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Antagonistic Activity of Indigenous Strains of *Pseudomonas fluorescens* Against Fungal Plant Pathogens

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Twelve indigenous strains of *Pseudomonas fluorescens* from different soils of Punjab were isolated to see the antagonistic activity against different fungal pathogens. Significant amount of IAA was produced by isolate PSF 1 (22.12 μgmL^{-1}) followed by PSF 4 (13.66 μgmL^{-1}), PSF 12 (12.41 μgmL^{-1}) and PSF 5 (12.09 μgmL^{-1}). All the isolates showed HCN positive reaction whereas PSF 1, PSF 3, PSF 8 and PSF 12 showed highest HCN production. Maximum siderophore production was by PSF 12 (18.7 mm) followed by PSF 5 (16.5 mm) and PSF 7 (16.0 mm). PSF 5 and PSF 12 showed positive chitinase production. Except PSF 1 all the culture were positive for starch hydrolysis with a maximum zone by PSF 12 (22.0 mm) followed by PSF 10 (21.0 mm). For protease production all the culture showed positive results with a maximum zone of solubilization by PSF 12 (30.5 mm) followed by PSF 5 (29.5 mm) and PSF 1 (29.3 mm). In general PSF 12 followed by PSF 5 and PSF 7 showed maximum inhibition percentage against the different fungal pathogens tested *in vitro* confirming *Pseudomonas fluorescens* a good antagonist.

Key words: *Pseudomonas fluorescens*, IAA, HCN, siderophore, antagonist.

Soil-borne, non-pathogenic bacteria have the ability to antagonize fungal pathogens and thus prevent plant diseases representing a realistic alternative to chemical fungicides. Scientific literature contains vast information on many soil bacteria with biocontrol abilities against different pathogens. These bacteria are known by different generic names, *viz*. biological control agents (BCAs), plant growth promoting rhizobacteria (PGPR) and biopesticides. Due to their catabolic versatility, excellent root-colonizing abilities and capacity to produce a wide range of antifungal metabolites, the soil-borne fluorescent *Pseudomonas* has received main attention. In addition, *Pseudomonas* spp. have shown to elicit disease-resistance response in crop species, known as induced systemic resistance (ISR)[1,2]. This dual activity of *Pseudomonas* spp. further highlights their potential as plant protection products (PPPs). Biological controls are non hazardous methods of disease control caused by plant pathogens[3,4,5,6]. *Pseudomonas fluorescens*, in recent years are important BCA's for suppression of root pathogenic fungi[7]. Beneficial pseudomonads rapidly colonize the root system, suppress pathogenic microorganisms, improve plant growth and crop yields[7,3]. The main mechanisms involved are production of diverse microbial metabolites including antibiotics, siderophores and volatile compounds such as HCN[8]. Fluorescent *Pseudomonads*, produce siderophores that act to fulfill the traces of Fe (III) in the soil and reduce the plant deleterious and pathogenic rhizospheric microorganisms[9,10]. Chitinase and lytic enzymes which degrade fungal walls, also act as biocontrol agent of pathogens[11,12].

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In the present study, the mechanisms involved in plant disease control by *Pseudomonas fluorescens* are discussed. Black scurf of potato (*Rhizoctonia solani*), sheath blight of rice (*Rhizoctonia solani*), foot rot of rice (*Fusarium moniliforme*) and muskmelon wilt (*Fusarium oxysporium* var *melonis*) are important seed and soil borne diseases which are difficult to control by other methods of plant disease control. Native strains of *Pseudomonas fluorescens* can play a major role and in these studies we isolated and used twelve strains of *Pseudomonas fluorescens* for their biochemical and biocontrol activities against above mentioned pathogens.

MATERIALS AND METHODS

Isolation and characterization of *Pseudomonas fluorescens*

Pseudomonas fluorescens strains were isolated from soil samples of vegetable and cereal crops from different locations of Punjab by using serial dilution method on petri dishes containing King's B medium. These dishes were incubated at 28°C overnight and colonies showing fluorescence under UV radiation were selected and sub cultured on King's B. Isolates that showed antagonistic activity *in vitro* were grouped on the basis of different characteristics *viz.* morphology, gram staining and standard biochemical procedures *i.e.* MRVP, citrate utilization, starch hydrolysis, nitrate reduction.

Determination of Indole acetic acid (IAA)

The production of IAA was determined according to the method of Bric *et al.*,[13]. All the cultures were inoculated on nutrient broth (peptone, 5g; yeast extract, 1.5g; beef extract, 1.5g; and NaCl, 5g; each per liter) with presence and absence of tryptophan (500 g/ml) and incubated at 30°C. A 5-ml culture was removed from each tube and centrifuged at 10,000 rpm for 15 minutes. An aliquot of two ml supernatant was transferred to a fresh tube in which 100 µl of 10 mM orthophosphoric acid and 4 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. The mixture was incubated at room temperature for 25 minutes, and the absorbance of pink colour developed was read at 530 nm. The IAA concentration in culture was determined by using a calibration curve of pure IAA as a standard following the linear regression analysis.

Detection of HCN

Production of HCN was observed according to the method of Lorck[14]. Freshly grown cells were spread on King's B containing glycine (4.5 g/L). A sterilized filter paper saturated with 1% solution of picric acid and 2% sodium carbonate was placed in the upper lid of the petri dish. The petri dish was then sealed with parafilm and incubated at 30°C for 4 days. A change in color of the filter paper from yellow to reddish brown as an index of cyanogenic activity was recorded.

Siderophore production

For testing qualitative production of siderophore, Chrome azurol S (CAS) agar was used[15]. For this purpose CAS dye of 60.5 mg was dissolved in 50 mL de-ionised water, and then mixed with 10 mL of a Fe (III) solution (1mmol $FeCl_3.6H_20^{-1}$ in 10 mmol $1HCl^{-1}$). This mixture was slowly mixed with 72.9 mg of hexadecyl-trimethylammonium bromide (HDTMA) dissolved in 40 mL water. The resulted mixture was autoclaved, cooled and mixed with 900 mL succinate media. The medium was poured into Petri dishes. The *Pseudomonas fluorescens* isolates were spot inoculated on the plates. Presence of siderophore production was indicated by presence of orange halos around the colony due to chelation of iron which bound to CAS dye.

Cell wall degrading enzyme production

Protease activity

Protease activity (casein degradation) was determined from clear zone on skimmed milk agar medium. A small amount of loopful of each isolates was stabbed in skimmed milk agar medium and incubated for 2-3 days at 28±2°C. Clear zone appeared on the periphery of the colonies indicated positive test[16].

Starch hydrolysis

Nutrient Agar supplemented with starch 0.5% was streaked with *Pseudomonas fluorescens* strains and incubated at 35±2°C for 2-3 days. The bacterial growth was then inundated with Lugol solution (Iodin + Potassium iodida). The positive test was showed by clear zone around the bacterial colony[17].

Chitinolytic activity

Colonies were screened for chitinolytic activity by plating on chitin agar. The agar plates were spot inoculated with test organism and

incubated at 30p C for 5 days. Development of halo zone around the colony indicated positive for cell wall degrading enzyme production[16].

In vitro* assessment of anti-fungal activity of *Pseudomonas fluorescens

The antagonistic ability of the twelve *Pseudomonas fluorescens* isolates was seen against *Rhizoctonia solani* (black scurf of potato and sheath blight of rice), *Fusarium moniliforme* (foot rot of rice) and *Fusarium oxysporum* var *melonis* (muskmelon wilt). Seven day old isolates of selected fungi disc were placed in one side on the King's B agar dish. Fresh isolates of *Pseudomonas fluorescens* were streaked on the other side of the dish. Percent growth inhibition after seven days was calculated by using the formula.

$$\% \text{ Inhibition} = \frac{C-T}{C} \times 100$$

Where,

C= Growth of the pathogen (mm) in control plate.

T= Growth of the pathogen (mm) in test plate.

RESULTS AND DISCUSSION

Characterization of isolates

The growth of the isolated bacterial strains which appeared on the surface of King's B medium was identified as *Pseudomonas fluorescens* based on morphological and biochemical reactions (Table 1). The identification of bacteria was done according to Bergey's Manual of Bacteriology[18].

Production of Indole acetic acid (IAA)

IAA is a plant growth hormone produced by fluorescent *Pseudomonas* to promote the plant growth and root enhancement. All the isolated *Pseudomonas fluorescens* strains were tested for their ability to produce IAA both in presence and absence of precursor L- tryptophan (Table 2). Significant differences were found among isolates in their ability to produce IAA by changing colour from pink to dark pink (Fig. 1). A low amount of IAA was produced by different isolates in the absence of L- tryptophan, which ranged from 1.25-9.44 µgmL^{-1}. In the presence of L-tryptophan the amount of IAA produced by *Pseudomonas fluorescens* found to be in the range of 3.19-22.12 µgmL^{-1} (fig. 2). Significant amount of IAA was in presence of tryptophan produced by PSF 1 (22.12 µgmL^{-1}) followed by PSF 4 (13.66 µgmL^{-1}), PSF 12 (12.41 µgmL^{-1}) and PSF 5 (12.09 µgmL^{-1}).

Pattern *et al.*,[19] found that wild type *Pseudomonas* produce significant amount of IAA (22.5 µgmL^{-1}) at 100 µgmL^{-1} L-tryptophan concentration. Results are also in confirmatory with Suzuki *et al.*,[20] who found that tryptophan test strain HP72 produced 11.8 µgmL^{-1} of IAA. The IAA overproducing strain of *Pseudomonas fluorescens* BSP53a stimulated root development of black currant and in sour cherry cuttings root development was also suppressed[21]. These findings were suggested that bacterial IAA affected the host plant, but its effect depend on the plant's sensitivity to IAA, the amount of IAA

Table 1. Morphological and biochemical characters of *Pseudomonas fluorescens*

Isolates	Morphological characters	Gram staining	Nitrate reduction	Methyl red	Voges proskauer
PSF 1	Greenish blue	-	-	-	-
PSF 2	Yellowish	-	-	-	-
PSF 3	Brownish	-	-	-	-
PSF 4	Yellow	-	-	-	-
PSF 5	Purplish	-	-	-	-
PSF 6	Greenish blue	-	-	-	-
PSF 7	Brownish	-	-	-	-
PSF 8	Brownish	-	-	-	-
PSF 9	Greenish blue	-	-	-	-
PSF 10	Greenish blue	-	-	-	-
PSF 11	Greenish blue	-	-	-	-
PSF 12	Greenish blue	-	-	-	-

- = Negative

produced from plant-associated bacteria and induction of other phytohormones[22]. Karnwal[23] also reported that IAA production of *Pseudomonas* in the presence of L-trptophan.

HCN production by *Pseudomonas fluorescens*

Change in color after incubation from yellow to light brown (indicated weak HCN production) whereas change in colour from yellow to reddish brown indicated strong HCN production (Fig. 3).

All the isolates showed positive HCN reaction, and of these PSF 1, PSF 3, PSF 8 and PSF 12 showed highest HCN production. Around 33.33% produced very good amount of HCN and 41% produced good amount of HCN, rest of them were poor HCN producers (Table 3).

Similar results were reported by Heydari *et al.*,[24] who observed that 37 % of fluorescent *Pseudomonas* isolates collected from different weeds rhizospere were efficient HCN producers. Bano and Mussarat[25] found that *Pseudomonas* strain NJ-15 had biocontrol potential against pathogenic fungi by HCN antibiosis. Change in color from yellow to reddish-brown with strain NJ-15 compared with the control NJ-10 indicated higher amount of HCN production.

Siderophore production by potential *Pseudomonas fluorescens* isolates

The maximum production of yellow halo signifying siderophore production was by PSF 12 (18.7 mm) followed by PSF 5 (16.5 mm) and PSF 7 (16.0 mm) on CAS plates (Table 3). The experiment was conducted without addition of excess iron in the plates (Fig. 4). Different isolates showed siderophore positive reaction due to iron specific compounds produced under low iron stress condition. These results are supported by Gupta *et al.*,[26] who observed antagonism of

Table 2. Quantitative measurement of Indole acetic acid (IAA) production by *P. fluorescens* in presence and absence of L-tryptophan

Isolates of	IAA production (μlmL^{-1}) [A]	
P. fluorescens	IAA (Trp^{-ve})	IAA (Trp^{+ve})
PSF 1	1.25±0.35	22.12±1.39
PSF 2	4.31±0.69	9.73±1.93
PSF 3	2.57±0.67	7.40±0.72
PSF 4	9.44±2.19	13.66±2.45
PSF 5	3.32±0.80	12.09±1.24
PSF 6	3.05±0.60	6.58±1.73
PSF 7	8.17±8.06	8.67±1.24
PSF 8	2.62±0.64	3.19±0.97
PSF 9	3.33±0.38	4.43±1.78
PSF 10	3.76±0.80	5.36±1.80
PSF 11	4.08±1.04	5.89±1.11
PSF 12	8.34±1.80	12.41±2.0
CD(p=0.05)	1.78	2.70

[A] Data were expressed as mean ± standard deviation.
Trp^{-ve}= In absence of tryptophan.
Trp^{+ve}= In presence of tryptophan.

Table 3. Qualitative measurement of siderophore and HCN production by *Pseudomonas fluorescens*

Isolates of *P. fluorescens*	HCN	Siderophore(mm) [A]
PSF 1	+++	+(12.8)±0.81
PSF 2	+	+(13.8)±0.72
PSF 3	+++	+(14.8)±0.87
PSF 4	++	+(13.5)±0.81
PSF 5	+	+(16.5)±1.75
PSF 6	++	+(11.3)±1.81
PSF 7	++	+(16.0)±0.55
PSF 8	+++	+(14.0)±1.51
PSF 9	++	+(13.3)±1.04
PSF 10	+	+(11.5)±0.26
PSF 11	++	+(12.6)±1.55
PSF 12	+++	+(18.7)±1.32

[A] Data were expressed as mean ± standard deviation
+ = Poor; ++ = Good; +++ = Very good

Table 4. Qualitative measurement of cell wall degrading enzymes production by *Pseudomonas*

Isolates of *P. fluorescens*	Chitinase production	Starch Hydrolysis (mm) [A]	Protease production (mm) [A]
PSF 1	-	-	+ (29.3)±3.76
PSF 2	-	+ (14.7)±2.70	+ (19.5)±1.50
PSF 3	-	+ (18.0)±2.00	+ (24.3)±2.60
PSF 4	-	+ (11.3)±1.67	+ (25.2)±1.40
PSF 5	+	+ (14.3)±0.60	+ (29.5)±3.73
PSF 6	-	+ (20.3)±2.06	+ (29.2)±2.52
PSF 7	-	+ (18.7)±1.57	+ (25.8)±1.08
PSF 8	-	+ (14.6)±1.44	+ (28.6)±1.38
PSF 9	-	+ (18.3)±0.62	+ (23.0)±2.22
PSF 10	-	+ (21.0)±2.64	+ (24.1)±1.20
PSF 11	-	+ (17.7)±1.12	+ (27.6)±1.99
PSF 12	+	+ (22.0)±2.00	+ (30.5)±1.51

[A] Data were expressed as mean ± standard deviation
+ = Positive; - = Negative

Table 5. Effect of *Pseudomonas fluorescens* strains on growth of fungal plant pathogens

Isolates of *P. fluorescens*	*Rhizoctonia solani* (Black scurf of Potato)		*Rhizoctonia solani* (Sheath Blight of rice)		*Fusarium moniliforme* (Foot rot of Rice)		*Fusarium oxysporum* var *melonis* (Muskmelon wilt)	
	Inhibition Zone (mm) [A]	Inhibition (%)	Inhibition Zone (mm) [A]	Inhibition (%)	Inhibition Zone (mm) [A]	Inhibition (%)	Inhibition Zone (mm) [A]	Inhibition (%)
PSF 1	10.0±2.00	70.0	2.0±1.0	68.7	9.7±1.52	66.7	0.0±0	25.9
PSF 2	0.0±0	50.0	0.0±0	31.8	6.7±4.16	55.9	0.0±0	26.6
PSF 3	0.0±0	50.9	3.3±2.30	70.0	8.7±1.15	52.2	12.0±2.00	62.0
PSF 4	10.0±2.00	75.0	5.7±2.30	70.4	3.7±1.15	69.6	0.0±0	27.7
PSF 5	8.7±1.15	72.5	8.7±2.51	70.4	10.0±2.00	74.0	11.7±3.05	69.8
PSF 6	10.0±2.00	70.0	9.7±1.52	72.6	9.7±1.52	44.9	7.3±1.52	75.9
PSF 7	0.0±0	45.4	10.0±4.35	78.7	7.7±2.51	65.5	0.0±0	28.2
PSF 8	10.6±1.52	70.0	5.3±2.51	68.7	17.3±1.15	63.1	16.3±2.51	68.7
PSF 9	6.3±1.52	69.6	3.7±1.52	68.7	0.0±0	18.7	9.3±0.57	67.5
PSF 10	10.6±1.15	71.3	5.7±2.51	70.9	10.0±2.00	61.8	0.0±0	24.6
PSF 11	3.3±1.52	67.9	7.3±2.08	70.4	10.7±0.57	59.5	11.3±3.05	66.9
PSF 12	10.3±1.52	75.4	10.3±2.08	76.9	9.7±1.52	66.0	11.7±3.51	75.9
Control	80.0	-	76.7	-	55.0	-	55.3	-
CD(p=0.05)	2.38		3.86		3.07		3.22	

[A] Data were expressed as mean ± standard deviation

Pseudomonas against *Microphomina* with a good amount of siderophore production. Singh *et al.*, [27] also found that maximum amount of siderophore was produced by LS I (1.9 cm) in iron stress condition and indicated antagonism activity of *Pseudomonas fluorescens*.

Cell wall degrading enzyme production

Sixteen percent of the isolates of *Pseudomonas fluorescens* showed chitinase activity. Only PSF 5 and PSF 12 showed positive chitinase production indicating *Pseudomonas* were poor chitin degraders. Except PSF 1 all the isolates of *Pseudomonas fluorescens* showed positive to starch hydrolysis with a maximum zone of PSF 12 (22.0 mm) followed by PSF 10 (21.0 mm). Regarding protease production all the isolates showed positive protease production with a maximum zone of solubilization by PSF 12 (30.5 mm) followed by PSF 5 (29.5 mm) and PSF 1 (29.3 mm) (Table 4 and Fig. 5).

Similar study was conducted by Chaiharn *et al.*,[16] and Nandakumar *et al.*,[28] who showed that population increase of 25% in chitin containing medium inoculated with FP7 strain, whereas PF1 and PB2 recorded 12.8% and 11.8% increases in population, respectively. FP7 also responded well with the addition of chitin and produced 31.2% increased chitinase in chitin-amended medium. Sokol *et al.*,[29] and West *et al.*,[30] also observed proteolytic activity by *Pseudomonas* strains in good manner.

***In vitro* evaluation of *Pseudomonas fluorescens* isolates against fungal pathogens**

Isolate PSF 12 with 10.3 mm zone of inhibition (ZOI) showed the maximum percent of

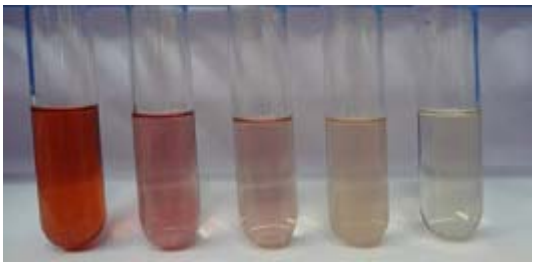

Fig. 1. IAA production by *Pseudomonas fluorescens* in presence of L-tryptophan

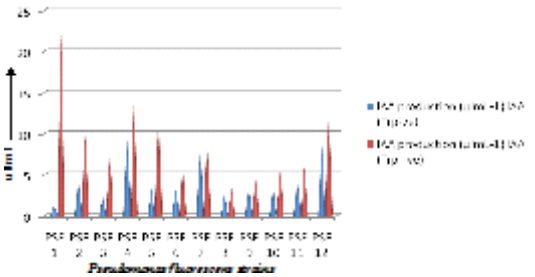

Fig. 2. Comparison of IAA production by *Pseudomonas fluorescens* strains in presence and absence of L-tryptophan

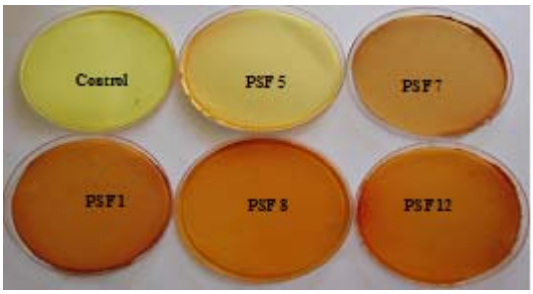

Fig. 3. Different strains of *Pseudomonas fluorescens* producing HCN production

Fig. 4. Siderophore production on CAS agar by *Pseudomonas fluorescens* (PSF 12)

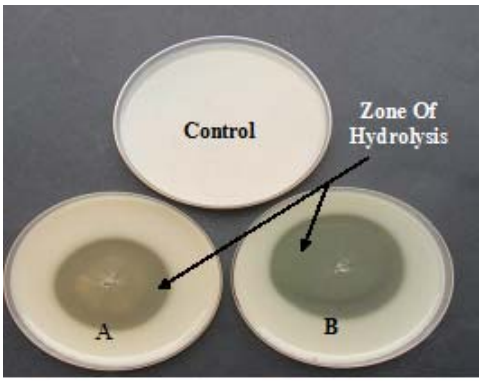

Fig. 5. Proteolytic activity of *Pseudomonas fluorescens* (A.PSF 5 and B.PSF 12) on skim milk agar

inhibition (75.4 %) against *Rhizoctonia solani* causing black scurf of potato followed by PSF 4 (75.0% of inhibition and 10.0 mm ZOI) and PSF 5 (72.5% of inhibition and 8.7 mm ZOI). For sheath blight of rice (*Rhizoctonia solani*), PSF 7 (78.7% of inhibition and 10.0 mm ZOI) and PSF 12 (76.9% of inhibition and 10.3 mm ZOI) were more effective (Fig. 6). Similarly maximum percent of inhibition (74.0%) of *Fusarium moniliforme* causing foot rot of rice was by PSF 5 followed by PSF 4 (69.6%). For muskmelon wilt maximum percentage of inhibition (75.9% of inhibition and 11.7 mm ZOI) of *Fusarium oxysporum* var *melonis* by PSF 12 isolate of *Pseudomonas fluorescens* (Fig. 7). It was followed by PSF 6 (75.9% of inhibition and 7.3 mm ZOI) (Table 5).

Rao *et al*.,[31] reported, five strains of fluorescent *Pseudomonas* which exhibited growth promotion and biocontrol of Fusarium wilt in lentil through siderophore production. Bano and Mussarat[25] also reported that *Pseudomonas* strain NJ-15 was a good biocontrol agent with high amount of IAA, HCN and siderophore production. The results are also supported by Singh *et al*.,[27] in case of *Rhizoctonia* spp.

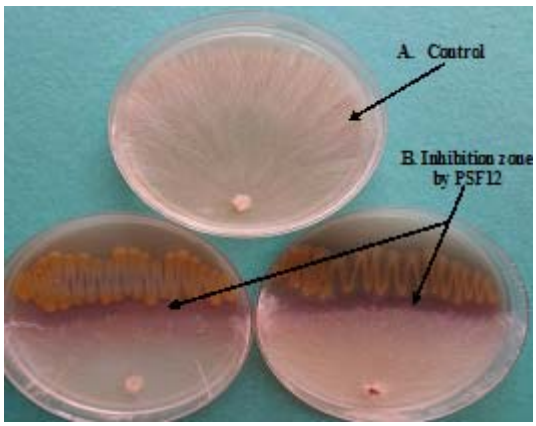

Fig. 6. Biocontrol activity of *Pseudomonas fluorescens* (PSF 12) against sheath blight of rice (*Rhizoctonia solani*)

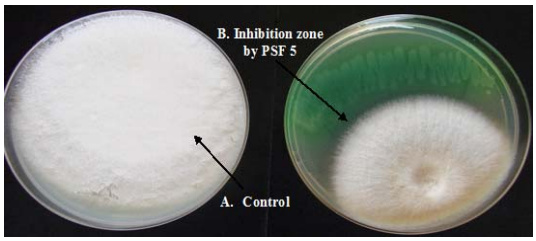

Fig. 7. Biocontrol activity of *Pseudomonas fluorescens* (PSF 5) against foot rot of rice (*Fusari* Sample Covering Letter

CONCLUSION

The present study suggests *Pseudomonas fluorescens* play a defensive role in plant disease management. Good evidence was found from experiments on the involvement of IAA, enzyme production, siderophores and HCN in the biocontrol of plant pathogens by *Pseudomonas fluorescens*. Isolates *viz.* PSF 12 (against black scurf of potato and muskmelon wilt), PSF 7 (against sheath blight of rice), PSF 5 (for foot rot of rice) can be explored for the management of these very important diseases under field conditions.

REFERENCES

1. Van Loon, L. C., Bakker, P.A.H.M., Pieterse, C.M.J. Systemic resistance induced by rhizosphere bacteria. *Ann. Rev. Phytopathol.*, 1998; **36**(1): 453-83.
2. Pieterse, C.M.J., Van Loon, L.C. Salicylic acid-independent plant defense pathways. *Trends. Plant. Sci.*, 1999; **4**(2): 52-8.
3. Weller, D.M. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopathol.*, 1988; **26**(1): 379-407.
4. O'Sullivan, D.J., O'Gara, F. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiol. Rev.*, 1992; **56**(4): 662-76.
5. Cook, R.J., Thomashow, L.S., Weller, D.M., Fujimoto, D., Mazzola, M., Bangera, G., Kim, D. Molecular mechanisms of defense by rhizobacteria against root disease. *Proceedings of the National Academy of Science* USA, 1995; **92**(10): 4197-201.
6. Lumsden, R.D., Lewis, J.A., Fravel, D.R. Formulation and delivery of biocontrol agents for use against soil borne plant pathogens. *In: Biorational Pest Control Agents*, (F. R. Hall and J. W. Barry ed). Washington DC: American Chemical Society, 1995; pp: 166-182.
7. Schippers, B., Bakker, A.W., Bakker, P.A.H.M. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Ann. Rev. Phytopathol.*, 1987; **25**(1): 339-58.
8. Thomashow, L.S., Weller, D.M. Current concepts in the use of introduced bacteria for

biological disease control: mechanisms and antifungal metabolites. *In Plant Microbe Interactions*, (G. Stacey and N. Keen, ed) New York: Chapman and Hall, 1995; pp: 187-235.

9. Singh, S.K., Sheeba., Gupta, R.D., Rajendra, S., Verma, S.K., Siddiqui, M.A., Mathur, A., Agarwal, P.K. Assessment of the role of *Pseudomonas fluorescens* as biocontrol agent against fungal plant pathogens. *Curr. Bot.*, 2011; **2**(3): 43-6.
10. Buysens, S., Heungens, K., Poppe, J., Hofte, M. Involvement of pyochelin and pyorerdin in suppression of Pythium-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NS K2. *Appl. Environ. Microbiol.,* 1996; **62**(3) : 865-71.
11. Fridlender, M., Inbar, J., Chet, I. Biological control of soilborne plant pathogens by a â-1, 3 glucanase- producing *Pseudomonas cepacia*. *Soil. Biol. Biochem.*, 1993; **25**(9): 1211-21.
12. Chernin, L., Ismailov, Z., Haran, S., Chet, I. Chitinolytic *Enterobacter agglomerans* antagonistic to fungal plant pathogens. *Appl. Environ. Microbiol.*, 1995; **61**(5): 1720-26.
13. Bric, J.M., Bostock, R.M., Silversone, S.E. Rapid *in situ* assay for indole acetic acid production by bacteria immobilization on a nitrocellulose membrane. *Appl. Environ. Microbiol.*, 1991; **57**(2): 535–38.
14. Lorck, H. Production of hydrocyanic acid by bacteria. *Physiol. Plant.*, 1948; 1(2): 142–6.
15. Schwyn, B., Neilands, J.B. Universal assay for the detection and determination of siderophores. *Anal. Biochem.*, 1987; **160**(1): 47-56.
16. Chaiharn, M., Chunhaleuchanon, S., Kozo, A., Lumyong, S. Screening of rhizobacteria for their plant growth promoting activities. *Sci. Tech. J.*, 2008; **8**(1): 18-23.
17. Cappucino, J.G. *Microbiology: A Laboratory Manual*. Addison Wesley Publishing Company, 1983.
18. Jordan, D. C., Family, L.L.L. Rhizobiaceae Conn 1938, 321AT3. *In Bergey's Manual of Systemic Bacteriology*. (N R Krieg and J G Holt ed) Baltimore: Williams & Wilkins, 1984; pp: 234-256.
19. Patten, C.L., Glick, B.R. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Appl. Environ. Microbiol.*, 2002; **68**(8): 3795-801.
20. Suzuki, S., He, Y., Oyaizu, H. Indole-3-Acetic Acid Production in *Pseudomonas fluorescens* HP72 and Its Association with Suppression of Creeping Bentgrass Brown Patch. *Curr. Microbiol.*, 2003; **47**(2): 138-43.
21. Dubeikowsky, A.N., Mordukhova, E.A., Kochetkov, V.V., Polikarpova, F.Y., Boronin, A.M. Growth promotion of blackcurrant softwood cuttings by recombinant strain *Pseudomonas fluorescens* BSP53a synthesizing an increased amount of indole-3-acetic acid. *Soil. Biol. Biochem.*, 1993; **25**(9): 1277-81.
22. Peck, S.C., Kende, H. Sequential induction of the ethylene biosynthetic enzymes by indole-3-acetic acid in etiolated peas. *Plant. Mol. Biol.*, 1995; **28**(2): 293–301.
23. Karnwal, A. Production of indole acetic acid by fluorescent *Pseudomonas* in the presence of L-tryptophan and rice root exudates. *J. Plant. Pathol.*, 2009; **91**(1): 61-3.
24. Heydari, S., Moghadam, P. R., Arab, S.M. Hydrogen Cyanide Production Abilty by *Pseudomonas fluorescence* Bacteria and their Inhibiton Potential on Weed. In Procedings "Competion for Resources in a ChangingWorld: New Drive for Rural Development", Tropentag, Hohenheim, 2008.
25. Bano, N., Musarrat, J. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Curr. Microbiol.*, 2003; **46**(5): 324–8.
26. Gupta, C.P., Dubey, R.C., Maheshwari, D.K. Plant growth enhancement and suppression of *Macrophomina phaseolina* causing charcoal rot of peanut by fluorescent *Pseudomonas*. *Biol. Fertil. Soils.*, 2002; **35**(6): 399–405.
27. Singh, N., Saini, P., Singh, P. Characterization and evaluation of Siderophore Producing Rhizopheric *Pseudomonas florescence* as *R. oryzae* and *R. solani* Antagonist. *J. Pure. App. Microbiol.*, 2014; **8**(1): 505-13.
28. Nandakumar, R., Babu, S., Raguchander, T., Samiyappan, R. Chitinolytic activity of native *Pseudomonas fluorescens* strains. *J. Agric. Sci. Technol.*, 2007; **9**(1): 61-8.
29. Sokol, P.A., Ohman, D.E. Iglewski, B.H. A more sensitive plate assay for detection of protease production by *Pseudomonas aeruginosa*. *J. Clin. Microbiol.*, 1979; **9**(4):538-40.
30. West, S.E.H., Kaye S.A., Hamood, A.N. Iglewski B.H. Characterization of *Pseudomonas aeruginosa* mutants that are deficient in exotoxin A synthesis and are altered in expression of regA, a positive regulator of exotoxin A. *Infect. Immun.*, 1994; **62**(3): 897-903.
31. Rao, C.V.S., Sachan, I.P. Johri, B.N. Influence of fluorescent *Pseudomonads* on growth and nodulation of lentil (*Lens esculentus*) in *Fusarium* infested soil. *Indian. J. Microbiol.*, 1999; **39**(1): 23–9.