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# Potential of *Pseudomonas* Isolates for the Production of Antifungal Activity against Phytopathogenic Fungi Associated with Replant Problem of Apple

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**ABSTRACT**

*Soil-borne plant pathogenic fungi are of major concern problem in agriculture and major cause of apple replant problem , which affects yield and quality of agricultural crops. In this study, antagonistic effects of Pseudomonas sp. isolated from normal and replant site of apple rhizosphere of Shimla district were evaluated against four plant pathogenic fungi i.e. Dematophora necatrix, Fusarium oxysporum, Phytophtora cactorum and Pythium ultimum. The ability of Pseudomonas isolates to inhibiting the growth of phytopathogenic fungi was tested by measuring the inhibition zone for the growth of the tested fungi using dual culture method. Pseudomonas isolates had significantly inhibited the radial growth of tested fungal pathogens.*

***Key words:*** *Antifungal activity,Rhizosphere, Phytopathogens, Pseudomonas sp., Replant problem*

## INTRODUCTION

In apple, replant disease is widespread and has been documented in all of the major fruit growing regions of the world[9]. Apple replant disease is characterized by uneven growth of young trees but, when severe disease pressure is encountered, poor growth may be exhibited by a majority of trees on the site and death of young trees may occur. Himachal Pradesh is also facing the replant problem of apple in Shimla and Kullu districts where apple orchards have lived their economic life and new plantation is being done at the same site. In Kullu district of H.P., 25-70 per cent incidence of apple replant problem has been observed[2]. Some fungi from the following genera *Pythium, Thielaviopsis, Rosellinia, Phytophthora, Cylindrocarpon, Fusarium, Rhizoctonia, Penicillium and Alternaria* responsible for causing the replant problem[1]. Among some beneficial rhizosphere microorganisms the fluorescent *Pseudomonas* are mainly known to stimulate the growth of several annual crops or perennial trees *Pseudomonas* sp. have also received much attention as biocontrol agents[3,11].

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Fluorescent *Pseudomonas* sp. are the most diverse and versatile group of indigenous micro flora of almost all the horticulture and forestry crops.

## MATERIAL AND METHODS

Antifungal activity of each test isolate of *Pseudomonas* sp. was checked by well plate assay method[10] using dual culture technique.

Fresh culture bits (7 mm dia) of 5 days old indicator fungi was placed with the help of sterile well borer and inoculating loop. On the other side of MEA plates, 7 mm well was cut with the help of sterile well borer. 100 µl of 72 h old cell free culture supernatant of each test bacterial isolates was added to each well (7 mm). Plates were incubated at 28±2°C for 5-7 days and for *Phytophthora cactorum*, plates were incubated at 28±2°C for 48h and observed for inhibition zone produced around the well. For control, culture bit of indicator fungus kept in the centre of MEA plate and incubated at 28±2°C for 5-7 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:

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

C : growth of mycelium in control

T : growth of mycelium in treatment

## RESULTS

In our study, isolates of *Pseudomonas* sp. were screened out for the production of antifungal activity by well plate assay method against four indicators test fungi viz. *Dematophora necatrix*, *Fusarium oxysporum*, *Phytophthora cactorum*, *Pythium ultimum* (Table 1, fig 1).

***Dematophora necatrix***

Out of eleven *Pseudomonas* isolates seven showed antifungal activity against *Dematophora necatrix* in the range of 11.11% to 31.11% inhibition. Maximum percent growth inhibition was shown by *Pseudomonas* isolate $Rn_3$ i.e. 31.11% inhibition, followed by isolate $Rn_4$ i.e. 28.88% inhibition of mycelial growth. Five *Pseudomonas* isolates $Rn_1$, $Rr_1$, $Mgn_1$, $Mgn_2$ and $Mgr_1$ showed inhibition of mycelia growth in the range of 11.11 %I to 22.22%I. No inhibition was observed in case of four isolates $Rn_2$, $Rr_2$, $Mgn_3$ and $Mgr_2$.

***Fusarium oxysporum***

Out of eleven *Pseudomonas* isolates six showed antifungal activity against *Fusarium oxysporum* in the range of 12.72% to 27.27% inhibition. Maximum percent growth inhibition was shown by *Pseudomonas* isolate $Mgn_3$ i.e. 27.27%I. Five *Pseudomonas* isolates ($Rn_1$, $Rn_3$, $Rn_4$, $Mgn_1$ and $Mgr_1$) showed percent growth inhibition in the range of 12.72%I to 20.00%I. No inhibition was observed in case of $Rn_2$, $Rr_1$, $Rr_2$, $Mgn_3$ and $Mgr_2$.

***Phytophthora cactorum***

Out of eleven *Pseudomonas* sp. only four isolates showed antifungal activity against *Phytophthora cactorum* in the range of 11.76%I to 15.29%I inhibition. Maximum percent growth inhibition was shown by *Pseudomonas* isolate $Rn_4$ i.e. 15.29%I. Three isolates i.e. $Rn_2$, $Rn_3$ and $Mgn_3$ showed (11.76%) inhibition of mycelia growth. Seven *Pseudomonas* isolates viz. $Rn_1$, $Rr_1$, $Rr_2$, $Mgn_1$, $Mgn_2$, $Mgr_1$ and $Mgr_2$ showed no inhibition against *Phytophthora cactorum*.

***Pythium ultimum***

Out of eleven isolates of *Pseudomonas* sp. nine showed antifungal activity against *Pythium ultimum* in the range of 10.00 to 42.85%. Maximum inhibition was shown by isolate $Rn_3$ i.e. 42.85%I followed by isolate $Rn_2$ i.e. 32.85%I. Six isolates ($Rn_1$, $Rn_4$, $Mgn_1$, $Mgn_2$, $Mgn_3$ and $Mgr_2$) showed inhibition of mycelia growth in the range of 11.42%I to 21.42%I. Minimum inhibition was shown by isolate $Rr_1$ 10.00%I. No inhibition was observed in two isolates i.e. $Rr_2$ and $Mgr_2$.

**Table 1: Potential of fluorescent *Pseudomonas* isolates for production of plant growth promoting activities: Antifungal activity against four plant pathogens**

| S.no. | Fluorescent *Pseudomonas* isolates | Percent inhibition of fungal pathogens | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Dematophora necatrix* (C=45mm) | | *Fusarium oxysporum* (C=55mm) | | *Phytophthora cactorum* (C=85mm) | | *Pythium ultimum* (C=70mm) | |
| | | mm dia | % Inhibition | mm dia | % Inhibition | mm dia | % Inhibition | mm dia | % Inhibition |
| 1 | $Rn_1$ | 38 | 15.55(23.21) | 48 | 12.72(20.88) | 0 | 0 | 60 | 14.28(22.19) |
| 2 | $Rn_2$ | 0 | 0 | 0 | 0 | 75 | 11.76(20.04) | 47 | 32.85(34.95) |
| 3 | $Rn_3$ | 31 | 31.11(34.05) | 47 | 17.02(24.35) | 75 | 11.76(20.04 | 40 | 42.85(40.87) |
| 4 | $Rn_4$ | 32 | 28.88(32.49) | 45 | 18.18(25.22) | 72 | 15.29(23.00) | 55 | 21.42(27.55) |
| 5 | $Rr_1$ | 40 | 11.11(19.46) | 0 | 0 | 0 | 0 | 63 | 10.00(18.41) |
| 6 | $Rr_2$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | $Mgn_1$ | 39 | 13.33(21.40) | 44 | 20.00(26.55) | 0 | 0 | 55 | 21.42(27.55) |
| 8 | $Mgn_2$ | 35 | 22.22(28.06) | 0 | 0 | 0 | 0 | 57 | 18.57(25.51) |
| 9 | $Mgn_3$ | 0 | 0 | 40 | 27.27(31.46) | 75 | 11.76(20.04) | 60 | 14.28(22.19) |
| 10 | $Mgr_1$ | 39 | 13.33(21.39) | 48 | 12.72(20.88) | 0 | 0 | 0 | 0 |
| 11 | $Mgr_2$ | 0 | 0 | 0 | 0 | 0 | 0 | 62 | 11.42(19.73) |
| | $CD_{0.05}$ | | 0.49 | | 0.50 | | 0.35 | | 0.57 |

*** Antifungal activity expressed in terms of mm diameter of clear zone of growth of mycelia (bit) on malt extract agar (MEA) which in turn also expressed as percent inhibition (% I) of mycelial growth of indicator test fungi by 100µl of 72 h old supernatant grown in nutrient broth for 72 h at $28^0$C under shaking condition by well plate.**

**Where, C: Growth of mycelia in** $\frac{C-Z}{C} \times 100$ **control; Z: Growth of mycelia in test**

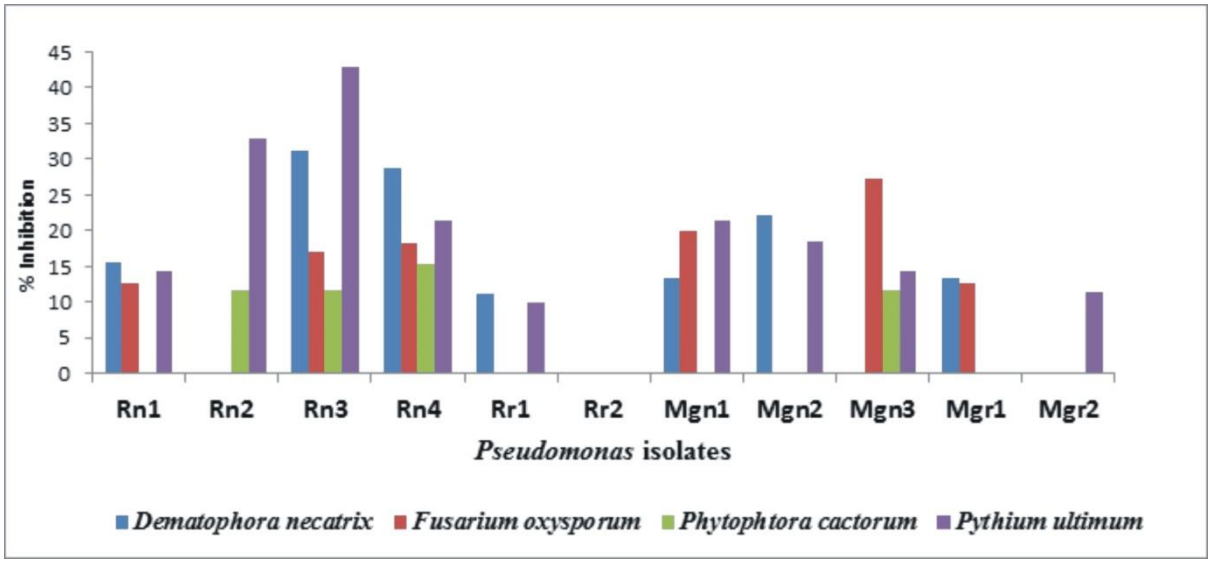


**Fig. 1: Antifungal activity against five fungal pathogens *viz, Dematophora necatrix, Fusarium oysporum, Phytophthora cactorum* and *Pythium ultimum***

## DISCUSSION

Some pseudomonads have been recognized as antagonists of plant fungal pathogens and antibiotic producers. This is probably due to the abundance of this diverse group of bacteria and their obvious importance in the soils[8]. A single strain of *Pseudomonas* can produce several different antibiotics. For instance, *Pseudomonas fluorescens* strain Pf-5 has been demonstrated to synthesize different antibiotics such as 2,4-diacetylphloroglucinol, pyoluteorin, and hydrogen cyanide. The *P. fluorescens* strain CHA0 has also been known to produce pyrrolnitrin, pyoluteorin, and 2,4-

diacetylphloroglucinol[5]. The biological protection of plants includes different types of amensalism, especially antibiosis as well as a competition between protective microorganisms and pathogens for nutrients, energy and habitat[6]. When biological methods are considered, special attention is paid to PGPR microorganisms (*plant growth promoting rhizobacteria*) which produce enzymes performing hydrolysis of the cell wall of pathogenic fungi resulting in its degradation and consequently termination of the pathogens. PGPR microorganisms include bacteria such as: *Pseudomonas, Bacillus, Paenibacillus, Brevibacillus, Agrobacterium,Burkholderia, Pantoea, Lysobacter*. Maleki *et al*.[4] reported that *P. fluorescence* CV6 had a broad spectrum antifungal activity against phytopathogens that can be used as an effective biological control candidate against devastating fungal pathogens that attack various plant crops. *P. fluorescens* strain possessing multiple mechanisms of broad spectrum antagonism and PGP activities can be explored as one among the best biocontrol agent[7].

## CONCLUSION

The biocontrol agents in the present study were *Pseudomonas* isolates. The production of antifungal metabolites by biocontrol agents (BCA) and other plant growth promoting activities such as lytic enzyme production, phosphate solubilization, siderophores production, HCN and ammonia production are the important mechanisms for plant growth promotion and disease suppression. Thus, the necessity to obtain high quality crop yield, the concern for the environment and the human safety, the possibility to reduce and control the environmental pollution, the request to safeguard that impose reduction of chemical treatments, all that should induce the farmers to choose biological interventions. According to our experiment, it is concluded that *Pseudomonas* isolates are the best biocontrol agents against different phytopathogens of apple rhizosphere.

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