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

Article Info
Accepted: 18 March 2020
Available Online: 10 April 2020

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\textsubscript{2} fixation, solubilization of insoluble phosphate (P), production of siderophores, phytohormones production, lowering of ethylene concentration, production of antibiotics and antifungal metabolites (Braun and Fuller, 2006). 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-diacyetylphloroglucinol (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 aeruginosaa 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°C for 5-7 days and for Phytophthora cactorum, plates were incubated 28±2°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°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:

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

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$_{0.05}$                       | 1.322 | 1.054 | 1.081 | 1.101 | 0.344 |
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 subtilis-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.

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**How to cite this article:**
Neha Kaushal and Mohinder Kaur. 2020. Antagonistic Activity of Plant Growth Promoting Fluorescent *Pseudomonas* spp. against Major Fungal Pathogens Involved in Replant Problem of Apple. *Int.J.Curr.Microbiol.App.Sci.* 9(04): 2285-2291.
doi: [https://doi.org/10.20546/ijcemas.2020.904.273](https://doi.org/10.20546/ijcemas.2020.904.273)