In vitro antagonistic activity of fluorescent Pseudomonas isolates against Rhizoctonia solani and Sclerotium rolfsii

Amrotin, Vijay Kumar, Anil S Kotasthane and CP Khare

Abstract
Fluorescent Pseudomonas as a biocontrol agent offers a promising alternative to manage soil borne plant pathogens. However, the production of an antimicrobial compound varies among cultivars of the same species, and this has hampered the commercialization (Notz et al., 2001). Secondary metabolites produced by fluorescent Pseudomonas play key roles in the suppression of various soilborne plant pathogens. However, the performance of this biocontrol agent varies depending on the environment and host plant species. In this study, In vitro antagonistic activity against phytopathogens by fluorescens Pseudomonas of the 5 isolates of Pseudomonas showed different rates of growth inhibitions of R. solani and S. rolfsii, ranging from 39.995 to 65.275% and 34.995 to 68.325% respectively. Based on percentage of inhibition radial growth values, the antagonistic isolates in decreasing order were P141> P200> P66> P229> P260 and P66> P200> P141> P229> P260 against R. solani and S. rolfsii respectively. The isolates P141 and P66 were found to have the maximum inhibiting effect on the growth of R. solani (65.275%) and S. rolfsii (68.325%) respectively.

Keywords: fluorescent pseudomonas, rhizoctonia solani, sclerotium rolfsii, antagonistic activity, biocontrol agent, isolation

Introduction
Agriculture over the past few decades is heavily dependent on the application of chemical inputs. However, many chemical pesticides are very toxic and thus result in contamination of environment. Biological control is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture (Compant et al., 2005; Welbaum et al., 2004) \[1\]. The introduction of P. fluorescens as a biocontrol agent offers a promising alternative to manage soilborne plant pathogens. However, the production of an antimicrobial compound varies among cultivars of the same species, and this has hampered the commercialization (Notz et al., 2001) \[9\]. The studies of the ability to produce antibiotic secondary metabolites and their plant growth promoting potential are important not only for understanding their ecological roles in the rhizosphere and their interaction with plants, but also for any biotechnological applications. Biological control of plant pathogens by antagonistic microorganisms is a potential non-chemical means (Harman, 1991) \[3\] and is known to be a cheap and effective eco-friendly method for the management of crop diseases (Cook and Baker, 1983) \[2\]. Pseudomonas fluorescens is adapted to survival in soil and colonization of plant roots (Kielty et al., 2006) \[3\].

Material Methods
In vitro screening for antagonistic activity against soil borne pathogens
The five isolates were also tested for their efficacy as biocontrol agent against the phytopathogens Sclerotium rolfsii and Rhizoctonia solani isolated from rice. Equal volume of sterilized potato dextrose agar (PDA) and King’s B medium was mixed and poured in sterilized petri dishes. A heavy inoculum from an actively growing fluorescent Pseudomonas was streaked at 1 cm away from the edges of the plate and the mycelial disc of the pathogens were placed at the centre of petriplates. Control plates were inoculated only with phytopathogens but not with Pseudomonas isolates. Percent inhibition of pathogens by Pseudomonas isolates over control was calculated by using the formula of Vincent (1947):

\[
\text{Percent Inhibition} = \left(1 - \frac{\text{Growth of pathogen in control - Growth of pathogen with Pseudomonas isolate}}{\text{Growth of pathogen in control}}\right) \times 100.
\]

Results and Discussion
In vitro antagonistic potential of different isolates of Pseudomonas was studied against fungal
plant pathogens *Rhizoctonia solani* and *Sclerotium rolfsii*, following dual culture method was assessed after 5 days of growth. There were differences in the antagonistic abilities of isolates of *Pseudomonas* against both the plant pathogens. (Table 1 and Fig 1)

All of the 5 isolates of *Pseudomonas* showed different rates of growth inhibitions of *R. solani* and *S. rolfsii*, ranging from 39.995 to 65.275% and 34.995 to 68.325% respectively. Based on percentage of inhibition radial growth values, the antagonistic isolates in decreasing order were P141> P200> P66> P229> P260 and P66> P200> P141> P229> P260 against *R. solani* and *S. rolfsii* respectively. The isolates P141 and P66 were found to have the maximum inhibiting effect on the growth of *R. solani* (65.275%) and *S. rolfsii* (68.325%) respectively while isolate P260 and P229 showed the lowest inhibitory effect on *R. solani* (39.995%) and *S. rolfsii* (34.995%) respectively. The study revealed that different isolates have different capacities as biological weapons in inhibiting the pathogens, even though all were *Pseudomonas* isolates. Over all *Pseudomonas* isolates were found to be more efficient against *S. rolfsii* than *R. solani* indicating that these isolates could therefore be exploited as potential candidates for development of biopesticides. Tiwari (2005) [7] also reported that isolate isolated were from the rhizosphere of rice were found effective as antagonists to the fungal species of *Pyricularia grisea* and *Rhizoctonia solani*.

**Table 1:** Confrontation assay between *R. solani* and *S. rolfsii* with fluorescent *Pseudomonas* isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>% inhibition of <em>R. solani</em></th>
<th>% inhibition of <em>S. rolfsii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>P66</td>
<td>62.660±1.000</td>
<td>68.325±0.555</td>
</tr>
<tr>
<td>P141</td>
<td>65.275±0.835</td>
<td>48.885±1.115</td>
</tr>
<tr>
<td>P200</td>
<td>64.440±1.110</td>
<td>66.105±0.055</td>
</tr>
<tr>
<td>P229</td>
<td>49.440±2.780</td>
<td>39.995±1.115</td>
</tr>
<tr>
<td>P260</td>
<td>39.995±1.115</td>
<td>34.995±0.555</td>
</tr>
<tr>
<td>Max</td>
<td>65.275±0.835</td>
<td>68.325±0.055</td>
</tr>
<tr>
<td>Min</td>
<td>39.995±1.115</td>
<td>34.995±0.555</td>
</tr>
<tr>
<td>CV</td>
<td>3.871</td>
<td>2.268</td>
</tr>
<tr>
<td>CD 0.01%</td>
<td>8.791</td>
<td>4.703</td>
</tr>
<tr>
<td>CD 0.05%</td>
<td>5.604</td>
<td>3.009</td>
</tr>
<tr>
<td>Fcal</td>
<td>52.539</td>
<td>332.746</td>
</tr>
</tbody>
</table>

**Fig 1:** % Inhibition of fluorescent *pseudomonas* isolates against *R. solani* and *S. rolfsii*
Conclusion
All of the 5 isolates of Pseudomonas showed different rates of growth inhibitions of R. solani and S. rolfsii, ranging from 39.995 to 65.275% and 34.995 to 68.325% respectively. Based on percentage of inhibition radial growth values, the antagonistic isolates in decreasing order were P141 > P200 > P66 > P229 > P260 and P66 > P200 > P141 > P229 > P260 against R. solani and S. rolfsii respectively. The isolates P141 and P66 were found to have the maximum inhibiting effect on the growth of R. solani (65.275%) and S. rolfsii (68.325%) respectively.

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References