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Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India

Madhurama Gangwar[1]*, Sonam Dogra[1], Urmil Phutela Gupta[2] and Ravindra Nath Kharwar[3]

[1]Department of Microbiology, Punjab Agricultural University, Ludhiana-141004, Punjab, India.
[2]School of Energy Studies for Agriculture, Punjab Agricultural University, Ludhiana-141004, Punjab, India.
[3]Mycopathology and Microbial Technology Laboratory, Banaras Hindu University, Varanasi -221005, India.

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Three medicinal plants, *Aloe vera, Mentha arvensis* and *Ocimum sanctum* were explored for endophytic actinomycetes diversity, plant growth promoting and antimicrobial activity. Endophytic actinomycetes were most commonly recovered from roots (70% of all isolates) followed by stems (17.5%) and leaves (12.5%), respectively. Single genus *Streptomyces* ranked first (60% of all isolates) followed by *Micromonospora* (25%), *Actinopolyspora* (7.5%), and *Saccharopolyspora* (7.5%). The highest numbers of endophytic actinomycetes were isolated from *Ocimum sanctum* (45%). Out of 22 isolates tested, 12 showed the ability to solubilize phosphate in the range of 5.4-16.5 mg/100 ml, while 16 isolates produced indole-3 acetic acid (IAA) ranging between 8.3-38.8 µg/ml. Nine isolates produced the amount of hydroxamate- type of siderophore ranging between 5.9-64.9 µg/ml and only four isolates were able to produce catechol-type of siderophore in the range of 11.2-23.1 µg/ml. Of the nine, interestingly, eight endophytic actinomycetes (88.9%) showed a significant antagonistic activity against one or more phytopathogenic fungi indicating their possible role as plant biocontrol agents. An extended infection of root tissues of *Ocimum sanctum* by *Saccharopolyspora* O-9 was observed using transmission electron microscope (TEM).

Key words: Endophytic actinomycetes, antifungal activity, indole-3-acetic acid, medicinal plants, phytopathogenic fungi, siderophores.

INTRODUCTION

Actinomycetes represent a high proportion of the soil microbial biomass and have the potential to produce a diverse range of secondary metabolites including various antibiotics, anticancer and immunosuppressive agents and plant growth hormones (Strobel and Daisy, 2003; Fiedler et al., 2008; Schulz et al., 2009) that play an important role in agriculture and pharmaceutical industry. Development of pathogen resistance against existing medicines and emergence of new diseases compelled the search for novel secondary metabolites (Strobel and Daisy, 2003). Endophytic actinomycetes are the microbes that reside in healthy tissues of living plants without causing clinically detectable symptoms of disease and can be isolated from the surface-sterilized plant tissues (Nimnoi and Pongslip, 2009).

Endophytic actinomycetes have attracted attention in the search for novel bioactive natural compounds that can be used as new drugs replacing those against which pathogenic strains have rapidly acquired resistance. The association of actinomycetes with plants is found to confer many advantages such as the production of anti-microbials, extracellular enzymes, phytohormones and

*Corresponding author. E-mail: madhugangwar@yahoo.com.

siderophores. They also help in phosphate solubilization and plant protection against abiotic and biotic stresses (Bailey et al., 2006; Clegg and Murray, 2000). Endophytic actinomycetes have been isolated from stem and root interior of many plants, such as snakevine, tomato, banana, neem, wheat, etc (Castillo et al., 2002; Cao et al., 2004, 2005; Verma et al., 2009; Coombs and Franco, 2003). The endophytic *Streptomyces* NRRL 30562 obtained from the snakevine produced novel peptide antibiotic with wide-spectrum activity against many pathogenic fungi and bacteria (Castillo et al., 2002). Endophytic actinomycetes are considered to be potential biocontrol agents as they can colonize the interior of the host plant avoiding competition by other microbes in the soil. *Streptomyces* S30 isolate was found to be an effective biocontrol agent to the root pathogen *Rhizoctonia solani* (Cao et al., 2004; Kunoh, 2002). Many actinomycetes isolates of *Azadirachta indica* were strong inhibitors of potential filamentous fungal pathogens *Pythium* spp., and *Phytophthora* spp. (Verma et al., 2009). Many endophytic *Streptomyces* sp. can enhance crop yield through protection of their host against pathogens as in the case of 'take all disease' of wheat (Coombs, 2002; Coombs and Franco, 2003).

Actinomycetes are known for their ability to promote plant growth by producing indole-3-acetic acid (IAA) to help root development or by producing siderophore to bind Fe^{3+} from the environment and help to improve nutrient uptake (Leong, 1986). Medically important herbs and ethnopharmacologically used plants are wide spread in the Indian subcontinent. However, to the best of our knowledge, there are very few recent reports regarding the microbiological studies on the endophytic actinomycetes residing in the medicinal plants. Thus, this habitat deserves close examination for potential and novel microbes that could produce compounds with desired bioactivities. The present study was undertaken to isolate endophytic actinomycetes from selected medicinal plants and evaluate their potential for plant growth promoting and antimicrobial activity.

MATERIALS AND METHODS

Sampling

Thirty six healthy plants of *Aloe vera, Mentha arvensis* and *Ocimum sanctum* were randomly selected from herbal garden, Punjab Agricultural University, Ludhiana district (30.9° North and 75.85° East; Elevation, 247 m) of northern India. These plants species have medicinal value due to their rich contents of functional flavonoids, anthraquinones, saponins, sterols, phenols and essential oils. The samples were dug out carefully to ensure that maximal amount of root, shoot and leaf materials were collected. Samples were placed in plastic bags and brought to the laboratory in an ice-box and used to screen actinomycetes within 48 h of collection.

Surface sterilization and isolation

The root, stem and leaf segments were washed in running tap water to remove adhered epiphytes and soil debris. After drying in sterile conditions, tissue surfaces were sterilized by using 70% (v/v) ethanol for 5 min and sodium hypochlorite solution (0.9% w/v available chlorine) for 20 min. Surface-sterilized tissues were washed thrice in sterile distilled water. In order to reduce the opportunity for emergence of endophytic fungi from the tissue, the samples were soaked in 10% $NaHCO_3$ solution for 10 min to disrupt the growth of the fungi. Each root, shoot and leaf were cut into small pieces (0.5-1.0 cm) and placed on tryptic soy agar (TSA) medium and incubated at 28°C for 20-25 days to record the microbial growth. Effectiveness of surface sterilization was tested by the method of Schulz et al. (1993).

Identification of actinomycetes

Cultural and morphological characteristics, including presence of aerial mycelia, spore mass color, distinctive reverse colony color, color of diffusible pigments and spore chain morphology were used as identification characters (Cao et al., 2005). Visual observation of both morphological and microscopic characteristics using light microscopy and Gram-stain properties were also performed. All morphological characters were observed on TSA and the criteria used for classification and differentiation was as follows: (i) Aerial mass color: The mass color of mature sporulating aerial mycelium was observed following growth on TSA plates. The aerial mass was classified according to Bergey's manual of systematic bacteriology. (ii) Substrate mycelium: Distinctive colors of the substrate mycelium were recorded. (iii) Diffusible pigments: The production of diffusible pigment was also considered. (iv) Spores chain morphology: The shape of the spore chains observed under light microscope was also used as an important step in the identification. (v) Biochemical screening: Physiological criteria such as ability to degrade casein, starch, esculin, Tween 80, tyrosine, xanthine and hypoxanthine as substrates by the various actinomycetes strains were also used for genus confirmation.

Plant growth promoting traits of endophytic actinomycetes isolates

Phosphate solubilization

Endophytic actinomycetes isolates were inoculated on Pikovskaya medium supplemented with tricalcium phosphate and incubated at 28°C for seven days. The halo zone around the colony was presumptive confirmation of phosphate solubilization. Quantitative estimation of phosphate solubilization in broth was carried out using Erlenmeyer flasks (250-ml) containing 50 ml of Pikovskaya medium. The selected isolates were grown in liquid Pikovskaya medium for 7 days for the analysis of released phosphate content in the culture filtrate. The total amount of phosphate solubilzed by actinomycetes isolates was estimated by Jackson (1973) method.

Indole acetic acid (IAA) production

The production of IAA by 40 actinomycetes isolates was determined according to the method of Gordon and Weber (1951). The isolates were grown on yeast malt extract agar at 28°C for 5 days. Eight millimeter diameter agar discs were inoculated into 100 ml of yeast malt extract broth containing 0.2% L-tryptophan and incubated at 28°C with shaking at 125 rpm for seven days. Cultures were centrifuged at 11,000 rpm for 15 min. One milliliter of the supernatant was mixed with 2 ml of the Salkowski reagent. Development of pink color indicated IAA production. Optical density (OD) was taken at 530 nm using a spectrophotometer (ELICO, UV-VIS Spectrophotometer- SL 159). The level of IAA produced was

estimated by comparison with an IAA standard.

Siderophores production

The endophytic actinomycetes 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 siderophores were estimated by Arnow (1937) method and hydroxamate siderophores were estimated by the Csaky (1948) test.

***In vitro* antagonistic assay**

The endophytic actinomycetes isolates were evaluated for their antagonistic activity against nine pathogenic fungi *Aspergillus niger, Aspergillus flavus, Alternaria brassicicola, Botrytis cinerea, Fusarium oxysporum, Penicillium digitatum, Penicillium pinophilum, Phytophthora drechsleri and Colletotrichum falcatum* by dual-culture *in vitro* assay. Fungal discs (8 mm in diameter), 5 days old on PDA at 28°C were placed at the center of PDA plates. Two actinomycete discs (8 mm) 5 days old, grown on yeast malt extract agar were incubated at 28°C placed on opposite sides of the plates, 3 cm away from fungal disc. Plates without the actinomycete disc served as controls. All the plates were incubated at 28°C for 14 days and the 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. The zone of inhibition was measured between the pathogen and actinomycete isolates (Khamna et al., 2009).

Transmission electron microscopy (TEM)

Electron microscopy (TEM) of *Saccharopolyspora* O-9 inoculated root sample of *Ocimum sanctum* was carried out according to the method by Wescott et al. (1987). *Saccharopolyspora* O-9 was grown on plates containing TSA medium and incubated at 28°C for 7 to 10 days until luxuriant sporulation had occurred. Spores were harvested by scraping them off from the plate with a sterile loop and suspending them in 2 ml sterile H_2O. Approximately 30 *Ocimum sanctum* seeds were placed in a sterile Petri dish and treated separately with 2 ml of the actinomycete spore suspensions and water as a control. After mixing the seeds well in the spore suspensions, the Petri dish was left on an angle in the laminar flow cabinet overnight to evaporate the water and coat the spores onto the seeds. The seeds were grown in pots with approximately 110-120 g of sterilized field soil per pot and seeds were sown at a depth of 1 cm. Plants were grown for six weeks in a glasshouse with watering as required. Roots were then harvested and immersed in water so that adhering soil could be removed with a fine sable-hair brush. Pieces of roots (about 1 cm long) were selected and fixed for 24 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4°C. The tissue was rinsed in same buffer three times and then fixed in 2% (w/v) osmium tetroxide for 1 h. After three rinses in buffer, the tissues were dehydrated via a graded alcohol series and treated with propylene oxide. Infiltration with spur's resin mix was performed for two days using graded resin prepared in propylene oxide and cured for 16 h at 60°C. Thin sections (70-90nm) were cut on Leica ultra microtome and examined after staining with 2% uranyl acetate with transmission electron microscope (Hitachi H-7650, Tokyo, Japan).

RESULTS AND DISCUSSION

Isolation and identification of endophytic actinomycetes

A total of 40 isolates of endophytic actinomycetes were isolated from the root, stem and leaf tissues of three medicinal plants of India, *A. vera*, *M. arvensis* and *O. sanctum*. Most of the actinomycetes were identified as *Streptomyces* sp., by morphological characteristics, which was consistent with the other reports from different hosts (Coombs and Franco, 2003; Cao et al., 2004; Verma et al., 2009; Shenpagam et al., 2012). Out of the 40 isolates, the majority (70%) was recorded from roots followed by stems (17.5%) and leaves (12.5%) respectively.

Kafur and Khan (2011) isolated only one actino-mycete from leaf samples of *M. arvensis*. *Streptomyces* was the dominant genus (n=24, 60% of isolates) followed by *Micromonospora* sp. (n=10, 25%), *Actinopolyspora* sp. (n=3, 7.5%) and *Saccharopolyspora* sp. (n=3, 7.5%). Shenpagam et al. (2012) also isolated *Streptomyces* spp. from leaf and root samples of *O. sanctum*. The isolates were obtained most frequently from roots and less frequently from terrestrial parts, and this may be because of prevalence of actinomycetes in rhizospheric soil (Sardi et al., 1992). Based on colony and cultural characteristics, various subgroups were identified and among *Streptomyces* sp., the subgroup *Streptomyces cinereus* (n=4) was most frequently isolated followed by *Streptomyces albosporus, Streptomyces aureus, Streptomyces griseofuscus, Streptomyces roseosporus, S. viridis* (n=3 for each), respectively. *S. albosporus* and *S. griseofuscus* were obtained from the roots of all the three medicinal plants (Table 1).

The results also revealed that the surface treatment was adequate for the isolation of endophytic actinomycetes, as surface sterilized imprinted Petri plate (control) did not produce any growth. Thus, all the actinomycetes recorded in this experiment must have been endophytic and not the epiphytic.

Phosphate solubilization

Out of 40 isolates, twelve (30%) were observed to solubilize phosphate as they formed a clear zone around the colony on Pikovskaya medium. The amount of phosphate solubilized by actinomycete isolates obtained from medicinal plants fall in the range from 4.2 to 16.5 mg/100 ml (Table 2). The maximum amount of phosphate solubilization was shown by *S. albosporus* A4 (16.5 mg/100 ml). These results are in accordance with some earlier report (Hamdali et al., 2008) where 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/100

Table 1. Genera and number of endophytic isolates of actinomycetes recovered from root, stem and leaf tissues of *A. vera, M. arvensis* and *O. sanctum*.

Actinomycetes group	No. of endophytic actinomycetes isolated from								
	Aloe vera			*Ocimum sanctum*			*Mentha arvensis*		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Streptomyces albosporus	1	0	0	1	0	0	1	0	0
S. aureus	0	0	0	1	0	0	1	0	1
S. cinereus	1	0	0	1	1	0	0	0	1
S. globisporus	0	0	0	1	0	0	0	1	0
S. griseofuscus	1	0	0	1	0	0	1	0	0
S. roseosporus	0	0	0	1	0	0	0	1	1
S. viridis	1	0	0	1	0	0	0	1	0
S. griseorubruviolaceus	0	0	0	1	1	0	1	0	0
Actinopolyspora spp.	0	0	0	1	0	0	2	0	0
Micromonospora spp.	1	0	0	2	1	1	3	1	1
Saccharopolyspora spp.	1	0	0	1	0	0	1	0	0
Total	6	0	0	12	3	1	10	4	4

Table 2. Plant growth promoting traits of endophytic actinomycetes isolated from medicinal plants.

Isolate	IAA production (µg/ml)	Phosphate solubilization (mg/100 ml)
Saccharopolyspora A1	-	7.5
Streptomyces viridis A3	-	15.1
S. albosporus A4	22.4	16.5
S. cinereus A6	15.6	5.4
S. griseofuscus A8	-	12.5
Micromonospora A9	9.1	-
Streptomyces roseosporus M1	30.2	5.8
S. griseofuscus M2	34.5	-
S cinereus M5	8.3	-
S albosporus M7	18.8	8.4
Saccharopolyspora M13	11.1	-
Streptomyces cinereus O1	20.1	13.8
S. aureus O2	35.4	-
Micromonospora O6	9.0	4.2
Saccharopolyspora O9	17.2	14.9
Streptomyces albosporus O11	18.8	11.0
S. globisporus O13	10.7	
Micromonospora O14	-	11.6
Streptomyces viridis O15	15.7	-
S. griseorubroviolaceous O17	38.8	-
S. griseofuscus O18	14.2	-
S. aureus O20	19.6	-
CD@5%	0.97	0.61

ml) was reported. Microbial solubilization of mineral phosphate might be either due to the acidification of external medium or the production of chelating substances that increase phosphate solubilization (Welch et al., 2002; Whitelaw, 1999). Hence, P-solubilzing actinomycetes play an important role in the improvement of plant growth.

Indole acetic acid production

Eighteen (45%) out of 40 isolates produced the phyto-

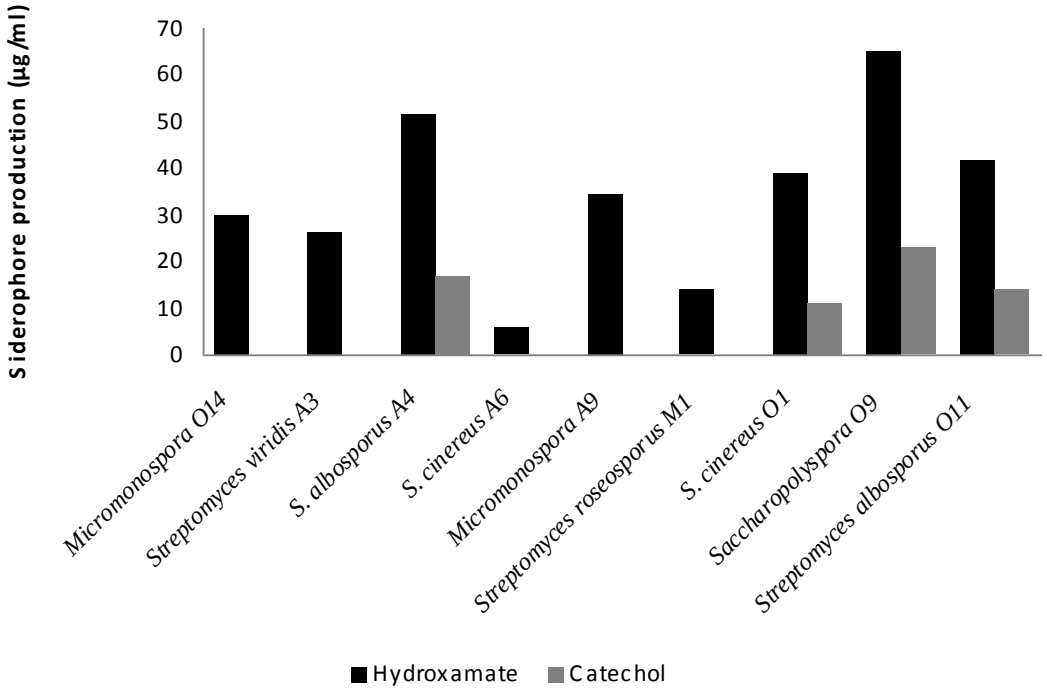

Figure 1. Siderophores production by different actinomycete isolates.

hormone indole acetic acid (IAA) and 14 of these belonged to *Streptomyces* sp. The range of IAA production was 9.0 - 38.8 µg/ml. The maximum IAA was produced by *S. griseorubroviolaceus* O-17. While *S. cinereus* M5 isolate produced the minimum yield of IAA (Table 2). Nimnoi et al. (2010) isolated endophytic actinomycetes from *Aquilaria crassna* and found that *Nocardiajiangxiensis* produced maximum IAA (15.14 µg/ ml) whereas *Actinomadura glauciflava* produced minimum yield of IAA (9.85 µg/ ml). The maximum IAA production was found to be greater in our study as compared to that reported by Nimnoi and Pongslip (2009). Soil and rhizosphere actinomycetes have shown potential to produce IAA and promoted the plant growth. Khamna et al. (2009) reported most active strains from the rhizospheres of *Cymbopagon citratus* and *Cymbopagon mangga*. *Streptomyces* CMU-HOO9 from *C. citratus* rhizosphere soil showed high ability to produce IAA. It is possible that high levels of tryptophan will be present in root exudates of medicinal plants and enhance IAA biosynthesis. Nimnoi and Pongslip (2009) demonstrated that the isolates of IAA synthetic bacteria enhanced root and shoot development of *Raphanus sativus* and *Brassica oleracea* more than fivefold when compared with the control. The IAA producing action-mycetes such as *Micromonospora*, *Nocardia*, *Actinomadura* and *Streptosporangium* have been reported to increase dry weight of corn, cucumbers, tomato, sorghum and carrot (El-Tarabily et al., 1997; Mishra et al., 1987). The presence of endophytic actinomycetes that produce IAA may have an important role in plant growth.

Siderophore production

Siderophore production was detected in nine actinomycetes isolates. The active isolates grew on CAS agar and an orange halo formed around the colonies. Most of them were *Streptomyces* sp. and *Saccharopolyspora* O-9 isolated from *Ocimum sanctum* showing high siderophore production ability. The 8 isolate *Saccharopolyspora* O-9 produced catechol (23.1 µg/ml) and hydroxamate (64.9 µg/ml) on glycerol yeast broth (Figure 1). This finding is in agreement with that obtained by Nimnoi et al. (2010) that eight endophytic actinomycetes isolates produced hydroxamate type of siderophore in the range of 3.21-39.40 µg/ml and one isolate produced catechol type siderophore (4.12 µg/ml). Khamna et al. (2009) showed that *Streptomyces* CMU-SK 126 isolated from *Curcuma mangga* rhizospheric soil exhibited high ability for siderophore production and produced catechol type (16.19 µg/ml) as well as hydroxamate type (54.9 µg/ml) siderophores. 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). Microbial siderophores may also be utilized by plants as an iron source (Bar-Ness et. al., 1991; Wang et. al., 1993; Robinson et al., 2002; Schulz et al., 2009). The role of siderophores produced by rhizospheric or endo-

Table 3. Antagonistic activity of endophytic actinomycetes isolated from medicinal plants against fungal plant pathogens.

Actinomycete isolates	Percentage inhibition (%)[a]								
	1	2	3	4	5	6	7	8	9
Micromonospora O14	0	0	0	62.2 ± 0.1	19.5 ± 0.1	12.6 ± 0.1	0	16 ± 0.1	16.1 ± 0.1
Streptomyces viridis A3	0	0	0	39.6 ± 0.2	22.8 ± 0.1	45.8 ± 0.1	0	0	17.4 ± 0.1
S. albosporus A4	11.4 ± 0.2	14.5 ± 0.1	0	63.5 ± 0.1	60.6 ± 0.1	19.8 ± 0.2	0	69.3 ± 0.3	57.9 ± 0.1
S. cinereus A6	0	0	0	42.4 ± 0.08	0	17.6 ± 0.1	34.5 ± 0.2	0	0
Micromonospora A9	0	0	0	40.3 ± 0.2	0	48.5 ± 0.2	0	13.9 ± 0.1	0
Streptomyces cinereus O1	0	0	0	25.3 ± 0.1	15.2 ± 0.1	0	0	0	42.6 ± 0.1
Saccharopolyspora O9	13.2 ± 0.2	28.6 ± 0.1	26.5 ± 0.2	71.4 ± 0.2	30.5 ± 0.1	51.4 ± 0.1	17.8 ± 0.1	56.4 ± 0.1	49.9 ± 0.1
Streptomyces albosporus O11	16.7 ± 0.1	17.5 ± 0.2	0	44.6 ± 0.2	18.6 ± 0.1	59.5 ± 0.2	0	53.5 ± 0.2	53.4 ± 0.2

[a]Mean ± standard error from triplicate samples. 1, *Aspergillus niger*; 2, *C. falcatum*; 3, *Aspergillus flavus*; 4, *Alternaria brassicicola*; 5, *Penicillium digitatum*; 6, *Fusarium oxysporum*; 7, *Penicillium Pinophilum*; 8, *Phytophthora drechsleri*; 9, *Botrytis ciner*.

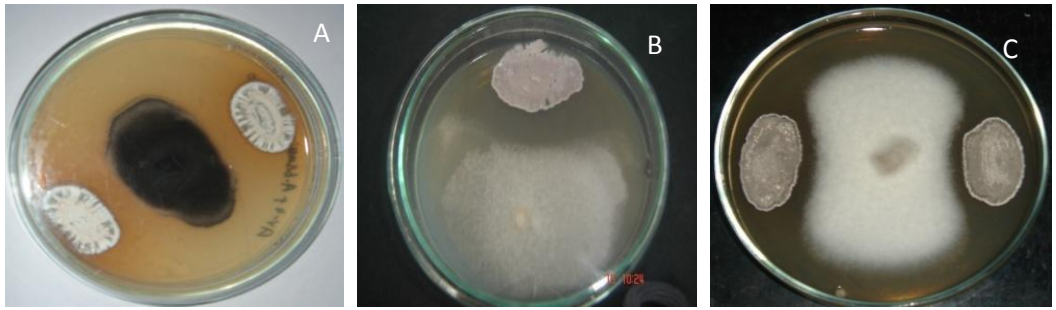

Figure 2. Antagonism of *Saccharopolyspora* O9 against (A) *Alternaria brassicicola* (B) *Botrytis cinerea* (C) *Fusarium oxysporum*.

phytic microbes has drawn more attention, for the metabolites that may be involved in promoting the growth substances and antagonise the phytopathogen (Bailey et al., 2006).

Antifungal activities

A total of eight endophytic actinomycetes isolates have strong inhibitory activity against *Alternaria brassicicola* and *Fusarium oxysporum* (Table 3). *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* exihibited antagonism against either one or more than one tested phytopathogens. *Streptomyce albosporus* O-11 and *S. albosporus* A-4 antagonized all the tested fungi except *A. flavus* and *P. pinophilum* (Figure 2). Taechowisan and Lumyong (2003) isolated 59 endophytic actinomycetes from roots of *Zinger officinale* and *Alpinia galanga* and tested against *Candida albicans* and phytopathogenic fungi. *Streptomyces aureofaciens* CMUAc130 was the most effective in antifungal activity. Biocontrol effects of endophytic actinomycetes both *in vitro*

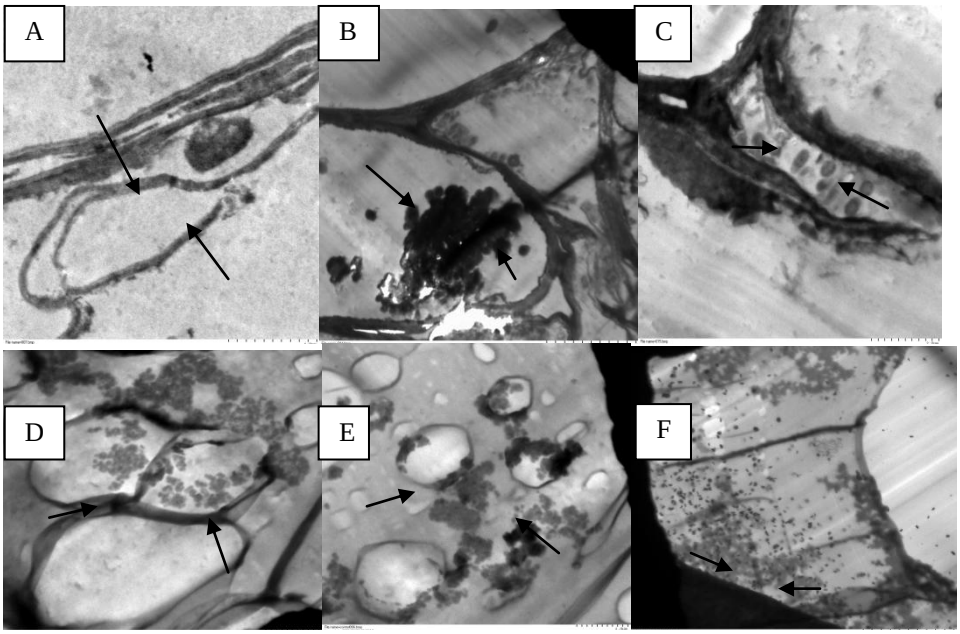

Figure 3. Electron micrographs of *Ocimum sanctum* roots infected with *Saccharopolyspora* O-9. A-C: Infection into middle lamella; D-E: hypervacuolation and vesicle formation; F: presence of spores inside the cell.

and *planta* have been reported. In another study, thirty eight strains of endophytic actinomycetes isolated from surface sterilized wheat and barley roots were tested for their antagonistic activity to wheat root pathogens *Gaeumannomyces graminis*, *Rhizoctonia solani* and *Pythium* sp. It was observed that 17 of 38 isolates displayed statistically significant activity *in planta* against *G. garminis* (Coombs et al., 2004). Zhao et al. (2011) interestingly reported that 59 out of 60 strains showed antagonistic activity against at least one of the 11 indicator organisms. Thirty eight *Streptomyces* isolates inhibited the growth of at least five indicator organisms. SAUK6015, a strain most similar to *Nonomuraea roseola*, was a very potent inhibitor of pathogens *Exerohilum turcicum* and *Curvularia lunata*, whereas SAUK6030, a strain most similar to *Micromonospora chokoriensis* was a potent inhibitor of *Curvularia lunata* (Zhao et al., 2011). The medicinal plants host numerous *Streptomyces* strains expected to produce a wide variety of bioactive metabolites. The antagonistic activity displayed in this study further indicates that endophytic *Streptomyces* hosted by medicinal plants are a key source of bioactive compounds. The ability of isolates to inhibit the growth of fungal pathogens is implication of the diffusible secondary metabolites secreted by actinomycetes.

Transmission electron microscopy of *Saccharopolyspora* O-9 inoculated *O. sanctum*

Transmission electron micrographs depicted an extended infection of the root tissues of *O. sanctum* by *Saccharopolyspora* O-9. The infection had been observed beneath the cell membrane as well as deep inside the cell. An elaborate vacuole and vesicle formation has also been observed in the infected tissue. The hyphae of *Saccharopolyspora* O-9 were also found within the middle lamella of infected root tissues (Figure 3). This finding corroborates the result obtained by Clark and Matthews (1987) and de Almeida et al. (2009) who had observed migration of the microorganism from the cytoplasm to inside of the vacuole and the vesicles formation.

The present study reveals that medicinal plants provide a rich source of diversity of endophytic actinomycetes. The presence of actinomycetes inside these plants confer many advantages to host plants such as production of phytohormone (IAA), siderophores, ability to solubilize the phosphate as well as protection against plant pathogens.

The metabolites produced by endophytic acinomycetes may enhance the fitness and growth of the host and indirectly affect the harmful microbial population. Further investigations are required to understand the other forms of relationship between endophytic actinomycetes and host plant that may be useful in pharmacological and agricultural fields in the future.

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