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RESEARCH ARTICLE

The diversity, plant growth promoting and anti-microbial activities of endophytic actinomycetes isolated from *Emblica officinalis* Gaertn.

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Abstract

A total of 36 endophytic actinomycetes were isolated from roots, stems and leaves of *Emblica officinalis* Gaertn (Gooseberry) and identified as *Streptomyces* sp. (17), *Micromonospora* sp. (17) and *Microbispora* sp. (2). The distribution of endophytic actinomycetes in roots was (50%), stem (25%) and leaves (25%). Twenty five (69.4%) isolates were observed to produce indole-3-acetic acid ranging between 1.93±0.3 to 125.7±0.6 µg/ml and sixteen isolates (44.4%) were able to solubilize phosphate in the range of 0.014±0.005 to 0.45±0.004 mg/ml. *Streptomyces griseofuscus* AL5 was producing maximum amount of IAA and *Streptomyces cinereus* AR3 solubilized maximum amount of phosphate. Thirteen isolates were producing catechol type of siderophores and fifteen were producing hydroxamate type of siderophores. The catechol siderophores production ranged from 6.2±0.3 to 55.6±0.4 µg/ml while hydroxamate siderophore were in the of range 8.0±0.2 to 91.3±0.5µg/ml. *Streptomyces griseofuscus* AL5 produced maximum catechol type siderophore (55.6±0.4 µg/ml) and maximum hydroxamate- type of siderophore production was also observed in *Streptomyces griseorubroviolaceus* AR3 (88.6±0.7 µg/ml). Fifteen isolates were active against one or the other phytopathogenic fungi. *Streptomyces cinereus* AR16 was displaying maximum antagonistic activity against five plant pathogenic fungi (*Fusarium oxysporum*, *Rhizoctonia solani*, *Aspergillus niger*, *Alternaria brassicicola* and *Phytophthora dresclea*). Twenty nine isolates were displaying the antibacterial activity against pathogenic bacteria (*Aeromonas hydrophila*, *Yersinia enterocolitica*, *Staphylococcus aureus* and *Klebsiella* sp.).

INTRODUCTION

Endophytes are defined as microorganisms that are found in an endosymbiotic relationship with plants which increase the ability of host to tolerate the abiotic stresses and also help in plant growth promotion (Hasegawa et al., 2000). Endophytic actinomycetes can promote the growth of field crops by producing plant growth-promoting substances and by fixing nitrogen from the atmosphere (Kumar et al., 2011). Endophytes are also known to stimulate plant growth due to the production of phytohormones (Nimnoi et al., 2010) and keep a check on the growth of phytopathogens through production of enzymes, antibiotics or siderophores (Quecine et al., 2008). Endophytes infected plants often grow faster than the non-infected ones due to the production of the phytohormone

Indole-3-acetic acid (IAA) (Lee et al., 2004), Pteridic acid A and B, which has auxin like activity (Igarashi et al., 2002), production of siderophores (Costa and Loper, 1994), cytokinin, phosphate solublizing activity (Wakelin et al., 2004), antibiotic production (Igarashi, 2004), production of enzymes such as amylases, cellulases, chitinases, hemicellulases and gluconases (Yuan and Crawford, 1995) and other plant growth promoting attributes by the infecting endophytes. Most of those actinomycetes belonged to the genera, *Streptomyces*, *Microbispora*, *Micromonospora*, *Actinosynnema*, *Actinomadura* and *Nocardia* (Coombs and Franco, 2003).

These endophytic actinomycetes can be used as potential biocontrol agents of various soil borne and root disease as they are able to colonize inner tissues of healthy plants and produce antibiotic *in situ* (Kunoh, 2002). Various genera of endophytic actinomycetes exhibit suppression of different soil borne plant pathogens including *Fusarium oxysporum*, *Rhizoctonia solani*, *Colletotrichum arbiculare*, *Phythium* sp. and *Verticillium alboatrum*, indicating their potential use as biological control agents (El-Tarabily et al., 2009). Since these are potent as phytopathogen suppressors, these may also be tested for their human pathogen suppressing abilities.

Keeping in view their extensive utility, the present investigation has been undertaken to test the potential of endophytic actinomycetes from *Emblica officinalis* Gaertn. (amla) for their potential as antimicrobial agents against various phytopathogenic fungi and human pathogenic bacteria as well as production of plant growth promoting substances.

MATERIALS AND METHODS

Collection of samples

The roots, stems and leaves of fifteen trees of *Emblica officinalis* were collected from different locations in Ludhiana district of northern India. Roots were obtained by digging the soil adjacent to the main trunk up to 40cm away and collected sections of approximately 0.5cm diameter and 3–5cm in length.

Isolation of endophytic actinomycetes

The plants were thoroughly washed with running water to remove soil particles, adhered epiphytes and soil debris. Tissue pieces were sterilized by sequential immersion in 70% (v/v) ethanol for 5 minutes and sodium hypochlorite solution for 20 minutes followed by washing in sterile water. The samples were soaked in 10% (w/v) sodium bicarbonate solution for 10 minutes to retard the growth of endophytic fungi. Then macerated tissue suspension (1 ml) was spread on to Petri plates containing starch casein agar (SCA) and incubated at 28^0C for 7-10 days. Isolated colonies were subcultured on slants and stored at 4^0C (Tian et al., 2004).

Identification of actinomycetes

Identification of endophytic actinomycetes had been achieved by studying their colonial and biochemical characteristics and also by their microscopic morphology as described by Bergey's Manual of Systematic Bacteriology and with published descriptions (Tan et al., 2006; Cao et al., 2004; Yan, 1992; Ruan et al., 1990).

Characterization of actinomycetes isolates for Plant Growth Promoting traits

Phosphate solubilization

The halo zone around the colony on Pikovskaya medium supplemented with tricalcium phosphate was presumptive confirmation of phosphate solubilization. Quantitative estimation of phosphate solubilization in broth was carried out by growing the presumptively selected cultures in 50 ml of Pikovskaya medium for 7 days. The total amount of phosphate solubilized by actinomycetes isolates was estimated by Jackson method (Jackson, 1973).

Indole acetic acid production

Characterization of isolates for the production of IAA was done by method of Gordon and Weber (1951) and estimated by comparison with an IAA standard.

Siderophore production

The endophytic actinomycete isolates were inoculated on chrome azurol S (CAS) agar medium and incubated at 28°C for five days (Schwyn and Neilands, 1987). The colonies with orange zones were considered as siderophore producing isolates. The active isolates (width of orange zone on CAS plate > 20 mm) were cultured on glycerol yeast broth and incubated at 28°C with shaking at 125 rpm for 10 days. Catechol type of siderophores were estimated by using Arnow's method (Arnow, 1937) and hydroxamate type of siderophores were estimated by the method of Csaky (1948).

***In vitro* antagonistic bioassay**

Antagonistic effect against pathogenic fungi

The actinomycetes isolates were evaluated for their antagonistic activity against six phyto pathogenic fungi: *Alternaria brassicicola, Aspergillus niger, Fusarium oxysporum, Helminthosporium oryzae, Phytophthora dresclea* and *Rhizoctonia solani* by dual-culture *in vitro* assay.

Five days old fungal discs (8 mm in diameter) at 28ºC were placed at the center of the plates containing PDA media. 5 days old actinomycetes discs (8mm) grown on nutrient agar at 28ºC were placed on opposite sides of

the Petri plates. Plates without the actinomycetes disc serve as controls. All the plates were incubated at 28ºC for 14 days and colony growth inhibition (%) was calculated by using the formula: C – T/C x 100, where C is the colony growth of pathogen in control and T is the colony growth of pathogen in dual culture.

Antagonistic effect against pathogenic bacteria and yeast

Spread plate method was used for detecting antagonism against 5 pathogenic bacterial cultures: *Escherichia coli*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Klebsiella* sp. and *Staphylococcus aureus*. A lawn of these pathogenic cultures was prepared on nutrient agar plates by spreading 0.1 ml of these pathogenic cultures but *Candida albicans* was spread on Sabouraud's dextrose agar. A well was made in the center of each nutrient agar plate. Culture filtrate (50µl) of the actinomycetes isolates was put in that well and incubated at 37^0C.

Optical microscopy

Optical research microscopy was performed with fungal culture of *Fusarium oxysporum* to observe the changes in cell wall of the fungus due to the effect of secondary metabolites produced by endophytic actinomycetes isolates (*Streptomyces cinereus*) using lactophenol cotton blue (LBCB) stain.

Scanning electron microscopy (SEM)

SEM was employed to evaluate the effect of *Streptomyces cinereus* (AR16) on the fungal cell wall of *Fusarium oxysporum* culture using chemical fixation and liquid osmium fixation technique (Bozzola and Russell, 1996).

RESULTS AND DISCUSSION

Isolation and characterization of endophytic actinomycetes diversity

A total of 36 isolates of endophytic actinomycetes were obtained from tissues of roots, leaves and stems of 15 amla trees (Table 1). Out of 36 isolates, the majority (n=18) was recorded from roots, followed by stems and leaves, (n=9) each. As we know that plants uptake water and nutrients via roots, so these roots are an ideal substrate for actinomycete colonization. Endophytic actinomycetes may enter into roots, when epidermal layer was damaged by lateral roots growing from the existing root. These results are in agreement with the reports of Verma et al. (2009) who reported that majority (n=30) of the isolates were recorded from roots, followed by stem (n=13) and leaves (n=12) respectively. Kumar et al. (2011) also reported that out of five isolates recovered from *Emblica officinalis*, three were isolated from *Emblica* twig and two from the leaves. Besides *Streptomyces* sp. other genera like *Micromonospora* sp. *Microbispora* sp. and *Nocardia* sp. were also reported. Similarly, Gangwar et al. (2014) obtained 40 isolates of endophytic actinomycetes from the root, stem and leaf tissues of *A. vera*, *M. arvensis* and *O. sanctum* out of which most of the actinomycetes were identified as *Streptomyces* sp.

Identification of actinomycetes

Based on colony and cultural characteristics, various subgroups were identified (Table 2) and among *Streptomyces* sp., the subgroups *Streptomyces grieseofuscus* (n=8) was dominating species followed by *S. albosporus* (n=4), *S. griseorubroviolaceus* (n=2), *S. cinereus* (n=2) and *S. flavus* (n=1). The studies revealed that actinomycetes such as *Streptomyces* sp., *Micromonospora* sp. and *Microbispora* sp. were able to colonize internal tissues of a wide range of host plants. Differences in the distribution of endophytic actinomycetes in the roots and in the leaves were observed. *Streptomyces griseofuscus* was obtained from all the plant parts, like, roots, stems and leaves, but *S. albosporus* was recovered only from roots.

Characterization of actinomycetes isolates for Plant Growth Promoting traits

Phosphate solubilization

Out of 36 isolates, sixteen (44.4%) were observed to solubilize phosphate as they formed a clear zone around the colony on Pikovskaya medium. The amount of phosphate solubilized by the isolates ranged from 0.014±0.005 to 0.45±0.004 mg/ml. The maximum amount of phosphate solubilized by *Streptomyces cinereus* AR3 (0.45±0.004 mg/ml), followed by *S. griseofuscus* strain AL4 and one *Micromonospora* isolate, AR15 (0.38±0.004mg/ml each). Hamdali et al. (2008) also reported high amount of phosphate solubilizing activity by *Streptomyces cavourensis* (83.3 mg/100 ml) followed by *Streptomyces griseus* (58.9 mg/100 ml) and *Micromonospora aurantiaca* (39 mg/100ml). The results were also in accordance with Gangwar and Kataria (2013), who reported that forty endophytic actinomycetes from medicinal plants solubilized phosphorus in the range of 0.02-0.68 mg/ml. The maximum amount of phosphate solubillized by *S. albosporus* A4 (16.5 mg/100 ml). It is suggested that these actinomycetes could be used as phosphate solubilizers as these microbes help in solubilizing the mineral phosphate might be either due to production of the chelating substances or acidification of the external medium (El-Tarabily and Sivasithamparam, 2006).

Indole acetic acid (IAA) production

Twenty five (69.4%) out of 36 isolates produced phytohormone IAA in the range of 1.93±0.3 to 125.7±0.6 µg/ml, whereby *Streptomyces griseofuscus* AL5 was observed to produce highest amount of IAA (Table 3). Nimnoi et al.

(2010) isolated endophytic actinomycetes from *Aquilaria crassna* and found that *Nocardia jiangxiensis* produced maximum IAA (15.14 µg/ml) whereas *Actinomadura glauciflava* produced minimum yield of IAA (9.85 µg/ ml). Our findings are in accordance with Dochhil et al. (2013) who isolated 30 endophytic actinomycetes from *Centella asiatica* and IAA production by strains CA10 and CA26 was quantified as 71µg/ml and 197µg/ml respectively. It could be inferred that IAA, a plant growth hormone can promote plant growth in rhizosphere soils. The root exudates are natural sources of tryptophan for rhizospheric microorganisms which can enhance auxin biosynthesis in actinomycetes. It was demonstrated by Nimnoi and Pongsilp (2009) that isolates of IAA synthetic bacteria enhanced root and shoot development of *Raphanus sativus* and *Brassica oleracea* more than fivefold when compared with control.

Siderophore production

Out of 36 isolates, twenty (55.2%) produced distinct orange halo on CAS plates. Thirteen isolates produced catechol type of siderophores ranging from 6.2±0.3 to 55.6±0.4 µg/ml and maximum production was observed by *Streptomyces griseofuscus* AL5 (55.6±0.4 µg/ml). On the other hand, 15 isolates produced hydroxamate type siderophores ranging from 8.0±0.2 to 91.3±0.5 µg/ml, maximum being produced by *Streptomyces griseorubroviolaceus* AS7 (91.3±0.5 µg/ml) and minimum being produced by *S. cinereus* AR3 (8.0±0.2 µg/ml) (Fig. 1). These results are in accordance with Gangwar and Kataria (2013) who reported that out of 40 endophytic actinomycetes from medicinal plants, 17 isolates produced catechol-type siderophore ranging between 2.68-51.6 µg/ml, while 22 isolates produced hydroxamate-type siderophores ranging from 8.71-144.21 µg/ml. Siderophores are produced by various soil microbes to bind Fe^{3+} from the environment, transport it back to microbial cell and make it available for growth (Neilands and Leong, 1986; Mishra et al., 1987).

***In vitro* antagonistic bioassay**

Antagonistic effect against pathogenic fungi

Fifteen (41.6%) out of 36 isolates were active against different plant pathogenic fungi as shown in figure 2. Most of the isolates displaying antagonistic activity belonged to the *Micromonospora* sp. (n=7) and *Streptomyces* sp. (n=6) and rest belonged to *Microbispora* sp. (n=2). All the isolates were tested for having antifungal activity against six phytopathogenic fungi *viz. Fusarium oxysporum, Alternaria brassicicola, Phytophthora dresclea, Helminthosporium oryzae, Aspergillus niger* and *Rhizoctonia solani.* In present study, the two isolates *Streptomyces cinereus* AR16 and *S. flavus* AL7 were showing maximum antifungal activity against all the phtopathogenic fungi tested (50.75, 40.75; 33.25, 32.50; 81.75. 68.75; 42.75, 33.25; 31.25, 27.75% respectively for *A. niger*, *A. brassicicola*, *F. oxysporum*, *P. dresclea* and *R. solani*). These were followed by isolate *Streptomyces cinereus* AR3, which was showing maximum antifungal activity against *Fusarium oxysporum, Phytophthora dresclea* and *Rhizoctonia solani* (80.50, 50 and 33.25% respectively). The isolates of *Micromonospora* sp. were observed to have antifungal activity against all the pathogenic fungi tested (ranging from 27.75-72.50%). The isolates of *S. griseofuscus* i.e. AR4 and AR13 were active against *Alternaria brassicicola* (28.75%), *Rhizoctonia solani* (38.75%) and *Aspergillus niger* (43.75%). The isolates of *Microbispora* sp. were observed to have antifungal activity against *Alternaria brassicicola* (27.75%) and *Aspergillus niger* (42.50%). The results are in accordance with Verma et al. (2009) who reported that 32 out of the 55 endophytic actinomycetes isolates from *Azadirachta indica* were found to have broad spectrum significant antimicrobial activity, while about 4% of them showed strong and acute inhibition to pathogenic fungi and bacteria. Similarly, in the study by Gangwar et al. (2014), it was found that 8 endophytic actinomycetes were having strong inhibitory activity against *A. brassicicola* and *F. oxysporum*. *Saccharopolyspora* O-9 from *O. sanctum* strongly inhibited all the pathogenic fungi, and maximum percent inhibition was observed against *A. brassicola* (71.4%). Many isolates of endophytic *Streptomyces* exhibited antagonism against either one or more than one tested phytopathogens. *Streptomyces albosporus* O-11 and *S. albosporus* A-4 antagonized all the tested fungi except *A. flavus* and *P. pinophilum.*

The actinomycetes isolates have tendency to cease or inhibit the growth of phyto pathogenic fungi due to the secretion of secondary metabolites by them and this shows that endophytic actinomycetes can be developed as potential biocontrol agents. Loper and Henkels (1999) demonstrated that the siderophores have ability to sequester and binding of iron by chelation. The fungal siderophores have lower affinity for iron, so these siderophores chelates the iron and deprive pathogenic fungi of this essential element. The antagonistic test revealed that mostly the isolates from roots showed antagonistic activity against phytopathogenic fungi and lesser from stems and leaves. This study signifies an innovative plant-microbe interaction with its involvement in biotechnological application of these endophytic actinomycetes.

Antagonistic effect against pathogenic bacteria and yeast

Twenty nine isolates out of 36 (80.50%) were displaying the antimicrobial activity against human pathogens. In the present study, isolates of *Streptomyces albosporus* were observed to have antimicrobial activity against *Staphylococcus aureus* and *Aeromonas hydrophila,* while the isolates of *S. griseofuscus* were active against *Yersinia*

enterocolitica, Klebsiella sp., *Staphylococcus aureus* and *Aeromonas hydrophila.* The isolates of *Streptomyces cinereus* were observed to have antimicrobial activity against *Staphylococcus aureus* and *Klebsiella* sp., while isolates of *S. griseorubroviolaceus* were active against *Klebsiella* sp., *S. aureus* and *A. hydrophila.* An isolate of *S. griseorubroviolaceus* AS7 was showing a very good antimicrobial activity against *A. hydrophila* (Fig. 2). The isolate from species *Streptomyces flavus* was active against *Y. enterocolitica, S. aureus* and *A. hydrophila,* while the isolate belonging to *Microbispora* sp. was active only against *A. hydrophila.* The *Streptomyces albosporus* isolates were observed to have antimicrobial activity against *Y. enterocolitica, Klebsiella* sp., *S. aureus* and *A. hydrophila.* Twenty nine isolates of amla were not demonstrating antimicrobial activity against *E. coli, Pseudomonas aeruginosa* and *Candida albicans*.

The results were supported by Verma et al. (2009), who reported that different isolates of *Streptomyces* sp. displayed an array of activity against bacteria, particularly isolate AzR036, which had an acute activity against *Pseudomonas fluorescens*, while AzR032 seriously inhibited the growth of *Escherichia coli*. Isolates AzS005, 030, and 023 were equally but moderately effective against all bacteria tested. Among non-identified isolates, AzR022, 053, and 019 were found to be antagonistic to *Pseudomonas fluorescens, E. coli* and *Staphylococcus aureus.*

Optical microscopy

The results revealed the presence of intact hyphae and numerous spores of *Fusarium oxysporum* in the control fungus (Fig. 3: **A**) as compared to the *Fusarium oxysporum* inoculated with *Streptomyces cinereus* AR16 (Fig. 3: **B**). The result indicated that potent antagonists inhibited the growth of the test fungi by releasing extracellular diffusible metabolites that inhibited the hyphal growth of *Fusarium oxysporum* and spore number, spore size was also reduced alongwith thinning of hyphae. Our results are in accordance with Taechowisan et al. (2005), who reported that fungal mycelium along the edges of the colonies facing CMUAc130 appeared thickened, with bulbous-like formations along the ends under a dissecting microscope.

Scanning electron microscopy

Results obtained from SEM showed the degradation of cell walls of *Fusarium oxysporum* mycelia growing towards *Streptomyces cinereus* AR16 as compared to control. The single culture plate of *Fusarium oxysporum* served as control, which showed the regular vegetative cells (Fig. 4: **A**), whereas fungal colony inoculated with *Streptomyces cinereus* AR16 (Fig. 4: **B**) revealed that hyphae got disrupted and reduced in size, whereas in control intact cells had a smooth surface with overall intact morphology. The cell wall surface of treated fungus seemed to be shrunken, indicating that the cytoplasmic structures were flushed out of the cells and spores were partially deformed and reduced in size. Many cells were enlarged and elongated, but having extremely low viability. Our results are similar to He et al. (2009) who reported that endophytic bacteria obtained from *Epimedium brevicornu* Maxim degraded hyphae of *Sclerotinia sclerotiorum* and the cytoplasm was extravagated outside from the fungal walls. Our results are also in corroboration with Tang-um and Niamsup (2012) who reported that breakage of the cell walls of *Fusarium oxysporum f.sp.lycopersici* mycelia growing towards *Streptomyces* sp. P4 as compared to control *F. oxysporum*. It is suggested that the actinomycetes isolate produced extracellular secondary metabolites and/or hydrolytic enzymes including chitinase, which play a crucial role in fungal growth inhibition.

Table 1: Distribution of endophytic actinomycetes isolates from amla trees

Types	**No. of isolates**		
	Root	Stem	Leaf
Streptomyces albosporus	4	-	-
S. griseofuscus	3	1	4
S. grieseorubroviolaceus	-	2	-
S. cinereus	2	-	-
S. flavus	-	-	1
***Micromonospora* sp.**	7	6	4
***Microbispora* sp.**	2	-	-
Total	18	9	9

Table 2: Morphological and biochemical characterization of endophytic *Streptomyces* sp.

Property	***Streptomyces albosporus***	***S. griseofuscus***	***S. griseorubroviolaceus***	***S. cinereus***	***S. flavus***
Colony color	white	grey	purplish-pink	grey	yellow
Pigmentation	-	grey	brown	-	yellow
Spore arrangement	chain	chain	chain	chain	chain
Hydrolysis of Casein	+	-	-	+	-
Starch	+	+	+	+	+
Decomposition of Esculin	-	+	+	-	+
Tween-80	+	-	+	+	+
Xanthine	+	+	+	+	+
Hypoxanthine	+	-	+	+	-
Tyrosine	+	+	+	+	+
Effect on growth in the presence of Sodium azide	+	+	-	+	+
NaCl (5%)	+	+	+	+	+
NaCl (10%)	-	+	+	+	-

(+) = Growth detected

(-) = Growth not detected

Table 3: Production of Indole acetic acid (IAA) by *E. officinalis* isolates after 10 days of incubation

Genus	**Isolate**	**IAA production (g/ ml)**
Streptomyces albosporus	**AR8**	21.30 ± 0.4
S. albosporus	**AR12**	71.16 ± 0.6
S. griseofuscus	**AR4**	9.46 ± 0.5
S. griseofuscus	**AR13**	23.93 ± 0.5
S. griseofuscus	**AR17**	49.83 ± 0.7
S. griseofuscus	**AS4**	38.79 ± 0.6
S. griseofuscus	**AL5**	125.70 ± 0.6
S. griseofuscus	**AL6**	75.43 ± 0.3
S. griseofuscus	**AL8**	107.65 ± 0.6
S. cinereus	**AR16**	73.26 ± 0.5
S. griseorubroviolaceus	**AS7**	13.74 ± 0.5
Micromonospora sp.	**AS1**	80.65 ± 0.7
Micromonospora sp.	**AS5**	12.20 ± 0.4
Micromonospora sp.	**AS6**	53.46 ± 0.5
Micromonospora sp.	**AS8**	15.76 ± 0.5
Micromonospora sp.	**AS9**	22.23 ± 0.6
Micromonospora sp.	**AL2**	1.93 ± 0.3
Micromonospora sp.	**AL9**	87.72 ± 0.3
Micromonospora sp.	**AR5**	20.73 ± 0.6
Micromonospora sp.	**AR9**	71.50± 0.5
Micromonospora sp.	**AR11**	8.40 ± 0.7
Micromonospora sp.	**AR15**	20.30 ± 0.4

Micromonospora sp.	**AR18**	57.31 ± 0.3
Microbispora sp.	**AR6**	80.53 ± 0.6
Microbispora sp.	**AR14**	42.72 ± 0.7
	CD (5%)	**1.51**

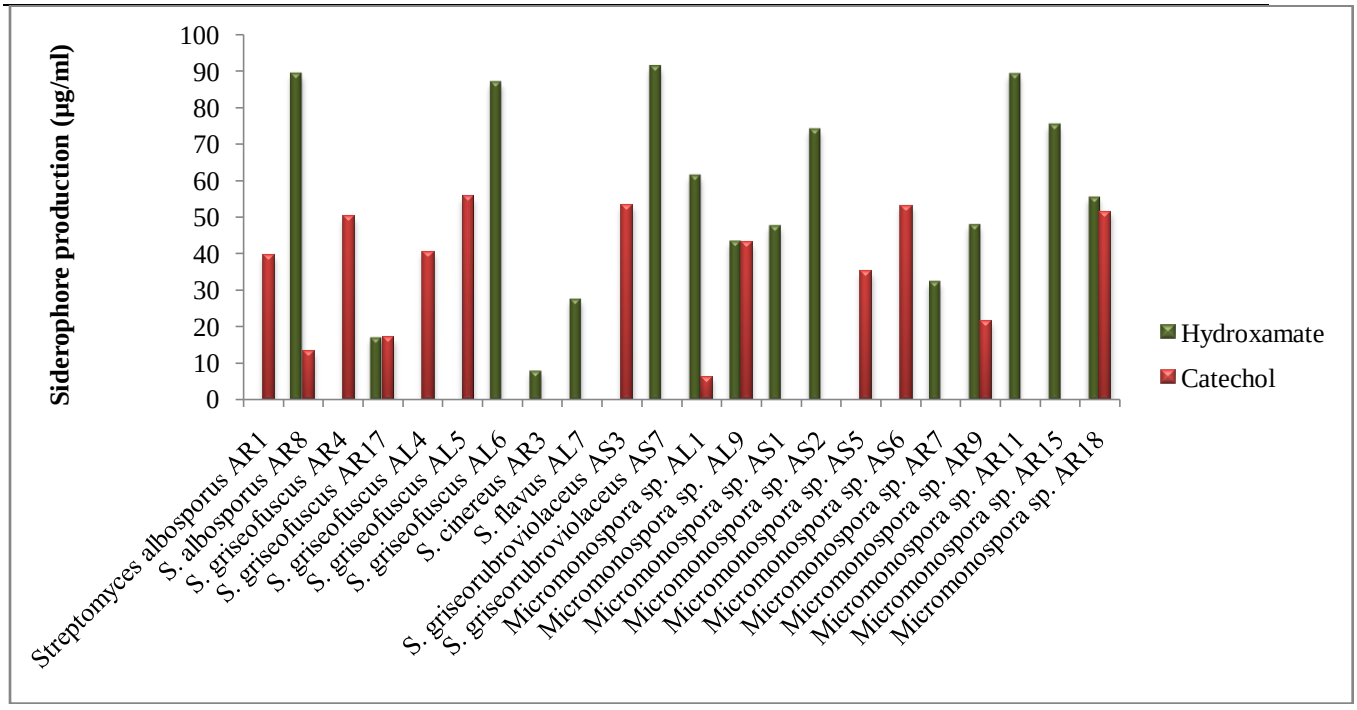

Figure 1: Siderophores production by different actinomycete isolates

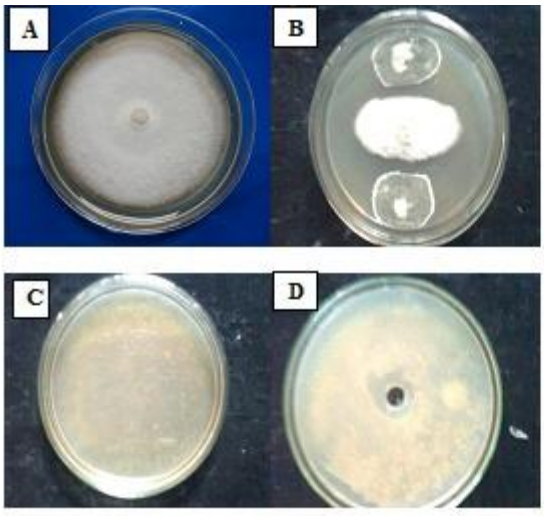

Figure 2: (A) *Fusarium oxysporum*-control
(B) Inhibition effect of *Streptomyces* against *F. oxysporum*
(C) *Aeromonas hydrophila*-control
(D) Inhibition effect of *Streptomyces griseorubroviolaceus* AS7

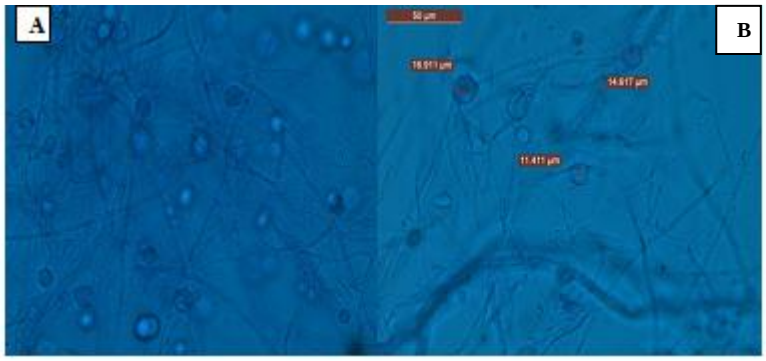

Figure 3: (A) Control
(B) *Fusarium oxysporum* infected with *Streptomyces cinereus* (AR16) shows reduction in the spore size, number of spores and thinning of hyphae

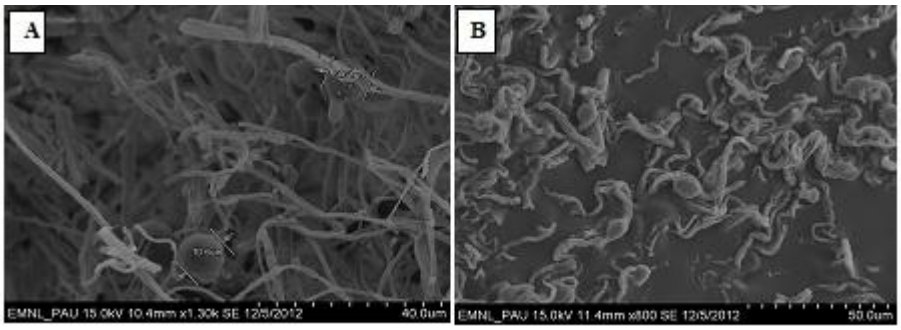

Figure 4: (A) Scanning electron microscopic analysis of *Fusarium oxysporum* grown alone showing regular, radial growth
(B) Co-cultured with *Streptomyces cinereus* (AR16) showing reduction in number and size of spores alongwith hyphal degradation

CONCLUSION

The study revealed that endophytic actinomycetes isolated from *E. officinalis* have broad-spectrum antimicrobial activity and are also the valuable reservoirs of novel bioactive compounds. Not only the *Streptomyces*, but also the other genera like *Micromonospora* sp. acted as the antagonistic agents.

Conflict of interests

The author(s) have declared no conflict of interests.

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