

## Evaluation of Antagonistic Potential of Actinomycetes against Phytopathogenic Fungi

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### ABSTRACT

Present investigation was carried out to isolate actinomycetes from soil as biocontrol agent to control the growth of phytopathogenic fungi *Macrophomina phaseolina* and *Fusarium oxysporum*. Total 40 isolates were obtained from soils of various agricultural fields as well as waste dumping sites. Out of total 40, three isolates showed maximum inhibition of mycelium growth of phyto-pathogenic fungi viz. *M. phaseolina* and *F. oxysporum*. From morphological, biochemical and molecular characterization through 16S rDNA sequencing, isolates were identified as *Streptomyces* sp. strain AAUBC I 14 (NCBI Accn. No. MN577354), *Streptomyces asenjonii* AAUBC M 1 (NCBI Accn. No. MN577357) and *Streptomyces cavourensis* AAUBC M 14 (NCBI Accn. No. MN577358). All the three isolates were capable of producing antifungal metabolites like fungal cell wall degrading enzyme (protease, lipase, cellulase, chitinase and amylase) as well as secondary metabolites like salicylic acid, siderophore and volatile organic compounds in variable pattern. In vitro plant infection studies revealed that all the isolates were capable of restricting charcoal rot in soybean and *Fusarium* wilt in chickpea caused by *M. phaseolina* and *F. oxysporum*, respectively. All the results clearly indicates, actinomycetes isolates were capable of restricting growth of phytopathogenic fungi *M. phaseolina* and *F. oxysporum* causing charcoal rot and wilt, respectively and can be explored as potential biocontrol agent.

**Keywords:** Biocontrol, Actinomycetes, *Macrophomina*, *Fusarium*, Enzymes, Secondary metabolites.

### INTRODUCTION

At present, more than sixty percent of the world's population mainly rely on agriculture for their survival. However, the growth and

production of most important agricultural crops continue to be hampered because of various abiotic and biotic factors (Elumalai & Rengasamy, 2012).

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A major concern to worldwide agricultural production is fungal plant diseases, which reduce yield up to 25% in western countries, and up to 50% in developing countries (Gohel et al., 2006; & Evangelista-Martinez, 2014). The ever-increasing demand for enhanced food production, due to a rapidly increasing population in 21<sup>st</sup> century, has pressed the need for controlling plant diseases in agriculture (Emmert & Handelsman, 1999). For reducing the infection of pathogen and good crop production, farmers depends on fungicides and pesticides. Such agro-chemicals have significantly contributed to reduction in disease incidence during past many decades.

Many of the chemical pesticides cause serious side effects for the environment and human health. The main reason of these harmful effects is the extensive use of these chemical pesticides which are used to kill the phytopathogens. Thus, it is an urgent need to search eco-friendly and safe alternative of chemical pesticides.

Actinomycetes, are aerobic spore producing gram positive bacteria with high GC content (55%). The actinomycetes have both fungal and bacterial characteristics. Actinomycetes having potential to produce various antagonistic compounds for getting reliable control of phytopathogens are relatively less explored as biocontrol agents. Actinomycetes especially those belonging to genus *Streptomyces sp.*, inhibit several phytopathogens therefore actinomycetes hold a prominent place due to their antimicrobial diversity and ability to produce new compounds. The well-known fact is that *Streptomyces* species produce wide spectrum of antibiotics, bioactive compounds and screened for new biological drugs.

The actinomycetes cover a high proportion of microbial biomass and capable to produce a wide range of antibiotic and enzyme like products. Actinomycetes are found widely in the soil as well as in rhizosphere so they can protect roots of plants by inhibiting the development of fungal infection in roots. Due to this action, plant is protected by fungal infection. This is only

because of production of any bioactive compound which is present in the actinomycetes like enzyme which can degrade the cell wall of fungi and known as antifungal compound (Khamna et al., 2009).

Actinomycetes derived enzymes such as chitinase, proteinase, xylanase and cellulase are also known as antifungal agent. Several researchers have proved that actinomycetes is a promising group for the isolation of new bioactive compound especially antifungal compounds which protect the plant from a high degree infection. This is the most appropriate reason that the scientists are still interested in the actinomycetes as an agent to control the fungal infection of mostly plants and crops (Sowndhararajan & Kang, 2012).

In this present study, actinomycetes isolated from soils of various agricultural fields and waste dumping sites were characterized and evaluated for biocontrol strategies to inhibit the growth of phytopathogenic fungi viz. *Macrophomina phaseolina* and *Fusarium oxysporum*.

## MATERIALS AND METHODS

### Standard strain used in study

Standard fungus strain of *M. phaseolina* and *F. oxysporum* were obtained from Department of Agricultural Microbiology and Biofertilizer Projects, B.A. College of Agriculture, Anand Agricultural University, Anand.

### Isolation of Actinomycetes from cultivated soil

#### Collection of soil samples

Different soil samples were collected from different sites of Agricultural field viz. experimental fields of Anand agricultural university, dumping site behind khodiyar mata temple, Anand. Soil samples were collected in sterile labeled plastic bags by using clean spatula and stored at 4°C temperature for further use.

#### Isolation of Actinomycetes

All the collected soil samples were air dried at room temperature before isolation and subjected to serial dilution agar plate method in which 10 gram of soil sample was weighed and suspended in 90 ml of sterile distilled

water. The suspension was properly mixed on the orbital shaker for 30 minutes at 150 rpm and isolation was performed by serial dilution and spreading method on Glycine Asparagine Agar (GAA) media consisting of antifungal agent cyclohexamide (50µg/ml). After inoculation the plates were incubated at 28±2°C for 24-48 hours. Single colony showing characteristics of actinomycetes were picked up and purified on nutrient agar plates using streak plate method.

### Screening of actinomycetes for antifungal activity

The actinomycetes isolates were further screened against two fungal plant pathogens

*M. phaseolina* and *F. oxysporum* by dual culture method. Each fungal pathogen was grown on a Potato Dextrose Agar (PDA) plate till it covered the whole surface of agar plate. With the help of sterile cup borer, a disc agar having fungal growth from plate was taken and placed on dual culture plate. Test culture suspension (50µl) was inoculated in the well 3.5 cm away from fungal disc and kept for incubation at 28±2°C for 7 days. Inhibition of fungal growth was recorded at 5<sup>th</sup> and 7<sup>th</sup> days after incubation and compared with fungal growth in control plates. The radial growth of mycelium was measured and percent inhibition (PI) was calculated as follows.

$$\text{Percent inhibition (PI)} = (C-T)/C \times 100$$

Where, C = the growth of pathogen (cm) in the absence of antagonistic isolate.

T = the growth of pathogen (cm) in the presence of antagonistic isolate.

### Characterization of the potential actinomycete isolates

Selected isolates were characterized on the basis of morphological, biochemical and molecular characteristics using Burgey's manual of Systematic Bacteriology (Williams et al., 1989).

#### Morphological characterization

Morphological characteristics of actinomycete isolates were recorded by two means after obtaining pure cultures on nutrient agar medium:

- 1.) Cultural characteristics of microorganisms viz. size, shape, elevation, margin, texture, opacity, and color were studied.
- 2.) Microscopic characteristics viz. size, shape, arrangement and Gram's reaction were studied.

#### Molecular characterization

Three actinomycetes isolates were grown in Luria broth for 24 h, and genomic DNA was extracted by CTAB method. The integrity and concentration of purified DNA was determined by agarose gel electrophoresis. The total genomic DNA extracted was dissolved in sterile distilled water and stored at 4°C. Primers for 16S *rRNA* genes were selected from standard scientific literature (Perez et al., 2007; & Mavrodi et al., 2001). Sequences of

the primers used to amplify and detect 16S *rRNA* genes are "27 F-AGAGTTTGATCCTGGCTCAG and 1492 R-GGTTACCTTGTTACGACTT". The oligonucleotides were synthesized at MWG Bio-tech Pvt. Ltd., Germany. Each pair of primers was highly specific and gave a PCR product of known size that was easily identified by electrophoresis on agarose gel.

#### DNA sequencing

Partial 16S *rRNA* gene sequencing was carried out for promising isolates' and was performed using the ABI PRISM® BigDye™ Terminator cycle sequencing kit on the ABI PRISM 3100 genetic analyser. The 16S *rRNA* gene sequences were assembled using MEGA 4 software, compared with other strains using NCBI BLAST analysis for identification purpose and comparison of homologies of isolated strains with previously characterized strains.

#### Antifungal metabolite production by isolates

##### Production of fungal cell wall degrading enzymes

##### Amylase

Amylase production was determined by using 1% starch agar plates. The isolates were spot inoculated and incubated at 28±2°C for 2 days.

After incubation, flood the plates with iodine solution for 30 sec and pour of the excess iodine. Clear zone around the colonies indicates the presence of amylolytic activity.

#### **Protease**

Protease production was determined using milk casein agar. The isolates were spot inoculated on milk casein agar and incubated for 2 days at 28±2°C. Proteolytic activities were identified by appearance of clear zone surrounding their growth.

#### **Lipase**

Lipolytic activity was determined by spot inoculated isolates on Tributyrin agar plates (Lawrence et al., 1967).

#### **Cellulase**

Cellulase activity was determined using cellulose agar plate and the isolates were spot inoculated on plate and after incubation, zone of clearance around colony indicates positive for cellulolytic activity.

#### **Chitinase**

Chitinase activity was done using colloidal chitin agar. The isolates were spot inoculated on plates. All the plates were incubated at 28±2°C for 2 days. Isolates showing clear zone around the colony considered as chitinolytic activity.

#### **Production of secondary metabolites**

##### **Salicylic acid production**

For determination of salicylic production, the strain were grown in the succinate medium at 28±2°C for 48 h. Cells were collected by centrifugation at 10,000 g for 5 minutes. One ml of cell free culture filtrate was acidified with 1N HCL to pH 2.0 and salicylic acid was extracted with equal volume of chloroform. Two ml of 2M FeCl<sub>3</sub> was added to the pooled chloroform phases. The absorbance of purple ion salicylic acid complex developed in aqueous phase, was read at 527nm. A standard curve prepared with salicylic acid in succinate medium and quantify of salicylic acid produced was expressed as µg/ml.

##### **Siderophore production**

###### **Catecholate nature**

FeCl<sub>3</sub>Test – To 0.5 ml of cell free culture suspension was added from nutrient broth to 0.5 ml of 2% aqueous FeCl<sub>3</sub> solution.

Appearance of orange or red wine colour indicated the presence of Catecholate nature of siderophore.

###### **Hydroxamate nature**

Tetrazolium salt test – to a pinch of tetrazolium salt, was added 1-2 drops of 2 N NaOH and 0.1 ml of the test culture supernatant. Instant appearance of a red to dip red color indicated the presence of hydroxamate siderophores.

###### **Volatile organic compound production**

To determine antifungal role of bacterial volatiles, an assay was carried out in two compartmentalized petridish. One compartment of petridish was filled with sterile nutrient agar medium and another with PDA medium. The compartment containing nutrient agar medium was streaked with actinomycete strains, whereas compartment filled with PDA medium was inoculated with 3 mm plug of fungal pathogen previously grown on PDA plates. After inoculation the petri dishes were sealed tightly to avoid escape of volatiles and incubated at 28±2°C in an incubator and the plates were observed for inhibition of fungal growth (mycelial or sporulation), if any. Control plates received fungal inoculation but was not inoculated with bacteria.

###### **In vitro efficacy of selected isolates for biocontrol of fungal pathogens**

The promising isolates were further tested for their efficacy for biocontrol of *F.oxysporum* and *M. phaseolina* in chickpea and soybean, respectively under *in vitro* conditions. The *In vitro* efficacy of isolates was tested on solid water agar (1 % agar) in tubes. Seeds were surface sterilized by treatment with 0.5% HgCl<sub>2</sub> for 30 sec (three times) followed by wash with sterilized distilled water.

One set of treatment received simultaneous inoculation of fungal pathogens and actinomycetes suspension (50 µl) keeping uninoculated controls. Another set receives sequential application, actinomycetes culture (50 µl) by seed treatment followed by fungal inoculation (50 µl) 5 days after inoculation of actinomycetes. The tubes were observed daily for disease development up to 15 days.

## RESULTS AND DISCUSSION

### Isolation of actinomycetes

Total 10 soil samples were collected from different area of agriculture fields and dumping sites. Total 40 actinomycetes were isolated on Glycine Asparagine Agar media consisting of antifungal agent cyclohexamide (50µg/ml) by serial dilution method. Out of 40 actinomycetes, 28 cultures were isolated from agriculture fields while 12 cultures were obtained from dumping sites. The colonies showing typical characteristics of the actinomycetes like forming branched network of hyphae and sexual spores were selected, purified and stored at 4°C temperature in refrigerator for further studies.

### Screening of actinomycetes for antifungal activity

Screening of all the isolates for their anti-pathogenic activity against soil borne fungal pathogens viz. *M. phaseolina* and *F. oxysporum* was performed by dual culture method. All isolates under the test were screened by their potentiality to check the mycelial growth of soil borne fungal pathogens *M. phaseolina* and *F. oxysporum*. After 7 days of incubation the mycelial growth was measured and the inhibition of mycelial growth due to presence of antagonistic bacteria was recorded as percentage inhibition.

Data in **Table 1** shows that isolate I 14 was found significantly superior as compared to other isolates showing maximum inhibition of mycelial growth i.e. 50% followed by isolate M 1 48% inhibition and isolate M 14 showing 44% of inhibition of *M. phaseolina*. Similarly Isolates M 1, M 14 and I 14 showed 48.89 %, 44.44 % and 35.56 % inhibition of mycelial growth of *F. oxysporum*, respectively (**Figure 1**).

Microorganisms have tendency of antagonism for their survival in specific niche. Phytopathogenic fungi are major threat for crop production which could be inhibited by the antagonistic microbes present in the same habitat which ultimately proved to be beneficial for plant health. Gopalakrishnan et al. (2011) isolated 137 actinomycetes cultures, from 25 different herbal vermicomposts, and

screened out them on the basis of their antagonistic potential against *F. oxysporum* f. sp. ciceri by dual-culture assay.

### Morphological characterization

Colonial characteristics of all the three isolates were studied by growing them on NA plate and observed after 72 h of incubation. All the isolates showed variable growth patterns on NA as narrated in **Table 2**. All the isolates were also found to produce spores and earthy odor. Further the isolates were subjected to the microscopic characterization through wet mount and Gram's staining. Microscopic examination revealed that all the isolates were Gram positive appear as filamentous growth and produced spores which is the characteristic feature of the actinomycetes. *Streptomyces* are gram-positive found predominantly in soil and decaying vegetation, mainly streptomycetes produce spores and are noted for their distinct "earthy" odour which results from production of a volatile metabolite, geosmin (Madigan & Martinko 2005).

### Molecular characterization

#### 16S rRNA gene sequencing and identification of promising isolates

16S rRNA partial gene sequence of ~ 1500 bp was carried out (with technical support of Eurofins genomics, Bangalore) and the output data were stored in FASTA format. The output sequences were subjected for BLAST (Basic Local Alignment Search Tool) analysis to identify the cultures and to find out the nearest match of the cultures (<http://www.ncbi.nlm.nih.gov/>). The sequence analysis of partial 16S rRNA gene of isolate I 14, M 1 and M 14 have been deposited in NCBI, GenBank under accession numbers MN577354, MN577357 and MN577358 respectively. The analysis named isolate I 14 as *Streptomyces* sp. strain AAUBC I 14, Isolate M 1 as *Streptomyces asenjonii* AAUBC M 1 and Isolate M 14 as *Streptomyces cavourensis* AAUBC M 14 (**Figure 2 to 4**).

Molecular identification of the actinomycetes is done by the nucleic acid sequencing method. The sequence of 16S ribosomal DNA is being used by various researchers for accurate identification of the

actinomycetes up to genus level. With help of the 16S rDNA sequences phylogenetic relationship of different actinomycetes can be confirmed by Jami et al. (2015).

### **Antifungal metabolite production by isolates**

#### **Production of fungal cell wall degrading enzymes**

Metabolites like hydrolytic enzymes viz. amylase, protease, lipase, cellulase, and chitinase secreted by many microorganisms helps in degradation of the fungal cell wall and thereby restrict their growth. Moreover, secondary metabolites like siderophores, salicylic acid and volatile compounds also help to inhibit fungal growth and thereby limit the pathogen attack to the crops.

Present study shows that all selected isolates were capable of producing amylase, protease, lipase, cellulase, and chitinase enzymes. (Table 3) secretion of these enzymes by isolates can result in the suppression of plant pathogen activity.

Thus, they play a vital role in the biological control of many plant diseases by degrading the cell walls of phytopathogens. It affects fungal growth by its lytic action on cell walls, hyphal tips, and germ tubes (Kim et al., 2003; & Sayyed et al., 2015) and partial swelling in the hyphae and at the hyphal tip leading to hyphal curling or bursting of the hyphal tip (Someya et al., 2000).

#### **Production of secondary metabolites**

All three isolates were capable of produced salicylic acid in succinate media. M 14 showed the highest salicylic acid production i.e. 50 µg/ml followed by M 1 (34.04 µg/ml) and I 14 (10.00 µg/ml) (Table 4).

Salicylic acid, a phenolic compound, is getting attention as plant stress regulator and induced both local and systemic plant defence response. It plays an important role in the regulation of plant growth and inhibits the growth of pathogen.

Biological control of soil borne pathogens is often attributed to improved nutrition that boosts host defences or to direct inhibition of pathogen growth and activity. Amendment with certain abiotic factors

(inducers) appears to stimulate the disease resistance by indirectly stimulating indigenous populations of microorganism that are beneficial to plant growth and antagonistic to pathogen.

Many studies have indicated that salicylic acid (SA) plays an important role in plant defence response against pathogen attack and is essential for the development of SAR (Ryals et al., 1996; & Yalpani et al., 1993). Ward et al. (1991) found that this increased resistance was correlated with accumulation of pathogenesis-related (PR) proteins, which are generally considered to be markers of SAR.

Exogenous application of SA or its functional analogs, such as 2, 6-dichloroisonicotonic acid and benzo- (1, 2, 3) -thiadiazole-7-carbothioic acid S-methyl ester (BTH), induces SAR in plants, resulting in resistance to certain pathogens (Ryals et al., 1996).

#### **Production of siderophores**

##### **FeCl<sub>3</sub> test for catecholate type of siderophore**

During the present study, all isolates failed to produce catecholate type of siderophore. As started by Neilands (1981) absence of wine color proves absence of catecholate type of siderophore in sample.

##### **Tetrazolium test for hydroxamate type of siderophore**

The test is based on capacity of hydroxamic acids to reduce tetrazolium salt by hydrolysis of hydroxamate groups using strong alkali. The reduction and release of alkali shows a deep red color. Tetrazolium test was carried out to find out presence of hydroxamate type of siderophore. All isolates produced hydroxamate type of siderophore because of instant appearance of deep red color in tubes. Similar results have been reported by Dave and Dube (2000).

The antagonism depends on the amount of iron available in the medium, siderophore produced by a biocontrol agent and sensitivity of target pathogens. Siderophore producing rhizobacteria improve plant health at various levels: they improve iron nutrition, inhibit growth of other

microorganisms with release of their antibiotic molecule and hinder the growth of pathogens by limiting the iron available for the pathogen, generally fungi, which are unable to absorb the iron siderophore complex.

This finding is in agreement with Nimnoi et al. (2010) who reported that *Pseudonocardia halophobica* isolated from roots of *Aquilaria crassna* exhibited high ability for siderophore production and produced 39.30µg/ml. Khamna et al. (2009) also reported that *Streptomyces* CMU-SK 126 isolated from *Curcuma manggarhizos* spheric soil exhibited high ability for siderophore production and produced catechol type (16.19µg/ml) as well as hydroxamate type (54.9µg/ml) siderophores.

#### **Production of volatile compounds**

In addition to diffusible substances, bacteria emit a wide range of volatile compounds into the atmosphere. Over the last ten years, evidence has been accumulated that these volatiles are not only able to promote plant growth, but also strongly inhibit fungal growth. Production of volatile compounds by biocontrol agents is one of the mechanisms to bring about inhibition of fungal pathogens. To evaluate the role of volatile compounds produced by all the individual isolates for inhibition of phytopathogenic fungi, dual culture assay in two compartmentalized petridish was carried out. Here, due to compartments there was no possibility of direct contact of microorganism and pathogens and thereby inhibition which we observed may solely due to effect of volatile compounds produced by microbes.

Data indicated that, all the individual strains showed inhibition of *M. phaseolina* and *F. oxysporum*. Major impact is seen on the sporulation efficiency of the fungi as there was reduction in fungal sporulation observed in the plates which received bacterial inoculation as compared to uninoculated control indicating the impact of volatile compounds on sporulation efficiency of the fungi which in turn affects the proliferation of fungal pathogens by inhibiting its reproduction (Figure 5).

#### **In vitro efficacy of selected isolates for biocontrol of fungal pathogens**

Charcoal rot caused by the fungus, *M. phaseolina*, have emerged as serious concern for cultivation of soybean. *M. phaseolina* causes huge annual losses to the crop and can survive in the soil mainly as microsclerotia for 2 years or longer and; germinate repeatedly during the crop-growing season. The pathogen generally attacks the young plants of the soybean. The results of the study clearly indicate that all the isolates were capable of inhibiting the growth of *M. phaseolina* when inoculated simultaneously. The plants showed better root and shoot development as well as no disease symptoms of *M. phaseolina* infection up to 15 days (Figure 6A). The plants inoculated with isolates and fungi simultaneously were as healthy as the uninoculated control plants which do not receive inoculation of the fungi. These results prove that all the isolates were capable of restricting growth of the *M. phaseolina* and thereby prevent disease outbreak in soybean plants.

Fusarium wilt caused by *F. oxysporum* is the major soil-borne fungus affecting chickpeas globally. Fusarium wilt epidemics can devastate crops and cause up to 100% loss in highly infested fields and under favourable conditions. The fungus can be transmitted by seed and may survive in plant debris in soil. It was demonstrated that the fungus chlamydospore was found free in soil, in the seed, in cotyledons and axis. Results of present study indicated that tubes receiving inoculation of isolates do not show any disease symptoms up to 15 days after inoculation and plants were found healthy as compared to the tubes which received fungal inoculation alone (Figure 6B).

The overall results of the *in vitro* biocontrol studies indicated that, all the isolates were capable of restricting the disease development by *M. phaseolina* and *F. oxysporum* in soybean and chick pea, respectively.

**Table 1: Screening of isolates against plant pathogenic fungi**

Sr. No.	Isolates	% growth inhibition of test pathogenic fungi	
		<i>M. phaseolina</i>	<i>F. oxysporum</i>
1	I1	38.00	-
2	I2	36.00	-
3	I3	-	-
4	I4	-	-
5	I5	-	-
6	I6	34.00	17.78
7	I7	-	-
8	I8	-	31.11
9	I9	38.00	13.33
10	I10	42.00	-
11	I11	-	-
12	I12	-	-
13	I13	-	33.33
14	I14	50.00	35.56
15	I15	-	-
16	I16	-	-
17	I17	-	-
18	I18	-	-
19	M1	48.00	48.89
20	M2	-	-
21	M3	-	28.89
22	M4	36.00	35.56
23	M5	40.00	33.33
24	M6	42.00	-
25	M7	40.00	-
26	M8	36.00	31.11
27	M9	-	-
28	M10	-	26.67
29	M11	-	-
30	M12	-	-
31	M13	40.00	33.33
32	M14	44.00	44.44
33	M15	-	-
34	M16	-	-
35	M17	-	-
36	M18	-	-
37	M19	38.00	-
38	M20	36.00	-
39	M21	40.00	-
40	M22	36.00	-

**Table 2: Colonial characterization of isolates on Nutrient agar plates**

Isolates	I 14	M 1	M 14
Size	Medium	Medium	Medium
Shape	Round	Round	Round
Margin	Rough	Rough	Rough
Elevation	Umbilicate	Umbilicate	Umbilicate
Texture	Dry	Dry	Dry
Opacity	Cretaceous	Cretaceous	Cretaceous
Pigment	White	Gray	Brownish white
Odor	Earthy	Earthy	Earthy

**Table 3: Enzyme activity of isolates**

Sr.No.	Isolates	Amylase	Protease	Lipase	Cellulase	Pectinase	Chitinase
1	I14	+	+	+	+	+	+
2	M1	+	+	+	+	+	+
3	M14	+	+	+	+	+	+

**Table 4: Production of salicylic acid by isolates**

Isolates	Concentration (µg/ml)
I 14	10.00
M 1	34.04
M14	50.00



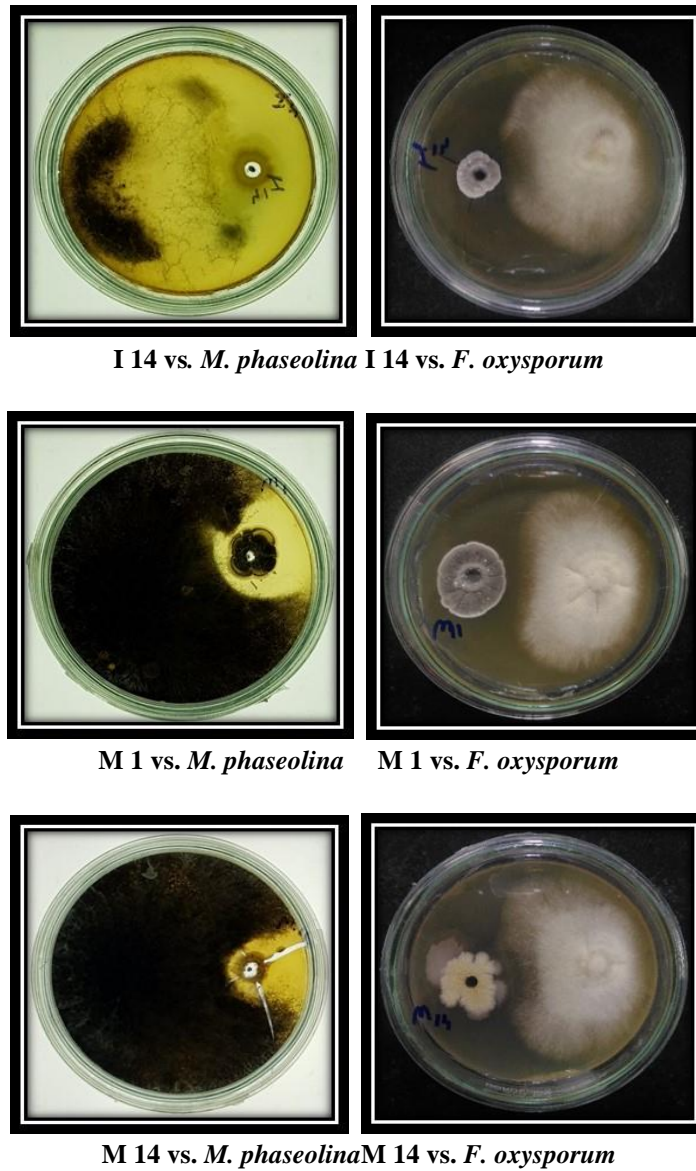


Figure 1: *In vitro* antifungal activity of isolates against plant pathogenic fungi

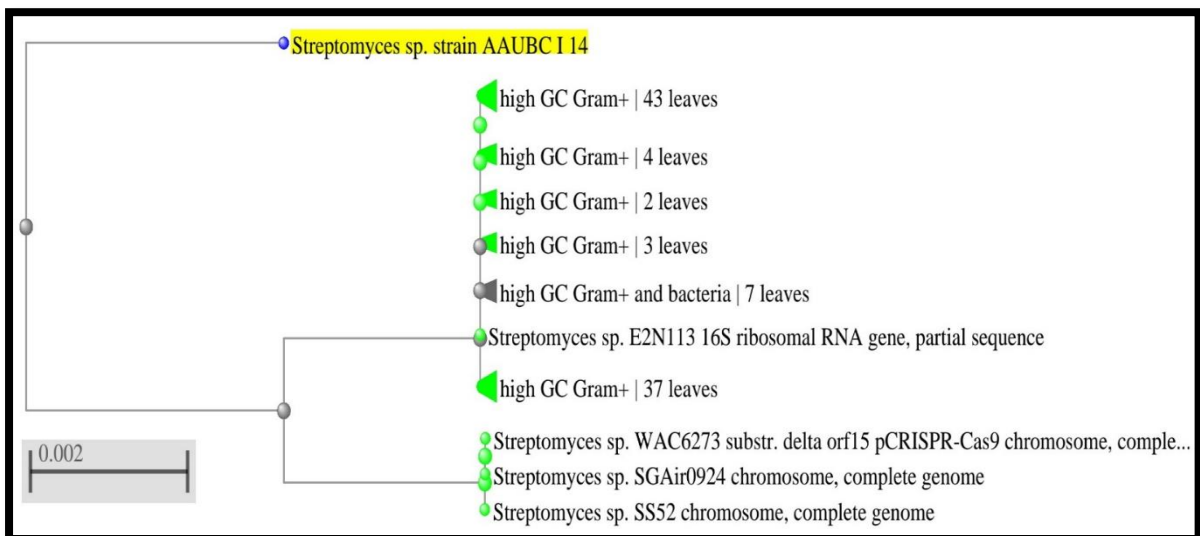


Figure 2: Phylogenetic tree based on 16S rDNA sequence of *Streptomyces* sp. strain AAUBC I 14

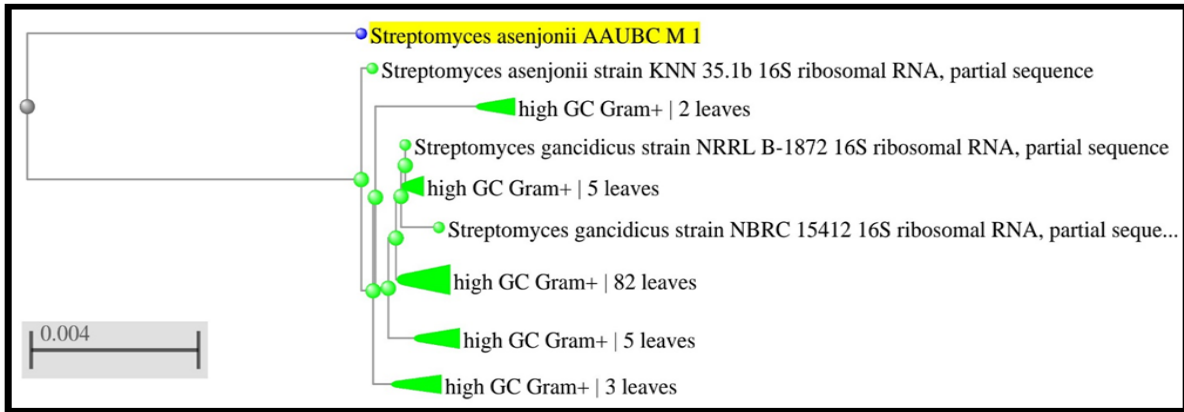


Figure 3: Phylogenetic tree based on 16S rDNA sequence of *Streptomyces asenjonii* AAUBC M 1

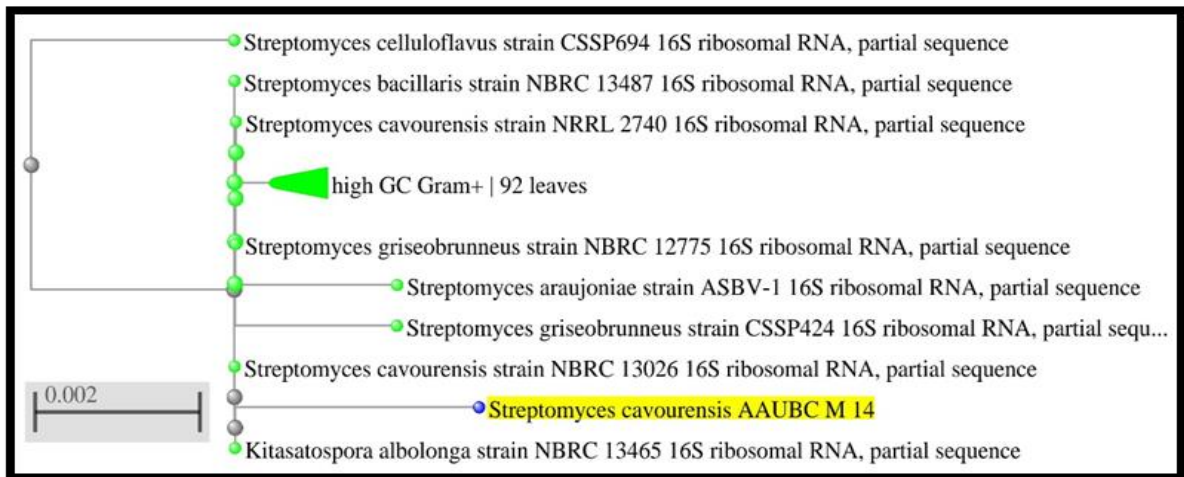


Figure 4: Phylogenetic tree based on 16S rDNA sequence of *Streptomyces cavourensis* AAUBC M 14

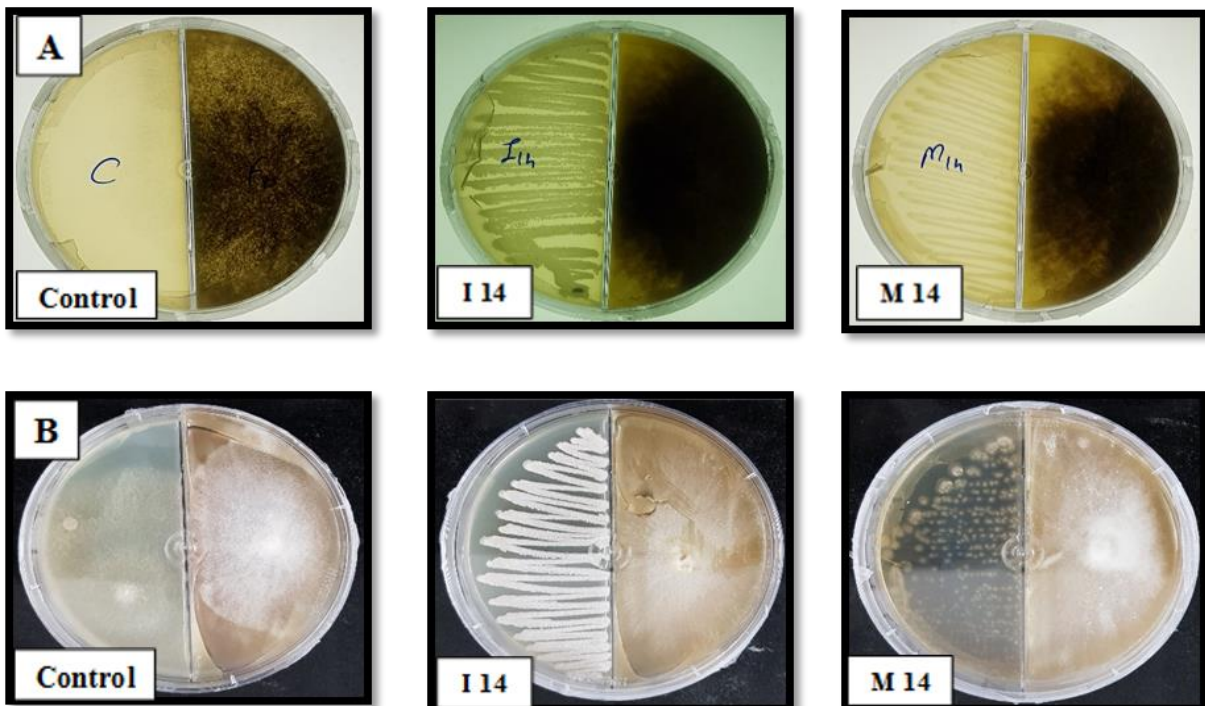
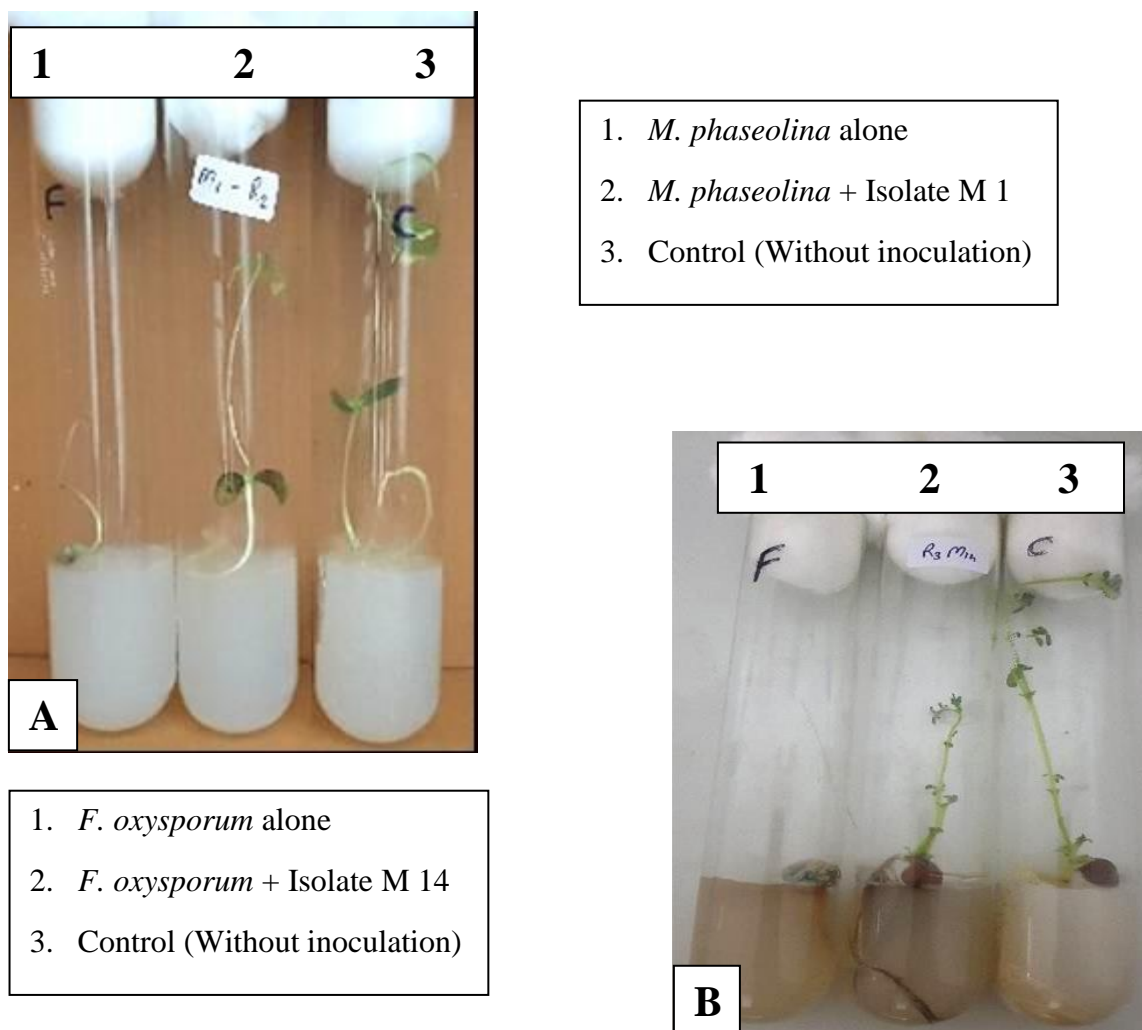


Figure 5: Reduction in fungal sporulation due to the production of volatile compound by isolates  
A. *M. phaseolina* B. *F. oxysporum*



**Figure 6: *In vitro* efficacy of selected isolates for biocontrol of fungal pathogens**

**A. *M. phaseolina* in soybean**

**B. *F. oxysporum* in chickpea**

### CONCLUSION

In this study, the selected actinomycetes, *Streptomyces* sp., *Streptomyces asenjonii* and *Streptomyces cavourensis* were showed good antagonistic property in suppressing the mycelial growth of both fungi *M. phaseolina* and *F. oxysporum*. Hence, further investigation with these potential isolates may help to control charcoal rot in soybean and Fusarium wilt in chickpea caused by *M. phaseolina* and *F. oxysporum* respectively. Thus, it can be concluded from results of present investigation, actinomycetes isolates belonging to the genus *Streptomyces* can be explored as potential source of novel biological control agent.

### REFERENCES

- Dave, B. P., & Dube, H. C. (2000). Regulation of siderophore production by iron Fe (III) in certain fungi and fluorescent pseudomonads. *Ind. J. Exp. Biol.*, 38(3), 297-9.
- Elumalai, L. K., & Rengasamy, R. (2012). Synergistic effect of seaweed manure and *Bacillus* sp. on growth and biochemical constituents of *Vigna radiata* L. *J. Biofertil. Biopest.*, 3, 121-128.
- Emmert, E. A. B., & Handelsman, J. (1999). Biocontrol of plant disease: A (Gram-) positive perspective. *FEMS Microbiol. Lett.*, 171, 1-9.

- Evangelista-Martínez, Z. (2014). Isolation and characterization of soil *Streptomyces* species as potential biological control agents against fungal plant pathogens. *World J. Microbiol. Biotechnol.*, 30(5), 1639–1647.
- Gohel, V., Singh, A., Vimal, M., Ashwini, P., & Chhatpar, H. S. (2006). Bioprospecting and antifungal potential of chitinolytic microorganisms. *Afr. J. Biotechnol.*, 5, 54-72.
- Gopalakrishnan, S., Kiran, B. K., Humayun, P., Vidya, M. S., Deepthi, K., Jacob, S., Vadlamudi, S., Alekhya, G., & Rupela, O. (2013). Biocontrol of charcoal-rot of sorghum by actinomycetes isolated from herbal vermicompost. *Afr. J. Biotechnol.*, 10(79), 18142-1815.
- <http://www.ncbi.nlm.nih.gov/>
- Jami, M., Ghanbari, N., Kneifel, W., & Domig, K. J. (2015). Phylogenetic diversity and biological activity of culturable Actinobacteria isolated from freshwater fish gut microbiota. *Microbiol. Res.*, 175, 6-15.
- Khamna, S., Yokota, A., & Lumyong, S. (2009). Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compound, indole-3-acetic acid and siderophore production. *World J. Microbiol. Biotechnol.*, 25, 649-655.
- Khamna, S., Yokota, A., & Lumyong, S. (2009). Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compound, indole-3-acetic acid and siderophore production. *World J. Microbiol. Biotechnol.*, 25, 649-655.
- Kim, K. J., Yang, Y. J., & Kim, J. G. (2003). Purification and characterization of Chitinase from *Streptomyces* sp. M-20. *J. Biochem. Mol. Biol.*, 36(2), 185–189.
- Lawrence, R. C., Fryer, T. F., & Reiter, B. (1967). Rapid method for quantitative estimation of microbial lipase. *Nature* (Lond.), 213, 1264–1265.
- Madigan, M., & Martinko, J. (2005). Brock Biology of Microorganisms. 11<sup>th</sup> Edn., prentice Hall, New Jersey, USA.
- Mavrodi, O. V., McSpadden Gardener, B. B., Mavrodi, D. V., Bonsall, R. F., Weller, D. M., & Thomashow, L. S. (2001). Genetic diversity of pHLD from 2, 4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. *Phytopathology*, 91(1), 35-43.
- Neilands, J. B. (1981). Microbial iron transport compounds (siderophores) as chelating agents. *Development of Iron Chelators for Clinical Use. New York: Elsevier/North Holland*, 13-31.
- Nimnoi, P., Pongsilp, N., & Lumyong, S. (2010). Endophytic actinomycetes isolated from *Aquilaria crassna* Pierre ex Lec and screening of plant growth promoters production. *World J. Microbiol. & Biotechnol.*, 26(2), 193-203.
- Perez, E., Sulbaran, M., Ball, M., & Yarzabal, L. A. (2007). Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biol. Biochem.*, 39, 2905–2914.
- Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H. Y., & Hunt, M. D. (1996). Systemic acquired resistance. *Plant Cell*, 8, 1809–1819.
- Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H. Y., & Hunt, M. D. (1996). Systemic acquired resistance. *Plant Cell*, 8, 1809–1819.
- Sayyed, R. Z., Patel, P. R., & Shaikh, S. S. (2015). Plant growth promotion and root colonization by EPS producing *Enterobacter* sp. RZS5 under heavy metal contaminated soil. *Ind. J. Exp. Biol.*, 53, 116–123.
- Someya, N., Kataoka, N., Komagata, T., Hirayae, K., Hibi, T., & Akutsu, K.

- (2000). Biological control of cyclamen soil borne diseases by *Serratia marcescens* strain B2. *Plant Dis.*, 84, 334–340.
- Sowndhararajan, K., & Kang, S. C. (2012). In vitro antagonistic potential of *Streptomyces* sp. AMS1 against plant and human pathogens. *J. Agricul. Chem. Environ.*, 1(1), 41-47.
- Ward, E. R., Uknes, S. J., Williams, S. C., Dincher, S. S., Wiederhold, D. L., Alexander, D. C., Ahl-Goy, P., Meetraux, J. P., & Ryals, J. A. (1991). Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell*, 3, 1085–1094.
- Williams, S. T., Goodfellow, M., & Alderson, G. (1989). Genus *Streptomyces* Waksman and Henrici 1943.339AL. In: Bergey's Manual of Systematic Bacteriology. *Williams & Wilkins Company, Baltimore*, 4, 2452-2492.
- Yalpani, N., Silverman, P., Wilson, T. M. A., Kleier, D. A., & Raskin, I. (1991). Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. *Plant Cell*, 3, 809–818.