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In vitro evaluation of antagonistic activity of *Pseudomonas fluorescens* against fungal pathogen

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ABSTRACT

The present investigation was undertaken to isolate different strains of *Pseudomonas fluorescens* from various agroecological zones or crop's rhizosphere like moong, brinjal, rice, chilli, mustard, chirchida and tomato. Totally eight micro flora resembling *Pseudomonas fluorescens* were isolated and three isolates were confirmed as *P. fluorescens* (strain P.f.01, strain P.f.05 and strain P.f.07). *Pseudomonas fluorescens* strains P.f 07 were found most effective with the highest antagonistic activity against three fungal pathogen and show maximum inhibition of mycelial growth of *Fusarium moniliforme* (65.45%), *Rhizoctonia solani* (68.23%), and *Alternaria alternate* (48.13%).

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INTRODUCTION

Pseudomonas fluorescens encompasses a group of common, gram negative, rod shaped, non pathogenic saprophytes bacteria that colonize in soil, water and plant surface environments. Since they are well adapted in soil, *P. fluorescens* strains are being investigated extensively for use in biocontrol of pathogens in agriculture (Ganeshan and Kumar, 2006). Biological control of plant pathogens by antagonistic micro organisms is a potential non-chemical means (Harman,1991) and is known to be a cheap and effective eco-friendly method for the management of crop diseases (Cook and Baker, 1983). *Pseudomonas fluorescens* is adapted to survival in soil and colonization of plant roots (Kiely *et al*., 2006) and this applies also to the particular case of biocontrol agents from this species. Biocontrol strains have noticeably been observed at the root surface, (i.e. the rhizoplane) often forming microcolonies or discontinued biofilms in the grooves between epidermal cells. Certain strains are also capable of endophytic colonization. Within root tissues, they are mostly found in the intercellular spaces of the epidermis and the cortex (Duijff *et al*., 1997). Many biocontrol agents from *P. fluorescens* and closely related species are well characterized for their ability to produce antimicrobial compounds, including 2,4-diacetylphloroglucinol (DAPG), phenazines, hydrogen cyanide and surfactants (Haas and De´fago, 2005).The use of biological control agents as an alternative to fungicides is increasing rapidly in the present day agriculture, due to the deleterious effects of chemical pesticides on human health and environment.

MATERIALS AND METHODS

Present investigations on *Pseudomonas fluorescens*, an antagonistic bacterium with special reference to evaluation of antagonistic activity of *P. fluorescens* against fungal pathogen were carried out in the laboratory of the Department of Plant Pathology of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.).

Isolation

For isolation, one gram of soil sample was placed in a 250 mL conical flask containing 100 mL of sterilized distilled water (SDW) and mixed thoroughly. Different dilutions of working samples were prepared by serially diluting the stock solution upto10^{-8}. One ml of last serial dilution *i.e.*, 10^{-8} was spread on *P. fluorescens* selective King's B Medium (King's *et al.*, 1954) for isolation of *P. fluorescens.* The plates were incubated for 2 days at 28±2ºC and after incubation, pure culture was grown; colour of bacterial colony was initially yellow but turned yellow green as pigmentation were produced (Bonds, 1957).

Isolation of *Pseudomonas fluorescens* from different rhizosphere

To isolate the *P. fluorescens* from different rhizosphere, the soil samples were collected from different location as given below:

Isolates No.	Vegetation	Location	Tentative Identification
P.f.01	Moong	PCP SVP UNI. Meerut	*P.fluorescens*
P.f.03	Brinjal	HRC SVP UNI. Meerut	*P.fluorescens*
P.f.05	Rice	CRC Chiraudi SVP UNI.Meerut	*P.fluorescens*
P.f.07	Chilli	Farmer field Meerut	*P.fluorescens*
P.f.09	Chilli	PCP SVP UNI. Meerut	*P.fluorescens*
P.f.11	Mustard	Farmer field Meerut	*P.fluorescens*
P.f.12	Chirchida	Meerut	*P.fluorescens*
P.f.13	Tomato	Farmer field Ambedkar Nagar	*P.fluorescens*

Culture and maintenance

Medium consists of peptone 20gm, agar-agar 20gm, potassium monophosphate (K_2HPO_4) 1.5gm, magnesium sulphate (MgSo4) 1.5gm, glycerol 10mL and distilled water 1000 mL (King *et al.*, 1954)**.** After mixing all the ingredients with distilled water, media was placed into a stainless steel pan and stirred with glass rod for proper mixing of all the ingredients. Now the medium was filtered through a muslin cloth by squeezing out whole liquid. 200 mL medium was placed in each 500 mL capacity flasks. Flasks were tightly plugged with non-absorbent cotton plug and wrapped with butter paper and rubber band. Medium was autoclaved at 15 psi (121.6ºC) for 20 min and cooled before pouring into Petri plates.

Another medium contain agar–agar 20.0g, dextrose 20.0g, potato (peeled and sliced) 200.0g, distilled water 1 L. 250g of potato was peeled and cut into small and fine sliced pieces. Exactly 200g of potato pieces were weighed and placed into a stainless steel pan. 500 mL of water was added to potato pieces and boiled gently for such a period until they are easily mashed by a glass rod. The decoction was filtered through muslin cloth and squeezed out all the liquid in a measuring cylinder and potato pieces were discarded. Now sufficient amount of water was added to make the volume 1000 mL. Now pre-weighed agar agar was added (20g) bit by bit to the boiling solution to dissolve it. At the same time dextrose (20g) was also added in boiling solution (melted with agar) and final volume made up to 1 L. It was poured @ about 200 mL in each of four conical flasks of 500mL 2ycelia2 and 10 mL per culture tube to prepare the PDA slants. Both, flasks and culture tubes were tightly plugged with non-absorbent cotton and wrapped with butter paper and rubber bands. The culture tubes and flasks were placed vertically (mouths up) in wire baskets and then autoclaved at 15 psi (121.6ºC) for 20 min. The bacteria, initially isolated in a pure culture on King's B media and sub cultured on PDA slants were allowed to grow at 28 ± 2^0C temperature. The culture thus obtained was stored in refrigerator at 5^0C for further studies and was sub cultured periodically.

Antagonism of *P. fluorescens*

The antagonistic activity of *P. Fluorescens* against *Fusarium moniliforme*, *Rhizoctonia solani* and *Alternaria alternata* were tested by dual culture technique. Bacterial isolates were streaked at one side of Petri dish (one cm away from the edge) containing PDA. 9 mm mycelial disc from seven days old PDA culture of *F. moniliforme*, *R. solani*, and *A. alternata* were placed at the opposite side of Petri dishes perpendicular to the bacterial streak respectively and incubated at 27 ± 2^0C for 5-7 days. Petri dishes inoculated with fungal discs alone served as control. Three replications were maintained for each isolate. Observations on width of inhibition zone and mycelial growth of test pathogen were recorded and per cent inhibition of pathogen growth was calculated by using the formula proposed by Vincent (1927).

Per cent inhibition (I) = C-T/C ×100

Where,

C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual culture plate.

RESULTS AND DISCUSSION

Isolation of *P. fluorescens*

Eight microflora resembling *P. fluorescens* were isolated from the rhizhosphere of different crops/plants.On the basis of colony characters and pigmentation etc, out of eight microflora, three isolates were confirmed as *P. fluorescens* and tested against *Fusarium moniliforme*, *Rhizoctonia solani*, and *Alternaria alternata*. The three *Pseudomonas*

fluorescens isolates isolated and confirmed as *Pseudomonas fluorescens* during present investigation were designated as given below:

P.f. Isolates	Source (Rhizosphere)	Location
P.f.01	Moong	P.C.P. SVPUAT,Meerut.
P.f.05	Rice	C.R.C. Chiraudi,SVPUAT,Meerut.
P.f.07	Chilli	Vill. Siwaya Jamalullahpur, Meerut.

Dual culture technique

The results of the dual culture technique indicated that the three isolates inhibited growth of tested fungi significantly. In case of *Fusarium moniliforme,* a maximum inhibition of 65.45% was recorded by PF 07 and minimum of 45.45% was recorded with the isolate P.f.05. In case of *Rhizoctonia solani,* the maximum inhibition of 68.23% was exhibited by PF 07 and minimum, 55.8% was recorded with the P.f.05 isolate, where as in case of *Alternaria alternata* maximum inhibition 48.13% was recorded by by P.f.07 and minimum, 44.40% was recorded with isolate P.f.05 (Table 1).

Table 1. Antagonistic activity of *Pseudomonas fluorescens* against chosen fungi

P.f. Isolates	Fungi	Mycelial growth (mm)	Growth inhibition (%)
P.f.01	*F. moniliforme*	11.00	60.00
P.f.05		15.00	45.45
P.f.07		9.50	65.45
Control		27.50	0
CD @ 5 % =			5.139
P.f.01	*R. solani*	34.50	59.41
P.f.05		37.50	55.88
P.f.07		27.00	68.23
Control		85.00	0
	CD @ 5% =		12.011
P.f.01	*A. alternata*	49.00	45.14
P.f.05		49.66	44.40
P.f.07		46.33	48.13
Control		89.33	0
	CD @ 5 % =		5.736

Attempst were made to isolate and maintain different strains of *Pseudomonas fluorescens* from different agro-ecological zones, so that they can be used in future for stress management in various crops in varying agro-ecological situations. In this direction eight microflora were isolated on King's B medium and further they were subjected to identification on the basis of antagonistic characteristics exhibited against some pathogenic microflora. Out of eight microflora isolated on King's B medium, only three were confirmed as *P. fluorescens* (isolate P.f 01, isolate P.f 05 and isolate P.f 07), which neither showed fluorescent pigment production nor they were able to inhibit any of the fungal pathogen when subjected to dual culture. The isolate P.f 01 was isolated from the rhizosphere of Moong (*Vigna radiata*) from the field of P.C.P. SVPUAT, Meerut. The isolate P.f.05-was isolated from the rhizosphere of rice crop from the field C.R.C. Chiraudi of SVPUAT, Meerut. The isolate P.f.07-was isolated from the rhizosphere of chilli crop from a farmer field of village Siwaya Jamalullahpur district of Meerut.

The results of the dual culture technique indicated that the three isolates were able to inhibit growth of tested fungal pathogen significantly. In case of *F. moniliforme* a maximum and minimum inhibition was exhibited by P.f 07 and P.f 05 respectively. In case of *Rhizoctonia solani*, the maximum inhibition of 68.23% was exhibited by P.f 07 and minimum, 55.8% was exhibited by P.f 05. where as in case of *Alternaria alternata* maximum inhibition 48.13% was exhibited by P.f 07 and minimum, 44.40% was exhibited with isolate PF05. Vanitha *et al*. (2007) screened six isolates of *Pseudomonas fluorescens* against *Alternaria chlamydospora* Mouchacca causing leaf blight diseases in *Solanum nigrum* L under *in vitro* conditions. The experimental results showed that Pf MMP of *Pseudomonas* showed 100 per cent inhibition of mycelial growth and culture filtrate of *P. fluorescens* (PfMMP) significantly reduced the mycelial growth and resulted in minimum spore germination of *A. Chlamydospora.* Adhikari *et al*. (2013) reported that among seventy isolates, antagonistic twenty one representing biovars of *P. fluorescens* (biovars I, II, III, and V) were collected from the rhizosphere of okra, chilli, ground nut, brinjal, cabbage and tomato from different agroecological regions of West Bengal and were subjected to evaluation for their antifungal activity under *in vitro* condition against *Rhizoctonia solani*, the most important soil-borne plant pathogen. Two isolates of *P. fluorescens* PF-8 and PF-7 effectively inhibited the mycelial growth of *Rhizoctonia solani* in dual culture method. The vigour index of okra was also recorded maximum for the isolate P.f 08 than by P.f 07.

Different strains of *P. fluorescens* from different agro-ecological zones, so that they can be used in future for stress management in various crops in

varying environment. The isolates confirmed as *P. fluorescens* were subjected to testing of antagonistic activity of *Pseudomonas fluorescens* against three fungal pathogen. Among three tested isolates of *Pseudomonas fluorescens*, strain P.f 07 was exhibited maximum antagonistic activity against fungal pathogen, *Fusarium moniliforme, Rhizoctonia solani* and *Alternaria alternata.*

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