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Research Article

Antagonistic Bioactivity of Endophytic Actinomycetes Isolated from Medicinal Plants

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Abstract: Endophytic actinomycetes are promising biocontrol agents for use in agriculture and have been isolated from various plant species. In the present study, 40 endophytic actinomycetes were isolated from roots, stems and leaves of three medicinal plants viz. *Aloe vera*, *Mentha arvensis* and *Ocimum sanctum*. The identification revealed that the majority of the isolates were *Streptomyces* spp. and the rest were identified as *Saccharopolyspora* spp., *Micromonospora* spp. and *Actinopolyspora* spp. The dual tests revealed that nine endophytic actinomycete isolates displayed a wide spectrum activity against nine fungal phytopathogens. Out of 8 isolates, 90% inhibited the growth of at least one or more phytopathogenic fungi and *Saccharopolyspora* 0-9 (Out of 8 isolates, 90% inhibited the growth of at least one or more phytopathogenic fungi and *Saccharopolyspora* 0-9 exhibited antagonistic activity against *Aspergillus niger*, *Aspergillus flavus*, *Alternaria brassicicola*, *Botrytis cinerea*, *Penicillium digitatum*, *Fusarium oxysporum*, *Penicillium pinophilum*, *Phytophthora dresclea* and *Colletotrichum falcatum*.

Keywords: Endophytic actinomycetes, Phytopathogenic fungi, Biocontrol agents, Antagonistic activity.

1. Introduction

Actinomycetes are known as producers of antibiotics and other biologically active substances with high commercial value such as vitamins, alkaloids, plant growth factors, enzymes and enzyme inhibitors (Tanaka and Omura, 1993). Approximately two-thirds of naturally occurring antibiotics, including some of agricultural importance, have also been isolated from these soil microorganisms. Evidence indicated that actinomycetes are important in the rhizosphere because they can influence plant growth and protect plant roots against invasion by root pathogenic fungi (Crawford *et al*., 1993; Tokala *et al*., 2002). Actinomycetes are found also as endophytes that colonize the plant tissues. Actinomycetes have been isolated from surface sterilized roots of Italian native plants (Sardi *et al*., 1992), from roots and leaves of maize (De-Araujo *et al*., 2000), from roots and leaves of banana plants (Cao *et al*., 2004) and from surface sterilized wheat roots (Coombs and Franco, 2003). *In vitro* and *in vivo* antagonistic activities of endophytic actinomycetes against plant pathogens have been reported (Cao *et al*., 2005; Taechowisan *et al*., 2003; Tian *et al*., 2004). The introduction of endophytic actinomycetes into plants with the ability to colonize the internal tissue would further enhance the stability and increase their potential effectiveness as biocontrol agents (Coombs *et al*., 2004).

In order to find effective biocontrol agents for fungal plant pathogens, endophytic actinomycetes were isolated from surface-sterilized roots, stems and leaves of medicinal plants. The present study was undertaken with a view to testing the potential of endophytic actinomycetes as biocontrol agents against various phytopathogenic fungi.

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2. Materials and Methods

2.1 *In vitro* antagonistic bioassay

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

3. Results and Discussion

3.1 Antifungal activities

Eight (20%) of actinomycete isolates were active against at least one of the nine phytopathogenic fungi (Table 1).

Different isolates of *Streptomyces* spp. displayed an array of activity against pathogenic fungi, particularly *S. albosporus* A4, an *Aloe vera* isolate and O-11, an *Ocimum sanctum* isolate. This is in conformity with the results of several studies carried out by other investigators (Crawford *et al.,* 1993; Taechowisan and Lumyong, 2003; Tian *et al.,* 2004; Verma *et al*., 2009). *Saccharopolyspora* O-9 from *Ocimum sanctum* strongly inhibited all of the pathogenic fungi (Fig. 1) with maximum percent inhibition was observed against the fungus *Penicillium digitatum* (71.4%).

Actinomycetes-fungus antagonism has been demonstrated for a variety of plant pathogens such as *Alternaria, Rhizoctonia, Verticillium, Fusarium, Phytophthora* and *Pythium* spp. (Yuan and Crawford, 1995; Aghighi *et al.,* 2004). The ability of isolates to inhibit the growth of fungal pathogens is implication of the volatile secondary metabolites secreted by actinomycetes. So, in the present study, the potential of endophytic actinomycetes to inhibit the growth of pathogens has been studied. Dual-culture assays showed that some actinomycetes isolated can be developed as potential biocontrol agents. Therefore, further studies are necessary to assess the ability of the isolates to confer protection against pathogens and their role in enhancing growth and yield of plants under field conditions.

In conclusion, all but one of our Streptomyces isolates tested displayed antifungal activity. In addition, to Streptomyces, the medicinal plants carried rare actinomycetes all of which displayed antifungal activity with *Saccharopolyspora* being the potent antagonist. Our survey suggested that medicinal plants are a potent source of endophytic actinomycetes with wide biological activity against pathogenic fungi.

Table 1. Antifungal activity of actinomycete isolates.

	Percentage (%) inhibition[a]								
Actinomycete isolates	***Aspergillus niger***	***Colletotrichum falcatum***	***Aspergillus flavus***	***Alternaria brassicicola***	***Penicillium digitatum***	***Fusarium oxysporum***	***Penicillium pinophilum***	***Phytophthora dresclea***	***Botrytis cinerea***
***Micromonospora* O-14**	0	0	0	62.2 ± 0.1	19.5 ± 0.1	12.6 ± 0.1	0	16 ± 0.1	16.1 ± 0.1
***S. 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
***S. cinereus* O-1**	0	0	0	25.3 ± 0.1	15.2 ± 0.1	0	0	0	42.6 ± 0.1
***Saccharopolyspora* O-9**	13.2 ± 0.2	28.6 ± 0.1	26.5 ± 0.2	30.5 ± 0.1	71.4 ± 0.2	51.4 ± 0.1	17.8 ± 0.1	56.4 ± 0.1	49.9 ± 0.1
***S. albosporus* O-11**	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]Average ± standard error from triplicate samples

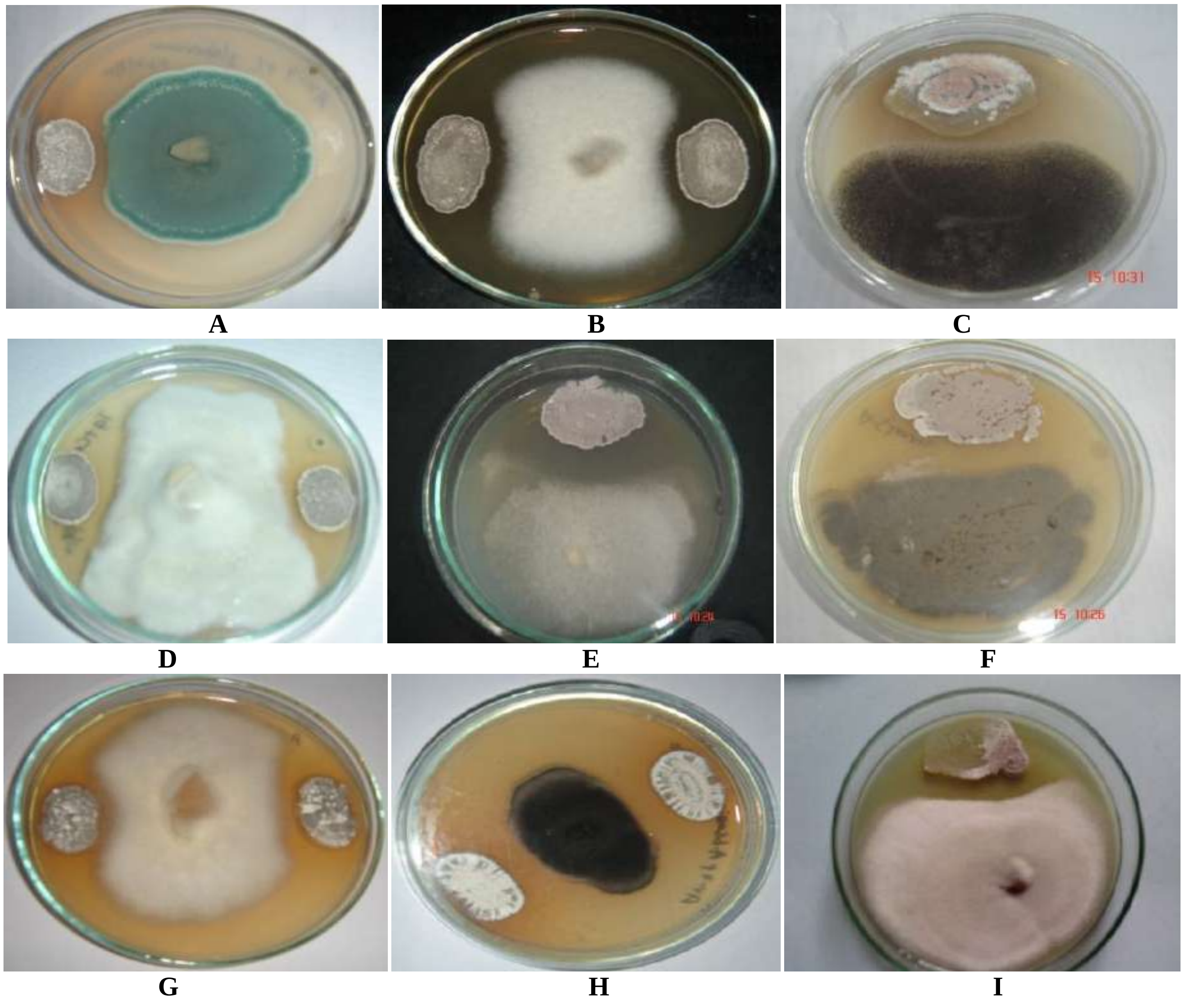

Fig. 1. Inhibition effect of *Saccharopolyspora* O-9 against *Penicillium digitatum* (A), *Fusarium oxysporum* (B), *Aspergillus niger* (C), *Colletotrichum falcatum* (D), *Botrytis cinerea* (E), *Aspergillus flavus* (F), *Phytophthora dresclea* (G), *Alternaria brassicicola* (H) and *Penicillium pinophilum* (I), *Penicillium digitatum*

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