inhibition 28.80% against

Fusarium oxysporum pv. *ciceri*. The *Streptomyces* sp. showed inhibition and ranged from 10.44% to 28.80%

The cellulose degradation ability of 72 *Streptomyces* sp. isolates was tested for cellulose degradation activity and result indicated that 56.94% *Streptomyces* sp. have good cellulose degradation activity and 40.28% *Streptomyces* sp. have moderate cellulose degradation activity.

Among the 72 *Streptomyces* sp., it was observed that 21 isolates were not form the zone around the antibiotic disc which indicates that these 21 isolates were resistant and 51 were sensitive to antibiotics R₅, S₁₀ and T₃₀. All the 72 *Streptomyces* sp. isolated from soils of Maharashtra were found resistant to NS₁₀₀.

Among the 72 *Streptomyces* sp., only 22.22% isolates showed the P solubilization ability in which less than 1cm P solubilization zone was observed and 77.78% isolates does not showed P solubilization activity.

The PCR amplification products of the 18 strains of *Streptomyces* sp. with respect to each of the 23 random primers were separated by agarose gel electrophoresis. A total of 1124 bands were produced by the 23 primers. The number of bands produced per primer varied from 36 to 72. The average number of bands per primer was 48.87. Polymorphic bands 88.52% and unique bands 11.48% were observed and no monomorphic band observed in all 23 primers. Most of the primers produced highly polymorphic bands varied from 2 to 24. Highly polymorphic bands observed maximum (24) in primer RBA-14 followed by RBA-16 (18) and minimum highly polymorphic bands observed in RBA-7. The primers RBA 10 produced maximum polymorphic bands (55). The per cent polymorphism ranged from 73.33 to 97.43% and average polymorphism was observed 87.72%.

The genetic similarity matrix based on the pooled data of RAPD profiles from all the 23 primers revealed that highest genetic similarity index of 0.65 between the isolates RHR S-40 and RHR S-41 and RHR S 41 and RHR S 48.

The minimum genetic similarity index was observed in the isolates RHR S-5 and RHR S-130 (0.13). The genetic similarity index ranged from 0.13 to 0.65 among the all isolates. On the basis of RAPD analysis, the dendrogram constructed from the pooled data *Streptomyces* sp. had two major clusters. The first cluster consisted of five strains of *Streptomyces* (S-28, S-138, S-116 S-23 and S-21) and the second cluster consisted of 13 strains of *Streptomyces*.

Out of 150 locations, *Streptomyces* sp. were found in 48% of soils of Maharashtra. It was distributed in 72 location of Maharashtra soils. *Streptomyces* were prominently distributed maximum in Vidarbha (27.78%) followed by Western Maharashtra (19.44%), Western Konkan coast (15.28), Marathwada region (13.89%) , Western Ghat (13.89%) and least was found in soils of North Maharashtra region (9.72).

In Vidarbha region, Nagpur, Wardha, Yeotmal, Akola, Washim and Gadchiroli districts having highly efficient *Streptomyces* spp. in soil. In Western Maharashtra, Solapur, Satara, Sangli, Pune and Ahmednagar district having efficient *Streptomyces* spp. in soil. In Western Konkan coast region, Ratanagiri and Raigad district having efficient *Streptomyces* sp. Whereas, Ratnagiri, Sindudurg , Satara, Pune and Kolhapur district have efficient *Streptomyces* sp. in Western Ghat region soil. In Marathwada region, Latur, Nanded and Parbhani districts having efficient *Streptomyces*. In North Maharashtra, Jalgaon and Nashik district having efficient *Streptomyces* spp.

Sulphur oxidizer : The occurrence of sulphur oxidizer microorganisms was found 14.67% of the soils of Maharashtra. Out of 22 locations, maximum found in Vidarbha (9 location) followed by Western Maharashtra (7), Western ghat (3) and Western konkan coast (2) and least was observed in Marathwada region (1 location) from soils of Maharashtra.

All the *Acidithiobacillus* sp. have small yellow colour colonies and grown in thiosulphate agar slants. They were found in short rods and size ranged

from 0.3-0.7 x 0.9-1.6 μm . All the isolates were gram -ve in reaction. The pH reduction was observed in all the *Acidithiobacillus* sp. from 8.0 to ≤ 5.0 .

The PCR amplification products of the 9 isolates of *Acidithiobacillus* sp. with respect to each of the 15 random primers were separated by gel electrophoresis. A total of 484 bands were produced by the 15 random primers. The number of bands produced per primer varied from 18 to 48. On an average 32.2 bands were produced per primer. Polymorphic bands 58.68 % and unique bands 41.32% were observed and no monomorphic band observed in all 15 primers. The amplified DNA fragment ranged from 289 to 6021 bp. The number of polymorphic loci amplified varied with different primers and isolates.

The genetic similarity matrix based on the pooled data of RAPD profiles from all the 15 primers revealed that highest genetic similarity index of 0.65 between the isolates SOB-113 and SOB-115. The minimum genetic similarity index was observed in the isolates SOB-44 and SOB-115 (0.13). The genetic similarity index ranged from 0.13 to 0.65 among the all isolates. From the genetic similarity coefficient, it is observed that there was very high genetic diversity in the SOB isolated from soils of Maharashtra on the basis of geographical region.

Acidithiobacillus sp. occurred in 8 locations which contributes 5.33% of soils of Maharashtra.

The results indicated that *Acidithiobacillus* was distributed in Western Maharashtra (37.5%) and Vidarbha (37.5%) whereas less observed in Marathwada (12.5%) and Western Ghat (12.5%). *Acidithiobacillus* sp. occurred 3 locations viz., Rahuri, Kalwan and North Solapur of Western Maharashtra and Vidarbha (3 location) viz, Umred, Kuhi in Nagpur, Chandrapur and least was observed in Latur of Marathwada region and Kolhapur in Western ghat region.

The highly efficient *Acidithiobacillus* sp. was observed in Kolhapur, Solapur and Nagpur districts from Western Ghat, Western Maharashtra and Vidarbha regions, respectively.

Pseudomonas fluorescens : The microbial population of fluorescent pseudomonads was found 43% of the soils of Maharashtra. The microbial population of fluorescent pseudomonads varied from 9×10^{-3} to 6×10^{-6} cfu/g in soils of Maharashtra. Microbial population were observed maximum in Western Maharashtra (18 locations) followed by Western ghats (13 locations) and Vidarbha region (13 locations), respectively from soils of Maharashtra. The less fluorescent pseudomonads were observed in the soils of konkan coast region.

The maximum population of fluorescent pseudomonads FP-57 (6×10^{-6} cfu/g of soil) was recorded in Kolhapur district of Western Maharashtra followed by FP-52 and FP-43 isolates (4×10^{-6} cfu/g of soil) from Nasik and Sindudrurg districts of North Maharashtra and Western Ghat region, respectively.

Out of the 65 isolates, 21 produced green colored colonies and 44 produced light green coloured colonies. Forty one isolates had round and non-spreading type colonies whereas the remaining 24 isolates produced irregular shaped spreading type colonies.

All the 65 isolates were rod shaped bacteria and gram negative in reaction. Among these Fluorescent pseudomonads 65 isolates grouped on the basis of habitat, 40 isolates were rod shape living in clustering, 8 isolates were rod shape in isolate and 17 isolates were rod shape in pair and chain habitat. The size of these bacterial isolates are categorized into three on the basis of size. The results indicated that 29, 31 and 5 isolates were measured upto $1.0 \mu\text{m}$, 1.01 to $2.00 \mu\text{m}$ and more than $2.01 \mu\text{m}$ length, respectively. The colonies showed wide variation in the intensity of fluorescence. Out of 65 isolates, 11

showed high fluorescence, 33 showed medium fluorescence and the remaining 21 isolates showed weak fluorescence.

Based on the morphological and biochemical characteristics, 34 isolates were identified as *Pseudomonas fluorescens*, 31 as *Pseudomonas aeruginosa* from soils of Maharashtra. Out of 18 isolates from soils of Western Maharashtra, 8 were identified as *Pseudomonas fluorescens* and 10 as *Pseudomonas aeruginosa*. Out of 13 isolates from Western Ghat, 10 were identified as *P. fluorescens* and three as *P. aeruginosa*. Six out of 13 isolates from Vidarbha were identified as *P. fluorescens* and seven as *P. aeruginosa*. Among the 9 isolates of Marathwada, three isolates belonged to *Pseudomonas fluorescens* and six to *Pseudomonas aeruginosa*. Among the 4 isolates of Western Konkan coast region, two isolates belonged to *Pseudomonas fluorescens* and two *Pseudomonas aeruginosa*. In North Maharashtra, out of 8 isolates, 5 isolates were identified as *Pseudomonas fluorescens* and three as *P. aeruginosa*.

The colony growth of 34 *Pseudomonas fluorescens* on King's B medium was yellow in colour whereas colony was white in colour on Nutrient Agar and Pikovskaya's medium.

The *Pseudomonas fluorescens* were found to be resistant to NS₁₀₀ and S₁₀ whereas were found sensitive to R₅ and T₃₀ antibiotics.

Among 34 *Pseudomonas fluorescens*, P-51 isolate was found having more growth inhibition per cent (47.50%) followed by P-31 (45.62%), P-56 (45.62%) and P-121 (45.00%). Seven strains of *Pseudomonas fluorescens* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 were found more growth inhibition per cent than the commercial *Pseudomonas* against the *Macrophomina phaseolina*.

The *Pseudomonas fluorescens* isolates viz., P-121 (45.86%), P-51 (41.49%), P-134 (39.37%), P-136 (37.24%), P-31 (35.11%) and P-56 (34.04%)

were found significantly superior in inhibition growth per cent over the commercial *Pseudomonas* (24.31%) against the *Fusarium oxysporum*.

By dual plate and dual inoculation method, 7 strains of *Pseudomonas fluorescence* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 showed antagonistic activity superior than commercial *Pseudomonas* against soil borne plant pathogens viz., *Macrophomina phaseolina* and *Fusarium* sp. The efficient *Pseudomonas fluorescens* viz., P-31, P-51, P-56, P-121, P-134, P-136 and P-145 isolated from soils from the locations Sawantwadi, Malegaon, Ahmedpur, Sevagram, Ramtek, and Kuhl, respectively.

The PCR amplification products of the 14 isolates of *Pseudomonas fluorescens* with respect to each of 24 random bacterial primers were separated by gel electrophoresis. A total of 801 bands were produced by the 24 primers. The number of bands produced per primer varied from 12 to 50. The average number of bands per primer was 33.33. Polymorphic bands were observed 73.53 % and unique bands were observed 26.34% and only one monomorphic band observed in RBA-1 primer. Most of the primers produced highly polymorphic bands varied from 1 to 27. The amplified DNA fragment ranged from 199 to 3848 bp. The number of polymorphic loci amplified varied with different primers and isolates.

The genetic similarity matrix based on the pooled data of RAPD profiles from all the 24 primers revealed that highest genetic similarity index of 0.68 between the isolates P-40 and P-41 followed by 0.65 for the isolates P-91 and P-119. The genetic similarity index ranged from 0.17 to 0.68 among the all isolates. From the genetic similarity coefficient, the results indicated that there was very distinct diversity (coefficient 0.0 to 0.35) observed in the *P. fluorescens* isolated from soils of Maharashtra on the basis of functional diversity and ecological regions.

On the basis of RAPD analysis, dendrogram constructed from the pooled data of *Pseudomonas fluorescens* isolates had major two clusters. The first

cluster consist five strains of *Pseudomonas fluorescens* (P-31, P-134, CP, P-121 and P-136) and second cluster is sub-clustered into three groups.

Out of 150 locations, *Pseudomonas fluorescens* was distributed in 34 location of Maharashtra which contributes 22.67% of soils of Maharashtra. *Pseudomonas fluorescens* was found maximum in soils of Western Ghats (29.41%) followed by Western Maharashtra (23.53%), Vidarbha(17.65%), North Maharashtra (14.71%), Marathwada region (8.82%) and least was found in Western Konkan region (5.88%).

Pseudomonas fluorescence strains P-31, P-51, P-56, P-121, P-134, P-136 and P-145 showed antagonistic activity superior than commercial *Pseudomonas fluorescens* from different soils of Maharashtra. The *P. fluorescens* was prominently observed from soils of Nagpur followed by Wardha district from Vidarbha followed by Sindudurg, Nasik and Latur from Western Ghat, North Maharashtra and Marathwada respectively. The occurrence of P-124, P-136 and P-145 isolate was observed from Ramtek, and Kuhl from Nagpur district. The P-121 isolate was observed in the soils of Sewagram from Wardha district. The occurrence of *Pseudomonas fluorescence* strains P-31, P-51, P-56 was observed in soils of Sindudurg, Nasik and Latur from Western Ghat, North Maharashtra and Marathwada, respectively.

It was evident from the study that a great diversity exist among the *Pseudomonas fluorescens* in the different soils of Maharashtra with respect to colony morphology, biochemical characters, cultural and beneficial functions and genetic makeup. Many of the isolates were having beneficial functions like biocontrol potential against one or more pathogens, siderophore and HCN production, Intrinsic antibiotic resistance.

Out of 150 soil samples from the Maharashtra, 72 isolates of *Streptomyces* sp., 8 isolates of *Acidithiobacillus* sp. and 34 isolates of *Pseudomonas fluorescens* were found. Out of 72 isolates of *Streptomyces* sp., 8 isolates of *Acidithiobacillus* sp. and 34 isolates of *Pseudomonas fluorescens*, 40,

3 and 7 were found highly efficient isolates, respectively. The efficiency of *Streptomyces* sp. for cellulose degradation and antimicrobial activity, *Acidithiobacillus* sp. for reduction in pH and *Pseudomonas fluorescens* for biocontrol activity was considered.

From soils of Maharashtra, it was concluded that efficient *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* was found in Nagpur district of Maharashtra. While efficient isolates of *Streptomyces* sp., and *Pseudomonas fluorescens* were observed in Wardha, Latur, Nashik and Sindudurg districts of Maharashtra. The efficient strains of *Streptomyces* sp. and *Acidithiobacillus* sp. were observed in Kolhapur and Solapur district of Maharashtra

Only *Streptomyces* sp. was observed in Yavatmal, Gadchiroli, Washim, Akola, Nanded, Parbhani, Sangali, Satara, Ratnagiri, Raigad, Thane, Pune, Ahmednagar and Jalgaon districts of Maharashtra. While none of the efficient *Streptomyces* sp., *Acidithiobacillus* sp. and *Pseudomonas fluorescens* were observed in Gondia, Chandrapur, Amaravati, Buldhana, Jalna, Beed, Osmanabad, Aurangabad and Nandurbar districts of Maharashtra. Such isolates could be useful in agriculture and industry. There is a need to conserve this treasure for the future.

7. LITERATURE CITED

- Ahmadzadeh, M. and Sharifi-Tehrani, A., 2009, Evaluation of fluorescent pseudomonads for plant growth promotion, antifungal activity against *Rhizoctonia solani* on common bean, and biocontrol potential. *Biol. Control.*, **48** (2) : 101-107.
- Ahmadzadeh, M., Afsharmanesh, H., Javan-Nikkhah, M. and Sharifi-Tehrani, A., 2006, Identification of some molecular traits in fluorescent pseudomonads with antifungal activity. *Iranian J. Biotechnol.*, **4** (4) : 245-253.
- Anonymous, 1957, *Manual of Microbiological Methods*, McGraw Hill Book Company Inc., New York, pp. 127.
- Antoun, H., Bordeleau, L. M. and D. Prevost. 1982. Strains identification in *Rhizobium meliloti* using the antibiotic disc susceptibility test. *Plant and Soil.*, **66**: 45-50.
- Arora. A., Nain and Gupta G. K. 2005. Solid-state fermentation of wood residues by *Streptomyces griseus* B1, a soil isolate, and solubilization of lignins. *World J. of Microbiology & Biotechnology.* **21** : 303-308.
- Arunachalam. R., Wesely, E.G., George, J. and Annadurai. 2010. Novel Approaches for identification of *Streptomyces noboritoensis* TBG-V-20 with cellulase production, *Curr. Res. In Bacteriol.* **3**(1): 15-26.
- Athalye M, Lacey, J., Goodfellow M. 1981. Selective isolation and enumeration of actinomycetes using Rifamycin. *J. Appl. Bacteriol.* 51: 289-297.
- Babana A.H., Samake F., and K. Maiga. 2011. Characterization of some agricultural soils ; Prescence and activity of tilemsi rock phosphate-solubilizing Thiobacilli. *British Microb. Res. J.* **1** (1) : 1-9.

- Bakker, A.W. and Schippers, B., 1987, Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. *Soil Biol. Biochem.*, **19** : 451-457.
- Bakker, P.A.H.M., Lamer, J.G., Bakker, A.W. and Schippers, B., 1986, The role of siderophores in potato tuber yield increase by *Pseudomonas putida* in a short rotation of potato. *Neth. J. Pl. Path.*, **92** : 249-256.
- Barea, J.M. And Brown, M.E., 1974, Effects of plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. *J. of Applied Bacteriology*, **37**: 583-593.
- Barett, E. L., Solanes, R. E., Tang, J. S. and Palleroni, N. J., 1986, *Pseudomonas fluorescens*, its resolution into distinct component groups and relationship of these to other *P. fluorescens* biovars to *P. putida* and to psychrophilic *pseudomonads* associated with food spoilage. *J. Gen. Microbiol.*, **130** : 2709-2721.
- Bartholomew, J. W. and Mittewar, T., 1950, A simplified bacterial stain. *Stain Tech.*, **25** : 153.
- Bashan, Y., 1986, Migration of the rhizosphere bacteria, *Azospirillum brasilense* and *Pseudomonas fluorescens* towards wheat roots in the soil. *J. Gen. Microbiol.*, **132** : 3407-3414.
- Bassam, B.J., Caetano-Anolles, G. and Gresshoff, P.M., 1992, DNA amplification, finger printing of bacteria. *Appl. Microbiol. Biotechnol.*, **38** : 70-76.
- Basu Chaudhary, K. C. 1964. Isolation, biochemical activity and some physical properties of a new strain of *Streptomyces griseus*. *J. Indian Bot. Soc.*, **43**(1): 158-159.
- Battu, P. R.; Y. R. Reddy and M. S. Reddy. 2009. Biochemical and PCR-RAPD characterization of *Pseudomonas fluorescens* produced antifungal

- compounds inhibit the rice fungal pathogen *in vitro*. *J Pure and Applied Microbiology.*, **3** (1) : 347-350.
- Baur, A. W.; W. M. M.Kirby, J. C. Sherris and M. Turch. 1966. Antibiotic susceptibility testing by standardized single disc method. *American J. Clinical Pathology.* **45**: 493-496.
- Becker, J.O. and Cook, R.J., 1988, Role of siderophores in suppression of *Pythium* species and production of increased growth response of wheat by fluorescent pseudomonads. *Phytopath.*, **78** : 778-782.
- Bendict, R. G. and Lindenfelser, L. A. 1951. Production of soluble pigments by certain strains of *S. griseus*. *Antibiot. and Chemo.*, **1** : 512-517.
- Benerjee, A. K., S.K. Mukherjee and P. Nandi. 1954. Production of antifungal substances by *Streptomyces* species isolated from *Indian soils*. *Sci. and Cult.*, **20** (3) : 141-143.
- Bhatia, S., Dubey, R. C. and Maheshwari, D. K., 2005, Enhancement of plant growth and suppression of collar rot of sunflower caused by *Sclerotium rolfsii* through fluorescent pseudomonads. *Indian Phytopath.*, **58** (1) : 17-24.
- Borichewski, R. M. 1967. Keto acids as growth limiting factors in autotrophs growth of *Thiobacillus thiooxidans*. *J. Bacteriol.* **93** : 547-599.
- Borkar, S. G., V. K. Mandhare, K. N. Chaudhari, B. S. Patil and B. Varma, 1999. *Indian J. Plant Pathol.* **17**(1-20; 78-81.
- Borneman, J., Skroch, P. W., O'Sullivan, K. M., Palus, J. D., Ramjanek, N. G., Jansen, J. L., Nionhuiz, J. and Triplett, E. R., 1996, Molecular microbial diversity of an agricultural soil of Wisconsin. *Appl. Environ. Microbiol.*, **62** : 1935-1943.
- Botelho, G.R. and Mendonça-Hagler, L.C., 2006, Fluorescent pseudomonads associated with the rhizosphere of crops: An overview. *Braz. J. Microbiol.*, **37** (4) : 401-416.

- Bowman, J.P. and McCuaig, R.D., 2003, Biodiversity, community structure shifts and bioagents of prokaryotes within Antarctic continental shelf sediment. *Appl. Environ. Microbiol.*, **69** : 2463-2483.
- Brown, V. I. and Lowbury, E. J. L., 1968, Use of an improved cetrimide agar medium and other culture methods for *Pseudomonas aeruginosa*. *J. Clinical Path.*, **18** : 752-756.
- Buchanan, R. E. and M. E. Gibbons. 1974. Bergey's Manual of Determinative Bacteriology, 8th Edn., The Williams and Wilkins Company, Baltimore, U.S.A., pp. 456-460.
- Busaya A, A. Josef and B. Heinrich. 2005. Isolation and identification of *Streptomyces fradiae* SU-1 from Thailand and protoplast transformation with the chitinase B gene from *Nocardioopsis prasina* OPC-131. *Current Biology*. **51** : 116-121.
- Buyer, J. S. and Leong, J., 1986, Iron transport mediated antagonism between plant growth promoting and plant deleterious *Pseudomonas* strains. *J. Biol. Chem.*, **261** : 791-794.
- Campbell, M., Mahenthiralingam, E. and Speert, D. P., 2000, Evaluation of random amplified polymorphic DNA typing of *Pseudomonas aeruginosa*. *J. Clinical Microbiol.*, **38** : 4614-4615.
- Cappuccino, J. G. and Sherman, N., 1992, *Microbiology: A Laboratory Manual*, The Benjamin / Cummins Publishing Company Inc., California.
- Cartwright, D. K., Chilton, W. S. and Benson, D. M. 1995. Pyrrolnitrin and phenazine production by *Pseudomonas cepacia*, strain 5.5 B, a biological agent of *Rhizoctonia solani*. *Appl. Microbiol. Biotechnol.* **43** : 211-221.
- Castrillo, L. A. and Brooks, W. M., 1998, Differentiation of *Beauveria bassiana* isolated from the darkling beetle *Alphitoblus diaperinus* using isozyme and RAPD analysis. *J. Invert. Path.*, **72** : 190-196.

- Challa, O., Gajbhiye, K.S. and Velayutham, M. 1999. "Soil Series of Maharashtra" NBSS Publ. No. 79, NBSS & LUP, Nagpur, 428p.
- Chapman, S. J., 1990. *Thiobacillus* population in some agricultural soils. *Soil Biol. Biochem.* **22**: 479-482.
- Charan, A. Ravi., V. Prathap Reddy; S. Sokka Reddy and S. Sivaramkrishnan. 2010 Assessment of Genetic Diversity in *Pseudomonas fluorescens* using PCR-based Methods. *Bioremediation, Biodiversity and Bioavailability.* **5**(1): 10-16.
- Chohen, Y. and J. G. Kuenen. 1976. Growth yields and excretion products of *T. neapolitanus* growth in a Chemostat. Abst. Anl. A.S.M., pp. 20 (cited from Prokarotes. 1: 1023-1037, 1981).
- Choudhary, D. K., Prakash, A., Wray, V. and Johri, B. N., 2009, Insights of the fluorescent pseudomonads in plant growth regulation. *Curr. Sci.*, **97** (2) : 170.
- Conn, . E. 1947. The pigment production of *Actinomyces coelicolor* and *A. violaceus rubber*. *J. Bact.* **54**: 213-218.
- Cornellis, P. and Matthijs, S., 2002, Diversity of siderophore mediated iron uptake systems in fluorescent pseudomonads: not only pyoverdines. *Environ. Microbiol.*, **12**: 987-998.
- Couillerot, O., Prigent-Combaret, C., Caballero-Mellado, J. and Moenne-Loccoz, Y., 2009, *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. *Letters in Appl. Microbiol.*, **48** (5) : 505-512.
- Da Silva Gildo Almeida and Erik Amazonas de Almeida. 2006. Production of Yellow-Green Pigment by *Pseudomonas fluorescens*. *Brazilian Archives of Biology and Technology.* **49** (3): 411-419.
- Das, I. K., Indira, S., Annapurna, A., Prabhakar, Seetharama, N., 2008, Biocontrol of charcoal rot in sorghum by fluorescent

- pseudomonads associated with the rhizosphere. *Crop Prot.*, **27** (11) : 1407-1414.
- Deepa, R., 2000, Mineral phosphate solubilization by fluorescent pseudomonads. *M. Sc (Agri.) Thesis*, Univ. Agric. Sci., Dharwad (India).
- Dehnad, Alireza., Lalle Parsa., Rouhollah Bakshi., Samad Abdi Soofiani and Ahad Mokhtarzadeh. 2010. Investigation and antibacterial activity of *Streptomyces* isolates from soil samples, West Iran. *Afr. J. Microbiol. Res.* **4**(14) : 1542 -1549.
- Deleij, F.A.A.M., Whips, J.M. And Lynch, J.M., 1993, The use of colony development for characterization of microbial communities in soil and roots. *Microbial Ecology*, **27**: 81-97.
- Demain A. L. 1999. Pharmaceutically active secondary metabolites of microorganism. *Applied Microbiology and Biotechnology*. **52** : 455-463.
- Dhanya M. K. and V. P. Potty. 2007. Siderophore production by *Pseudomonas fluorescens* from the rhizosphere of *Solenostemon rotundifolius*. *J. Root Crops*. **33** (2): 138-140.
- Dice, L. R., 1945, Measures of the amount of ecological association between species. *Ecology*, **26** : 293-302.
- Dileep Kumar, B. S. and Dubey, H. S., 1993, Siderophore production by a plant growth promoting fluorescent pseudomonad, RBT13. *Indian J. Microbiol.*, **33** : 61-65.
- Dobereiner, J. And Baldani, V.L.D., 1979, Selective infection of maize roots by streptomycin resistant *Azospirillum lipoferum* and other bacteria. *Canadian J. Microbiology*, **25**: 1264-1269.

- Dwivedi, D. and Johri, B. N., 2003, Antifungals from fluorescent pseudomonads: Biosynthesis and regulation. *Curr. Sci.*, **85** : 1693-1703.
- Egorova, A. A. and Z. P. Deryugina. 1963. The spore forming Thermophilic *Thiobacterium*. *T. thermophilica* Imsche netskli Microbiologia. 32 : 439-446 (cited from Prokarotes. 1: 1023-1037, 1981).
- Elmer, W. H., 2009, Influence of earthworm activity on soil microbes and soilborne diseases of vegetables. *Pl. Dis.*, **93** (2) : 175-179.
- Frobisher, M. 1961. The sulphur bacteria. In : Fundamentals of Microbiology. 6th Edn., W. B. Saynders Co., London, pp. 378-383.
- Ganesan, P. and Gnanamanickam, S. S., 1987, Fungal antagonistic bacteria in rhizosphere. *Soil Biol. Biochem.*, **19** : 35.
- Gharaibeh, R.; Saadoun I. and Mahasneh A. 2003. Genotypic and phenotypic characteristics of antibiotic producing soil Streptomyces investgated by RAPD-PCR. *J. Basic Microbiol.* **43** (1) :18-27.
- Ghule, H. B. 1968. Studies on some *Streptomyces* species from soil. M.Sc. (Agri.) Thesis, Univ., Poona.
- Girvan, M. S., Bullimore, J., Pretty, J. N., Osborn, M. and Ball, A. S., 2003, Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Appl. Environ. Microbiol.*, **69** : 1800-1809.
- Goodfellow M. 1989. Suprageneric classification of actinomycetes. In Bergey's Manual of Systemic Bacteriol. Williams and Wilkins Company, Baltimore, 4. pp. 2333-2339.
- Goodfellow M., C. Lonsdale, A. L. James and O. C. MacNamara. 1987. Rapid biochemical tests for characterisation of streptomycetes. *FEMS Microbiology Letters.* **43** (1) : 39-44.

- Guay, R. and M. Silver. 1975. *Thiobacillus acidophilus* sp. nov; isolation and some physiological characteristics. *Can. J. Microbiol.*, **21**: 281-288.
- Gupta S. G. and Agate, A. D. 1986. Preservation of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* with activity check. *Antonie van Leeuwenhoek*. **52** : 121-127.
- Hadrys, H., Balick, M. and Schierwater, B., 1992, Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecol.*, **1** : 55-63.
- Hassanin, S. M.; A. A. El-Mehalawy, N. M. Hassanin and S. A. Zaki .2007. Induction Of Resistance And Biocontrol of Rhizoctonia In Cotton Damping-off Disease By Rhizosphere Bacteria And Actinomycetes . *The Internet Journal of Microbiology*. **3** (2).
- He ZG, Hu Y H., Hu W.X., Zhong H., Xu J and Zhu M. 2004. Studies of polymorphisms of *Thiobacillus ferrooxidans* using RAPD. *PMID:15626671*. **26**(1) 69-74.
- Hemming, B. C., 1986, Siderophore receptors of root colonizing *Pseudomonas* sp. *J. Pl. Nutrition*, **9** : 505-507.
- Hildebrand , D. C., Schroth, M. N. and Sand , D. C., 1992, *Pseudomonas*. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, Ed. N. W. Schad, 2nd Edition, International Book Distributing Co. Lucknow, pp. 60-80.
- Hill, G.T., 2000, Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Microbiology*, **15**: 25-36.
- Holt, J. G., Kreig, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T., 1984, *Bergey's Mannual of Determinative Bacteriology*, Williams and Wilkins, Baltimore, London.

- Howell, C. R. and Stipanovic, R. D., 1979, Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopath.*, **69** : 480-482.
- Howell, C. R. and Stipanovic, R. D., 1980, Suppression of *Pythium ultimum*-induced damping off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic pyoleuteorin. *Phytopath.*, **70** : 712-715.
- Hugenholtz, P., Goebel, B. M. and Pace, N. R., 1998, Impact of culture independent studies on the emerging phylogenetic view of bacterial diversity. *J. Bacteriol.*, **180** : 4765-4774.
- Hultberg, M., Bergstrand, K., Khalil, S. and Alsanius, B., 2008, Production of biosurfactants and antibiotics by fluorescent pseudomonads isolated from a closed hydroponic system equipped with a slow filter. *Antonie Van Leeuwenhoek*, **93** (4) : 373-380.
- Ibrahaim, H. A. 2006. A biological and biochemical studies of actinomycetes isolated from Kuwait saline soil. *Kuwait J. Applied Sci. Res.* **2** : 809-815.
- Isaac Juan Luna- Romero; Magda, C- Moreno, A.F. Martinez and R. F. Cerrato. 2000. Possibility of Biocontrol of *Aspergillus flavus* with *Pseudomonas fluorescens* on maize ear. *Revista Mexicana de FITOPATOLOGIA*. **18** (1) : 50-54.
- Iwatsuka, H and T. Mori. 1960. Studies on the metabolism of sulphur oxidizing bacterium. I. Oxidation of sulphur. *Plant and Cell Physiol.* **1**: 163-172.
- Iwatsuka, H. 1962. Studies on metabolism of sulphur oxidizing bacterium. II. Post oxidative fixation of CO₂. *Plant and Cell Physiol.* **3**: 167-173.

- Jackson, J. G., D. J. W. Moriarty and D. T. O. Micholow. 1968. Deoxyribonucleic acid base composition and nitrifying bacteria. *J. Gen. Microbiol.*, **53**: 53-60.
- Jackson, M. L., 1973, *Soil Chemical Analysis*, Prentice Hall of India Pvt. Ltd., New Delhi.
- Jain P. K. and P. C. Jain. 2007. Isolation, characterization and antifungal activity of *Streptomyces sampsonii* GS 1322. *Indian J. Exp. Biol.* **45** : 203-206.
- James, B., Paul, W. S., Katherine, M. O., James, A. P., Norma, G. R., Jennifer, L. J., James, N. and Eric, W. T., 1996, Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl. Environ. Microbiol.*, **62** : 1935-1943.
- James, G. C. 1978. Native Sherman Rockland Community College, State University of New York. The Benjamin / Coming Pul. Co. , Inc. pp. 75-80.
- Jayaswal R. K., M. A. Fernandez and R. G. Schroeder. 1990. Isolation and characterization of a *Pseudomonas* strain that restricts growth of various phytopathogenic fungi. *Appl. Environ. Microbiol.*, **56**(4): 1053-1058.
- Jeffrey, L. S. H. 2008. Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. *Afr. J. Biotechnol.* **7** (20) : 3697-3702.
- Jeffrey, L. S. H., Sahilah, A. M., Son R., Tosiah. 2007. Isolation and screening of actinomycetes from Malaysian soil for their enzymatic and antimicrobial activities. *J. Trop. Agri. & Food. Sci.* 35: 159-164.
- Jimenez-Salgado, T., Fuenter-Ranirez, L. E., Tapia-Hernandez, A., Mascarna-Esparez, M. A., Marting-Romeo, E. and Caballero-Mellado, J., 1997, *Coffea arabica* L. a new host plant for *Acetobacter*

diazotrophicus and isolation of other nitrogen fixing *Acetobacter*. *Appl. Environ. Microbiol.*, **63** : 3676-3683.

Johnson, J. L. and Palleroni, N. J., 1989, Deoxyribonucleic acid similarities among *Pseudomonas* species. *Int. J. Syst. Bacteriol.*, **39** : 230-235.

Johri, B. N., Ganguly, B. N., Goel, S. K., Viridi, J. S., Tripathi, A. K., Jain, R. K., Kamra, D. N. and Bhatnagar, A., 2005, Microorganism diversity: strategy and action plan. *Curr. Sci.*, **89** : 151-154.

Johri, B. N., Rao, C. V. S. and Goel, R., 1997, Fluorescent pseudomonads in plant disease management. In : *Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production*, Ed. Dadarwal, K. R., Scientific Publishers, Jodhpur, India, pp. 193-221.

Joseph B, Ranjan Patra R, Lawrence R. 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.) *Intl. J. of Pl. Production*. **1** : 141-151.

Joshi, G. S. 2010. Interaction between *Trichoderma* spp. and other soil microflora. M.Sc. (Agri.) Thesis, M.P.K.V., Rahuri (Maharashtra), India. pp. 1-83.

Joshi, S. P., Ranjekar, P. K. and Gupta, V. S., 1999, Molecular markers in plant genome analysis. *Curr. Sci.*, **6**: 230-240.

Kannan, R.R. and S.G.P. Vincent, 2011. Molecular characterization of antagonistic streptomyces isolated from a mangrove swamp. *Asian J. Biotechnol.*, **3**: 237-245.

Kapoor, K. K. and M. M. Mishra. 1985. Studies on oxidation of sulphur and pyrite in soil. Proc. Natl. Seminar on Sulphur in agriculture, TNAU, Coimabatore (India), Oct., 18-19, pp. 186.

Karavaiko, G. I., Tat yana P., Turova, T. F. Kondrat'eva, A. M. Lysenko, T. V. Kolganova, S. N. Ageeva, L. N. Muntyan and T. A. Pivoverova. 2003.

- Phylogenetic heterogeneity of the species *Acidithiobacillus ferrooxidans*. *Int. Syst. Evol. Microbiol.* **53** : 113-119.
- Kari, E. and James, J. G., 2001, Diversity of bacterial communities in the rhizosphere and root interior of field grown genetically modified *Brassica napus*. *Microb. Ecol.*, **38** (1) : 1-9.
- Kellerman, K. F. and I. G. McBeth. 1912. The fermentation of cellulose. *Centrbl. Bact.*, 2 Abst., 34 : 485-494 (In *Mycologia*, 19: 248-266, 1927)
- Kelly D. P. and Wood A. P. 2000. Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. *Int. J. Syst. Evol. Microbiol.* **50** : 511-516.
- Kelly, D. P. 1969. Transformation of sulphur and its compounds in soil. *Annals agronomique, numero horaggrerie*, pp. 217-237. (Cited from *Soils and Fert. Abst.* Pp. 35: 2524, 1969).
- Kelly, D. P. and Harrison, A. P. 1988. Genus *Thiobacillus* Beijerinck. In : *Bergey's manual of Systemic Bacteriology*, G. T. Staley, N. Pfennig, J. G. Holt (Eds.), Vol. 3 Baltimore: Williams and Wilkins Co. , 1842-1871.
- Kelly, W. D. 1972. Evaluation of *Trichoderma harzianum* impregnated calygranules as a biocontrol for *Phytophthora cinnamoni* causing damping off in pine seedlings. *Phytopath.* **66** : 1023-1027.
- Kempner, E. S. 1966. Acid production by *Thiobacillus thiooxidans*. *J. Bacteriol.* **92** : 1842-1843.
- Khamna, S.; A. Yokota; J. F. Peberdy and S. Lumyong. 2009. Antifungal activity of *Streptomyces* spp. isolated from rhizosphere of Thai medicinal plants. *IJIB.*, **6** (3) ; 144 -147.

- King, E.O., Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluoresceins. *J. Laboratory and Clinical Medicine*, **44**: 301-307.
- Kishore, P. 2010. Isolation, characterization and identification of Actinobacteria of mangrove ecosystem, Bhitarkanika Odisha. M.Sc. Thesis submitted to National Institute of Technology, Rourkela, Odisha. Pp. 1-74.
- Kloepper, J. W. and Schroth, M. N., 1981, Relationship of *in-vitro* antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopath.*, **71** : 1020-1024.
- Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N., 1980a, Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature*, **286** : 885-886.
- Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N., 1980b, *Pseudomonas* siderophores : A mechanism explaining disease suppressive soils. *Curr. Microbiol.*, **4**: 317-320.
- Koh, J. and Nou, I. S., 2002, DNA markers for identification of *Pseudomonas syringae* pv. *actinidae*. *Molecular Cells*, **16** (2) : 309-314.
- Konde B. K. 1974. Studies on Streptomyces from the soils of Maharashtra State. Ph. D. thesis submitted to M.P. K. V., Rahuri (M.S.) India. P: 1-145.
- Kovacs N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature*. **178** : 703.
- Kuenen, J. G. 1975. Sulphur oxidation by Heterotrophic micro-organisms. *Plant and Soil*. **43** : 49-76 (Cited from Adv. In Agron. 37 : 344-395, 1984).

- Kuenen, J. G. and O. H. Thovinen. 1981. The Genera *Thiobacillus* and *Thiomicrospira*. In : The Prokaryotes : A hand book on Habitatas, Isolation and Identification of Bacteria (Ed. M. P. Starr) Berlin Heidelberg, New York, U. S. A., pp. 1023-1036.
- Kumar, A., Sachdev, A., Balasubramanyam, S. D., Saxena, A. K. and Lata, A., 2002, Optimization of conditions for production of neutral and alkaline protease from species of *Bacillus* and *Pseudomonas*, *Indian J. Microbiol.*, **42** : 233-236.
- Kumari K. Krishna, P. Ponmurugan and N. Kannan. 2006. Isolation and characterization of *Streptomyces* sp. from soil samples for secondary metabolite production. *Biotechnology*. **5** (4) : 478-480.
- Kurek, E. and Jaroszuk-Scise, J., 2003, Rye (*Secale cereale*) growth promotion by *Pseudomonas flourescens* strains and their interactions with *Fusarium culmorum* under various soil conditions. *Biol. Control*, **26** : 48-56.
- Kuster, E. and Williams, S. T. 1964. Selection of media for isolation of streptomycetes. *Nature (London)*. **202** :928-929.
- Kwon, S.W., Kim, J.S., Crowley, D.E. and Lim, C.K., 2005, Phylogenetic diversity of fluorescent pseudomonads in agricultural soils from Korea. *Letters in Appl. Microbiol.*, **41** (5) : 417-423.
- Lakshmipathy Deepika and K. Kannabrian. 2010. Antibacterial and antifungal activity of *Streptomyces* sp. VITDDK-3 isolated Ennore Coast, Tamil Nadu, India. *JPRHC* **2**(2) : 1880-196.
- Lakshmipathy Deepika T. and K. Kannabrian. 2009. A morphological, biochemical and biological studies of hallophilic *Streptomyces* sp. isolated from Saltpan Environment. *Am. J. Infect. Dis.*, **5**(3) : 200-206.

- Lancey, J. 1997. Actinomycetes in compost. *Ann. Agric. Environ. Med.* 4 : 113-121.
- Lawongsa, P., Boonkerd, N., Wongkaew, S., O'Gara, F. and Teaumroong, N., 2008, Molecular and phenotypic characterization of potential plant growth-promoting *Pseudomonas* from rice and maize rhizospheres. *World J. Microbiol., Biotechnol.*, **24** (9) : 1877-1884.
- Lawrence, J. R. and J. J. Germida. 1985. Microbial population involved in the oxidation of elemental sulphur in Saskatchewan Agricultural soils (Cited from Agron. Abst., pp. 95, 1985).
- Lechevalier, H. A. 1989. The actinomycetes III. A practical Guide to Generic Identification of Actinomycetes. In Bergey's Manual of Systemic Bacteriology. Williams and Wilkins Company, Baltimore, 4. pp. 2344-2347.
- Lee, E. Y., K. S. Cho, and H. W. Ryu. 2000. Characterization of sulfur oxidation by an autrophic sulfur oxidizer, *Thiobacillus* sp ASWW-2. *Biotechnol. Bioprocess Eng.*, **5** : 48-52.
- Lee, L. S. and Henry, J. J., 2001, Commercial application of plant genotyping, In: *Plant Genotyping – The DNA Finger Printing of Plants*. Ed. Henry, R.J., CABL Publishing, CAB International, Oxford, UK.
- Leong, J. 1986. Siderophores: their biochemistry and possible role in biocontrol of plant pathogens. *Ann. Rev. Phytopathol.* **24**: 187-209.
- Locci, R. 1989. Goodfellow M. 1989. Streptomyces and related genera. Bergey's Manual of Systemic Bacteriol. Williams and Wilkins Company, Baltimore, 4. pp. 2451-2508.
- Loganathan, P., Sunita, R., Parida, A. K. and Nair, S., 1999, Isolation and characterization of two genetically distant growth of *Acetobacter diazotrophious* from a new host plant *Eleusina corocana* L. *J. Appl. Microbiol.*, **87** : 167-172.

- Luciana Campos Paulino, Rogério Faria Bergamo, Maricilda P. de Mello, Oswaldo Garcia, Gilson P. Manfio and Laura M. M. Ottoboni. 1995. Molecular characterization of *Acidithiobacillus ferrooxidans* and *A. thiooxidans* strains isolated from mine wastes in Brazil. Biomedical and Life Sciences *Antonie van Leeuwenhoek* **80**(1): 65-75, DOI: 10.1023/A:1012247325537
- Lyric, R. M. and I. Suzuki. 1970. Enzymes involved in the metabolism of thiosulphate by *T. thioparus*. III. Properties of thiosulphate oxidizing enzyme and proposed pathway of thio sulphate oxidation. *Canad. J. Biochem.*, **48** : 355-363.
- Mahoney, Robert P. and Mercedes R. Edwards. 1966. Fine structure of *Thiobacillus thiooxidans*. *J. Bacteriol.*, **92**: 487-495.
- Malathi, P., Padmanaban, P., Viswanathan, R. and Mohanraj, D., 2008, Interaction between *Colletotrichum falcatum* pathotypes and biocontrol agents. *Arch. Phytopath., Pl. Prot.*, **41** (5) : 311-317.
- Maleki M., S. Mostafaei, L. Mokhtarnejad and M. Farzaneh. 2010. Characterization of *Pseudomonas fluorescens* strain CV6 isolated from cucumber rhizosphere in Varamin as a potential biocontrol agent. *AJS.*, **4**(9): 676-683.
- Malkawi, H. I.; Saadoun I.; Moumani, F. A.; Meqdam M. M. 1999. Use of RAPD-PCR fingerprinting to detect genetic diversity of soil *Streptomyces* isolates. *New Microbiol.* **22** (1) : 53-58.
- Manwar, A. V., Vaiganker, P. D., Bhonge, L. S. and Chincholkar, S. B., 2000, *In-vitro* suppression of plant pathogens by siderophores of fluorescent pseudomonads. *Indian J. Microbiol.*, **40** : 109-112.
- Martin, P.; Dary A. Andre A. and Decaris B. 2000. Identification and typing of *Streptomyces* strains : evaluation of interspecific, intraspecific and

- intraclonal differences by RAPD fingerprinting. *Res. Microbiol.* **151** (10) :853-864.
- McClelland, M. and Welsh, J., 1994, DNA finger printing by arbitrarily primed PCR. *PCR Methods and Applications*, **4** : 559-565.
- Megha, Y. J. 2006. Functional and molecular diversity of fluorescent pseudomonads from Western Ghats regions of Uttara Kannada district.M.Sc. Agri. Thesis submitted to Univ. Agril. Science, Dharwad (Karanataka), India.
- Megha, Y. J., Alagawadi, A. R. and Krishnaraj, P. U., 2007a. Diversity of fluorescent pseudomonads isolated from the forest soils of the Western Ghats of Uttara Kannada. *Curr. Sci.*, **93** (10) : 1433-1437.
- Megha, Y. J., Alagawadi, A. R. and Krishnaraj, P. U., 2007b. Multiple beneficial functions of fluorescent pseudomonads of Western Ghats of Uttara Kannada district. *Karnataka J. Agric. Sci.*, **20** (2) : 305-309.
- Mehnaz, S., Weselowski, B., Aftab, F., Zahid, S., Lazarovits, G. and Iqbal, J., 2009, Isolation, characterization, and effect of fluorescent pseudomonads on micropropagated sugarcane. *Canadian J. Microbiol.*, **55** (8) : 1007-1011.
- Mencher, J. R. and A. H. Hein. 1962. Melanin biosynthesis by *Streptomyces lavendulae*. *J. Gen. Microbiol.*, **28** : 665-670.
- Mercado-Blanco, J., Rodriguez-Jurado, D., Hervas, A. and Timnez-Diaz, M., 2004, Suppression of *Verticillium* wilt in olive planting stocks by root associated fluorescent pseudomonads. *Bicontrol*, **30** : 474-486.
- Meredith, C. H. 1943. The antagonism of *Actinomyces* to *Fusarium oxysporum* f. *cubense*. *Phytopath.* **33** : 403.
- Meredith, C. H. 1944. The antagonism of *Actinomyces* to *Fusarium oxysporun* f. *cubense*. *Phytopath.*, **34** : 426.

- Mittal Shilpi and Bhavdish N. Johri. 2007. Assessment of rhizobacterial diversity of *Triticum aestivum* and *Eleusine coracana* from northern region of India. *Curr.Sci.* **63** (11) : 1530-1537.
- Moenne-Loccoz, Y., Tichy, H., O'Donnell, A., Simon, R. and O'Gara, F., 2001, Impact of 2, 4-diacetylphloroglucinol – producing biocontrol strain *Pseudomonas fluorescens* F113 on intraspecific diversity of resident culturable fluorescent pseudomonads associated with roots of field grown sugarbeet seedlings. *Appl. Environ. Microbiol.*, **67** : 3418-3425.
- Moura, M. L., Rocha, M., Fischer-Le Saux, M., Lima, A. and Duclos, J., 2008, Genetic diversity of non-fluorescent pseudomonads associated with tomato pith necrosis in Portugal. *Acta Hort.*, **789** : 285-292.
- Mukherjee, S. K. and P. Nandi. 1955. Studies on the antagonistic actinomycetes from the soils of West Bengal. *Indian Phytopath.*, **8** : 64-71.
- Munira Al-Kahtani, Al-Khalil Abdullah, Al-Kadeeb Siham and Hassan H. Z. 2008. Molecular Genetic Fingerprint of some *Streptomyces* Isolated from Riyadh city. *Saudi J. Biological Sciences.* **15** (2) : 243-251.
- Muthukumaraswamy, R., Revathi, G. and Vadivellu, M., 2000, Antagonistic potential of N₂-fixing *Acetobacter diazotrophicus* against *Colletotrichum falcatum*. Went, a casual organism of red rot of sugarcane. *Curr. Sci.*, **78** (9) : 1063-1065.
- Mythili B. and M. P. Ayyappa Das. 2011. Studies on antimicrobial activity of *Streptomyces* spp. isolates from Tea plantation soil. *Res. J. Agril. Sci.*, **2** (1): 104-106.
- Naik P.R., Raman G, Narayan K. B., Sakhivel N (2008a). Assesment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizosphere soil. *BMC Microbiology.* **8**: 230-235.

- Naik, P. R., Sahoo, N., Goswami, D., Ayyadurai, N. and Sakthivel, N., 2008b. Genetic and functional diversity among fluorescent pseudomonads isolated from the rhizosphere of banana. *Microb. Ecol.*, **56** (3) : 492-504.
- Nakhimovskaia, M. I. 1937. The antagonism between actinomycetes and soil bacteria. *Mikrobiologiya*, **6** : 131-157.
- Nareshkumar, R., R. Nagendram and K. Parvathi. 2007. Microbial solubilization of heavy metals from soil using indigenous sulfur oxidizing bacterium : Effects of sulfur / soil ratio. *J. of Scientific and Industrial Res.* **66** : 680-683.
- Negi, Y.K., S.K. Garg and J. Kumar. 2005. Cold-tolerant fluorescent *Pseudomonas* isolates from Garhwal Himalayas as potential plant growth promoting and biocontrol agents in pea. *Curr. Sci.* **89**(12). 2151-2156.
- Nei, M. and Li, W. H., 1979, Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. National Academy of Science, USA* **70** : 5269-5273.
- Neilands, J. B., 1981, Microbial iron compounds. *Annu. Rev. Biochem.*, **50** : 715-731.
- Nonoh, James Oluoch., Wilber Lwande¹., Daniel Masiga¹., Rafi Herrmann, James K. Presnail., Eric Schepers, Matilda Angela Okech, Richard Bagine, Paul Mungai, Aggrey Bernard Nyende and Hamadi Iddi Boga. 2010 Isolation and characterization of *Streptomyces* species with antifungal activity from selected national parks in Kenya. *Afr. J. Microbiol. Res.* **4**(9) : 856-864.
- O'Sullivan, D. J. and O'Gara, F., 1992, Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiol., Rev.*, **56** (4) : 662-676.

- Okonski, A., Levin, S. and McCreath, S., 2003, Influence of soil amendments on chemical and microbial properties of Cu-Ni smelter damaged Sudburg soil. www.ott.wrcc.osmre.gov/library/proceed/sudburg.
- Olembo, R., 1991, The biodiversity of microorganisms and invertebrates: Its role in sustainable agriculture, Ed. Hawksworth, D.L., pp. 7-15.
- Onczena, M., Daay, F., Jacques, P., Thonart, P., Benhamou, N., Paulitz, T. C., Cornelis, P., Koedam, N. M. and Belanger, R. R., 1999, Protection of cucumber against root rot by fluorescent pseudomonads; predominant role of induced resistance over siderophores and antibiosis. *Pl. Pathol.*, **48** : 66-76.
- Oskay, M.; Tamer, A. U. and Azeri, C. 2004. Antibacterial activity of some actinomycetes from farming soils of Turkey. www.scribd.com/doc/2192441.
- Overeas, L. and Torsvik, V., 1998, Microbial diversity and community structure in two different agricultural soil communities. *Microb. Ecol.*, **36** : 303-315.
- Pace, N. R., 1997, A molecular view of microbial diversity and the biosphere. *Science*, **276** : 734-740.
- Pal, V. and Jalali, I., 1998, Rhizosphere bacteria for biocontrol of plant diseases. *Indian J. Microbiol.*, **38** : 187-204.
- Palleroni, N. J., 1977, *Pseudomonas*. In: *Hand book of Microbiology*, Eds. Laskin, A. I. and Lechevalier, H. A., 2nd Edition, Vol.1, CRC Press Inc., Cleveland, pp. 247-258.
- Palleroni, N. J., Doudoroff, M., Stainer, R. Y., Solanes, R. E. and Manel, M., 1970, Taxonomy of the aerobic pseudomonads. The properties of the *Pseudomonas stutzeri* group. *J. Microbiol.*, **60** : 215-231.

- Palleroni, N. J., Kunisawa, R., Contopoulou, R. and Doudoroff, M., 1973, Nucleic acid homologies in genus *Pseudomonas*. *Int. J. Syst. Bacteriol.*, **23** : 333-339.
- Palleroni, N.J., 1984, Genus I *Pseudomonas nigula*, In: Bergey's Manual of Systematic Bacteriology. Krig, N.R. and Holt, J.G. (Ed.). Baltimore. Williams and Wilkins, pp. 141-199.
- Panse, V. S. and Sukhatme, P. V., 1985, Statistical Methods for Agricultural Workers, ICAR, New Delhi, pp. 152-155.
- Patel A. K. 2010. Isolation and characterization of facultatively autotrophic, acidiphillic, thermophilic bacterium, *Thiobacillus thermosulfatus* sp.nov. from coal mine overburden spoil. *Intl. J. Appl. Environ. Sci.* **5** (1)
- Paulsen, I.T., Press, C.M., Ravel, J. 2005. Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf- 5. *Nature Biotechnology*. **23**. p. 873-878.
- Peck, H. D. 1962. Comparative metabolism of sulphur compounds in micro-organisms. *Bacteriol. Rev.* **26** : 67-94.
- Picard, C. and Bosco, M., 2008, Genotypic and phenotypic diversity in populations of plant-probiotic *Pseudomonas* spp. colonizing roots. *Naturwissenschaften*, **95** (1) : 1-16.
- Picard, C., Cello, F. D., Ventura, M., Fan, R. and Guckert, A., 2000, Frequency and biodiversity of 2,4- diacetylphloroglucinol producing bacteria isolated from the maize rhizosphere at different stages of plant growth. *Appl. Environ. Microbiol.*, **66** : 948-955.
- Pikovskaya, R. I., 1948, Mobilization of phosphorous in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, **17** : 302-370.

- Prasanna Reddy, B. and Rao, K. S., 2009, Biochemical and PCR-RAPD characterization of *Pseudomonas fluorescens* produced antifungal compounds inhibit the rice fungal pathogens *in-vitro*. *Electronic J. Environ. Agricu. Food Chem.*, **8** (10) : 69-73.
- Price, P. W., 1988, An overview of organismal interaction in ecosystem in evolutionary and ecological time. *Ecosys. Environ.*, **24** : 369-377.
- Pridham, T. G., L. A. Lindenfelser, O.L. Shotwell, R. G. Benedict, C. Poley, R. W. Jackson, W. J. Zaumryer, W. H. Jr. Preston and J. W. Mitchell. 1956. Antibiotics against plant diseases I. Laboratory and greenhouse survey. *Pytopath.* **46** : 568-575.
- Raaijmakers, J. M. and Weller, D. M., 2001, Exploiting genotypic diversity of 2, 4-Diacetylphloroglucinol *Pseudomonas* spp., Characterization of superior Root-colonizing *P. fluorescens* strain Q8rI-96. *Appl. Environ. Microbiol.*, **67** (6) : 2545-2554.
- Rabbani, M., H. Mirmohammad Sadeghi and Z. Karjoo. 2007. Molecular Detection of *Streptomyces griseus* Isolated from Isfahan Soil. *Pak. J. Biol. Sci.*, **10** (19): 3374-3379.
- Rachid Djibaoui and Bensoltane Ahmed. 2005. Effect of iron and growth inhibitors on siderophores production by *Pseudomonas fluorescens*. *Afr. J. Biotechnol.*, **4** (7) : 697-702.
- Ramesh Kumar N., V. Tirumalai Arasu and P. Gunasekaran. 2002. Genotyping of antifungal compounds producing plant growth promoting rhizobacteria, *Pseudomonas fluorescens*. *Curr. Sci.*, **82**(12): 1463-1466.
- Ramette, A., Moenne-Loccoz Y. and Defago, G., 2006, Genetic diversity and biocontrol potential of fluorescent pseudomonads producing phloroglucinols and hydrogen cyanide from Swiss soils naturally suppressive or conducive to *Thielaviopsis basicola*-mediated black root rot of tobacco. *FEMS Microb. Ecol.*, **55** : 369-381.

- Rangarajan, S., Loganathan, P., Saleena, L. M. and Nair, S., 2001, Diversity of pseudomonads isolated from three different plant rhizospheres. *J. Appl. Microbiol.*, **91** : 742-749.
- Rangaswami, G. and P. Vidyasekaran 1963. Antibiotic production by *Streptomyces* sp. In corn rhizosphere. *Phytopath.*, **53** : 995-997.
- Rangaswami, G. and R. Venkatesan 1963. Microorganisms in paddy soil and rice rhizosphere. Intern. Symp. Global Impact Appl. Microbiol., Stockholm, July-August, 1963.
- Rangaswami, G. ; G. Oblisami and R. Swaminathan 1967. Antagonistic actinomycetes in the soils of South India. Univ. Agri. Sci. Bangalore, India and U. S. Department Agri. 1967.
- Rao, M. G. and V. Subramanyan 1929. Studies on soil actinomycetes. Part III. Standardization of plate method of counting soil actinomycetes. *J. Indian Inst. Sci.* **12** A : 253-273.
- Rao, V. S., Sachan, I. P. and Jhori, B. N., 1999, Influence of *Pseudomonas* on growth and nodulation of lentil (*Lens esculentus*) in *Fusarium* infested soil. *Indian J. Microbiol.*, **39** : 23-29.
- Reddy N. G., D.P.N. Ramakrishna and S.V. Raja Gopal. 2011. A morphological, physiological and biochemical studies of marine *Streptomyces rochei* (MTCC 10109) showing antagonistic activity against selective human pathogenic microorganisms. *Asian J. Biol. Sci.* DOI : 10.3923/ajbs. 1-14.
- Rehm H. J. and U. Rehm (1953) Investigations on the microflora of the soil at Gaterslaber (Loess region of central Germany) I. Investigations on the population of some fungi and bacteria in the soil, from antibiotic and plant pathological stand points. *Kulturpffanze*, **1** : 111-121 (Soil and Fert., 17 : 339, 1954)

- Rekha, V., S. Ahmed John and T. Shankar. 2010. Antibacterial activity of *Pseudomonas fluorescens* isolated from Rhizosphere soil. *Intl. J. Biological Tech.* **1**(3): 10-14.
- Rifaat Hala M. and Amay L. Kanosh. 2005. *Streptomyces virginiae* : Taxonomy, identification and biological activities. *Arab. J. Biotech.*, **8** (1) : 29-34.
- Rini C. R. and Sulochana, K. K., 2007, Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *J. Trop. Agric.*, **45** : 21-28.
- Robert, S. B., 1975, Regulation of extracellular protease secretion in *Pseudomonas maltophilia*. *J. Bacteriol.*, **123** : 954-961.
- Roberts Mark A. and Don L. Crawford. 2000. Use of Randomly Amplified Polymorphic DNA as a Means of Developing Genus- and Strain – specific *Streptomyces* DNA Probes. *Appl. Environ. Microbiol.*, **66**(6): 2555-2564.
- Rupela, O. P. and P. Tauro. 1973. Isolation and characterization of *Thiobacillus* sp. from alkali soils. *Soil Biol. Biochem.* **6** : 891-897.
- Saadoun, I. R., R. Rifaat, D. Tasnim, A. Qotaiba and M. Amjad. 2007. Isolation, characterization and screening for fiber hydrolytic enzymes - producing streptomycetes of Jordanian forest soils. *Biotechnology*, **6** : 120-128.
- Sambrook, J. and Russell, D. W., 2001, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.
- Sambrook, J. Fritsch, E. F. and Maniatis, T. 1989. *Molecular Cloning : A Laboratory Manual* 2nd Edn. USA : Cold Spring Harbor Laboratory Press. ISBN 0-879693096.

- Sardi, P., M. Saracchi, S. Quaroni, B. Petrolini. G. E. Borgonovi and S. Merli. 1992. Isolation of endophytic *Streptomyces* strains from surface sterilized root. *Appl. Environ. Microbiol.* **58** (8) : 2691-2693.
- Sayyed, R.Z.; Badgujar, M.D.; Somawane H.M.; Mhaske, M. M. and S. B. Chincholkar. 2005. Production of microbial iron chelator (siderophores) by fluorescent pseudomonads. *Indian Journal Biotechnology.* **4** : 484-490.
- Schalk, I. J., 2008, Metal trafficking via siderophores in gram-negative bacteria: Specificities and characteristics of the pyoverdine pathway. *J. Inorg. Biochem.*, **102** (5-6) : 1159-1169.
- Schreffer, W. J. and W. W. Umbreit . 1983 Phosphoty dilinositol as a wetting agent in sulphur oxidation by *Thiobacillus thiooxidans*. *J. Bacteriol.* **85** : 492-493.
- Selenska-pobell, Otto and Kutschke. 2002. Identification and discrimination of thiobacilli using ARDREA, RAPD and rep-RAPD. *J. Appl. Microb.* **84** (6): 1085-1091.
- Seung Bum Kim, Christine Falconer, Edwin Williams and Michael Goodfellow. 1998. *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxyus* sp. nov., two moderately thermophillic carboxydotrophic species from soil. *Intl. J. Syst. Bacteriology.* **48**:59-68.
- Sharifi-Tehrani, A. and Omati, F., 1999, Biocontrol of *Phytophthora capsici* the causal agent of pepper damping off by antagonistic bacteria. *Medede Lingen-Faculteit Land Bouwkundige in Toegepaste Biologische Watensohappen, Universiteit Gent* **64** : 419-423.
- Sheath, P. H. A., Stevens, M. and Sackin, M. J., 1981, Numerical taxonomy of *Pseudomonas* based on published records of substrate utilization. *Antonie Van Leeuwenhoek*, **47** : 423-448.

- Shinde D. B. , P. L. Patil and B. R. Patil. 1996. Potential use of sulphur oxidizing micro organism as soil inoculant. *Crop. Res.* **11** : 291-295.
- Shinde D. B. 1993. Studies on Sulphur Oxidising Micro-organisms. Ph.D. Thesis, M.P.K.V., Rahuri (M. S.) India.
- Shinobu, R. (1958) Physiological and cultural study for the identification of soil actinomycete species. *Mem. Osaka Univ. B. Nat. Sci.* **1** : 1-76.
- Siddiqui and Shakeel. 2009. Biocontrol of wilt disease complex of pigeon pea (*Cajanus cajan* (L.) Millsp.) by isolates of *Pseudomonas* spp. *African J. Pl. Sci.* **3**(1) 001-012.
- Siddiqui Shazia, Zaki A. Siddiqui and Iqbal Ahmad. 2005. Evaluation of Fluorescent *Pseudomonads* and *Bacillus* isolates for the control of a wilt disease complex of pigeonpea. *World J. Microbiology & Biotech.* **21** : 729-732.
- Siddiqui, Z.A., 2007, Biocontrol of *Alternaria triticina* by plant growth promoting rhizobacteria on wheat. *Arch. Phytopathology, Pl. Prot.*, **40** (4) : 301-308.
- Silby MW, Cerdeno-Tarranga AM, Vernikos GS, Giddens SR, Jackson RW, Preston GM, Zhang XX, Moon CD, Gehrig SM, Godfrey SA, Knight CG, Malone JG, Robinson Z, Spiers AJ, Harris S, Challis GL, Yaxley AM, Harris D, Seeger K, Murphy L, Rutter S, Squares R, Quail MA, Saunders E, Mavromatis K, rettin SD, Hothersall J, Stephens E, Thomas CM, Parkhill J, Levy SB, Rainey PB and Thomson NR 2009. Genomics and genetic analysis of diversity and plant interactions of *Pseudomonas fluorescens* . *Genomic Biology.* **10**, R51.
- Singh, J. 1937. Studies in soil bacteria of the subtropical region - Punjab, North India, *Proc. Indian Acad. Sci.*, **6**(13) : 121-129.

- Singh, R. and Sinha, A. P., 2009, Biological control of rice sheath blight with antagonistic bacteria. *Ann. Pl. Prot. Sci.*, **17** (1) : 107-110.
- Sneath, P.H.A. and Sokal, R. R. 1973. Numerical taxonomy – the principles and practice of numerical classification (W. H. Freeman : Francisco).
- Sokolova, C. A. and G. I. Karavaiko. 1968. Physiological and geochemical activity of *Thiobacilli*. In : Prokaryotes (Ed. M. P. Starr), 1 : 1023-1037, 1981.
- Somasegaran, P. And Hoben, H.J., 1994, Hand Book of Rhizobia – Methods in legume *Rhizobium* technology Springer – Verlaug, New York, Inc. p. 58.
- Sorheim, R., Torsvik, V. L. and Goksoyr, J., 1989, Phenotypic divergences between populations of soil bacteria isolated on different media. *Microb. Ecol.*, **17** : 181-192.
- Sottero, A. N., Freitas, S., Melo, A. and Trani, P. E., 2006, Rhizobacteria and lettuce: Root colonization, plant growth promotion and biological control. *Revista Brasileira de Ciencia de Solo*, **30** (2) : 225-234.
- Spiers, A. J., Bukling, A. and Rainey, P. B., 2005, The causes of *Pseudomonas* diversity. *Microbiol.*, **146** (10) : 2-9.
- Srivastav, S., Yadav, K. S. and Kundu, B. S., 2004, Phosphate solubilizing pseudomonads suppress damping-off disease in tomato. *J. Mycol. Pl. Pathol.*, **34** : 662-664.
- Srivastava, P., Shukla S., Choubey S. B. and Gomase V. S. 2010. Isolation, purification and characterization of Glucose isomerase enzyme from Strptomyces species isolated from parbhani region. *Journal Enzyme Research*. **1** (1) : 1-10.
- Srivastava, R. and Shalini. 2009. Antifungal Activity of *Pseudomonas fluorescens* Against Different Plant Pathogenic Fungi. *The Internet Journal Microbiology*. **7** No. 2

- Stahl, D. A. and Flesher, B., 1987, *Application of Ribosomal RNA Sequencing to Studies of Microbial Ecology*. Gray Freshwater Biological Institute Summer Course, URBAna III.
- Starkey, R. L. 1966. Oxidation and reduction of sulphur compounds. *Soil Sci.*, **101** : 297-306.
- Starkey, R. L., G. E. Jones and L. R. Fredrick. 1956. Effect of medium agitation and wetting agents on oxidation to sulphur by *Thiobacillus thiooxidans*. *J. Gen. Microbiol.*, **52** (2) : 329-334.
- Stefka Antonova- Nikolova; Nikolova Tzekova and Ljubomira Yocheva. 2004-2005 . Taxonomy of *Streptomyces* sp. strain 3 B. *J. Culture Collections*. **4**: 36-42.
- Subramanyan, V. and R. V. Noris 1929. Studies on soil actinomycetes. Part II. Their mode of occurrence in the soil. *J. Indian Inst. Sci.*, **12** : 53-68.
- Suneesh, K., 2004, Biodiversity of fluorescent pseudomonads in soils of moist-deciduous forests of Western Ghats of Uttar Kannada district. *M. Sc (Agri.) Thesis*, Univ. Agric. Sci., Dharwad (India).
- Suryakala, D., Maheshwaridevi, P. V. and Lakshmi, K. V., 2004, Chemical characterization and *in-vitro* antibiosis of siderophores of rhizosphere fluorescent pseudomonads. *Indian J. Microbiol.*, **44** (2) : 105-108.
- Sutra, L. Risede, J. M. and Gardan, L., 2000, Isolation of fluorescent pseudomonads from the rhizosphere of banana plants antagonistic towards root necrotising fungi. *Letters in Appl. Microbiol.*, **31** (4) : 289–293.
- Swaby, R.J., Fedel. R. 1973. Microbial production of sulphate and sulphide in some Australian soils. *Soil Biol. Biochem.* **5** : 773-781.
- Taddei Antonieta., Maria Jose Rodriguez., Ernesto Marquez-Vilchez., Cristina Castelli. 2006. Isolation and identification of *Streptomyces* spp.

- from Venezuelan soil : Morphological and biochemical studies.I. *Microbiological Research*. **161** : 222-231.
- Tandon, H. L. S. 1986. Sulphur research and development in Indian agriculture. *Fert. News*. **31**(9) : 9-16.
- Tandon, S. M. 1966. Bacterial flora of laterite soils of Maharashtra, Ph. D. Thesis, Univ., Poona (M.S.), India.
- Tanveer Akbar, Kalsoom Akhtar, Muhmmad A. Ghauri, Munir A. Anwar, Moazur Rehman, Mehboobur Rehman, Yusuf Zafar and Ahmad M. Khalid. 2005. Relationship among acidophilic bacteria from diverse environments as determined by randomly amplified polymorphic DNA analysis (RAPD). *World J. Microbiol and Biotech*. **21** : 645-648.
- Taylor, B. F. and D. S. Horare. 1971. *Thiobacillus denitrificans* as an obligate chemolithotroph. II Cell suspension and enzymic studies. *Archiv fur Mikrobiologia* **80** : 262-276 (Cited from Prokaryotes, 1 : 1023-2037, 1981).
- Throne, J. and Cloete, 2000, Molecular techniques for determining microbial diversity and community structure in natural environments. *Critical Rev. Microbiol.*, **26** : 37-57.
- Tran, H., Kruijt, M. and Raaijmakers, J. M., 2008, Diversity and activity of biosurfactant-producing *Pseudomonas* in the rhizosphere of black pepper in Vietnam. *J. Appl. Microbiol.*, **104** (3) : 839-851.
- Tresner, H. D. and M. S. Hamlin. 1964. Differential detection of Hydrogen sulphide and melanin pigments produced by streptomycetes. *Bact. Proc.*, G 51 : 22.
- Tripathi, M. and Johri, B. N., 2002, *In-vitro* antagonistic potential of fluorescent pseudomonads and control of sheath blight of maize caused by *Rhizoctonia solani*. *Indian J. Microbiol.*, **42** : 207-214.

- Tsao, P. H., C. Leben and G. W. Keitt. 1960. An enrichment method for isolating actinomycetes that produce diffusible antifungal antibiotics. *Phytopath.* **50** :88.
- Umashankar, M.E., Kumar, G., Karthik L. and Bhaskara Rao K.V. 2010. Exploration of antagonistic actinobacteria from Amirthi forest. *Intl. J Curr. Pharmaceutical Res.* **2** (1) : 16-19.
- Upadhyay, R. S. and Rai, B. 1987. Studies on antagonism between *Fusarium udum* and root region microflora of pigeon pea, *Plant and Soil.* **101**: 79-83.
- Varshney, S. and Chaube, H.S. 1999. Comparative rhizosphere competence of Fluroscent pseudomonads and *Trichoderma* spp. *Indian J. Pl. Path.* **17**(1 & 2) : 27-29.
- Varshney, S., Chaube, H.S. and Singh, H.B. 2000. Interaction between *Fluroscent pseudomads* and *Trichoderma harzianum*. *Indian Journal of Plant Pathology.* **18**(1 & 2) : 40-43.
- Venkatachalam, P., J. Ronald and K. Sambath. 2010. Effect of soil Streptomyces on seed germination. *Intl. J. of Pharma and Bio Sci.* **1**(4) : 145-155.
- Venkatesan, R. 1964. The influence of soil depth on the microbial population of paddy soil. *Annamalai Univ. Agri. Mag.*, **4** : 53-54.
- Vidyalakshmi R. and R. Sridar. 2006-2007. Isolation and characterization of sulphur oxidizing bacteria. **5** : 73-77.
- Vidyalakshmi R., R. Paranthaman and R. Bhakyaraj. 2009. Sulphur oxidizing bacteria and pulse nutrition - a review. *World J. Agril. Sci.* **5**(3): 270-278.
- Vinod Kumar, Anuj Kumar and R. N. Kharwar. 2007. Antagonistic potential of fluorescent pseudomonads and control of charcoal rot of chickpea

caused by *Macrophomina phaseolina* *Journal Environmental Microbiology*. **28** (1): 15-20.

Vishniac, W. and M. Santer. 1957. The *Thiobacilli* Bact. Reviews **21** : 195-213.
(In : Oxidation of sulphur in soils (Ed : G. R. Burus, 1967).

Vitgeet, A. E. 1960. The antagonistic action of actinomycetes against the causal agents of cotton wilt. *Antibiotiki*. **5** : 111-113.

Voisard, C., Keel, O., Haas, P. and Defago, G., 1989, Cyanide production by *Pseudomonas fluorescens* helps to suppress black root rot of tobacco under gnotobiotic condition. *European Microbiological Journal*, **8**: 351-358.

Von Plotho, O. 1940. Beitrage zur Kenntis der Morphologie u. Physiologis der Actinomyceten. *Arch. Mikrobiol.*, **11** : 33-72.

Waksman S. A. 1922. Micro-organisms encountered in the oxidation of sulphur in soil. III. Media used for the isolation of the sulphur oxidizing bacteria from the soil. *Soil Sci.*, **13** : 329-336.

Waksman S. A. 1950. The actinomycetes. Their nature, occurrence, activities, and importance. Chronica Botanica Co., Waltham Mass.

Waksman S. A. 1957. Species concept among the actinomycetes with special reference to the genus *Streptomyces*. *Bact. Rev.* **21** : 1-29.

Waksman S. A. 1959. The actinomycetes. Vol. I. Nature, Occurrence and Activities. Williams and Wilkins Co., Baltimore, U.S.A.

Waksman S. A. 1961. The Actinomycetes, Classification, Identification and Description of Genera and Species. Baltimore. The Williams and Wilkins Company. Vol. 2.

Waksman S. A. and A. Schatz. 1946. Soil enrichment and development of antagonistic microorganisms. *J. Bacteriol.*, **51** : 305-316.

- Waksman S. A. and J. S. Joffe. 1922. Microorganisms concerned in the oxidation of sulphur in soil. II. *Thiobacillus thiooxidans*, a new sulphur oxidizing organism isolated from the soil. *J. Bacteriol.* **7** (2) : 239-256.
- Waksman S. A., Elizabeth, Welch M. and Woodruff, H. B. 1942. Distribution of antagonistic actinomycetes in nature. *Soil Sci.* **54** : 281-296.
- Waltenbury Danielle R. , L. G. Leduc and G. D. Ferroni. 2005. The use of RAPD genomic fingerprinting to study relatedness in strains of *Acidithiobacillus ferrooxidans*. *Journal Microbiological method.* **62** : 103-112.
- Wei, G., Kloepper, J. W. and Tuzun, S., 1991, Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by selected strains of plant growth promoting rhizobacteria. *Phytopath.* **81** : 1508-1512.
- Welch, M. 1942. Bacteriostatic and bacteriolytic properties of actinomycetes. *J. Bacteriol.* **44** : 571- 588.
- Weller, D. M. 2007. *Pseudomonas* biocontrol agents of soilborne pathogens : Looking back over 30 years. *Phytopath.* **97**:250-256.
- Weller, D. M. and Thomashow, L.S. 1993. Use of rhizobacteria for biocontrol. *Curr. Opin. Biotechnol.* **4** : 306-311.
- Weller, D. M., Howke, W. J. and Cook, R. J., 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Phytopathology*, **38** : 1094.
- Weller, D. M., Raaijmakers, J. M., Mc Spadden, G. B. B. and Thomashow, L. S., 2002, Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.*, **40** : 309-348.
- Welsh, J. and McClelland, M., 1990, Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.*, **18** : 7213-7218.

- Welsh, J., Pretzman, C., Postic, D., Saint Girons, M., Baranton, G. and McClelland, M., 1992, Genomic fingerprinting by arbitrarily primed polymerase chain reaction resolves *Borrelia blirgdorferi*, into three distinct phyletic groups. *Int. J. Syst. Bacteriol.*, **42** : 370-377.
- Whaley J. M. 1965. The effect of nutrition on antibiotic production of rhizosphere actinomycetes antagonistic to soil borne plant pathogenic fungi. *Phytopath.* **55** : 1083.
- Wiedmann, M., Weilmeier, D., Dineen, S. S., Ralyea, R. and Boor, K. J., 2000, Molecular and phenotypic characterization of *Pseudomonas spp.* isolated from milk. *Appl. Environ. Microbiol.*, **66** : 2085-2095.
- Williams S.T., Goodfellow, M., Wellington EMH, Vickers JC, Alderson G, Sneath, PHA. et al. 1983. A probability matrix for identification of some Streptomyces. *J. of Gen. Microbiology.* **129**: 1815-1830.
- Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V., 1990, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, **18** : 6531-6535.
- Williams, R. A. D. and D. S. Hoare. 1972. Physiology of a new facultatively autotrophic thermophilic *Thiobacilli*, *J. Gen. Microbio.* **70** : 555-566.
- Williams, S. T., M. Goodfellow and G. Alderson, 1989. Genus *Streptomyces* Waksman and Henrici. 1943, 399AL. In: Bergey's Manual of Systematic Bacteriology. Williams and Wilkins Baltimore. pp: 2452-2492.
- Yang Yu, Qian Lin, Shi Wu-yang, Peng Hong, Qiu Ghan-zhon. 2008. Isolation and characterization of acidophilic bacterium from Gaofeng Mine in China. *Trans. Nonferrous Met. Soc. China.* **18** : 1253-1257.
- Yongqing Ni, Dongshi Wan and Kaiyu He 2008. 16S rDNA and 16S-23S internal transcribed spacer sequence analyses reveal inter- and

intraspecific *Acidithiobacillus* phylogeny. *Microbiology* **154** : 2397-2407.

You, J. L., L. X. Cao, G. F. Liu, S. N. Zhou, H. M. Tan and Y. C. Lin. 2005. Isolation and characterization of actinomycetes antagonistic to pathogenic *Vibrio* spp. from nearshore marine sediments. *World J. Micro. And Biotech.* **21** : 679-682.

Yusran Y., Weinmann, M., Neumann, G., Romheld, V., and Muller T. 2008. Isolation and screening of *Pseudomonas fluorescens* isolates from Inonesian soils against *Fusarium oxysporum* Schlect f.sp. *radicislycopersici* Jarvis and Shomkar "Competition for Resources in a changing World: Drive for Rural Development " held on 7-9 Oct., 2008 at Hohenheim.

Zehnder, G. W., Murphy, J. F., Sikora, E. J. and Kloepper, J. W., 2001, Application of rhizobacteria for induced resistance. *Eur. J. Plant Pathol.*, **107** : 39-50.

Zhou, J., Xia, B., Treves, D.S., Wu, L.Y., Marsh, T.L., O'Neill, R.V., Palumbo, A.V. And Tiedge, T.M., 2002, Spatial and resource factors influencing high microbial diversity in soil. *Appl. Environ. Microbiol.*, **68**: 326-334.

8. APPENDIX

Appendix-I

Details of *Pseudomonas fluorescens*, *Streptomyces* sp. and *Aciditibacillus* sp. microorganisms isolated from the soil samples collected from different physiographic regions of Maharashtra for studying microbial diversity

I) Western Konkan Coast (23)

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
1	Actinomycetes	Village: Pural Tahsil: Devgarh Dist: Sindhudurg	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	4 cm	Yellowish brown	16°55'20"N lat. 73°20'30"E long.
2	Actinomycetes	Village: Ratnagiri Tahsil: Ratnagiri Dist: Ratnagiri	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	8 cm	Reddish brown	17°00'00"N lat. 73°16'19"E long.
3	Actinomycetes(<i>Streptomyces</i>)	Village: Bhurapada Tahsil: Palghar Dist: Thane	Fine, mont. (cal), isohyperthermic, Vertic Halaquepts	Moderately shallow	53 cm	Dark grayish brown	19°32'00"N lat. 72°45'30"E long.
4	Actinomycetes	Village: Virthan Tahsil: Palghar Dist: Thane	Fine, mont. (cal), isohyperthermic, Entic Haplusterts	Moderately deep	>85cm	Brown & dark gray brown	19°32'20"N lat. 72°44'30"E long.
5	Actinomycetes(<i>Streptomyces</i>)	Village: Nivli Tahsil: Chiplun Dist: Ratnagiri	Fine, loamy, mixed, isohyperthermic, Udic Rhodustalfs	Moderately shallow	65 cm	Reddish brown	17°22'00"N lat. 73°27'30"E long.
6	Actinomycetes(<i>Streptomyces</i>)	Village: Harchil Tahsil: Ratnagiri Dist: Ratnagiri	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	18 cm	Yellowish red	16°55'30"N lat. 70°26'20"E long.
7	Actinomycetes(<i>Streptomyces</i>)	Village: Nandgaon Tahsil: Chiplun Dist: Ratnagiri	Clayey-skeletal mix isohyperthermic Typic Haplustepts	Moderately shallow	56 cm	Dark reddish brown	17°21'00"N lat. 73°33'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
8	Actinomycetes	Village: Janvali Tahsil: Kankauli Dist: Sindhudurg	Fine, loamy, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	50 cm	Dark reddish brown	16°16'21"N lat. 73°43'53"E long.
9	Actinomycetes	Village: Adeli Tahsil: Vengurla Dist: Sindhudurg	Fine, mixed, isohyperthermic, Ultic Haplustalfs	Moderately shallow	55 cm	Dark reddish brown	15°35'13.6"N lat. 73°41'29.6"E long.
10	Actinomycetes(<i>Streptomyces</i>)	Village: Vatora Tahsil: Vengurla Dist: Sindhudurg	Fine, mixed, isohyperthermic, Ultic Haplustalfs	Very deep	140 cm	Yellowish red	15°54'40"N lat. 73°39'6"E long.
11	Actinomycetes	Village: Tikali Tahsil: Ratnagiti Dist: Ratnagiti	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	15 cm	Reddish brown	16°55'30"N lat. 73°20'40"E long.
12	Actinomycetes	Village: Achra Tahsil: Malvan Dist: Sindhudurg	Loamy, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	65 cm	Brown	16°13'3"N lat. 73°1'3"E long.
13	Actinomycetes(<i>Streptomyces</i>)	Village: Gavalwadi Tahsil: Chiplun Dist: Ratnagiti	Fine-loamy, mixed, isohyperthermic, Udic Rhodustaf	Deep	>100 cm	Reddish brown	17°25'00"N lat. 73°28'30"E long.
14	Actinomycetes	Village: Naringre Tahsil: Devgarh Dist: Sindhudurg	Fine, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	75 cm	Reddish brown	16°17'16"N lat. 73°27'20"E long.
15	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i> , SO	Village: Rajurbahula Tahsil: Nasik Dist: Nasik	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	7 cm	Brown	19°53'0"N lat. 73°41'30"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
16	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Gunde Tahsil: Shahapur Dist: Thane	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	23 cm	Dark brown	19°26'10"N lat. 73°38'10"E long.
17	Actinomycetes(<i>Streptomyces</i>)	Village: Brahmanwadi Tahsil: Kankauli Dist: Sindhudurg	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	12 cm	Strong brown	16°17'10"N lat. 73°46'50"E long.
18	Actinomycetes	Village: Nane Tahsil: Wada Dist: Thane	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	56 cm	Dark brown	19°38'30"N lat. 72°58'30"E long.
19	Actinomycetes <i>Pseudomonas aeruginosa</i>	Village: Ambet Tahsil: Man Dist: Raigad	Clay-skeletal, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	70 cm	Brown	18°5'00"N lat. 73°16'00"E long.
20	Actinomycetes SO	Village: Tamhane Tahsil: Mahad Dist: Raigad	Clay-skeletal, mixed, isohyperthermic, Typic Rhodustalfs	Moderately shallow	70 cm	Yellowish red	17°55'00"N lat. 73°10'00"E long.
21	Actinomycetes	Village: Sakhar Tahsil: Poladpur Dist: Raigad	Fine, mixed, isohyperthermic, Udic Haplustalfs	Moderately deep	90 cm	Reddish brown	18°00'00"N lat. 73°34'00"E long.
22	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Kenjule Tahsil: Khed Dist: Ratnagiri	Loamy-skeletal, mixed, isohyperthermic, Typic Haplustepts	Moderately deep	76 cm	Dark red	17°48'00"N lat. 73°34'00"E long.
23	Actinomycetes(<i>Streptomyces</i>)	Village: Bhorghar Tahsil: Mangaon Dist: Raigad	Clay-skeletal, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	56 cm	Dark reddish brown	17°14'40"N lat. 73°12'10"E long.

Appendix-I contd...

II) Western Ghats (27)

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
24	Actinomycetes(<i>Streptomyces</i>)	Village: Ojhar Budruk Tahsil: Sangameshwar Dist: Ratnagiri	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Shallow	40 cm	Dark reddish brown	17°01'44"N lat. 73°47'19"E long.
25	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Ojhar Tahsil: Rajapur Dist: Ratnagiri	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	16 cm	Yellowish red	16°44'24"N lat. 73°37'40"E long.
26	Actinomycetes(<i>Streptomyces</i>)	Village: Harkul Tahsil: Kankauli Dist: Sindhudurg	Loamy-skeletal, mixed, isohyperthermic, Typic Haplustepts	Shallow	45 cm	Reddish brown	16°19'12"N lat. 73°50'11"E long.
27	SO	Village: Manivili Tahsil: Murbad Dist: Thane	Fine-loamy, mixed isohyperthermic, Typic Haplustepts	Shallow	33 cm	Dark reddish brown	19°17'00"N lat. 73°18'00"E long.
28	Actinomycetes(<i>Streptomyces</i>)	Village: Golevane Tahsil: Sahuwadi Dist: Kolhapur	Fine-loamy, mixed isohyperthermic, Udic Rhodustalfs	Deep	100 cm	Reddish brown	17°01'52"N lat. 73°52'06"E long.
29	Actinomycetes(<i>Streptomyces</i>)	Village: Ambarde Tahsil: Ajra Dist: Kolhapur	Fine, mixed isohyperthermic, Udic Rhodustalfs	Deep	125 cm	Dark reddish brown	16°05'59"N lat. 70°01'56"E long.
30	Actinomycetes	Village: Manjra Tahsil: Sahuwadi Dist: Kolhapur	Loamy-skeletal, mixed, isohyperthermic, Udic Haplustalfs	Shallow	40 cm	Yellowish red	16°49'57"N lat. 73°49'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
31	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Amboli Tahsil: Sawantwadi Dist: Sindhudurg	Fine-loamy mixed, isohyperthermic, Typic Haplustepts	Moderately deep	90 cm	Red & dark red	15°58'00"N lat. 73°59'00"E long.
32	Actinomycetes	Village: Amba Tahsil: Sawantwadi Dist: Sindhudurg	Fine-loamy mixed, isohyperthermic, Udic Rhodustalfs	Deep	125 cm	Reddish brown	17°0'48"N lat. 73°50'43"E long.
33	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Balkawadi Tahsil: Gargoti Dist: Kolhapur	Fine, mixed, isohyperthermic, Udic Rhodustalfs	Moderately deep	95 cm	Dark reddish brown	16°11.43'48"N lat. 74°01'57.7"E long.
34	Actinomycetes <i>Pseudomonas aeruginosa</i>	Village: Kodekhurd Tahsil: Gaganbavda Dist: Kolhapur	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Shallow	27 cm	Reddish brown	16°38'40"N lat. 73°54'30"E long.
35	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Dahisar Tahsil: Palghar Dist: Thane	Clayey-skeletal, mixed, isohyperthermic, Typic Ustorthents	Very shallow	13 cm	Black gravely clay	19°38'30"N lat. 72°53'15"E long.
36	Actinomycetes	Village: Haloli Tahsil: Palghar Dist: Thane	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately deep	76 cm	Dark brown	19°39'15"N lat. 72°53'00"E long.
37	Actinomycetes	Village: Verla Tahsil: Sawantwadi Dist: Sindhudurg	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Shallow	36 cm	Reddish yellow	15°59'04"N lat. 73°58'52"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
38	-	Village: Nirguda Tahsil: Peint Dist: Nasik	Clayey-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	8 cm	Yellowish red & dark reddish brown	20°10'00"N lat. 73°25'00"E long.
39	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Mahakoshi Tahsil: Bhore Dist: Pune	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	13 cm	Brown	18°03'30"N lat. 73°02'05"E long.
40	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Barsewadi Tahsil: Wai Dist: Satara	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	12 cm	Very dark grayish brown	18°00'30"N lat. 73°56'15"E long.
41	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i> SO	Village: Mahakoshi Tahsil: Jawahar Dist: Thane	Clayey, mixed, isohyperthermic, Typic Ustorthents	Very shallow	16 cm	Yellowish brown & dark yellowish brown	19°59'00"N lat. 73°10'30"E long.
42	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Chinchoda Tahsil: Peint Dist: Nasik	Clayey-skeletal, mixed, isohyperthermic, Typic Ustorthents	Shallow	30 cm	Reddish brown & dark reddish brown	27°10'30"N lat. 73°26'30"E long.
43	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Shirange Tahsil: Sawantwadi Dist: Sindhudurg	Loamy, mixed, isohyperthermic, Lithic Haplustepts	Shallow	42 cm	Dark red	15°44'35"N lat. 74°06'18.5"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
44	Actinomycetes <i>Pseudomonas fluorescens</i> SO(A)	Village: Ramghat Tahsil: Chandgad Dist: Kolhapur	Fine-loamy, mixed, isohyperthermic, Typic Haplustepts	Moderately deep	90 cm	Reddish brown & dark reddish brown	15°50'3"N lat. 74°06'18"E long.
45	Actinomycetes	Village: Khaprle Tahsil: Sinnar Dist: Nasik	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	13 cm	Brown	19°48'15"N lat. 73°54'30"E long.
46	Actinomycetes	Village: Indalkarwadi Tahsil: Purandar Dist: Pune	Clayey-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	8 cm	Dark reddish brown	18°25'45"N lat. 73°13'24"E long.
47	-	Village: Sonmbre Tahsil: Sinnar Dist: Nasik	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	18 cm	Brown	19°48'00"N lat. 73°55'00"E long.
48	Actinomycetes(<i>Streptomyces</i>)	Village: Tarwade Tahsil: Dhule Dist: Dhule	Clayey-skeletal, mixed, isohyperthermic, Typic Ustorthents	Very shallow	12 cm	Brown & dark grayish brown	20°24'40"N lat. 73°38'30"E long.
49	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Chimanipada Tahsil: Navapur Dist: Nandurbar	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	55 cm	Very dark grey & very dark grayish brown	21°31'36"N lat. 74°30'20"E long.
50	Actinomycetes <i>Pseudomonas aeruginosa</i>	Village: Kolada Tahsil: Navapur Dist: Nandurbar	Clay, mixed, isohyperthermic, Typic Haplustepts	Shallow	36 cm	Very dark grayish brown	21°08'30"N lat. 73°53'30"E long.

Appendix-I contd...

III) North Deccan: Maharashtra Plateau

A) North Deccan Maharashtra Upper Plateau (42)

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
51	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Girnare Tahsil: Malegaon Dist: Nasik	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	8-15 cm	Brown	20°23'15"N lat. 74°20'15"E long.
52	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Girnar Tahsil: Malegaon Dist: Nasik	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	18-22 cm	Brown & dark brown	20°22'15"N lat. 74°20'00"E long.
53	Actinomycetes(<i>Streptomyces</i>)	Village: Pimple Tahsil: Kalvan Dist: Nasik	Clayey-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	10-15 cm	Dark reddish brown	20°21'20"N lat. 73°56'30"E long.
54	<i>Pseudomonas aeruginosa</i>	Village: Mohabari Tahsil: Sakri Dist: Dhule	Clayey-skeletal, mixed, isohyperthermic, Typic Ustorthents	Shallow	41 cm	Dark brown	20°34'00"N lat. 73°55'20"E long.
55	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Karkata Tahsil: AUSA Dist: Latur	Clayey, montmorillonitic, isohyperthermic, Typic Haplustepts	Shallow	43 cm	Dark brown	18°23'00"N lat. 76°17'15"E long.
56	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Laman Tanda Tahsil: Ahmadpur Dist: Latur	Clayey, montmorillonitic, isohyperthermic, Lithic Ustorthents	Very Shallow	15-22 cm	Dark brown	18°26'00"N lat. 76°46'15"E long.
57	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Wadkudi Tahsil: Chandgad Dist: Kolhapur	Fine, mixed isohyperthermic, Udic Rhodustalfs	Deep	135 cm	Reddish brown & dark reddish brown	15°56'04"N lat. 74°14'26"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
58	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Khodal Tahsil: Junnar Dist: Pune	Loamy-skeletal, mixed (cal), isohyperthermic, Lithic Ustorthents	Very shallow	17 cm	Dark reddish brown	19°5'00"N lat. 74°41'00"E long.
59	Actinomycetes	Village: Devargaon Tahsil: Niphad Dist: Nasik	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	5-10 cm	Brown	20°12'30"N lat. 74°9'30"E long.
60	Actinomycetes(<i>Streptomyces</i>)	Village: Rokdwadi Tahsil: Ambegaon Dist: Pune	Loamy, mixed (cal), isohyperthermic, Lithic Ustorthents	Very shallow	9-21 cm	Dark yellowish brown	18°56'00"N lat. 74°8'00"E long.
61	-	Village: Sarole Tahsil: Mohol Dist: Solapur	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	6-18 cm	Yellowish brown	17°48'35"N lat. 75°33'00"E long.
62	Actinomycetes(<i>Streptomyces</i>)	Village: Nandgaon Tahsil: Karjat Dist: Ahmednagar	Clayey, montmorillonitic, isohyperthermic, Lithic Ustorthents	Very shallow	7-18 cm	Brown & dark reddish brown	18°35'00"N lat. 74°55'30"E long.
63	Actinomycetes <i>Pseudomonas aeruginosa</i>	Village: Ambawadi Tahsil: Shirala Dist: Sangli	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	12-20 cm	Dark reddish brown	17°3'20"N lat. 74°4'50"E long.
64	Actinomycetes(<i>Streptomyces</i>)	Village: Nimbhore Tahsil: Rahuri Dist: Ahmednagar	Loamy-skeletal, mixed (cal), isohyperthermic, Typic Ustorthents	Very shallow	10-22 cm	Brown	19°28'00"N lat. 74°29'00"E long.
65	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Karvali Tahsil: Mohol Dist: Solapur	Clayey-skeletal, montmorillonitic (cal) isohyperthermic, Typic Ustorthents	Extremely shallow	9 cm	Brown	17°35'40"N lat. 75°42'30"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
66	-	Village: Jhikri Tahsil: Jamkhed Dist: Ahmednagar	Clayey, montmorillonitic, isohyperthermic, Lithic Haplustepts	Very shallow to Shallow	12-36 cm	Very dark grayish brown	18°39'30"N lat. 74°19'00"E long.
67	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Ambulga Tahsil: Ahmadpur Dist: Latur	Clayey, montmorillonitic, isohyperthermic, Typic Haplustepts	Shallow	42 cm	Very dark grayish brown	18°27'15"N lat. 76°46'30"E long.
68	<i>Pseudomonas aeruginosa</i> , SO	Village: Karlewadi Tahsil: Ambegaon Dist: Pune	Fine, mont (cal), isohyperthermic, Typic Haplustepts	Moderately deep	76-90 cm	Dark brown	18°57'49"N lat. 73°52'00"E long.
69	-	Village: Kameri Tahsil: Valva Dist: Sangli	Fine, mont (cal), isohyperthermic, Typic Haplustepts	Deep	105-150 cm	Very dark grayish brown	17°17'70"N lat. 74°56'00"E long.
70	SO	Village: Bumne Tahsil: Gargoti Dist: Kolhapur	Fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	160 cm	Brown & dark brown	16°16'5.24"N lat. 74°14'17.9"E long.
71	Actinomycetes	Village: Parpoli Tahsil: Ajra Dist: Kolhapur	Fine, mixed, isohyperthermic, Udic Haplustepts	Moderately deep	80 cm	Brown & reddish brown	16°6'8"N lat. 74°5'11"E long.
72	SO(A)	Village: Bamni Tahsil: Latur Dist: Latur	Very fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	110-150 cm	Very dark grayish brown	18°28'0"N lat. 76°41'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
73	Actinomycetes(<i>Streptomyces</i>)	Village: Deothan Tahsil: Akole Dist: Ahmednagar	Fine-loamy, mixed, calcareous, isohyperthermic, Typic Ustifluvents	Deep	150 cm	Brown	19°38'00"N lat. 74°4'00"E long.
74	Actinomycetes	Village: Ranjani Tahsil: Ambegaon Dist: Pune	Loamy, mixed (cal), isohyperthermic, Lithic Ustorthents	Very shallow	13-23 cm	Brown & dark brown	17°0'58"N lat. 74°30'00"E long.
75	Actinomycetes <i>Pseudomonas aeruginosa</i>	Village: Ambade Tahsil: Bhore Dist: Pune	Loamy, mixed isohyperthermic, Lithic Ustorthents	Very shallow	12 cm	Strong Brown	18°3'00"N lat. 73°51'30"E long.
76	Actinomycetes(<i>Streptomyces</i>)	Village: Patharwadi Tahsil: Atpadi Dist: Sangli	Clayey, mixed isohyperthermic, Lithic Ustorthents	Extremely shallow	7-14 cm	Dark reddish brown	17°17'00"N lat. 74°54'45"E long.
77	<i>Pseudomonas fluorescens</i>	Village: Dudhebari Tahsil: Phaltan Dist: Satara	Loamy-skeletal, mixed (cal), isohyperthermic, Typic Ustorthents	Very shallow	18-20 cm	Very dark grayish brown	17°56'00"N lat. 74°30'00"E long.
78	Actinomycetes	Village: Sonawadi Tahsil: Phaltan Dist: Satara	Fine, montmorillonitic (cal), isohyperthermic, Vertic Haplustepts	Moderately deep	73-86 cm	Dark brown	17°57'00"N lat. 74°29'00"E long.
79	Actinomycetes	Village: Penjalwadi Tahsil: Bhore Dist: Pune	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	55-65 cm	Dark brown	18°11'00"N lat. 74°01'00"E long.
80	Actinomycetes <i>Pseudomonas fluorescens</i> , SO(A)	Village: Kolyachiwadi Tahsil: Rahuri Dist: Ahmednagar	Fine, mixed (cal), isohyperthermic, Fluventic Haplustepts	Moderately Deep	83 cm	Brown & dark brown	19°23'00"N lat. 74°27'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
81	Actinomycetes	Village: Kurli Tahsil: Vite Dist: Sangli	Fine, mont. (cal), isohyperthermic, Typic Calcustepts	Moderately deep	75 cm	Brown & dark brown	17°14'00"N lat. 74°33'45"E long.
82	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas</i> <i>aeruginosa</i>	Village: Wagha Tahsil: Kaij Dist: Beed	Fine, mont. (cal), isohyperthermic, Typic Haplustepts	Deep	88-150 cm	Dark brown	18°44'00"N lat. 75°50'30"E long.
83	Actinomycetes	Village: Targaon Tahsil: Koregaon Dist: Satara	Fine, mont. (cal), isohyperthermic, Typic Haplustepts	Deep	165 cm	Brown to dark brown	17°31'00"N lat. 74°09'00"E long.
84	-	Village: Shiware Tahsil: Shahuwadi Dist: Kolhapur	Very fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	165 cm	Dark brown	16°56'52"N lat. 74°01'12"E long.
85	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas</i> <i>fluorescens</i>	Village: Wathar Tahsil: Koregaon Dist: Satara	Fine, mont. (cal), isohyperthermic, Typic Haplustepts	Deep	150 cm	Very dark grayish brown	17°32'00"N lat. 74°11'00"E long.
86	-	Village: Kondvan Tahsil: Shahuwadi Dist: Kolhapur	Fine, mixed, isohyperthermic, Udic Rhodustalfs	Moderately Deep	98 cm	Reddish brown	17°02'30"N lat. 73°54'40"E long.
87	Actinomycetes	Village: Ghospuri Tahsil: Ahmednagar Dist: Ahmednagar	Clayey-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	5-15 cm	Dark reddish brown	18°54'45"N lat. 74°40'20"E long.
88	Actinomycetes (<i>Streptomyces</i>)	Village: Nizampur Tahsil: Sangola Dist: Solapur	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	5-20 cm	Yellowish brown & brown	17°19'00"N lat. 75°8'20"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
89	Actinomycetes	Village: Sulaiwadi Tahsil: Vite Dist: Sangli	Clayey, mon., isohyperthermic, Lithic Ustorthents	Very shallow	12-24 cm	Dark reddish brown	17°14'30"N lat. 74°34'00"E long
90	Actinomycetes <i>Pseudomonas aeruginosa</i>	Village: Davri Tahsil: Patan Dist: Satara	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	12-28 cm	Dark reddish brown	17°16'5"N lat. 73°55'59"E long.
91	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Virgaon Tahsil: Akole Dist: Ahmednagar	Loamy-skeletal, mixed (cal), isohyperthermic, Typic Ustorthents	Very shallow	14 cm	Dark yellowish brown	19°37'00"N lat. 74°3'00"E long.
92	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Bhoirepathar Tahsil: Ahmednagar Dist: Ahmednagar	Fine, mont. (cal), isohyperthermic, Vertic Haplustepts	Moderately shallow	60-75 cm	Dark brown / Very dark brown	19°36'00"N lat. 74°2'00"E long.

III) B) North Deccan Maharashtra Lower Plateau (37)

93	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i> , SO	Village: Ancholi Tahsil: Biloli Dist: Nanded	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	5-20 cm	Brown dark brown	18°53'10"N lat. 77°27'00"E long.
94	Actinomycetes <i>Pseudomonas aeruginosa</i>	Village: Gulvanch Tahsil: Sinnar Dist: Nasik	Loamy, mixed, calcareous, isohyperthermic, Lithic Ustorthents	Very shallow	19 cm	Brown & dark brown	19°52'00"N lat. 74°7'00"E long.
95	Actinomycetes(<i>Streptomyces</i>) <i>P. aeruginosa</i>	Village: Beed Tahsil: Beed Dist: Beed	Clayey, mont. (cal), isohyperthermic, Typic Haplustepts	Shallow	34 cm	Dark brown	18°58'15"N lat. 75°48'00"E long.
96	<i>Pseudomonas fluorescens</i>	Village & Tal. : Kavthe Mahankal Dist: Sangli	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	5 cm	Reddish brown	16°59'55"N lat. 74°55'30"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
97	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Jamthi Babala Tahsil: Hingoli Dist: Parbhani	Loamy-skeletal, mixed (cal), isohyperthermic, Lithic Ustorthents	Very shallow	14-20 cm	Very dark grayish brown	19°49'07"N lat. 77°5'4"E long.
98	Actinomycetes (<i>Streptomyces</i>)	Village: Rajur Babala Tahsil: Nasik Dist: Nasik	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	10-22 cm	Strong Brown	19°54'55"N lat. 73°42'00"E long.
99	Actinomycetes	Village: Bhalvani Tahsil: Malsirus Dist: Solapur	Clayey, montmorillonitic, isohyperthermic, Lithic Ustorthents	Very shallow	8-20 cm	Dark brown	17°42'00"N lat. 75°7'30"E long.
100	Actinomycetes <i>Pseudomonas aeruginosa</i>	Village: Kumbhoj Tahsil: Karmala Dist: Solapur	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	8-22 cm	Dark brown	18°17'30"N lat. 75°11'3"E long.
101	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Chandhai Tahsil: Mangrulpir Dist: Akola	Clayey, montmorillonitic, isohyperthermic, Lithic Haplustepts	Shallow	27-36 cm	Very dark grayish Brown	20°17.8'00"N lat. 73°24'00"E long.
102	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Satana Tahsil: Vaijapur Dist: Aurangabad	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	49-73 cm	Very dark grayish Brown	19°52'00"N lat. 74°47'00"E long.
103	SO	Village: Nandgaon Tahsil: Gondpipri Dist: Chandrapur	Fine-loamy, mixed, isohyperthermic, Typic Haplustepts	Shallow	40 cm	Brown & dark brown	19°51'30"N lat. 79°44'15"E long.
104	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Ghospuri Tahsil: Ahmednagar Dist: Ahmednagar	Clayey, mont. (cal.), isohyperthermic, Typic Haplustepts	Shallow	28-45 cm	Very dark grayish brown	18°55'25"N lat. 74°40'50"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
105	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Dhulgaon Tahsil: Kavthe Mahankal Dist: Sangli	Fine, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Moderately deep	35-100 cm	Dark yellowish brown	16°57'30"N lat. 74°52'35"E long.
106	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Wadachiwadi Tahsil: Jintur Dist: Parbhani	Fine, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Deep	100-150 cm	Dark grayish brown & dark brown	19°34'00"N lat. 76°44'30"E long.
107	Actinomycetes	Village: Sewagram Tahsil: Wardha Dist: Wardha	Fine, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Deep	140-150 cm	Dark grayish brown & dark brown	20°44'00"N lat. 78°39'30"E long.
108	Actinomycetes(<i>Streptomyces</i>)	Village: Umrer Tahsil: Umrer Dist: Nagpur	Fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	99-150 cm	Dark grayish brown & very dark grayish brown	20°52'00"N lat. 79°22'00"E long.
109	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Ranvad Tahsil: Niphad Dist: Nasik	Clayey, montmorillonitic, isohyperthermic, Lithic Haplustepts	Shallow	30-42 cm	Dark brown	20°09'15"N lat. 74°07'00"E long.
110	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i> , SO	Village: Nandurdi Tahsil: Niphad Dist: Nasik	Clayey, montmorillonitic (calcareous), isohyperthermic, Lithic Haplustepts	Shallow	44 cm	Dark brown	20°08'00"N lat. 74°05'30"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
111	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i> , SO	Village: Purandarwada Tahsil: Malshirus Dist: Solapur	Fine, montmorillonitic (calcareous), isohyperthermic, Vertic Haplustepts	Moderately deep	80-110 cm	Very dark grayish brown	17°51'45"N lat. 74°51'00"E long.
112	Actinomycetes(<i>Streptomyces</i>)	Village: Bandhalwadi Tahsil: Malshirus Dist: Solapur	Clayey, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Shallow	25-50 cm	Dark grayish brown & dark brown	17°44'00"N lat. 75°06'00"E long.
113	Actinomycetes(<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i> , SO(A)	Village: Masala Tahsil: North Solapur Dist: Solapur	Fine, montmorillonitic (calcareous), isohyperthermic, Vertic Haplustepts	Moderately deep	100 cm	Dark grayish brown	17°51'30"N lat. 75°45'30"E long.
114	Actinomycetes	Village: Thejoda Tahsil: Satana Dist: Nasik	Fine, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Moderately shallow to deep	50-125 cm	Brown & dark brown	20°00'32"N lat. 74°14'00"E long.
115	Actinomycetes(<i>Streptomyces</i>) SO(A)	Village: Belbare Tahsil: Kalwan Dist: Nasik	Fine, montmorillonitic, isohyperthermic, Udic Haplustepts	Deep	105 cm	Very dark grayish brown	20°21'30"N lat. 73°56'30"E long.
116	Actinomycetes(<i>Streptomyces</i>)	Village: Dhondrai Tahsil: Gevarai Dist: Beed	Fine, montmorillonitic, (calcareous), isohyperthermic, Entic Haplustepts	Deep	80-160 cm	Brown	19°18'00"N lat. 75°41'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
117	-	Village: Mendki Tahsil: Katol Dist: Nagpur	Fine, montmorillonitic, (calcareous), hyperthermic, Typic Haplustepts	Deep	125 cm	Very dark grayish brown	21°21'00"N lat. 78°38'00"E long.
118	<i>Pseudomonas aeruginosa</i>	Village: Sirpur Tahsil: Deoli Dist: Wardha	Fine, montmorillonitic, (calcareous), hyperthermic, Vertic Haplustepts	Deep	90-150 cm	Dark brown	20°52'00"N lat. 78°23'00"E long.
119	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Mehunbara Tahsil: Chalisgaon Dist: Jalgaon	Fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	150 cm	Dark brown & very dark grayish brown	20°35'10"N lat. 73°55'20"E long.
120	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Dahiwad Tahsil: Chalisgaon Dist: Jalgaon	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	10-15 cm	Brown & dark brown	20°31'10"N lat. 73°55'00"E long.
121	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Sewagram Tahsil: Wardha Dist: Wardha	Loamy, mixed, hyperthermic, Lithic Ustorthents	Extremely shallow	8 cm	Yellowish brown & brown	19°44'30"N lat. 78°40'00"E long.
122	Actinomycetes (<i>Streptomyces</i>)	Village: Koregaon Tahsil: Wani Dist: Yeotmal	Clayey, montmorillonitic, isohyperthermic, Lithic Haplustepts	Shallow	25 cm	Very dark grayish brown	19°56'00"N lat. 78°36'00"E long.
123	Actinomycetes (<i>Streptomyces</i>)	Village: Wanjri Tahsil: Kelapur Dist: Yeotmal	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	56 cm	Dark grayish Brown & dark brown	19°57'00"N lat. 78°34'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
124	Actinomycetes (<i>Streptomyces</i>)	Village: Garpit RF Tahsil: Karanja Dist: Wardha	Loamy-skeletal, mixed, hyperthermic, Lithic Ustorthents	Very shallow	18 cm	Very dark grayish brown	21°4'00"N lat. 78°28'00"E long.
125	Actinomycetes	Village: Talegaon Tahsil: Karanja Dist: Wardha	Clayey, montmorillonitic, hyperthermic, Lithic Ustorthents	Shallow	28 cm	Very dark grayish brown	21°08'00"N lat. 78°28'00"E long.
126	Actinomycetes	Village: Mulgi Tahsil: Akkalkuwa Dist: Dhule	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	10-15 cm	Reddish brown	21°34'30"N lat. 73°55'10"E long.
127	Actinomycetes (<i>Streptomyces</i>)	Village: Phulwari Tahsil: Kinwat Dist: Nanded	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	<10 cm	Dark brown	19°43'00"N lat. 78°7'00"E long.
128	Actinomycetes	Village: Bodla Tahsil: Dhudgaon Dist: Dhule	Loamy-skeletal, mixed, isohyperthermic, Typic Ustorthents	Extremely shallow	7 cm	Brown & dark brown	21°34'30"N lat. 73°55'10"E long.
129	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Umri Tahsil: Yeotmal Dist: Yeotmal	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately deep	64-82 cm	Dark grayish brown	20°00'00"N lat. 79°00'00"E long.

III) C) North Deccan Maharashtra Lower Plateau (Metamorphic) (21)

130	Actinomycetes (<i>Streptomyces</i>) SO(A)	Village: Manori Tahsil: Umrer Dist: Nagpur	Fine-loamy, mixed, hyperthermic, Typic Ustorthents	Shallow	28-50 cm	Dark grayish brown & Very dark grayish brown	20°42'00"N lat. 79°5.5'00"E long.
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Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
131	<i>Pseudomonas aeruginosa</i>	Village: Abola Tahsil: Ramtek Dist: Nagpur	Fine-loamy, mixed, hyperthermic, Typic Haplustepts	Moderately deep	80 cm	Dark brown	21°23'20"N lat. 79°20'45"E long.
132	Actinomycetes (<i>Streptomyces</i>) SO	Village: Bamhni Tahsil: Sindewahi Dist: Chandrapur	Fine, montmorillonitic, hyperthermic, Entic Haplustepts	Deep	140 cm	Yellowish brown	20°45'15"N lat. 79°59'30"E long.
133	Actinomycetes (<i>Streptomyces</i>) SO	Village: Kinhala Tahsil: Umrer Dist: Nagpur	Fine, mont. hyperthermic, Typic Haplustepts	Deep	125-150 cm	Dark grayish brown	20°44'0"N lat. 79°9'00"E long.
134	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i> , SO	Village: Hatodi Tahsil: Ramtek Dist: Nagpur	Coarse-loamy, mixed, hyperthermic, Typic Haplustepts	Shallow	26 cm	Dark brown	21°20'30"N lat. 79°22'00"E long.
135	Actinomycetes SO(A)	Village: Phutki Tahsil: Sindewahi Dist: Chandrapur	Fine-loamy, mixed, hyperthermic, Typic Haplustepts	Deep	>100 cm	Yellowish brown	20°14'00"N lat. 79°42'00"E long.
136	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i>	Village: Takali Tahsil: Kuhu Dist: Nagpur	Fine, montmorillonitic, hyperthermic, Typic Haplustepts	Deep	> 125-150 cm	Brown	21°2'00"N lat. 79°29'55"E long.
137	Actinomycetes SO	Village: Sawarkhanda Tahsil: Kuhu Dist: Nagpur	Fine, mixed, hyperthermic, Typic Haplustepts	Moderately shallow	60 cm	Brown and dark brown	21°3'20"N lat. 79°29'30"E long.
138	Actinomycetes (<i>Streptomyces</i>)	Village: Elgartola RF Tahsil: Ahiri Dist: Gadchiroli	Fine, mixed, hyperthermic, Typic Ustorthents	Shallow	35 cm	Brown and dark brown	19°31'00"N lat. 80°7'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
139	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Nalikasa (Dalli) Tahsil: Kurkheda Dist: Gadchiroli	Fine, mixed, hyperthermic, Vertic Haplustepts	Deep	100-125 cm	Brown and dark brown	20°32'5"N lat. 80°21'46"E long.
140	Actinomycetes	Village: Sitagondi Tahsil: Parseoni Dist: Nagpur	Coarse-loamy, mixed, hyperthermic, Typic Haplustepts	Shallow	50 cm	Very dark brown	21°32'00"N lat. 79°03'00"E long.
141	Actinomycetes (<i>Streptomyces</i>) SO	Village: Tekala Tahsil: Etapalli Dist: Gadchiroli	Fine-loamy, mixed, hyperthermic, Typic Haplustepts	Deep	100-110 cm	Brown & dark brown	19°28'30"N lat. 80°33'30"E long.
142	Actinomycetes	Village: Kasansur Tahsil: Etapalli Dist: Gadchiroli	Fine, mixed, hyperthermic, Typic Haplustepts	Moderately shallow	57 cm	Grayish brown & very dark grayish brown	19°28'00"N lat. 80°09'00"E long.
143	Actinomycetes (<i>Streptomyces</i>)	Village: Pediguradom RF Tahsil: Ahiri Dist: Gadchiroli	Fine-loamy, mixed, hyperthermic, Typic Haplustoll	Deep	125 cm	Dark reddish grey & dark reddish brown	19°28'30"N lat. 80°9'00"E long.
144	Actinomycetes (<i>Streptomyces</i>)	Village: Khatera Tahsil: Sindewahi Dist: Chandrapur	Fine, montmorillonitic, hyperthermic, Typic Haplustepts	Deep	125 cm	Brown & dark brown	20°44'30"N lat. 79°54'45"E long.
145	Actinomycetes <i>Pseudomonas fluorescens</i>	Village: Bhiwkund Tahsil: Kuhu Dist: Nagpur	Loamy-skeletal, mixed, hyperthermic, Lithic Ustorthents	Extremely shallow	9 cm	Brown and dark brown	20°14'00"N lat. 79°28'15"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
146	Actinomycetes (<i>Streptomyces</i>) <i>P. aeruginosa</i> , SO(A)	Village: Palora Tahsil: Ramtek Dist: Nagpur	Fine-loamy, mixed hyperthermic, Typic Haplustepts	Deep	100 cm	Brown	21°22'0"N lat. 79°10'00"E long.
147	Actinomycetes (<i>Streptomyces</i>)	Village: Andhali Tahsil: Kurkheda Dist: Gadchiroli	Fine, montmorillonitic, hyperthermic, Vertic Haplustepts	Deep	125 cm	Grayish brown & dark grayish brown	20°33'52"N lat. 80°16'45"E long.
148	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas aeruginosa</i>	Village: Palandur Tahsil: Deori Dist: Bhandara	Fine-loamy, mixed hyperthermic, Typic Haplustepts	Deep	145 cm	Brownish yellow & yellowish brown	20°51'3"N lat. 80°13'9"E long.
149	<i>Pseudomonas fluorescens</i>	Village: Bhugaon Tahsil: Nagpur Dist: Nagpur	Fine, montmorillonitic, hyperthermic, Typic Haplustepts	Deep	125 cm	Very dark grayish brown	20°6'00"N lat. 79°22'00"E long.
150	Actinomycetes (<i>Streptomyces</i>) <i>Pseudomonas fluorescens</i> , SO	Village: Bahadura Tahsil: Nagpur Dist: Nagpur	Fine, montmorillonitic, hyperthermic (cal), Vertic Haplustepts	Moderately deep	90 cm	Dark brown	21°05'55"N lat. 79°10'09"E long.

Figures in parentheses of different physiographic regions of Maharashtra indicates number of soil samples collected for studying the microbial diversity

Pa = *Pseudomonas aeruginosa*

Pf = *Pseudomonas fluorescens*

SO = Sulphur oxidizer

SO(A)= Sulphur oxidizer (*Acidithiobacillus*)

APPENDIX – II

Use of different media composition / indicators/ reagents

A) Glucose broth	
Peptone	5.00 g
Beef extract	3.00 g
Glucose	5.00 g
Distilled water	1000 ml
BCP solution	15 ml
B) Iodine solution (For starch hydrolysis)	
Iodine	0.50 g
Potassium iodide	1.00 g
Distilled water	1000 ml
C) King's B medium (King <i>et al.</i>, 1954)	
Peptone	20.00 g
MgSO ₄	1.50 g
K ₂ HPO ₄	1.50 g
Glycerol	10 ml
Agar	18.00 g
Distilled water	1000 ml
Adjust pH to 7.0	
D) Kovac's reagent	
P-Dimethylaminobenzaldehyde	5.0 g
Amyl alcohol	75.0 ml
Hydrochloric acid (Conc.)	25.0 ml
Store the reagent at refrigerator	
E) Luria Bertani agar (LB agar) (Miller,1987)	
Tryptone	10.00 g
Yeast extract	5.00 g
NaCl	5.00 g
Agar	15.00 g
Distilled water	1000 ml
pH	7.2

F) Kovac's reagent	
P- Dimethyl amino benzaldehyde	5.0 g
Amyl alcohol	75.0 ml
Hydrochloric acid (Conc.)	25.0 ml
Store the reagent at refrigerator	
G) Knight medium	
Glucose	1.0 g
Mono potassium phosphate	0.1 g
Sodium nitrate	0.1 g
Potassium chloride	0.1 g
MgSO ₄	0.1 g
Agar	15.0 g
Distilled water	1000 ml
pH 7.00 to 7.2	
H) Nitrate broth	
Peptone	5.00 g
Beef extract	3.00 g
KNO ₃	1.00 g
Distilled water	1000 ml
Adjust pH to 6.8-7.0	
I) Nutrient agar (Anon, 1957)	
Peptone	5.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Agar	18.00 g
Distilled water	1000 ml
pH : 6.8-7.2	
K) Nutrient gelatin (Cappuccino and Sherman, 1992)	
Peptone	5.00 g
Beef extract	3.00 g
Gelatin	120.00 g
Distilled water	1000 ml
Adjust pH to 6.8-7.0	

L) Pikovskaya's agar (Pikovskaya, 1948)	
Glucose	10.00 g
MgSO ₄ (2.5%)	10.00 ml
CaCl ₂ (1%)	10.00 ml
Tricalcium phosphate	5.00 g
Agar	18.00 g
Distilled water	1000 ml
Adjust pH to 7.0	
M) Potato dextrose agar (Okon et al., 1977)	
Peeled potato	200.00 g
Dextrose	20.00 g
Agar	18.00 g
Distilled water	1000 ml
N) SIM agar (Cappuccino and Sherman, 1992)	
Peptone	30.00 g
Beef extract	3.00 g
Ferrous amm. Sulphate	0.20 g
Sodium thisoulphate	0.025 g
Distilled water	1000 ml
Agar	18.00 g
Adjust pH to 7.3 before adding agar	
O) Skimmed milk agar (Bailey and Schott, 1970)	
Skim milk powder	10.00 g
Peptone	5.00 g
Distilled water	1000 ml
Agar	18.00 g
Adjust pH to 7.2 before adding agar	
P) Starch Casein Agar medium	
Starch	10 g
Casein	0.3 g
KNO ₃	2.0 g
NaCl	2.0 g
K ₂ HPO ₄	2.0 g

MgSO ₄	0.05 g
CaCO ₃	0.02 g
FeSO ₄	0.01 g
Agar	18.0 g
Distilled Water	1000 ml
Q) Starch agar (Bailey and Schott, 1970)	
Peptone	5.00 g
Beef extract	3.00 g
Starch solution	10.00 ml
Agar	18.00 g
Distilled water	1000 ml
Adjust pH to 7.2	
R) S isolation medium	
Ammonium sulphate	0.2 g
Potassium dihydrogen phosphate	3.0 g
Magnesium sulphate	0.5 g
Calcium chloride	0.2 g
Ferric sulphate	Trace
Elemental sulphur	10 g
Distilled water	1000 ml
Indicator : 5 ml bromocresol purple (0.25 %) per liter	
S) Thiosulphate medium (Waksman, 1922)	
Sodium thiosulphate	5.0 g
Ammonium chloride	0.1 g
Sodium bicarbonate	1.0 g
Disodium hydrogen phosphate	0.2 g
Magnesium sulphate	0.1 g
Distilled water	1000 ml

T) Trypticase soy agar(Frazier <i>et al.</i>, 1987)	
Tryptone	17.00 g
Soyatone	3.00 g
Glucose	2.50 g
Agar	18.00 g
Distilled water	
U) N-N-N-N- Tetra methyl – para – phenylenediamine dihydrochloride (Wurster's reagent) (for oxidase activity)	
N-N-N-N- Tetra methyl – para – phenylenediamine dihydrochloride	5.0 g
Distilled Water	50.0 ml
V) Urea broth (Frazier <i>et al.</i>, 1967)	
Yeast extract	0.10 g
KH ₂ PO ₄	9.10 g
Na ₂ HPO ₄	9.50 g
Urea	20.00 g
Phenol red	0.010 g
Distilled water	1000 ml
Adjust pH to 6.8	

Appendix – III

Molecular characterization reagents used for Genomic/ total DNA isolation :

Stock solutions

1M Tris (pH 8.0)

Tris	:	121.1 g
Water	:	8000 ml
pH (adjusted with concentrated HCl)	:	8.0
Total volume	:	1000 ml

EDTA 500 mM (pH 8.0)

EDTA	:	18.6 g
Water	:	80 ml
pH (adjusted with NaOH)	:	8.0
Total volume	:	100 ml

Proteinase K: 100 mg/ml

Dissolved 100 mg of proteinase K in 1 ml of sterilized milli Q water and stored at -20°C and used @ 5 μl /sample.

DNase free RNase: 10 mg/ml

Dissolved 10 mg of DNAase free RNAase in 1 ml of sterilized milli Q water and stored at -20°C and used @ 10 μl /sample.

Extraction buffer (2 per cent Sarkosyl in T₅₀E₂₀) (pH: 8.0)

Stock conc.	Chemical	Quantity	Final Conc.
1 M	Tris	2.5 ml	50 mM
0.5 M	EDTA	2.0 ml	20 mM
20%	Sarkosyl	5.0 ml	2 %
	Milli Q water	40.5 ml	
	Total	50.0 ml	

The extraction buffer used @ 500 μl /sample.

70 % Ethanol (Chilled)

70 ml Ethanol
30 ml Distilled Water

PCR reaction mix (20 µl tube⁻¹) for RAPD

10 x Taq. buffer A (Tris with 15 mM MgCl ₂)	2.0 µl
25 mM MgCl ₂	0.4 µl
dNTP Mix. (2.5 mM)	1.6 µl
Taq DNA polymerase (3U/µl)	0.33 µl
Primer	1.0 µl
Template DNA (5 ng)	1.0 µl
Sterile double distilled water	13.67 µl
Total volume	20 µl

Loading dye composition

Loading dye (6x)	:	0.25% bromophenol blue
Store at 4°C		in 40 % glycerol

Composition for 0.8 per cent agarose gel (100 ml)

Agarose	:	0.8 g / 100 ml
1x TBE	:	100 ml
EtBr (10 mg/ml)	:	7 µl / 100 ml

Composition for 1.2 per cent agarose gel (180 ml)

Agarose	:	1.2 g / 100 ml
1x TBE	:	180 ml
EtBr (10 mg/ml)	:	7 µl / 100 ml

10x TBE buffer composition –

Tris base	:	108 g
Boric acid	:	55 g
0.5 M EDTA (pH 8.0)	:	40 ml
Total volume 1000 ml with sterilized milli Q water		

Appendix – IV
Coding used for isolation of *Pseudomonas fluorescens*, *Streptomyces* sp. and *Acidithiobacillus* sp. from different soils of Maharashtra

Soil sample No.	Fluorescent Pseudomonads	<i>Pseudomonas fluorescens</i>	Actinomycetes	<i>Streptomyces</i> sp.	Sulphur oxidizing micro-organism	<i>Acidithiobacillus</i> sp.
1	-	-	A-1	-	-	-
2	-	-	A-2	-	-	-
3	-	-	A-3	RHR S-3	-	-
4	-	-	A-4	-	-	-
5	-	-	A-5	RHR S-5	-	-
6	-	-	A-6	RHR S-6	-	-
7	-	-	A-7	RHR S-7	-	-
8	-	-	A-8	-	-	-
9	-	-	A-9	-	-	-
10	-	-	A-10	RHR S-10	-	-
11	-	-	A-11	-	-	-
12	-	-	A-12	-	-	-
13	-	-	A-13	RHR S-13	-	-
14	-	-	A-14	-	-	-
15	FP-15	-	A-15	RHR S-15	SOM-15	-
16	FP-16	P-16	A-16	RHR S-16	-	-
17	-	-	A-17	RHR S-17	-	-
18	-	-	A-18	-	-	-
19	FP-19	-	A-19	-	-	-
20	-	-	A-20	-	SOM-20	-
21	-	-	A-21	RHR S-21	-	-
22	FP-22	P-22	A-22	-	-	-

Soil sample No.	Fluorescent Pseudomonads	<i>Pseudomonas fluorescens</i>	Actinomycetes	<i>Streptomyces</i> sp.	Sulphur oxidizing micro-organism	<i>Acidithiobacillus</i> sp.
23	-	-	A-23	RHR S-23	-	-
24	-	-	A-24	RHR S-24	-	-
25	-	-	A-25	RHR S-25	-	-
26	-	-	A-26	RHR S-26	-	-
27	-	-	-	-	SOM-27	-
28	-	-	A-28	RHR S-28	-	-
29	-	-	A-29	RHR S-29	-	-
30	-	-	A-30	-	-	-
31	FP-31	P-31	A-31	RHR S-31	-	-
32	-	-	A-32	-	-	-
33	FP-33	P-33	A-33	-	-	-
34	FP-34	-	A-34	-	-	-
35	FP-35	P-35	A-35	-	-	-
36	-	-	A-36	-	-	-
37	-	-	A-37	-	-	-
38	-	-	-	-	-	-
39	FP-39	P-39	A-39	RHR S-39	-	-
40	FP-40	P-40	A-40	RHR S-40	-	-
41	FP-41	P-41	A-41	RHR S-41	SOM-41	-
42	FP-42	P-42	A-42	-	-	-
43	FP-43	P-43	A-43	-	-	-
44	FP-44	P-44	A-44	-	SOM-44	SOB-44
45	-	-	A-45	-	-	-
46	-	-	A-46	-	-	-

Soil sample No.	Fluorescent Pseudomonads	<i>Pseudomonas fluorescens</i>	Actinomycetes	<i>Streptomyces</i> sp.	Sulphur oxidizing micro-organism	<i>Acidithiobacillus</i> sp.
48	-	-	A-48	RHR S-48	-	-
49	FP-49	P-49	A-49	-	-	-
50	FP-50	-	A-50	-	-	-
51	FP-51	P-51	A-51	-	-	-
52	FP-52	P-52	A-52	RHR S-52	-	-
53	-	-	A-53	RHR S-53	-	-
54	FP-54	-	-	-	-	-
55	FP-55	P-55	A-55	RHR S-55	-	-
56	FP-56	P-56	A-56	RHR S-56	-	-
57	FP-57	P-57	A-57	-	-	-
58	FP-58	-	A-58	RHR S-58	-	-
59	-	-	A-59	-	-	-
60	-	-	A-60	RHR S-60	-	-
61	-	-	-	-	-	-
62	-	-	A-62	RHR S-62	-	-
63	FP-63	-	A-63	-	-	-
64	-	-	A-64	RHR S-64	-	-
65	FP-65	-	A-65	RHR S-65	-	-
66	-	-	-	-	-	-
67	FP-67	-	A-67	RHR S-67	-	-
68	FP-68	-	-	-	SOM-68	-
69	-	-	-	-	-	-
70	-	-	-	-	SOM-70	-
71	-	-	A-71	-	-	-
72	-	-	-	-	SOM-72	SOB-72

Soil sample No.	Fluorescent Pseudomonads	<i>Pseudomonas fluorescens</i>	Actinomycetes	<i>Streptomyces</i> sp.	Sulphur oxidizing micro-organism	<i>Acidithiobacillus</i> sp.
73	-	-	A-73	RHR S-73	-	-
74	-	-	A-74	-	-	-
75	FP-75	-	A-75	-	-	-
76	-	-	A-76	RHR S-76	-	-
77	FP-77	P-77	-	-	-	-
78	-	-	A-78	-	-	-
79	-	-	A-79	-	-	-
80	FP-80	P-80	A-80	-	SOM-80	SOB-80
81	-	-	A-81	-	-	-
82	FP-82	-	A-82	RHR S-82	-	-
83	-	-	A-83	-	-	-
84	-	-	-	-	-	-
85	FP-85	P-85	A-85	RHR S-85	-	-
86	-	-	-	-	-	-
87	-	-	A-87	-	-	-
88	-	-	A-88	RHR S-88	-	-
89	-	-	A-89	-	-	-
90	FP-90	-	A-90	-	-	-
91	FP-91	P-91	A-91	-	-	-
92	FP-92	P-92	A-92	-	-	-
93	FP-93	-	A-93	RHR S-93	SOM-93	-
94	FP-94	-	A-94	-	-	-
95	FP-95	-	A-95	RHR S-95	-	-
96	FP-96	P-96	-	-	-	-
97	FP-97	-	A-97	RHR S-97	-	-

Soil sample No.	Fluorescent Pseudomonads	<i>Pseudomonas fluorescens</i>	Actinomycetes	<i>Streptomyces</i> sp.	Sulphur oxidizing micro-organism	<i>Acidithiobacillus</i> sp.
98	-	-	A-98	RHR S-98	-	-
99	-	-	A-99	-	-	-
100	FP-100	-	A-100	-	-	-
101	FP-101	-	A-101	RHR S-101	-	-
102	FP-102	-	A-102	RHR S-102	-	-
103	-	-	-	-	-	-
104	FP-104	-	A-104	RHR S-104	-	-
105	FP-105	P-105	A-105	RHR S-105	-	-
106	FP-106	P-106	A-106	-	-	-
107	-	-	A-107	-	-	-
108	-	-	A-108	RHR S-108	-	-
109	FP-109	-	A-109	RHR S-109	-	-
110	FP-110	P-110	A-110	RHR S-110	SOM-110	-
111	FP-111	-	A-111	RHR S-111	SOM-111	-
112	-	-	A-112	RHR S-112	-	-
113	FP-113	-	A-113	RHR S-113	SOM-113	SOB-113
114	-	-	A-114	-	-	-
115	-	-	A-115	RHR S-115	SOM-115	SOB-115
116	-	-	A-116	RHR S-116	-	-
117	-	-	-	-	-	-
118	FP-118	-	-	-	-	-
119	FP-119	P-119	A-119	-	-	-
120	FP-120	P-120	A-120	RHR S-120	-	-
121	FP-121	P-121	A-121	RHR S-121	-	-
122	-	-	A-122	-	-	-

Soil No.	Fluorescent Pseudomonads	<i>Pseudomonas fluorescens</i>	Actinomycet es	<i>Streptomyces</i> sp.	Sulphur oxidising mo	<i>Acidithiobacillus</i> sp.
123	-	-	A-123	RHR S-123	-	-
124	-	-	A-124	RHR S-124	-	-
125	-	-	A-125	-	-	-
126	-	-	A-126	-	-	-
127	-	-	A-127	RHR S-127	-	-
128	-	-	A-128	-	-	-
129	FP-129	-	A-129	RHR S-129	-	-
130	-	-	A-130	RHR S-130	SOM-130	SOB-130
131	FP-131	-	-	-	-	-
132	-	-	A-132	RHR S-132	SOM-132	-
133	-	-	A-133	RHR S-133	-	-
134	FP-134	P-134	A-134	RHR S-134	SOM-134	-
135	FP-	-	A-135	-	SOM-135	SOB-135
136	FP-136	P-136	A-136	RHR S-136	-	-
137	-	-	A-137	-	SOM-137	-
138	-	-	A-138	RHR S-138	-	-
139	FP-139	-	A-139	RHR S-139	-	-
140	-	-	A-140	-	-	-
141	-	-	A-141	RHR S-141	SOM-141	-
142	-	-	A-142	-	-	-
143	-	-	A-143	RHR S-143	-	-
144	-	-	A-144	RHR S-144	-	-
145	FP-145	P-145	A-145	-	-	-
146	FP-146	-	A-146	RHR S-146	SOM-146	SOB-146
147	-	-	A-147	RHR S-147	-	-
148	FP-148	-	A-148	RHR S-148	-	-
149	FP-149	P-149	-	-	-	-
150	FP-150	P-150	A-150	RHR S-150	SOM-150	-

9. VITA

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