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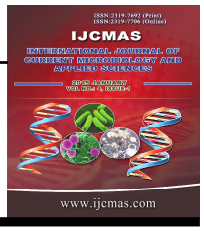

Original Research Article

Antifungal Activity of Actinomycetes from Rhizospheric Soil of Medicinal plants against phytopathogenic fungi

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A B S T R A C T

A total of 100 actinomycetes isolates were obtained from 30 rhizospheric soil samples of *Catharanthus roseus* and *Withania somnifera* in different locations in Ludhiana, India. The 100 actinomycetes isolates obtained were tested for their antifungal activity against seven phytopathogenic fungi *viz*: *Alternaria alternata*, *Fusarium oxysporum*, *Helminthosporium oryzae*, *Macrophomina phaseolina*, *Penicillium* sp., *Rhizoctonia solani* and *Sclerotium rolfsii.* A total of 39 isolates showed antifungal activity against one of the others seven fungus and none was found to show antifungal against *Sclerotium rolfsii.* Out of the 39 Isolate, 9 showed antifungal activity against *Alternaria alternata*, 19 against *Fusarium oxysporum*, 20 against *Helminthosporium oryzae*, 14 against *Macrophomina phaseolina*, 10 *against Penicillium* sp. and 16 *against Rhizoctonia solani.* It was concluded that actinomycetes from the rhizospheric soil of rhizospheric soil are the most promising candidates to be used as biocontrol agent against phytopathogenic fungi

Keywords

Catharanthus roseus, *Withania somnifera*, Antifungal activity, Rhizospheric soil, Phytopathogenic fungi

Introduction

Fungal phytopathogens cause serious problems worldwide in agriculture and food industry by destroying crops and economically important plants especially in the subtropical and tropical regions (2006), producing mycotoxins, which are harmful to humans and livestock. Fungicidal or synthetic compounds which are used to keep fungal infections at an acceptable level, are associated with several drawbacks such as their lack of specificity, accumulation if biodegradation is slow or even missing, and others are toxic not only to fungi but also to other beneficial life forms and also have led to environmental pollution and development of pathogen resistance (Pohanka 2006).

Due to worsening problems in fungal disease control, alternate methods for plant protection are needed which are less dependent on chemicals and more environmentally friendly which is potential use of biocontrol agents as replacements or supplements for agrochemicals (Shimizu *et al* 2000, Yang *et al* 2007).

Soil actinomycetes have revealed their wide antifungal activity (Tinatin and Nuzrat 2006). They have been shown to protect several plants to various degree of soil borne fungal pathogens. Actinomycetes as biocontrol agent, produce Urauchimycins which is a member of antimycin class, a set of well-identified antifungals, that

act by inhibiting the electron flow in the mitochondrial respiratory chain of a phytopathogenic fungus and have been identified in *Streptomyces* isolated from the integument of attine ants (Schoenian *et al* 2014, Seipke *et al* 2011, Seipke *et al* 2012). Furthermore, Actinomycetes produced lots of biofungicide like MYCOSTOP® for the control of seed- and soil-borne plant pathogens (*Fusarium, Alternaria, Phytophthora* and *Pythium*) which cause damping-off and root diseases, Actinovate® isolated from streptomycetes species. *Streptomyces lydicus* WYEC108 is a strain of this species which has been formulated to control fungal plant pathogens effectively for fresh market tomatoes, PRESTOP® for controlling of damping-off and root diseases (*Pythium, Fusarium, Phytophthora* and *Rhizoctonia*) as well as for the control of *Botrytis* grey mould and *Didymella* (*Mycosphaerella*) gummy stem blight cucumber (Yedidia 2011) etc. that are available commercially

The present study aimed to isolate actinomycetes from rhizospheric soil of medicinal plants and test the potential isolates against phytopathogenic fungal.

Materials and Methods

Collection of soil samples

Rhizospheric soil samples of *Catharanthus roseus* and *Withania somnifera* plants were collected from different locations *viz.*, Herbal garden, PAU, Central market, Raj Guru Nagar, Agar Nagar Sector B, Agar Nagar Sector A, In front of Hostel: 3, PAU, In front of COBS&H, PAU, Big Haibowal, Raj Guru Nagar, I block and Herbal Garden, PCTE of Ludhiana, Punjab, India. The samples were taken from the growing roots up to a depth of 5 cm after removing approximately 3cm of the soil surface. These samples were placed in polythene bags, closed tightly and analyzed for actinomycetes.

Isolation and enumeration

Actinomycetes population in the rhizospheric soil of *Catharanthus roseus* and *Withania somnifera* plants were determined by serial dilution method. Ten grams of soil sample was taken and transferred to an Erlenmeyer flasks containing 90 ml of sterile distilled water. The soil sample was serially diluted upto 10^{-5} levels. Aliquot (1ml) serial logarithmic dilution of each suspension was pipetted onto the surface of duplicated petri plates containing starch casein agar. The inoculum was spread evenly over the surface using glass spreader. Petri plates were incubated for 7-10 days at 28 ºC. The mean colony count was determined and recorded as colony forming unit (c.f.u/g) of each sample. Isolated colonies were streaked on Starch Casein Agar after purification and then sub cultured on slants and stored at 40°C. Sub culturing was done after every two months.

Antagonistic effect against phytopathogenic fungi

The actinomycetes isolates were evaluated for their antagonistic activity against seven phytopathogenic fungi: *Alternaria* sp, *Fusarium oxysporum*, *Helminthosporium oryzae*, *Macrophomina phaseolina*, *Penicillium sp*., *Rhizoctonia solani* and *Sclerotium rolfsii* by dual-culture *invitro* assay. Fungal discs (8mm in diameter), 5 days old on GYE at 28ºC were placed at the center of GYE plates. Two actinomycetes discs (8mm) 5 days old, grown on starch casein agar, incubated at 28ºC were placed on opposite sides of the plates, 3 cm away from fungal disc. 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. The zone of inhibition was measured between the pathogen and actinomycetes isolate.

Result and Discussion

Out of the 100 isolates obtained from rhizospheric soil of medicinal plants, 39 (21 from *Catharanthus roseus* and 18 from *Withania somnifera*) were displaying antagonistic activity against one or the other plant pathogenic fungi tested. In the present study, nine isolates from *C.*

roseus (CR-4, CR-5, CR-6, CR-11, CR-24, CR-27, CR-28, CR-31 and CR-40) and ten from *W. somnifera* (WS-10, WS-21, WS-23, WS-24, WS-28, WS-35, WS-40 and WS-48) were observed to have antifungal activity against *Fusarium oxysporum* respectively. The percent growth inhibition was found to be ranging between 20.71±0.1 to 73.78±0.5% in case of *F. oxysporum* (Table 1 and 2). As shown in Fig. 1, isolate CR-6 showed highest zone of inhibition (73.78±0.5%). Nine isolates (4 from *C. roseus* and 5 from *W. somnifera*) were found to exhibit antifungal activity against *Alternaria alternata* ranging from 13.9±0.3 to 57.37±0.4% (Fig 1, B). Maximum percent inhibition was produced by isolate WS-40 (57.37±0.4%). Khamna *et al* (2009) found that *Streptomyces spectabilis* CMU-PA101 from *Curcuma mangga* rhizosphere has the ability to exhibit high antifungal activity *in vitro* against hyphal growth of *Alternaria porri* on shallot plant. A total of eight endophytic actinomycetes isolates have strong inhibitory activity against *Alternaria brassicicola*, as reported by Gangwar *et al* (2014). *Saccharopolyspora* O-9 from *O. sanctum* strongly inhibited all the pathogenic fungi tested, and maximum percent inhibition was observed against *A. brassicola* (71.4%).

Similarly in case of *Rhizoctonia solani*, sixteen isolates (8 from each *C. roseus* and *W. somnifera*) exhibited antifungal activity ranging from 33.11±0.1 to 74.81±0.3%. Isolate WS-10 showed maximum percent inhibition i.e. 74.81±0.3% (Fig 1,). Robati and Mathivanan (2013) obtained similar results while using *Strptomyces* sp. MML1715 and indicated remarkable activity against test pathogen *R. solani*. Khendker and Deshpande (2014) collected a total of 48 actinomycete isolates from 19 rhizospheric soil samples of different plants (soybean, brinjal, okra, mango, turmeric, ginger, coconut, onion and wheat). Among those, 11 actinomycete isolates exhibited antagonistic activity against *Rhizoctonia bataticola*, displaying a zone of inhibition ranging from 2-5 mm.

None of the isolates were displaying antifungal activity against *Sclerotium rolfsii*.The results are in accordance with Madhuban *et al* (2010) who isolated actinomycetes from the soil of Division of Microbiology, IARI and name it strain SI (IARI 100) and S2. Reports showed that S1 and S2 exhibit inhibition against *Rhizoctonia bataticola, Colletotrichum falcatum, Fusarium oxysporum and Penicillium* but against *Sclerotium rolfsii.*

As evident from Fig 1 (D), CR-27 an isolate of *Catharanthus* was observed to have the maximum antifungal activity against *Macrophomina phaseolina*, which was exhibiting percent inhibition of 58.87±0.2%, followed by CR-25 (57.45±0.4) and WS-32 (57.37±0.4). The minimum inhibition was observed by WS-31 (29.75±0.3) when compared to control. The results are in corroboration with Gopalakrishnan *et al* (2011), who reported that out of the 137 actinomycetes obtained from the herbal vermicomposts, 79 (58%) were displaying the antagonistic potential (in the dual-culture assay) against *M. phaseolina*. This inhibition could be due to the production of hydrolytic enzymes or antibiotics by the actinomycetes which were disseminated through the media.

Ten isolates (5 from each *C. roseus* and *W. somnifera*) possessed moderate to strong antifungal activity against *Penicillium* sp. with inhibition zone ranging from 31.25±0.4 to 62.46±0.5%. Isolate WS-27 displayed maximum zone 62.46±0.5% followed by WS-35 (61.70±0.5%) and WS-23 (58.83±0.3%). Similar results were obtained by other workers as well. Kamble and Kulkarni (2014) obtained 5 isolates of *Streptomyces* from rhizospheric soil of *Curcuma longa* L., whereby all of those were able to resist growth of *Penicillium chrysogenum*, exhibiting a zone of inhibition ranging from 10-12 mm.

Twenty isolates (12 from *Catharanthus roseus* and 8 *Withania somnifera*, respectively) were found to exhibit broad spectrum antifungal activity against *Helminthosporium oryzae* ranging from 19.66±0.2 to 42.75±0.2%. Maximum inhibition being shown by isolate CR-18 i.e. 42.75±0.2% (Fig 3, I and J) and minimum being displayed by the isolate CR-34 viz. 19.66±0.2%.

Table.1 Antifungal activity (% inhibition) of *Catharanthus roseus* isolates

Isolates	*Alternaria alternata*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Macrophomina phaseolina*	*Penicillium* sp.	*Helminthosporium oryzae*
CR-1	-	-	-	35.3±0.2	-	25.5±0.3
CR-3	-	-	42.6±0.3	-	55.79 ± 0.2	28.0 ±0.5
CR-4	-	50.54±0.8	-	48.5±0.1	-	24.9± 0.1
CR-5	-	42.62±04	33.11±0.1	-	-	-
CR-6	46.0 ± 0.4	73.78± 0.5	-	-	-	-
CR-11	-	49.0 ± 0.5	43.73± 0.9	-	-	-
CR-13	-	-	68.35± 0.3	-	-	31.9± 0.6
CR-14	-	-	-	-	-	30.7±0.4
CR-16	-	-	52.61± 0.3	43.1±0.5	45.52 ± 0.7	-
CR-18	27.58±0.4	-	68.1± 0.1	-	-	42.75±0.2
CR-24	-	40.692±0.4	-	-	-	-
CR-25	28.59±0.7	-	-	57.45±0.4	-	-
CR-27	-	59.85±0.03	-	58.87±0.2	-	32.5 ±0.3
CR-28	-	37.4±0.8	-	-	-	-
CR-29	-	-	-	-	55.63± 0.4	40.30±0.2
CR-31	-	57.78±0.4	-	-	-	28.7±0.2
CR-32	-	-	-	57.02±0.6	55.17±0.2	-
CR-33	-	-	-	-	-	31.66±0.2
CR-34	-	-	-	37.70±0.5	-	19.66±0.2
CR-37	13.9±0.3	-	68.2± 0.1	-	31.25±0.4	38.13±0.2
CR-40	-	41.2±0.6	35.2± 0.2	50.70±0.5	-	-

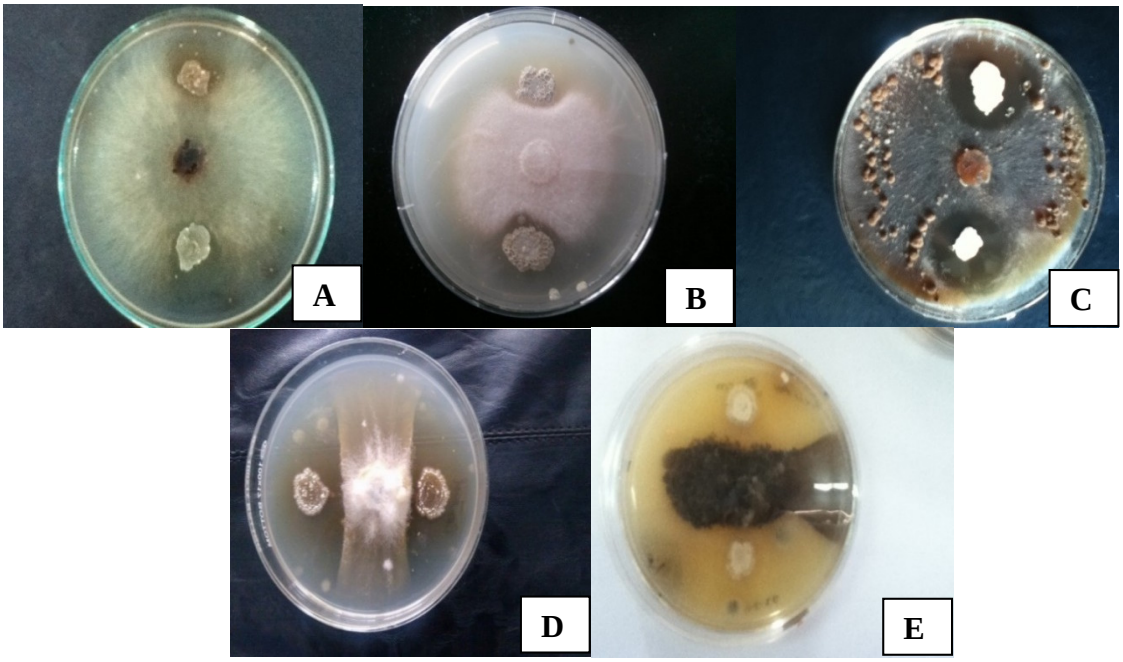
*Average ± standard error from three replicates

Table.2 Antifungal activity (% inhibition) of Withania somnifera isolates

Isolates	*Alternaria alternata*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Macrophomina Phaseolina*	*Penicillium* sp.	*Helminthosporium oryzae*
WS-10	-	47.34±0.5	74.81±0.3	-	-	-
WS-13	69.9 ± 0.9	-	65.01±0.4	-	-	-
WS-16	40.0 ± 0.6	-	-	-	-	-
WS-21	-	28.14±0.3	-	-	-	20.13±0.2
WS-23	-	31.93±0.3	66.32±0.3	-	58.83±0.3	-
WS-24	-	22.61±0.9	43.31± 0.4	-	-	36.25±0.2
WS-27	-	-	-	-	62.46±0.5	-
WS-28	-	20.71±0.1	67.52±0.2	-	-	-
WS-29	-	-	66.11±0.7	-	-	30.33±0.2
WS-31	-	-	-	29.75±0.3	39.9 ± 0.8	-
WS-32	22±0.4	-	-	57.37±0.4	-	22.13±0.2
WS-34	-	-	-	19.83±0.8	-	28.33±0.2
WS-35	32.36±0.8	39.5±0.4	74.25±0.3	35.26±0.4	61.70±0.5	38.47±0.3
WS-37	-	-	71.86±0.2	-	-	-
WS-40	57.37±0.4	68.52±0.3	-	50.17±0.8	55.0 ± 0.5	-
WS-43	-	71.1±0.3	-	42.65 ±0.4	-	30.46±0.3
WS-45	-	29.1±0.7	-	-	-	-
WS-48	-	58.41± 0.3	-	-	-	32.26±0.8

*Average ± standard error from three replicates

Figure.1 Inhibition effect of Actinomycetes isolates against Fusarium oxysporum (A), Alternaria alternate (B), Rhizoctonia solani (C), Macrophomina phaseolina and Helminthosporium oryzae



Elamvazhuthi and Subramanian (2013) also reported that 33.3% isolates from upland paddy of Jeypore, Odissa; were showing antagonism against *Helminthosporium oryzae* to more or less extent. Likewise, Poomthongdee *et al* (2014) isolated 351 actinomycetes from 21 rhizospheric soil samples. Of these actinomycetes, 32.5% showed antagonistic activity against rice pathogenic fungi *Helminthosporium oryzae*.

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