Responses of Bean to Root Colonization With *Pseudomonas putida* in a Hydroponic System

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**ABSTRACT**


Colonization of bean roots by *Pseudomonas putida* was maintained at 0.6-1.8 x 10^8 colony-forming units per gram of root tissue during growth for 18 days under hydroponic culture involving complete as well as iron- and boron-deficient media. Leaves from 18-day-old transplants colonized by *P. putida* had reduced iron contents compared with uninoculated seedlings, and roots had 17-93% higher lignin contents than did uninoculated seedlings. Plants with roots colonized by *P. putida* gained more weight after inoculation with *Fusarium solani* f. sp. *phaseoli* compared with plants grown without *P. putida*. In plants inoculated with both *P. putida* and *Fusarium*, foliar wilting and onset of lesion formation were delayed by 2-3 days. Colonization by *P. putida* decreased the amount of lignin normally generated at the lesioned root-shoot interface in plants infected by *Fusarium*. These data suggest that *P. putida* may afford some protection against *F. solani* in the early stage of disease development. Protection may involve alteration of the plant's defense potential through an increase in lignin in the root tissues.

Additional key words: nutrition, suppressive soil.

The colonization of plant roots by certain isolates of the group characterized by *Pseudomonas putida* and *P. fluorescens* may have a beneficial effect on plant performance (7,9,10,17,18). Increased growth and crop yields are reported for seedlings treated with *P. putida*. Suppression of Fusarium wilts and disease caused by *Gaeumannomyces graminis* var. *tritici* has been observed in pseudomonad-colonized plants (8,15,16).

The growth promotion and disease suppression attributed to pseudomonad colonization are affected by iron nutrition. Growth promotion was not observed for plants grown with adequate iron (7). Suppression of Fusarium wilt and take-all was negated by iron deficiency of chelates in the soils (7,16). The importance of iron nutrition may be related to bacterial production of siderophores in chelate Fe^3+ ions under iron-deficient conditions. These siderophores have higher chelation efficiencies and stabilities than siderophores produced by other soil microorganisms including *Fusarium* species (16). These observations suggest that the pseudomonad siderophores may inhibit growth of deleterious microorganisms in the rhizosphere by limiting their iron availability (18).

We have demonstrated agglutination of cells of certain strains of *P. putida* by a glycoprotein present on bean root surfaces (1). Cells agglutinated by this plant component include protective isolates used by Scher and Baker (15) and Kloeper, et al (7). Because agglutinability may enhance colonization of plant roots, we initiated studies of the interaction between bean seedlings and the isolate of *P. putida* routinely used for our agglutination investigations. Studies reported in this paper examined the ability of this isolate of *P. putida* to colonize bean roots under adequate or nutritionally stressed hydroponic conditions. Additionally, the effect of inoculation with *P. putida* on the severity of root rot caused in bean by *Fusarium solani* f. sp. *phaseoli* was examined. Our previous studies have demonstrated that plants grown under limited iron and boron nutrition displayed enhanced symptom formation (4). Consequently, effects of the pseudomonad and *F. solani* were studied under adequate nutrient supply as well as under iron- and boron-limited conditions. Hydroponic culture was used to obtain precision in providing defined nutrient conditions.

**MATERIALS AND METHODS**

**Culture of Pseudomonas and Fusarium.** *Pseudomonas putida* (Trevisan) Migula isolate Corvallis (1) was labeled by selecting for resistance to nalidixic acid and rifampicin (Sigma Chemical Company, P.O. Box 14508, St. Louis, MO) by using King's B medium containing the antibiotics at 50 μg/ml. The nalidixic acid- and rifampicin-resistant isolate, *P. putida* (nal^R^ rif^R^), was maintained on plates of King's B medium containing the antibiotics and was transferred at 3-wk intervals.

Inoculum of *P. putida* (nal^R^ rif^R^) was obtained by growth in liquid culture in King's B medium lacking antibiotics. Mid-log phase cells were obtained by centrifugation at 10,000 g, washed twice with sterile distilled water, and suspended in 10^-3 M MgCl2 before being used to inoculate bean seedlings.

*Fusarium solani* (Mor.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. and Hans. was cultured as described (3,4).

**Assessment of bacterial colonization.** A qualitative replicating procedure was used to assess the degree of colonization of bean seedling roots by colonies of *P. putida* (nal^R^ rif^R^). The seedling root was transferred to a sterile petri dish and pressed with a sterile velvet pad which was subsequently replica-plated onto plates of King's B medium containing antibiotics. The plates were incubated at 30 C for 3-5 days and the colony locations were noted.

A quantitative assay was used that involved dividing the root into an upper 5-cm portion adjoining the stem and a lower portion from 5 cm above the root tips. The material was weighed, and were possible 1 g of each portion was transferred to a tube containing 5 ml of sterile water. The root segments were vortexed for 2 min and the tube contents allowed to settle. Aliquots (0.1 ml) were serially diluted, 0.1-ml samples were plated on King's B medium containing the antibiotics, incubated at 30 C for 3-5 days, and the colonies were counted.

**Growth of bean seedlings.** Seedlings of *Phaseolus vulgaris* L. 'Dark Red Kidney' were grown as described (4). Inoculum of 1.5 X...
colonies of P. putida were assayed by agar plate assay. However, plating these samples onto King's B without antibiotics revealed a microflora, including some fluorescent pseudomonads, to be present.

**Effect of root colonization by P. putida on subsequent root infection by Fusarium.** Seedlings infected with F. solani reached greater weights when preinoculated with P. putida than did unoinoculated plants (Table 1). Each seedling infected with F. solani developed a brown lesion at the root-stem interface. Lesions were obtained even when the seedlings were preinoculated with P. putida although the reddish streaks first formed 2–3 days later than in the pseudomonad-nontreated plants. The presence of P. putida also delayed for 2–3 days the foliar wilting observed in all plants inoculated with F. solani. Preinoculation with P. putida of plants inoculated with F. solani had no significant effect on the lengths of the lesions obtained after 18 days at the stem-root interface. Lesion size was significantly greater for plants grown in 5 μM FeCl₃ without added borate (3.0 ± 0.4 cm) than for the other nutrient regimes (1.9 ± 0.3 cm).Iron contents of bean leaves.** The iron contents of bean leaves after 18 days in hydroponic culture were related to the nutrient media and the microbial inocula (Table 2). With 50 μM FeCl₃, infection by Fusarium increased the iron content of terminal leaves compared to those of unoinoculated plants. This effect was correlated with the noticeably greener leaves of the infected plants. With 5 μM FeCl₃, leaf chlorosis appeared in unoinoculated seedlings after 16 days and in infected seedlings after 18 days. Iron contents of leaves from these plants were similar. However, with 5 μM FeCl₃, the pseudomonad-inoculated plants developed chlorosis after 7 days with P. putida alone and after 12 days with the pseudomonad plus Fusarium treatment. Leaf iron contents were lower in the pseudomonad-inoculated plants than in unoinoculated plants. Boron deficiency did not consistently alter the iron levels detected in the leaves.

**RESULTS**

**Colonization of bean roots by P. putida.** Inoculation of bean seedlings with P. putida naf₉ rif₉ resulted in roots which, from imprints of the tissue, were colorized over all of the surfaces. The colonies of P. putida naf₉ rif₉ were present at 0.6 to 1.8 × 10⁶ cfu per gram fresh weight of root. Colonization was similar for the lower and upper root sections. This range of colonization was observed for plants removed from the flats 2 days after inoculation and for seedlings examined throughout the 18-day hydroponic growth period. The iron and boron status of the hydroponic solutions, or the presence of Fusarium inoculum, did not consistently influence bacterial numbers from the seedling in all cases. The weight gain of plants inoculated and uninoculated with P. putida was not statistically different under any of the growth conditions.

A comparison of bacterial growth in King's B and King's B plus antibiotics for samples from the pseudomonad-inoculated plants showed that P. putida naf₉ rif₉ accounted for 60–90% of the colonies obtained from the roots. Roots from plants not inoculated with P. putida possessed no naf₉ rif₉ colonies whether assayed by

| Table 1. The effect of Pseudomonas putida on weight of bean seedlings infected with Fusarium solani and grown with different levels of iron and borate⁠³
table

go | Fresh weight gain (g) and nutrient levels | Microorganism treatment² | 50 μM FeCl₃ | 5 μM FeCl₃ |
<table>
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<tbody>
<tr>
<td></td>
<td></td>
<td>50 μM FeCl₃</td>
<td>5 μM FeCl₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25 μM borate</td>
<td>Zero borate</td>
</tr>
<tr>
<td>P. putida plus F. solani</td>
<td>3.1 a</td>
<td>2.0 a</td>
<td>2.7 a</td>
<td>2.4 a</td>
</tr>
<tr>
<td>F. solani</td>
<td>0.3 b</td>
<td>0.5 b</td>
<td>0.6 b</td>
<td>0.3 b</td>
</tr>
</tbody>
</table>

⁠³Plants were preinoculated with P. putida grown in hydroponic culture at the different nutrient levels, and inoculated with F. solani as described in Materials and Methods. Means with a common letter within columns were not significantly different according to Duncan's new multiple range test, P = 0.05.

| Table 2. The effects of Pseudomonas putida and Fusarium solani and mineral nutrition on iron content of terminal leaves of bean seedlings | Leaf iron content (μg/g) and nutrient levels¹ |
|---|---|---|
| Microorganism treatment² | 50 μM FeCl₃ | 5 μM FeCl₃ |
| | 25 μM borate | Zero borate | 25 μM borate | Zero borate |
| Control | 370 a | 450 a | 379 a | 323 a |
| P. putida | 351 b | 360 b | 297 b | 200 b |
| P. putida plus F. solani | 730 b | 878 c | 242 c | 262 c |
| F. solani | 645 c | 720 d | 320 d | 310 a |

¹Plants were preinoculated with P. putida and challenged with F. solani as described in Materials and Methods.

²The iron content of leaves from plants grown under described iron treatment conditions was determined after 18 days of hydroponic growth. Means with a common letter within columns were not significantly different according to Duncan's new multiple range test, P = 0.05.

| Table 3. The effect of Pseudomonas putida and Fusarium solani and levels of iron and borate on the lignin contents at the root-shoot interface of bean seedlings | Lignin (mg/g dry wt.) and nutrient levels¹ |
|---|---|---|---|---|
| Microorganism treatment² | 50 μM FeEDDA | 50 μM FeCl₃ | 5 μM FeCl₃ |
| | 25 μM borate | Zero borate | 25 μM borate | Zero borate | 25 μM borate | Zero borate |
| Control | 177 a | 160 a | 160 a | 168 a | 157 a |
| P. putida | 192 a | 162 a | 185 a | 172 a | 152 a |
| P. putida plus Fusarium | 218 b | 188 b | 220 b | 153 a | 186 b |
| Fusarium | 259 c | 230 b | 246 b | 185 a | 189 b |

¹Lignin was extracted from dried tissue at the root-shoot interface of seedlings grown hydroponically for 18 days under the described nutrient conditions. Means with a common letter within a column were not significantly different according to Duncan's new multiple range test, P = 0.05.
6.5 to 3.8 between days 5 and 8 after transplanting. In contrast with 5 μM FeCl₃, the pH of media containing plants inoculated with either the pseudomonad or *Fusarium* alone, or both together, remained between 6.2 and 6.8. With 10 μM FeCl₃, the pH of the nutrient solutions ranged between 6.3 for the uninoculated plants and 7.2 for the plants inoculated with the pseudomonad plus *Fusarium*. Plants inoculated with the pseudomonad or with *Fusarium* alone did not alter the pH of the nutrient solutions from the initial value of 6.7.

The concentrations of o-dihydroxyphenols in the nutrient solutions were monitored simultaneously with pH because of a proposed role of these compounds in promoting iron reduction (14). Whether 5 or 10 μM FeCl₃ was used as the iron source, nutrient media containing uninoculated plants and plants inoculated with *P. putida* showed an increase in o-dihydroxyphenol concentration from zero at day 2 to 4.4 (± 0.1) μM at day 8. In contrast, 2 days after inoculation with *Fusarium*, with or without *P. putida*, the concentration of o-dihydroxyphenols was 6.1 (± 0.5) μM for 5 μM FeCl₃ and 8.6 (± 0.1) μM for 10 μM FeCl₃. These o-dihydroxyphenol concentrations declined daily to 5.0 (± 0.2) μM for 5 μM FeCl₃ and 7.0 (± 0.5) μM for 10 μM FeCl₃ media by day 8.

**Lignin formation.** Lignin contents of the root-shoot interface were not altered consistently by inoculation with the pseudomonad alone compared with control plants (Table 3). Lesion development caused by *F. solani* increased the amount of lignin extracted from the root-shoot interface. Plants grown in 5 μM FeCl₃ and inoculated with the pseudomonad plus *Fusarium* showed lignin contents that were reduced 18–22% compared to those of plants with *Fusarium* alone. With 10 μM FeCl₃, lignin contents of plants inoculated with *Fusarium* plus the pseudomonad were reduced 21% with borate but only 2% without borate when compared to plants inoculated with *Fusarium* alone.

The presence of both microorganisms also affected the lignin contents of the lower roots (Table 4). Inoculation with *Fusarium* stimulated lignin formation above that in uninoculated plants. The presence of *P. putida* reduced the amount of lignin formed in plants inoculated with Fusarium, although the difference was not always statistically significant. Inoculation with *P. putida* alone also enhanced lignification by 26–93% over that observed in uninoculated plants. To confirm this effect of colonization by *P. putida*, the lignin contents of lower roots from plants harvested at the time of transplanting into hydroponic culture were determined (Table 5). In three separate trials, the lignin contents of roots of plants inoculated with *P. putida* were higher by 9–50% than for the uninoculated plants.

**DISCUSSION**

The aggressive colonization of bean root surfaces by the *P. putida* isolate Corvallis agrees with reports of effective colonization of roots of other plant species by strains of the group characterized by *P. putida* and *P. fluorescens* (17, 18). Our data demonstrate that colonization occurred on lateral roots as well as the main root, and that it was maintained in the presence of *F. solani* and *Fusarium* under growth conditions varying in iron and boron availability. Under low iron availability, pseudomonad colonization of bean caused chlorosis and reduced foliar iron contents. The production of siderophores by the *P. putida* Corvallis isolate (F. Fekete and A. J. Anderson, unpublished) may be involved in this chlorotic response. The siderophores may chelate the iron in a form which is unavailable to the plant.

**Pseudomonas putida** as well as the challenging pathogen *F. solani* and *Fusarium* affected two other phenomena related to iron nutrition. Many plants respond to low iron availability with acidification of the rhizosphere (2). Although acidification of the nutrient solution was observed with low-iron nutrition for uninoculated plants, root colonization by *P. putida* and/or *Fusarium* alleviated this pH drop. The secretion by plant roots of dihydroxyphenolic compounds has been proposed as a mechanism to promote reduction of rhizosphere Fe³⁺ ions to Fe²⁺ ions (14). In our studies, production of dihydroxyphenolic compounds was affected more by the microbial populations than by the iron status. The enhanced phenolic accumulations from plants inoculated with *P. putida* and/or *F. solani* may have resulted from changes in root wall permeability and in microorganism-mediated changes in phenol metabolism.

Altered phenolic metabolism was demonstrated by increased lignin formation in roots colonized by *P. putida* or *F. solani*. Formation of lignin, whether stimulated by inocula of the pseudomonad or *Fusarium*, responded similarly to iron nutrition. Reduction in lignin content associated with low available iron may relate to an essential role of the stelidines containing heme iron in the synthesis of the polymer. The slight increase in lignin observed in boron-deficient plants when inoculated with pseudomonads or *Fusarium* is contrary to the hypothesis of Lewis (11). Lewis predicted that boron deficiency may impair polymerization of the monomeric precursors to lignin. Our observations would be consistent if the factors that control phenol metabolism differ in plants that are responding to a microbial challenge rather than being at equilibrium with the biotic environment.

Enhanced production of oxidized phenols has been documented for many plant-pathogen interactions including that of *F. solani* and bean. However, root colonization by the saprophytic *P. putida* alone caused increased production of ligninlike polymers. This observation suggests that *P. putida* may contribute to plant resistance to pathogens by increasing lignin levels. The subtle effects of colonization by *P. putida* on infection by *F. solani* support this suggestion. The time of onset of lesion formation was lengthened and the amount of lignin was reduced in plants inoculated with *P. putida* versus uninoculated plants challenged with *F. solani*. The failure of *P. putida* to prevent infection by *F. solani* agrees with the lack of suppression of this pathogen in natural soils suppressive to *Fusarium* wilts (R. Baker, personal communication). Results of greenhouse and field studies of suppression of take-all and of *Fusarium* wilts by *P. putida* demonstrated that a high level of protection occurred only when available iron was low. Scher and Baker (16) and Kloepper et al (8) suggest that the siderophores produced by the proteobacteria under low-iron conditions limited iron availability to the pathogen.

**TABLE 4.** Effect of microorganisms and mineral nutrition on lignin content of the lower roots of bean seedlings harvested after 18 days in hydroponic culture

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lignin (mg/g dry weight)</th>
<th>50 μM FeCl₃</th>
<th>50 μM FeCl₃</th>
<th>5 μM FeCl₃</th>
<th>5 μM FeCl₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>112 a</td>
<td>98 a</td>
<td>109 a</td>
<td>90 a</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas putida</em> plus <em>Fusarium</em></td>
<td>160 b</td>
<td>190 b</td>
<td>138 b</td>
<td>150 b</td>
<td></td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td>164 b</td>
<td>204 b</td>
<td>150 b</td>
<td>210 b</td>
<td></td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td>190 b</td>
<td>297 c</td>
<td>160 b</td>
<td>225 b</td>
<td></td>
</tr>
</tbody>
</table>

1Plants were preinoculated with *P. putida* and challenged with *F. solani* as described in Materials and Methods.

2Lignin was extracted from dried tissue of the lowest 5 cm of roots of seedlings grown hydroponically for 18 days under the described nutrient conditions. Means with a common letter within columns were not significantly different according to Duncan's new multiple range test, P = 0.05.

**TABLE 5.** The effect of microorganisms on the lignin content of the lower roots of greenhouse-grown bean seedlings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lignin (mg/g dry weight)</th>
<th>trial number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>86</td>
<td>128</td>
</tr>
<tr>
<td>Pseudomonad-inoculated</td>
<td>100</td>
<td>140</td>
</tr>
</tbody>
</table>

1Plants were preinoculated with *P. putida* as described in Materials and Methods.

2Lignin was extracted from dried tissue of the lowest 5 cm of roots of seedlings grown in vermiculite to the first trifoliolate leaf stage.
and consequently hindered fungal growth. In the system involving
F. solani and P. putida, some protective effects are apparent with
iron levels that should not stimulate the production of the bacterial
siderophores. Rather, results of our examination of lignin contents
of root tissue suggest that the interaction between P. putida and the
plant that enhances lignification also could be crucial in the
protection phenomenon. If lignin functions as a defensive barrier in
the plant (5), then the enhanced level associated with colonization
by P. putida may contribute to an impaired root-pathogen
interaction. Consequently, we suggest that in addition to direct
effects upon pathogens, beneficial isolates of P. putida also may
strengthen the defensive potential of the plant.

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