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Antagonistic Activity of Plant Growth Promoting Fluorescent *Pseudomonas* spp. against Major Fungal Pathogens Involved in Replant Problem of Apple

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A B S T R A C T

Replant disease is a major problem of apple orchards, which is a main cash crop in Trans-Himalayan region. The cause of this replant problem includes both biotic and abiotic factors, such as low nutrients, phytotoxins, actinomycetes, fungal complexes and nematodes. Fungi and oomycetes belonging to the well-known root rot complex are important factor of replant problems in apple. Application of plant growth promoting fluorescent *Pseudomonas* with antagonistic potential at replant site is one of the promising methods being a major component of rhizobacteria, promotes the plant growth and also act as biocontrol agents against fungal plant pathogens. In the present study, 14 fluorescent *Pseudomonas* isolates were screened out for *in-vitro* antagonistic activity by well plate assay method against five major fungal pathogens of apple replant problem *viz., Dematophora necatrics, Phytophthora cactorum, Pythium ultimum, Fusarium oxysporum* and *Rhizoctonia solani.* Out of these 14 fluorescent *Pseudomonas* isolates, maximum % inhibition was shown by *Pseudomonas* strain K (48.38 %) against *D. necatrics*, An-5-Jub (34.09 %) against *F. oxysporum*, isolate I (31.57 %) against *P. cactorum*, Ps-1-Sin (35 %) against *P. ultimum* and isolate J (28 %) against *R. solani*. These fluorescent *Pseudomonas* isolates with strong antagonistic potential against different fungal pathogens of replant problem not only reduce the replant problem by suppressing pathogens and improving plant growth through multifarious plant growth promoting activities but also helps in the maintenance of soil health by reducing indiscriminate use of chemicals and therefore can be helpful in solving the replant problem of apple.

Keywords

Apple replant problem, PGPR, Fluorescent *Pseudomonas*, Fungal pathogen, Biocontrol, Antifungal activity

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Introduction

Apple replant disease (ARD) is a disorder caused by a complex of fungal, bacterial and nematode pathogens that affect tree fruit crops when planted on sites where those crops were previously grown and directly affect the yield as compared to healthy trees (Winkelmann *et al.,* 2019). In past years, ARD was managed with preplant soil fumigation with methyl bromide or treatment with organophosphate biocides (Mai and Abawi, 1981) but those materials have been or are being phased out due to environmental concerns. Presently recommended practices to mitigate ARD in new orchards include preplant cover copping, multi-year fallow periods and use of resistant rootstocks (Merwin *et al.*, 2001), but those practices are not always effective in supporting optimum tree growth (Robinson, 2007).

To overcome this problem, application of plant growth promoting bacteria at replant site has been proved to be one of the promising approaches. Plant growth-promoting rhizobacteria (PGPR) occur naturally in soil and in the vicinity of roots of the plant where they colonize the roots and provide benefits to the plant by multifarious plant growth promoting activities (Braun and Fuller, 2006). Plant growth promoting rhizobacteria facilitate the plant growth through N_2 fixation, solubilization of insoluble phosphate (P), production of siderophores, phytohormones production, lowering of ethylene concentration, production of antibiotics and antifungal metabolites (Lugtenberg and Kamilova, 2009; Tank and Saraf, 2010; Zaidi *et al.*, 2009). Different PGPR's and endophytes have been reported useful to enhance the yield of different agricultural produce including different cereals, vegetables & spices etc. Presently, PGPR and endophytes are being used progressively individually as well as consortia of two or more strains also in combination with manures and fertilizers for improving crop yields and reducing the use of chemical fertilizers thus, contributing towards the advancement of sustainable agricultural systems.

Fluorescent *Pseudomonas* sp. are the most diverse and versatile group of indigenous micro flora of almost all the horticulture and forestry crops. Their potential to synthesize different secondary metabolites with diverse biological activities is the important function of soil fertility and sustainability of crops. These organisms may be ideal for use as plant growth promoting and disease suppressing bioagents. The integration of their important traits like production of antifungal antibiotics, iron chelating siderophores, lytic enzymes, plant growth regulators, phosphate solubilization, ammonia and HCN production with ecological fitness of the strains will be prerequisite for designing useful, efficient and effective novel bio agent (Malik, 1982; Kaur *et al.*, 2011; Kapoor *et al.*, 2012).

Pseudomonads possess many traits that make them well suited as biocontrol and plant growth-promoting agents. The *Pseudomonas* sp. are well known for their involvement in the biocontrol of several plant pathogens (Antoun and Prevost, 2005). The selection of effective strains of particular bacteria is of prime importance for the biocontrol of plant pathogens. Isolation of bacteria from pathogen suppressive soils may increase the chances of finding effective strains (Cook and Baker, 1983). The biocontrol mechanism *Pseudomonas* spp. normally involves the production of antibiotics, hydrolytic enzymes and secondary metabolites which suppress the fungal pathogens. *P. fluorescens* has a gene cluster that produces a suite of antibiotics, including compounds such as 2,4-diacetylphloroglucinol (DAPG), phenazine, pyrrolnitrin, pyoluteorin and biosurfactant antibiotics which have been reported among antifungal mechanisms by which *Pseudomonas* strains inhibited the fungal growth through damaging of cell walls (Angayarkanni *et al.*, 2005; Sindhu and Dadarwal, 2001). *Pseudomonas aeruginosa* produced several metabolites which were active against many pathogenic fungi and bacteria such as phenazine compound and its derivatives. There were more than 80 heterotrocyclic nitrogen-containing natural products of phenazines synthesized by fluorescent *Pseudomonas* spp. (Blankenfeldt *et al.*, 2004). Fluorescent *Pseudomonas* is uniquely capable of synthesizing many of these antibiotics, not only to enhance its own fitness but also to help in the maintenance of soil health and bioprotection of crops from pathogens (Dubey and Patel, 2001).Therefore to exploit the potential of fluorescent *Pseudomonas* isolates, the present study was aimed at *in- vitro* evaluation of antifungal activity of potential fluorescent *Pseudomonas* isolates against major plant pathogens involved in apple replant problem.

Materials and Methods

Plant growth promoting fluorescent *Pseudomonas* isolates isolated from apple replant sites were evaluated for antagonistic activity against major fungal pathogens involved in apple replant problem. 14 isolates of *Pseudomonas* sp. were tested for antifungal activity by standard well/bit plate assay method (Vincent, 1947). Fungal pathogens used in this study were isolated from apple replant sites. Fresh culture bits (10 mm dia) of 5 days old indicator fungi were cut with the help of sterile well borer and placed on the one side of prepoured malt extract agar (MEA) plates with the help of sterile inoculating needle. On the other side of plates, 10 mm well was cut with the help of sterile cork borer. 100 µl of 72 h old cell free culture supernatant of each test bacterial isolates was added to each well (10 mm). Plates were incubated at 28 ± 2^{o}C for 5-7 days and for *Phytophthora cactorum,* plates were incubated 28 ± 2^{o}C for 48h and observed for inhibition zone produced around the well. For control culture bit of indicator fungi kept in the centre of MEA plate and incubated at 28 ± 2^{0}C for 4 days. Antifungal activity expressed in terms of mm diameter of mycelial growth and that in turn expressed as per cent inhibition of fungal mycelia growth as calculating from equation:

$$\text{Percent Inhibition (\%I)} = \frac{C - T}{C} \times 100$$

where,
C : growth of mycelium in control
T : growth of mycelium in treatment

Results and Discussion

The present study showed that all bacterial isolates showed inhibition of fungal pathogens related to apple replant disease (Table 1 and Figure 1). Antifungal activity against plant pathogen *Dematophora necatrics* has been showed by nine isolates of *Pseudomonas* sp. in the range of 12.90 to 48.38 % inhibition. Maximum inhibition was shown by *Pseudomonas* strain K (48.38 %) followed by J (41.93 %).

Ten isolates showed antifungal activity against *Fusarium oxysporum* in the range of 9.09 to 34.09 % whereas no inhibition was observed in case of other four isolates against this test fungus. Maximum inhibition was shown by An-5-Jub (34.09 %) followed by four isolates i.e. J, Ar-2-Sh, An-4-Jub and Ps-1-Sin showed less inhibition of this plant pathogen i.e. (27.27 %). Eight isolates of *Pseudomonas* sp. out of fourteen showed inhibition against *Phytophthora cactorum* in the range of 7.89 to 31.57 %. Maximum inhibition was shown by isolate I (31.57 %) followed by Ar-4-De (26.31 %). Antifungal activity against plant pathogen *Pythium ultimum* has been shown by eight isolates of *Pseudomonas* sp. in the range of 22.5 to 35 %. Maximum inhibition was shown by Ps-1-Sin (35 %) followed by two isolates i.e. B (32.5 %) and I (30 %). Nine isolates showed antifungal activity against *Rhizoctonia solani* in the range of 8 to 28 % whereas no inhibition was observed in case of other five isolates against this test fungal pathogen. Maximum inhibition was shown by J (28 %) followed by two isolates i.e. K and M showed less inhibition against this plant pathogen i.e. (20 %).

Table.1 Potential of fluorescent *Pseudomonas* isolates for production of plant growth promoting activities: Antifungal against fungal pathogens:- *viz, Dematophora necatrix, Fusarium oxysporum, Phytophthora cactorum, Pythium ultimum* and *Rhizoctonia solani*

Fluorescent *Pseudomonas* Isolates	**Percent inhibition of fungal pathogens***									
	***Dematophora necatrix* (C=62mm)**		***Fusarium oxysporum* (C=44mm)**		***Phytophthora cactorum* (C=38mm)**		***Pythium ultimum* (C=40mm)**		***Rhizoctonia solani* (C=50mm)**	
	mm dia	**% Inhibition**	**mm dia**	**% Inhibition**	**mm dia**	**% Inhibition**	**mm dia**	**% Inhibition**	**mm dia**	**% Inhibition**
***P. aeruginosa* I**	38	38.70 (38.54)	37	15.90 (23.35)	26	31.57 (34.16)	28	30 (33.19)	44	12 (3.59)
***P. aeruginosa* J**	36	41.93 (40.22)	32	27.27 (31.46)	-	0 (0.00)	31	22.5 (28.30)	36	28 (5.38)
***P. aeruginosa* K**	32	48.38 (44.05)	40	9.09 (17.52)	30	21.05 (27.28)	29	27.5 (31.61)	40	20 (4.58)
***P. aeruginosa* B**	42	32.25 (35.00)	33	25 (29.98)	-	0 (0.00)	27	32.5 (34.74)	42	16 (4.12)
***P. putida* L**	-	0 (0.00)	33	25 (29.98)	-	0 (0.00)	31	22.5 (28.30)	-	0 (1.00)
***P. fluorescens* M**	-	0 (0.00)	-	0 (0.00)	-	0 (0.00)	30	25 (29.98)	40	20 (4.92)
***P. poae* Ar-2-Sh**	-	0 (0.00)	32	27.27 (31.46)	-	0 (0.00)	-	0 (0.00)	46	8 (2.48)
***Pseudomonas sp.* An-4-Jub**	54	12.90 (21.02)	32	27.27 (31.46)	34	10.52 (18.90)	-	0 (0.00)	-	0 (1.00)
***Pseudomonas sp.* An-5-Jub**	52	16.12 (24.14)	29	34.09 (35.70)	31	18.42 (25.39)	29	27.5 (31.61)	45	10 (3.30)
***Pseudomonas sp.* Ar-2-De**	48	22.58 (28.35)	-	0 (0.00)	29	23.68 (29.10)	-	0 (0.00)	-	0 (1.00)
***Pseudomonas sp.* Ar-4-De**	43	30.64 (33.59)	-	0 (0.00)	28	26.31 (30.84)	-	0 (0.00)	43	14 (3.87)
***Pseudomonas sp.* Ar-14-De**	-	0 (0.00)	-	0 (0.00)	-	0 (0.00)	-	0 (0.00)	41	18 (4.35)
***Pseudomonas sp.* Ps-1-Mgn**	48	22.58 (28.35)	33	25 (29.98)	35	7.89 (16.28)	-	0 (0.00)	-	0 (1.00)
***Pseudomonas sp.* Ps-1-Sin**	-	0 (0.00)	32	27.27 (31.46)	32	15.7 (23.33)	26	35 (36.25)	-	0 (1.00)
$C.D_{O.O5}$		**1.322**		**1.054**		**1.081**		**1.101**		**0.344**

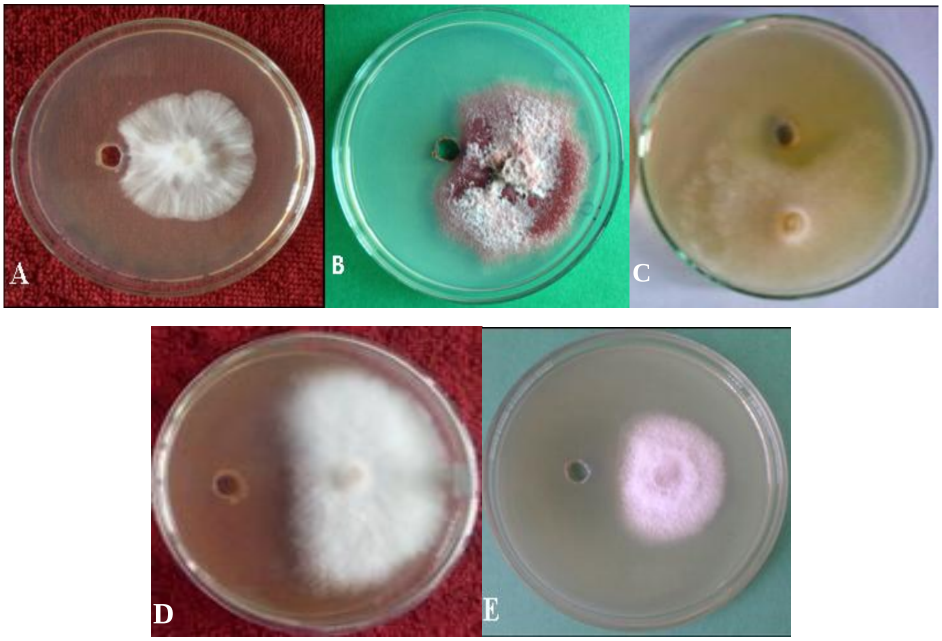

Figure.1 Antifungal activity shown by *Pseudomonas* isolates against different fungal pathogens at 28±2°C :- *viz.*,(A) *Dematophora necatrix,* (B) *Fusarium oxysporum,* (C) *Phytophthora cactorum,* (D) *Pythium ultimum* and (E) *Rhizoctonia solani*

Reddy *et al.,* (2007) also reported that crude compounds from *P. fluorescens* isolates metabolites completely inhibited the growth of *Magnaporthe grisea, Dreschelaria oryzae, Rhizoctonia solani* and *Sacrocladium oryzae* at 5%. The antifungal activity of fluorescent *Pseudomonas* sp. against phytopathogens *viz.*, *Fusarium* sp., *Pythium* sp., *Phytophthora* sp., *Dematophora* sp. and *Alternaria* sp. has a strong potential for plant growth promotion by inhibiting fungal pathogens and is also helpful in solving the replant problem of apple in field conditions (Sharma *et al.*, 2013).

Pseudomonas chlororaphis, Pseudomonas fluorescens and *Pseudomonas putida* isolated from the rhizospheres of healthy avocado trees showed antagonistic activity against *Dematophora necatrix,* the cause of avocado *Dematophora* root rot (also called white root rot) (Cazorla *et al.,* 2006). Berta *et al.*, (2004) studied the ability of *Glomus mosseae* BEG12 and *Pseudomonas fluorescens* A6RI to suppress *rhizoctonia* root rot (*Rhizoctonia solani*) of tomato and observed that both beneficial strains suppressed the soil borne disease.

This suppression was related to microbial antagonism and induced resistance for both strains. Sharma and Bhardwaj (2005) evaluated *Bacillus* spp. NA1, *Bacillus subtillus*-2, *E. aerogenes*-3 and *P. fluorescens* KB6 in controlling two important soil borne diseases of apple, viz. collar rot (*Phytophthora cactorum*) and root rot (*Dematophora necatrix*) and observed significant reduction in growth of these fungal pathogens further these isolates were effective against root rot pathogen under pot culture conditions and provided disease control of 72.3-82.1%.

All the plant growth promoting fluorescent *Pseudomonas* isolates showed antifungal activity against five test indicator plant pathogenic fungi isolated from replant sites of apple in the range of 7.8- 48.3 per cent. These plant growths promoting rhizobacteria can consequently act as an effective biological control agent against fungal plant pathogens since the rhizosphere provide the front line defense for roots against attack by pathogen. Thus these fluorescent *Pseudomonas* isolates can be helpful in solving the replant problem of apple in field conditions.

References

Angayarkanni T, Kamalakannan A, Santhini E and Predeepa D. 2005. Identification of biochemical markers for the selection of *Pseudomonas fluorescens* against *Pythium* spp. *In*: Asian conference on emerging trends in plant-microbial interactions. University of Madras, Chennai. pp. 295-303

Antoun H and Prevost D. 2005. Ecology of plant growth promoting rhizobacteria *In*: PGPR: biocontrol and biofertilization. Siddiqui Z A (*ed.*). Springer, Dordrecht: The Netherlands. pp. 1-38

Berta G, Sampo S, Gamalero E, Massa N and Lemanceau P. 2004. Suppression of *Rhizoctonia* root rot of tomato by *Glomus mosseae* BEG12 and *Pseudomonas fluorescens* A6RI is associated with combined modes of action. *Bulletin-OILB/SROP* 27(8): 99

Blankenfeldt W, Kuzin PA, SkarinaT, Korniyenko Y, Tong L, Bayer P, Thomashow SL and Mavordi DV. 2004. Structure and function of phenazine biosynthetic protein PhzF from *Pseudomonas fluorescence*. *Biochemistry* 101: 16431-16436

Braun PG and Fuller KK. 2006. Biological control of apple replant disease. Canadian Journal of Plant Pathology 28: 326

Cazorla F M, Duckett S B, Bergstrom E T, Noreen S, Odijk R, Lugtenberg B J J, Thomas-Oates J E and Bloemberg G V. 2006. Biocontrol of avocado *Dematophora* root rot by antagonistic *Pseudomonas fluorescens* PCL1606 correlates with the production of 2-hexyl 5-propyl resorcinol. *Molecular Plant Microbe Interaction* 19(4): 418-428

Cook RJ and KF Baker. 1983. The nature and practice of biological control of plant pathogens. *The American Phytopathological Society*. pp. 539

Dubey SC and Patel B. 2001. Determination of tolerance in *Thanatephorus cucumeris*, *Trichoderma viride*, *Gliocladium virens* and *Rhizobium* sp. to fungicides. *Indian Phytopathology* 54: 98-101

Kapoor R, Kumar A, Patil S, Thapa S and Kaur M. 2012. Evaluation of plant growth promoting attributes and lytic enzyme production by fluorescent *Pseudomonas* diversity associated with apple and pear. *International Journals of Scientific and Research Publication* 2(2): 1-8

Kaur M, Kumar S, Sharma S, Rana V and Sharma G. 2011. Potential of indigenous strains of *Pseudomonas* and *Bacillus* species for production of plant growth regulators viz., auxin, gibberellins and cytokinins from carnation and medicinal plants. *Progressive Horticulture* 43(1): 1-10

Lugtenberg B and Kamilova F. 2009. Plant growth promoting rhizobacteria. *Annual Review of Microbiology* 63: 541-556

Mai WF and Abwai GS. 1981. Controlling replant diseases of pome and stone fruits in Northeastern United States by a preplant fumigation. *Plant Diseases* 34: 254-267

Malik VS. 1982. Genetic and biochemistry of secondary metabolites. *Applied Microbiology* 28: 28-101

Merwin IA, Byard R, Robinson TL, Carpenter S, Hoying SA, Iungerman KA and Fargione M. 2001. Developing an integrated program for diagnosis and control of apple replant disease. *Horticultural Science* 23: 791

Reddy L, Moradi F and Koch C. 2007. Top-down biases win against focal attention in the fusiform face area. *NeuroImage* 38: 730-739

Robinson TL. 2007. Common mistakes in planting and establishing high-density apple orchards. *New York Fruit Quart* 15(4): 1-7

Sharma IM and Bhardwaj SS. 2005. Bacterial antagonists in management of collar rot and root rot diseases in apple. *Acta Horticulturae* 696: 349-354

Sharma S, Kumar M and Kaur M. 2013. Comparative study of the siderophores produced by *Pseudomonas* sp. isolated from apple rhizosphere of Trans-Himalayan region: potential utilization in replant problem and development of biosensor. *Asian Journal of Science and Technology* 4(12): 27-36

Sindhu SS and Dadarwal KR. 2001. Chitinolytic and cellulolytic *Pseudomonas* spp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* spp. cicer in chickpea. *Microbiology Reviews* 56: 662-676

Tank N and Saraf M. 2010. Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. *Journal of Plant Interactions* 5: 51-58

Vincent JM. 1947. Distribution of fungal hyphae in presence of certain inhibitors. *Nature* 150: 158-850

Winkelmann T, Smalla K, Amelung W, Baab G, Stöcker G, Kanfra X, Meyhöfer R, Reim S, Schmitz M, Vetterlein D, Wrede A, Zühlke S, Grunewaldt J, Wei S and Schloter M. 2019. Apple replant disease: causes and mitigation strategies. *Current Issues in Molecular Biology* 30: 89-106

Zaidi A, Khan MS, Ahemad M and Oves M. 2009. Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiological Et Immunological Hungarica* 56: 263-284.

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