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Endophytic Actinomycetes from Indian Medicinal Plants as Antagonists to Some Phytopathogenic Fungi

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Abstract

The present study is focused on the antifungal activities of 45 endophytic actinomycetes that were recovered from 4 medicinal plants from India (Tamilnadu). *In vitro* antifungal activity was determined using the dual culture bioassay against four fungal phytopathogens, *Fusarium solani, Phytophthora infestans, Macrophomina phaseolina, Rhytisma acerinum* and *Botrytis cinerea*, which are the causative agents of tomato's wilt, potato's late blight, soybean's charcoal rot, tar spot disease and eggplant fruit rot, respectively. Out of the 47 isolates, *Streptomyces sp. 5* and *Streptomyces sp.7* were prolific producers of fungal inhibitory bioactive compounds. Both isolates were investigated for the production of antifungal diffusible compounds, volatile compounds and enzymes (chitinase, cellulose, and CMCase). They showed *in vitro* activity against the five previously mentioned fungal pathogens as well as another pathogen (*Fusarium oxysporum*), with a probability of $p<0.05$. The actinomycete isolates employed various mechanisms for inhibiting the pathogens. The competitive growth between pathogens and antagonists on solid media revealed lesser suppression, comparing to that recorded for the diffusates on agar. Additionally, greater antagonistic effects were recorded for crude metabolites, membrane diffusible metabolite(s), and volatiles, respectively. However, both actinomycete isolates showing high ability to produce chitinases and cellulases. The potential of using *Streptomyces sp. 5* and *Streptomyces sp.7* as phytopathogen biocontrol agents was discussed.

Keywords: Endophytic actinomycetes; Antimicrobial activity; Indian Medicinal plants; Phytopathogenic fungi

Introduction

Endophytic microorganisms are microbes that colonized inside plant tissues with symptom less to their hosts and without causing immediate overt negative effect [1]. As a result of these long-held associations, endophytic microorganisms and plants have developed better information transfer; leading to the hypothesis that plants with an ethnobotanical history are more potent sources of endophytes producing active natural products than other plants [2,3].

Endophytic actinomycetes are particularly, considered as potential sources of bioactive compounds and various novel compounds [4]. Actinomycetes are the main source of antibiotics and endophytic actinomycetes isolated from medicinal plants has considerable development potential. From the present findings, some new actinomycetes from tissue of medicinal plants have been found constantly [5-8]. Moreover, most endophytic actinomycetes of medicinal plants can produce important compounds and some of them are new chemical structure [9-11]. The actinomycetes, especially *Streptomyces* species are valuable economical and biotechnological bacteria by providing over two third of antibiotics and bioactive compounds used these days [12]. Endophytic actinomycetes which associated with plants also play important role in protecting their host from phytopathogenic invasions.

Previous investigations proved that the endophytic actinomycetes are having high ability to inhibit phytopathogenic fungi is mainly by production of bioactive compounds, such as antibiotics and cell wall degrading enzymes and highlighted their importance as candidates for further investigation in the biocontrol of phytopathogens. The ability of endophytic actinomycetes to inhibit phytopathogenic fungi is mainly by production of bioactive compounds, such as antibiotics and cell wall degrading enzymes. In addition, endophytes are known to compete phytopathogens for nutrients [13]. Endophytic actinomycetes were also reported to hold the ability of triggering plant induced systemic resistance (ISR) [14].

The present study aimed to screen the endophytic actinomycetes from Indian medicinal plants and to identify their *in vitro* potential as biocontrol agents for selected phytopathogenic fungi.

Materials and Methods

Sample collections

Healthy leaves and roots of five different medicinal plants (*Phyllanthus niruri, Withania somnifera, Catharanthus roseus and Hemidesmus indicus*) were collected from Melpuram at EDU-TECH Educational and Research Institute Campus and Malankara Catholic College garden. Plant samples were kept in plastic bags and stored at 4°C until isolation.

Isolation of endophytic actinomycetes

Plant materials were cut into small pieces (2 × 2 cm for leaf and 2 cm long for root). The samples were washed by running tap water for 1-2 in to remove soil particles and then surface sterilized by 70% ethanol for 10 min and 1% sodium hypochlorite for 15 min modified from the methods of Coombs and Franco [15] and Cao et al. [16]. The plant

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materials were then rinsed with sterilized water for three times before soaking in 10% (w/v) $NaHCO_3$ solution for 5 min. At this point, the final washed solution was spread onto Starch Casein Agar containing 100 g/ml ampicillin, 2.5 U/ml penicillin G, 50 g/ml amphotericin B and 50 g/ml cyclohexamide; to validate the surface sterilized protocol. Surface sterilized leaf and root samples were crushed in ¼ Ringer's solution. The solution and the crushed materials were spread onto SCA supplemented with above antibiotics. Endophytic Actinomycetes were observed after incubation at 28°C for 1-4 weeks.

Identification of Actinomycetes

The isolates were picked from isolation plates and purified on Mannitol Soybean Agar and ISP 2 and ISP 3 media (International *Streptomyces* Project). The isolates were identified according to morphological criteria, including characteristics of colonies on plate, morphology of substrate and aerial hyphae, morphology of spores and pigment production [17]. The isolates were preserved in 20% (v/v) glycerol and stored at -80°C for subsequent investigation.

Source of phytopathogenic fungi

The tested fungi were phytopathogenic isolates (*Fusarium oxysporum, Phytophthora infestans*, *Fusarium solani*, *Macrophomina phaseolina* and *Botrytis cinerea*) provided by Mycology Lab, EDU-TECH Educational and Research Institute. All cultures were stored at 4°C as potato dextrose agar slants and at -15°C as spore suspensions in 20 % glycerol.

Unless mentioned, the incubation conditions in all experiments were 7 days at 28°C for actinomycetes, 5-7 days at 18°C for *B. cinerea* and 5-7 days at 28°C for the other fungi.

Screening of antifungal spectrum of the Actinomycetes

Antagonistic activity on agar was performed as described by Aghighi et al. [18]. A 5-mm agar disc of well-grown actinomycetes colony mass was aseptically transferred to potato dextrose agar (PDA) plates pre-inoculated with fungal isolates. Controls were included using plain discs from starch casein agar (SCA) medium and triplicates were conducted. After incubation, the bioactivity was evaluated by measuring the average diameter of the inhibition zones.

Antifungal abilities of *Streptomyces sp.5 and Streptomyces sp.7*

Dual culture bioassay was performed by transferring fungal mycelial-discs (5 mm) from growing margin of cultures on PDA to new PDA plates, positioned 3 cm away from each pre-grown actinomycete disc; triplicates were conducted and incubated. The fungal inhibition was calculated from the equation AB = B – A [19]. Where AB = activity to inhibit fungal growth, A = the distance of fungal growth in the direction of actinomycete colony and B = the fungal growth radius of a control culture. Rating of AB was as follows: <1 mm (no inhibition); 1-9 mm (+); 10-19 mm (++); >20 mm (+++).

Production of antifungal diffusible compounds

A modified single thickness dialysis membrane overlay technique was followed [20]. Actinomycetes were grown on cellophane removed from the plates after 7 days of incubation. The metabolite containing pates were inoculated with 5-mm diameter discs of the tested fungi and incubated as mentioned above. The colony diameters of the fungi were compared with controls. The tested fungi were sub-cultured on a fresh plate of PDA to determine fungicidal effect of metabolites.

Production of volatile compounds

The method described by El-Tarabily et al. [21] was employed. Lids were removed from 7-days old culture of Actinomycetes on SCA and replaced with PDA plates inoculated with actively growing pathogenic fungi mycelial 5-mm plugs; the bases were taped together with a double layer of parafilm. After the incubation period, the colony diameter of each pathogen growing in the presence of the Actinomycete isolates was measured and compared with that of the control. Exposed fungal cultures were sub-cultured on a fresh plate of PDA to determine whether the diffused metabolites were fungicidal or fungi-static. Control plates were prepared in the same way except that a non-inoculated plate was used instead of a plate containing the Actinomycete.

Production of active metabolites

Actinomycetes were grown in starch casein broth at 28°C, 120 rpm for 10 days. A 25ml of crude metabolites were concentrated by freeze drying, and then tested for the antifungal activity against phytopathogenic fungi at a concentration of 1/4, 1/2, 3/4 and full strength of the dry stock. Solvent-extractable metabolites were obtained using equal volumes of ethyl acetate for three successive times and pooled; this allowed organic molecules to be suspended in less polar solvent. The obtained extracts were tested at concentrations 5, 10, 30 mg/ml in distilled water. Both crude metabolites and solvent extractable metabolites were tested for the antifungal activity against the tested phytopathogenic fungi using disc technique as described by Aghighi et al. [18] after incubation at the above mentioned conditions.

Cell wall degrading enzyme production

Chitinase activity: Production of chitinase was investigated on colloidal chitin agar (CCA) as described by Gupta et al. [22]. Seven days old discs of grown isolates on SCA were transferred to CCA, incubated at 28°C and monitored for 28 days until zones of chitin clearing were seen around and beneath the colonies. Clear-zone diameters (mm) were measured and used as an indicator of chitinase activity. Large diameters (> 40 mm) represented high activity.

Cellulolytic activity (cellulases and CMC-ase): Cellulases production by Actinomycetes was performed on cellulose agar as described by Wollum. Cellulolytic ability was indicated by clearing of the medium around colonies after 7 to 28 days of incubation at 28°C. CMC-ase production by Actinomycetes was performed on basal medium containing soluble Carboxy Methyl Cellulose (CMC). Hydrolysis zones were detected by flooding the plates with 1% (wt/vol) aqueous solution of hexadecyl trimethyl ammonium bromide to precipitate the un-degraded CMC. The optical zone sizes were recorded after incubation for 10 days at 28°C.

Statistical analysis: Data means and standard errors of means were calculated using Microsoft Excel XP and Analysis of Variance (ANOVA) was performed to investigate significant differences between the antagonistic activities of different isolates using data analysis tools of Microsoft Excel XP. The differences were considered significant at $p < 0.05$.

Results

Isolation and identification of endophytic isolate

Several colonies of endophytic actinomycetes could be morphologically observed in SCA medium spreading with crushed root solution after 1-4 weeks of incubation. Hyphal growth of endophytic actinomycetes was also detected on the surface of root materials.

Genus / Sample Code	Source plant	Average inhibition zone (mm) ± SE				
		Fusarium solani	*Phytophthora infestans*	*Botrytis cinerea*	*Macrophomina Phaseolina*	*Rhytisma acerinum*
Streptomyces sps.						
Phy. Stp. 1	*Phyllanthus niruri*	13 ± 0	0.6 ± 0.3	0.6 ± 0.33	11.30 ± .88	9 ± 0.87
Phy. Stp. 2	*Phyllanthus niruri*	0.3 ± 0.3	0.6 ± 0.3	1.3 ± 0.67	17.3 ± 0.33	0.3 ± 0.3
Phy. Stp. 3	*Phyllanthus niruri*	0.6 ± 0.3	1.3 ± 0.7	1.6 ± 0.33	0.3 ± 0.33	0.6 ± 0.3
Phy. Stp. 4	*Phyllanthus niruri*	19 ± 0.9	17 ± 0.6	26 ± 0.58	14.3 ± 0.33	13.3 ± 0.3
Phy. Stp. 5	*Phyllanthus niruri*	6.7 ± 0.7	0.6 ± 0.3	13.3 ± 0.3	8.3 ± 0.33	8.6 ± 0.7
Phy. Stp. 6	*Phyllanthus niruri*	1 ± 0	1 ± 0.6	1 ± 0	1.6 ± 0.33	1.3 ± 0.7
Phy. Stp. 7	*Phyllanthus niruri*	1 ± 0	1.3 ± 0.7	1.3 ± 0.33	1.6 ± 0.33	18 ± 0.3
Phy. Stp. 8	*Phyllanthus niruri*	0.7 ± 0.3	0.3 ± 0.3	12 ± 1	1.6 ± 0.33	0.3 ± 0.3
Phy. Stp. 9	*Phyllanthus niruri*	8 ± 0.6	8.6 ± 0.7	18 ± 0.58	7.6 ± 0.33	2 ± 0.6
Phy. Stp. 10	*Phyllanthus niruri*	2.3 ± 0.3	1.3 ± 0.7	0.6 ± 0.33	0.6 ± 0.33	0.3 ± 0.33
Phy. Stp. 11	*Phyllanthus niruri*	17 ± 0.9	1.6 ± 0.3	11 ± 0.58	0.3 ± 0.33	1.3 ± 0.33
Phy. Stp. 12	*Phyllanthus niruri*	1.7 ± 0.3	2 ± 0.6	0.6 ± 0.33	0.6 ± 0.33	0.7 ± 0.3
Phy. Stp. 13	*Phyllanthus niruri*	0.7 ± 0.3	0.3 ± 0.3	12 ± 1	1.6 ± 0.33	0.3 ± 0.3
Hem.Stp.1	*Hemidesmus indicus*	1 ± 0	1.3 ± 0.7	1.3 ± 0.33	1.6 ± 0.33	18 ± 0.3
Hem.Stp.2	*Hemidesmus indicus*	8 ± 0.6	8.6 ± 0.7	18 ± 0.58	7.6 ± 0.33	2 ± 0.6
Hem.Stp.3	*Hemidesmus indicus*	2.3 ± 0.3	1.3 ± 0.7	0.6 ± 0.33	0.6 ± 0.33	0.3 ± 0.33
Cat.Stp.1	*Catharanthus roseus*	17 ± 0.9	1.6±0.3	11 ± 0.58	0.3 ± 0.33	1.3 ± 0.33
Cat.Stp.2	*Catharanthus roseus*	1.7 ± 0.3	2 ± 0.6	0.6 ± 0.33	0.6 ± 0.33	0.7 ± 0.3
Wit.Stp.1	*Withania Somnifera*	12 ± 0.6	13.6 ± 0.7	13.3 ± 0.8	6.3 ± 0.88	1.3 ± 0.7
Wit.Stp.2	*Withania Somnifera*	14 ± 0.6	21 ± 0.3	21.3 ± 0.3	15.3 ± 0.58	17 ± 0.6
Hem.Stp.3	*Hemidesmus indicus*	16 ± 1	1 ± 0	13 ± 0.58	0.6 ± 0.33	0.6 ± 0.3
Kibdelosporangium sps.						
Phy. Ksp.1	*Phyllanthus niruri*	1.6 ± 0.3	2 ± 0	0.3 ± 0.33	8 ± 0	18 ± 58
Phy. Ksp.2	*Phyllanthus niruri*	0.6 ± 0.3	1 ± 0.6	0.3 ± 0.33	16 ± 0.58	6.7 ± 0.7
Nocardioides sps.						
Phy.Nsp.1	*Phyllanthus niruri*	0.7 ± 0.3	1 ± 0	15.3 ± 0.3	7.6 ± 0.33	8.3 ± 0.3
Phy.Nsp.2	*Phyllanthus niruri*	15 ± 0.7	11 ± 0.6	9.3 ± 0.88	1.3 ± 0.33	9.3 ± 0.9
Wit. Nsp.1	*Withania Somnifera*	6.7 ± 0.7	0.6 ± 0.3	13.3 ± 0.3	8.3 ± 0.33	8.6 ± 0.7
Wit. Nsp.2	*Withania Somnifera*	1 ± 0	1 ± 0.6	1 ± 0	1.6 ± 0.33	1.3 ± 0.7
Hem. Nsp.1	*Hemidesmus indicus*	0.7 ± 0.3	1 ± 0.6	0.3 ± 0.33	1 ± 0.58	1.6 ± 0.3
Cat. Nsp.1	*Catharanthus roseus*	1 ± 0	1.3 ± 0.7	1.3 ± 0.33	1.6 ± 0.33	18 ± 0.3
Pseudonocardia sps						
Pse.Pse.1	*Phyllanthus niruri*	1 ± 0.6	0.6 ± 0.3	1 ± 0	7.3 ± 0.33	1.3 ± 0.6
Hem.Pse.1	*Hemidesmus indicus*					
Actinomadura sps.						
Wit.Act.1	*Withania Somnifera*	7.7 ± 0.7	8.6 ± 0.3	5.3 ± 0.88	7 ± 1.15	1.3 ± 0.33
Wit.Act.2	*Withania Somnifera*	0.6 ± 0.3	1 ±0	8 ± 0.58	10.3 ± 0.33	8.3 ± 0.33
Hem.Act.1	*Hemidesmus indicus*	2.3 ± 0.3	1.3 ± 0.7	0.6 ± 0.33	0.6 ± 0.33	0.3 ± 0.33
Kitasatosporia sps.						
Phy.Kit.1	*Phyllanthus niruri*	0.7 ± 0.3	0.3 ± 0.3	1 ± 0.58	1 ± 0	0.8 ± 0.3
Wit.Kit.1	*Withania Somnifera*	8.3 ± 0.3	1 ± 0	0.6 ± 0.33	17.3 ± 0.33	0.6 ± 0.3
Wit.Kit.2	*Withania Somnifera*	9.3 ± 0.9	0.6 ± 0.3	16.3 ± 0.8	13 ± 0.58	1.6 ± 0.3
Unknowns						
Unknown 1	*Phyllanthus niruri*	0.7 ± 0.3	0.3 ± 0.3	1 ± 0.58	1 ± 0	0.8 ± 0.3
Unknown 1	*Hemidesmus indicus*	0.3 ± 0.3	0.6 ± 0.3	1,6 ± 0.33	0.3 ± 0.33	1 ± 0
Unknown 1	*Hemidesmus indicus*	1 ± 0	1.3 ± 0.3	1 ± 0.58	0.3 ± 0.33	11 ± 0.6
Unknown 1	*Catharanthus roseus*	1 ± 0	0.6 ± 0.3	2 ± 0.58	1.3 ± 0.33	0.6 ± 0.3
Unknown 1	*Catharanthus roseus*	1 ± 0	0.6 ± 0.3	1 ± 0.58	0.3 ± 0.33	1 ± 0.6
Unknown 1	*Catharanthus roseus*	0.3 ± 0.3	17 ± 0.3	15.6 ± 0.3	14 ± 0.58	1 ± 0.6
Unknown 1	*Hemidesmus indicus*	0.3 ± 0.3	1 ± 0	0.6 ± 0.33	16.3 ± 0.88	1.3 ± 0.7
Unknown 1	*Withania Somnifera*	0.7 ± 0.3	0.3 ± 0.3	1 ± 0.58	1 ± 0	0.3 ± 0.3
Unknown 1	*Withania Somnifera*	0.3 ± 0.3	2.3 ± 0.3	1 ± 0.58	8 ± 0.58	2.3 ± 0.3
Unknown 1	*Withania Somnifera*	13 ± 0.6	2.6 ± 0.7	18.3 ± 0.3	8 ± 0.58	2.6 ± 0.7

Table 1: Isolated Endophytic Actinomycetes Generic Names, Source Plant and Average Width of Inhibition Zone Caused By Isolates Against the tested Phytopathogenic Fungi.

No endophytes were obtained from leaf samples. In addition, the last wash from all surface sterilized root and leaf samples showed no microbial growth which indicated that the surface sterilized protocol was exceptionally efficient. After purification of the endophytes, 37 isolates were identified and 10 isolates aren't identified (Table 1). The results indicated various types of endophytic actinomycetes obtained from four different plant samples. The isolates were labeled accordingly their plant name and genus names as shown in Table 1. These isolates were assigned to the genus level, which was sufficient to cover a broad spectrum of Actinomycete genera in the *in vitro* pilot study, as follows, 21 of the isolates were *Streptomyces*, 6 were *Nocardioides*, 3 were *Kitasatosporia*, 2 were *Pseudonocardia*, 3 were *Actinomadura*, 2 were *Kibdelosporangium*, ; in addition to 10 unidentified isolates.

In vitro antifungal spectrum of the actinomycetes

The initial assay showed that 18.5 % were highly antagonistic to *Fusarium solani*, 12.3 % to *Phytophthora infestans*, 30.7 % to *Macrophomina* and 32.3 % to *Botrytis cinerea,* 38.5 % to *Rhytisma acerinum*. Two of the isolates showed the highest activities towards all the five tested fungi (Table 1). These isolates were *Streptomyces sp.7* and *Streptomyces* sp.5. The two isolates were selected for further investigation, as they had the highest means of inhibition activities against all the tested fungal phytopathgens.

Antagonistic activities of *Streptomyces* sp.5 and *Streptomyces sp.7*

The two isolates, in dual cultures, strongly antagonized the tested fungi. *Streptomyces sp.5* inhibited 22.3 % of the *Fusarium oxysporum* growth, 19.6 % of the *Phytophthora infestans* growth, 30 % of the *Botrytis cinerea* growth and 16.2 % of the *Macrophomina phaseolina* culture growth, 36.5 % of the *Rhytisma acerinum* in tested plates, compared to the growth in control plates which was 100 %. Isolate *Streptomyces sp.7*, inhibited 21 % of the *Fusarium oxysporum* growth, 19.5 % of the *Phytophthora infestans* growth, 18.4 % of the *Botrytis cinerea* growth and 19.9% of the *Macrophomina phaseolina* culture growth and 28.3% of the *Rhytisma acerinum* culture growth.

Using cellophane membrane overlay technique, both isolates showed an effect on the tested fungi. Diffusible metabolites from *Streptomyces* sp.1, exhibited a fungicidal effect on *M. phaseolina*, *F.oxysporum* and *B. cinerea* and *Rhytisma acerinum* a fungicidal effect on *P. infestans*.

However, diffusible metabolites of *Streptomyces sp.7* were highly fungicidal to all the tested phytopathogens (Table 2). Volatiles produced by both actinomycete strains were highly effective against the tested fungi (Table 3). It caused mycelia discoloration and it showed a more pronounced effect on the sub-culturing capability of the phytopathogens regarding growth and morphological characters.

Concentrated metabolites of the *Streptomyces sp.5*and *Streptomyces sp.7* were moderately active against *P. infestans* and *M. phaseolina*, while highly active against *B. cinerea* and *F. oxysporum* and *R. acerinum*. The crude organic extracts of both isolates had no effect on the pathogen mycelial growth in test plates under all the used dilutions. In addition, both isolates did not show neither cellulolytic nor chitinolytic activities.

Discussion

In the present study, *Streptomyces sp.5* and *Streptomyces sp.7* was selected upon the preliminary screening of 47 isolates belonging to five morphologically different groups of Actinomycetes isolated from 4 different and unique Indian medicinal plants. Both endophytic isolates showed a wide spectrum of the antifungal activities against *Fusarium oxysporum, Phytophthora infestans, Botrytis cinerea, Rhytisma acerinum and Macrophomina phaseolina* with a probability of ($p< 0.05$).

Similar results were reported by Aghighi et al. [18] and Heba et al. [23], indicating that a small number of endophytic microorganisms had the capability of producing broad-spectrum, antifungal compounds. Mechanisms of action of endophytic actinomycetes are mainly focused on the production of bioactive compounds, such as antibiotics, cell wall degrading enzymes and competition for nutrients [13]. The obtained results demonstrated that *Streptomyces sp.5* and *Streptomyces sp.7* may use more than one mechanism in suppressing phytopathogens. The competitive growth between pathogens and antagonists in dual cultures revealed lesser suppression when compared with antibiotic production of different assays, diffusates on agar, crude metabolites, membrane diffusible metabolite(s), and volatiles, respectively.

Volatile organic compounds produced by *Streptomyces* spp. and other species of actinomycetes were reported to cause growth abnormalities in different fungi, including *Fusarium oxysporum*. Volatile organic compounds from the currently investigated actinomycetes significantly inhibited the fungal growth and completely prevented the pigment production. The pigments of pathogenic fungi, such as melanin, were reported to be interrelated with fungal

Antagonist	*Fusarium solani (cm)*	*Phytophthora infestans (cm)*	*Botrytis cinerea (cm)*	*Macrophomina phaseolina (cm)*	*Rhytisma acerinum (cm)*
Streptomyces Sps.5	10 (100%)	9 (100%)	10 (100%)	8 (35.3%)	11 (100%)
Streptomyces Sps.5	10 (100%)	8 (100%)	9 (98.5 %)	10 (100%)	10 (100%)

Table 2: *In vitro* antifungal activity of diffusible metabolites.

Actinomycetes	Phytopathogens	Inhibition (%)	Mycelial Growth	Sporulation
Streptomyces sps.5	*Fusarium solani*	12.3	Scarce	Scant sporulation
	Phytophthora infestans	85.3	Scarce	Scant sporulation
	Macrophomina phaseolina	67.5	Normal	No sporulation
	Rhytisma acerinum	98.6	Scarce	Scant sporulation
	Botrytis 0cinerea	60.3	Scarce	Scant sporulation
Streptomyces sps.7	*Fusarium solani*	96.4	Normal	No sporulation
	Phytophthora infestans	48.6	Scarce	Normal
	Macrophomina phaseolina	94.8	Scarce	No Sporulation
	Rhytisma acerinum	96.4	Normal	No sporulation
	Botrytis cinerea	65.3	Scarce	No Sporulation

Table 3: Inhibition percentage and morphological changes in testing fungi subjected to volatile compounds produced by Actinomycete isolates.

pathogenicity and could endow fungi some special recovery function, such as anti-radiation, anti-oxidation and scavenging free radical. Therefore, it seems that the volatiles produced by *Streptomyces sp.5* and *Streptomyces sp.7* would be possible to play a significant role in reducing the pathogenic fungal infection ability. So far, information on the efficacy of volatile substances from *Streptomyces* species in suppression of plant diseases is meager. The present study did not include microscopic observation of the inhibited fungi; therefore, detailed study of the Volatile organic compounds, produced by *Streptomyces sp.5* and *Streptomyces sp.7* should be conducted to elucidate their mode of inhibition.

According to the *in vitro* evaluation, *Streptomyces sp.5* antagonistic activity depends mainly on its metabolites. The antagonistic activity of this isolate was best in diffused metabolites on agar, followed by membrane diffusates and crude metabolites assays, respectively. In regard to pathogen sensitivity, *Fusarium oxysporum, Botrytis cinerea, Rhytisma acerinum* and *Macrophomina phaseolina* were highly sensitive to diffused metabolites on agar and on cellophane followed by volatiles. On the contrary *Phytophthora infestans* was less sensitive than the previous three pathogens. *Streptomyces sp.7* antagonistic activity was similarly dependent on its metabolites, according to the *in vitro* evaluation.

The present results also showed that chitinolytic enzymes were involved in the inhibition of fungal growth in any of the selected Actinomycete isolates. This disagrees with the studies of Gupta et al. [22] and El-Tarabily et al. [21] who reported that actinomycetes produce chitinases can lyse living fungal mycelia. From application point of view, the ability of *Streptomyces sp.5*and *Streptomyces sp.7* can be produce cellulytic enzymes is of significant importance.

Conclusion

In conclusion, endophytic actinomycetes from medicinal plants in India could represent a potential biocontrol agent for the phytopathogens *P. infestans*, *M. phaseolina*, *B. cinerea R. acerinum* and *F. oxysporum*. The antagonistic activities of *Streptomyces sp.5* and *Streptomyces sp.7* are likely dependent on volatile and diffusible metabolites. Further research is on the two actinomycetes gene amplification and sequencing, phylogenetic analysis and efficacy and their mode of action is in progress.

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