# Inhibition of the Sugarbeet Pathogens *Phoma betae* and *Rhizoctonia solani* by Bacteria Associated with Sugarbeet Seeds and Roots

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## ABSTRACT

**Bacteria that inhibited growth of the sugarbeet fungal pathogens *Phoma betae* and *Rhizoctonia solani* were isolated from sugarbeet *(Beta vulgaris)* seeds and roots. These bacteria include fluorescent pseudomonads, and species of *Serratia, Enterobacter, and Bacillus.* Inhibition of growth of *P. betae* and *R. solani* on solid medium was dependent upon growth medium. Inhibition under both low-iron and iron-sufficient conditions suggests that siderophore production by the fluorescent pseudomonads was not a major factor. Inhibition was not correlated with hydrogen cyanide production by the bacteria. Growth inhibition was associated with production of phenazine-1-carboxylic acid by certain fluorescent pseudomonads. In greenhouse trials, some of the bacteria that displayed the strongest level of *in vitro* antagonism suppressed sugarbeet seedling disease caused by *P. betae* or *R. solani.* Certain pseudomonads and strains of *Serratia* and *Enterobacter* suppressed both diseases.**

**Additional Key Words:** *Beta vulgaris,* biological control, siderophores, phenazines, pseudomonads

Seedling stand in sugarbeet (*Beta vulgaris* L.) is influenced by both soil-and seedborne pathogens. Soilborne *Rhizoctonia solani* Kühn is associated with both aerial and root symptoms on beet, depending on the anastomosis group (Ruppel, 1972; Windels and Nabben, 1989). In comparison, *Phoma betae* A.B. Frank is seedborne and causes preemergence damping-off and severe hypocotyl infections of emerged seedlings in cool soils (Bugbee, 1974; El-Nashaar and Bugbee, 1981; Kober and Gallian, 1988; Leach and MacDonald, 1976). If the seedling survives, the plant may suffer loss in vigor and reduced sugar content. Bugbee and Cole (1981) correlated superficial hypocotyl lesions and storage rot after beet harvest with *P. betae* infection (El-Nashaar and Bugbee, 1980).

Control of *P. betae* and *R. solani* in sugarbeet by fungicides and breeding for resistance is insufficient (Campbell and Bugbee, 1988; Durrant et al., 1988; Gallian and Kober, 1988; Gallian et al., 1989) and biological control may be an alternative strategy (Davison 1988; Kloepper et al., 1989; Weller, 1988). The mechanisms involved in biocontrol are diverse. Certain biocontrol agents produce chemicals that directly impair pathogen growth. Fluorescent pseudomonads and bacilli produce antibiotics (Gueldner et al., 1988; Thomashow and Weller, 1988; Thomashow et al., 1990). Hydrogen cyanide (HCN) production by fluorescent pseudomonads has also been implicated in protection of tobacco against *Thielaviopsis* (Ahl et al., 1986; Voisard et al., 1989). Restriction of available iron by highly effective siderophores produced by fluorescent pseudomonads was correlated to suppression of other pathogens (Kloepper et al., 1980, Scher and Baker, 1980).

The plant may be involved in biological control by favoring the establishment of sufficiently large populations of microorganisms that produce an effective dose of fungal growth inhibitors. Other mechanisms may also operate. Competition between the pathogen and biological control agent for colonization sites and for nutrients from the root may occur. Competition has been implicated in the suppression of ice-nucleating *Pseudomonas syringae* isolates by non-nucleating strains (Lindow, 1988). Paulitz (1991) suggested that utilization of seed volatiles by beneficial pseudomonads may be important in suppression of infection by *Pythium*.

Isolates of *Bacillus* species suppressive to *P. betae* previously were isolated from sugarbeet (Dunleavy, 1955; Krezel and Stankiewicz, 1984; Vesely, 1986). Biological control of *R. solani* also was demonstrated in other crops (Howell and Stipanovic, 1978). Consequently, to detect isolates that may function as biocontrol agents in Idaho, we isolated and tested bacteria from sugarbeet seedlings grown in

Idaho and from sugarbeet seeds for traits associated with pathogen suppression. Initially, we screened for direct growth inhibition of *P. betae* and *R. solani* in vitro and for the production of fluorescent siderophores and phenazines (Thomashow et al., 1990). Selected isolates displaying these properties were subsequently screened for their ability to suppress disease caused by *P. betae* or *R. solani* in sugarbeet seedlings. Single bacterial isolates were sought that would suppress both seedborne *P. betae* and soilborne *R. solani.*

## MATERIALS AND METHODS

**Bacterial sources.** Bacteria were isolated from the internal tissues of seeds of *B. vulgaris* 'WS-88' from Oregon seed fields. Seeds were treated with 5% sodium hypochlorite for 30 min at 50°C, before being rinsed with sterile water at 50°C for 30 min. After air drying in a sterile transfer hood, the seeds were placed on King's medium B (KB) agar (King et al., 1954). Bacteria growing from the seeds were restreaked on KB agar and single colonies isolated.

Bacteria associated with sugarbeet roots were obtained from 1-mo- and 2-mo-old WS-88 seedlings that were collected from fields in Idaho. Individual roots and associated soil particles were immersed in water (10 ml/root) and vortexed for 30 sec to obtain isolates which we term rhizosphere isolates. The suspensions were streaked on KB and bacterial colonies were purified by single-colony isolation.

Bacteria which we term rhizoplane isolates were obtained from roots of other 2-mo-old plants. A root was thoroughly washed in sterile water, then immersed in 10 ml 0.05 M sodium ethylenediamine tetraacetic acid (EDTA), pH 6.5, and vortexed for 1 min. The root was removed and 0.5 ml of 1.0 M $MgCl_2$ was added to the tube. These procedures were to remove bacteria bound to the root through cationic binding (Anderson et al., 1988). The suspension was dilution-plated on KB agar and single colonies were isolated and purified. Bacteria associated with the rhizoplane and internal tissues were isolated from the 2 mo-old plants by grinding 1 g of water-washed root in 10 ml of 0.05 M sodium EDTA (pH 6.5) with a sterile pestle and mortar. The slurry was amended with 0.5 ml of 1.0 M $MgCl_2$, dilution-plated onto KB, and single colonies were isolated and purified.

Bacteria from internal tissues were obtained after surface treating roots by immersion into alcohol and flaming twice. The roots were washed in sterile water and ground with a sterile pestle and mortar in 0.05 M EDTA, pH 6.5 (10 ml/g tissue). The slurry was

amended with 0.5 ml of 1.0 M $MgCl_2$ and dilution-plated to obtain single colonies.

Bacteria were maintained on minimal medium (Anderson and Guerra, 1985) and stored at 6°C with monthly transfers. Stock suspensions were prepared in 20% glycerol and stored at −70°C. Selected bacteria were identified by gas liquid chromotographic-mass spectral analysis of lipids (performed by MDI, Newark, Delaware, and the Plant Pathology Department, Auburn University, Auburn, AL).

**Hydrogen cyanide production.** HCN production by the bacteria on potato-dextrose agar (PDA) (Difco, Detroit MI) was detected upon growth of a colony from 5$\mu$l of a logarithmic culture grown in rich broth medium (Anderson and Guerra, 1985). The colorimetric detection method for HCN reported by Castric and Castric (1983) was used.

**Phenazine production.** Phenazine production by the pseudomonads was examined by methods of Thomashow et al. (1990). Cultures (100 ml) were grown in KB broth at 22°C for 24 h with shaking at 100 rpm. Cells were removed by centrifugation at 10,000 g for 10 min. The culture fluid was acidified to pH 2 with HCl before extraction with an equal volume of benzene for two, 2 h intervals. The benzene fractions were dehydrated with $Na_2SO_4$ and evaporated to dryness in a rotary evaporator at 50°C. The material was redissolved in benzene (500 $\mu$l) and fractionated using 50 $\mu$l aliquots on silica gel G plates (Sigma Chemical Co., MO) with ethyl acetate in butyl ether: acetic acid (150:150:10 v/v/v). The thin layer chromatographic (TLC) plates were examined under visible and UV light. Yellow material (with $R_f$ 0.28 ± 0.1) was removed from the TLC plates, redissolved in benzene, and its UV absorbance recorded. Copurification of the components from the sugarbeet isolates with products from *P. fluorescens* 2-79, which is known to produce phenazine-1-carboxylic acid, was performed. Isolate *P. fluorescens* 2-79 was obtained as a gift from L. Thomashow (USDA-ARS, Pullman, WA).

**Growth inhibition studies.** Bacteria were screened for growth inhibition of *P. betae* and *R. solani.* A virulent *P. betae* isolate was obtained from sugarbeet seeds by J.J. Gallian; *R. solani* AG-2-2, which causes seedling disease, was isolated by E. G. Ruppel (USDA-ARS, Fort Collins, CO). Fungi were maintained on PDA at 20°C, with passage through sugarbeet and reisolation at 2-mo intervals to retain pathogenicity.

Growth inhibition *in vitro* was assayed by transferring mycelial

plugs (1 X 1 cm) from the growing edge of the *P. betae* or *R. solani* colonies to the middle of KB agar or PDA plates. Four 5$\mu$l droplets of bacteria from late logarithmic cultures grown in rich medium (Anderson and Guerra, 1985) were applied 5 cm from the fungal inoculum. Growth of the bacteria and the fungal colonies was observed after 5 days of growth at 22°C in darkness. Inhibition of fungal growth was recorded by use of the scale: – no inhibition (fungus grew to and over the bacteria); + slight inhibition (fungus stopped growing 1 to 3 mm from bacteria); + + moderate inhibition (fungus stopped growing 4 to 7 mm from the edge of bacteria); and + + + + strong inhibition (fungal growth stopped at least 8 mm from bacteria).

**Disease suppression.** Sugarbeet seeds were immersed into a suspension of *P. betae* conidia ($10^3$/ml) and infiltrated for 15 min under three cycles of applied vacuum produced by a water-driven pump. The *P. betae*-treated seed was vacuum infiltrated with bacteria at $10^{10}$ colony-forming units (CFU)/ml, or sterile water as a control treatment. Treated seeds were suspended in sterile water, vortexed for 5 min, and after another 15 min samples were withdrawn for dilution-plating to determine CFU. This method indicated that each seed retained $10^4$ - $10^5$ CFU of the bacterium. Four seeds were planted within 1 wk of treatment into sterilized vermiculite (200 ml vermiculite and 30 ml water) in Magenta boxes (Magenta Corporation, Chicago IL). Seed was covered with sterile vermiculite and the boxes incubated at 26°C for 7 days with a 16 h light - 8 h darkness regime in a randomized block design. Emergence and incidence of disease was recorded over a 10 day period. Seedlings that were black and wilted were an indication of infection by *P. betae.*

Inoculation with *R. solani* involved treating seed for 15 min under three cycles of an applied vacuum to infiltrate a suspension of hyphal fragments obtained by 10-fold dilution of water washes (50 ml) of two, 5-day old plate cultures. Bacteria were added to the fungal suspension at $10^{10}$ CFU /ml, where appropriate. Seeds were planted and grown as described above. Emergence was recorded at day 7 and incidence of disease at day 10.

## RESULTS

Bacteria isolated from the sugarbeet seeds and roots were categorized by appearance on KB medium into three groups (Table 1). Two groups (I and II) were pseudomonads which produced fluorescent pigments when observed under UV light. Group III was an assortment of cream, white, or yellow-pigmented bacteria. Group

**Table 1.** Numbers and properties of bacteria isolated from sugarbeet seed and roots.

| Isolate source and property[†] | Number of Bacteria: | | |
|---|---|---|---|
| | Group I | Group II | Group III |
| **Rhizosphere** (40 1-mo-old plants) | | | |
| Total number | 14 | 14 | 17 |
| Number with: | | | |
| HCN production[‡] | 5 | 5 | 11 |
| Strong *Phoma* inhibition[‡] | 5 | 14 | 3 |
| Strong *Rhizoctonia* inhibition[‡] | 3 | 8 | 2 |
| **Rhizoplane** (3-mo-old roots) | | | |
| Total number | 4 | 0 | 8 |
| Number with: | | | |
| HCN production | 2 | 0 | 2 |
| Strong *Phoma* inhibition | 2 of 3 tested | 0 | 5 of 6 tested |
| Strong *Rhizoctonia* inhibition | 0 | 0 | 2 of 6 tested |
| **Rhizoplane and Internal** (6 2-mo-old roots) | | | |
| Total number | 1 | 0 | 6 |
| Number with: | | | |
| HCN production | 0 | 0 | 1 |
| Strong *Phoma* inhibition | 1 | 0 | 0 |
| Strong *Rhizoctonia* inhibition | 1 | 0 | 0 |
| **Internal** (5 2-mo- old roots) | | | |
| Total number | 0 | 0 | 5 |
| Number with: | | | |
| HCN production | 0 | 0 | 0 |
| Strong *Phoma* inhibition | 0 | 0 | 0 |
| Strong *Rhizoctonia* inhibition | 0 | 0 | 0 |
| **Seed** (30 seeds) | | | |
| Total number | 4 | 0 | 30 |
| Number with | | | |
| HCN production | 0 | 0 | 1 |
| Strong *Phoma* inhibition | 0 | 0 | 6 of 21 tested |
| Strong *Rhizoctonia* inhibition | 1 | 0 | 2 of 21 tested |

[†]Bacteria were isolated from the root surface or internal tissues of seedlings as described in Materials and Methods. The bacteria were classified by appearance on King's medium B into three groups I, II, and III according to appearance: Group I are fluorescent, Group II are fluorescent and produce green or brown pigments, and Group III are nonfluorescent and diverse in pigmentation.
[‡]Production of hydrogen cyanide (HCN) and inhibition of growth of *Phoma betae* and *Rhizoctonia solani* on solid media were measured for each bacterium as described in Materials and Methods.

II fluorescent pseudomonads, unlike Group I, developed dark lines of pigmentation along colony edges when incubated in the presence of the fungal pathogens. In most isolates, pigmentation was dark brown-green although in three strains the pigmentation was green (Table 2).

Yellow-pigmented, mucoid bacteria listed in Group III in Table 1 were the dominant bacteria isolated from seeds, whereas fluorescent pseudomonads were the most prevalent isolates from the rhizosphere. Group II fluorescent pseudomonads were detected only from the rhizosphere of 1-mo-old seedlings (Table 1). Rhizoplane isolates were fluorescent pseudomonads and cream and white bacteria (Table 1).

**Inhibition of growth of *P. betae* and *R. solani*.** Inhibition of growth of the beet pathogens *P. betae* and *R. solani* on solid media was detected with certain Group I and Group II fluorescent pseudomonads and Group III isolates (Tables 1 and 2). Strong growth inhibition was not displayed by the yellow mucoid isolates from seed (Table 2).

Of 18 Group I fluorescent pseudomonads, four isolates (P1C, P3B, P5B, R1-I-1) strongly inhibited growth of *P. betae* on both KB and PDA medium. KB medium was used to evaluate inhibition under conditions of iron limitation when fluorescent siderophores are produced by the Group I and Group II isolates. PDA was used as an iron-sufficient medium that is known to support production of phenazines from pseudomonads (Thomashow et al. 1990). Five other isolates (P8E, R1U-2, R3U-2, R6, J4B10) displayed intermediate inhibitory activity dependent on the medium. Three of the 18 isolates (P3C, P8E and R6) were strong inhibitors and eight other strains (P1C, P3B, P5B, R1-I-1, R2U, R3U-2, R7-3 and S4D) were weaker inhibitors of *R. solani* growth. Inhibition of the growth of *R. solani* was observed on both KB and PDA media.

All of the 14 Group II fluorescent pseudomonads, which produced pigmentation in the presence of the pathogen, consistently inhibited growth of *P. betae* and most were inhibitory to *R. solani*. Inhibition of growth of *P. betae* with seven of the 14 isolates was greater on PDA than on KB agar while inhibition of six other isolates was similar on both media. No consistent relationship was observed between medium and inhibition of *R. solani*. The strong growth inhibition of *P. betae* by the Group II pseudomonads changed the colony morphology with hyphae becoming more fluffy and aerial.

Group III cream-colored bacteria (P4D, R3L-2 and J7A1) and white-colored J1A8, J2B3 and J6B1 from seed and white-colored

**Table 2.** Hydrogen cyanide (HCN) production and in vitro growth inhibition of *Phoma betae* and *Rhizoctonia solani* displayed by bacteria isolated from sugarbeet seeds and roots.

| Isolate[†] and source | HCN[‡] production | Growth inhibition[§] *Phoma betae* on: KB | Growth inhibition[§] *Phoma betae* on: PDA | Growth inhibition[§] *Rhizoctonia solani* on: KB | Growth inhibition[§] *Rhizoctonia solani* on: PDA |
|---|---|---|---|---|---|
| **Group I (fluorescent pseudomonads)** | | | | | |
| Rhizosphere | | | | | |
| P1C | + | +++ | ++++ | ++ | - |
| P3B | + | ++++ | ++++ | ++ | - |
| P3C | - | ++ | + | ++++ | ++++ |
| P5B | + | ++++ | ++++ | ++ | - |
| P8E | - | ++++ | + | ++ | ++++ |
| P10A | ++ | + | ++ | + | - |
| P10D | + | ++ | ++ | + | + |
| P10E | - | + | - | - | - |
| Rhizoplane | | | | | |
| R1-I-1 | ++ | +++ | ++++ | ++ | - |
| R1U-2 | - | - | ++++ | + | - |
| R2U | - | + | + | ++ | - |
| R3U-2 | - | ++ | +++ | ++ | + |
| Rhizoplane plus internal | | | | | |
| R6 | - | +++ | +++ | ++++ | ++++ |
| R7-3 | - | ++ | - | ++ | - |
| Seed | | | | | |
| S4D | - | ++ | ++ | ++ | ++ |
| J4B10 | - | ++ | +++ | + | - |
| J4D8 | - | + | +++ | + | - |
| J5B1 | - | + | + | + | - |
| **Group II (rhizosphere fluorescent pseudomonads with dark pigmentation)** | | | | | |
| P1D (brown) | ++ | ++++ | ++++ | +++ | +++ |
| P1E (brown) | + | ++++ | ++++ | ++ | - |
| P2B (brown) | - | +++ | ++++ | ++ | - |
| P2E (brown) | - | ++++ | ++++ | ++ | ++++ |
| P5A (brown) | - | ++++ | ++++ | ++ | +++ |
| P6B (brown) | - | ++++ | ++++ | ++ | ++ |
| P6C (green) | - | +++ | +/- | ++++ | ++ |
| P6D (brown) | - | ++ | ++++ | ++ | ++++ |
| P6E (brown) | - | ++ | ++++ | +/- | ++ |
| P7D (green) | - | +++ | +++ | +/- | +/- |
| P8C (green) | - | ++ | +++ | + | - |
| P9A (brown) | - | ++ | +++ | +/- | +++ |
| P9B (brown) | - | ++ | ++++ | +++ | - |
| P9D (brown) | + | + | +++ | ++ | ++++ |
| **Group III (Cream colored isolates)** | | | | | |
| Rhizosphere | | | | | |
| P3D | - | ++ | + | ++++ | +++ |
| P4D | - | ++ | ++++ | + | + |
| P9C | - | + | + | + | - |
| Rhizoplane | | | | | |
| R3L-2 | - | ++ | ++++ | - | ++ |
| Rhizoplane plus Internal | | | | | |
| R72 | - | + | - | + | + |
| R78 | - | + | + | - | - |

**Table 2.** *(Continued)*

| Isolate[†] and source | HCN[‡] production | Growth inhibition[§] *Phoma betae* on: KB | PDA | *Rhizoctonia solani* on: KB | PDA |
|---|---|---|---|---|---|
| **Group III (Cream colored isolates)** *(continued)* | | | | | |
| Internal | | | | | |
| RF1 | - | + | + | ++ | - |
| RF2 | - | + | + | ++ | - |
| Seed | | | | | |
| ST12 | - | - | - | NA | NA |
| J4B8 | - | + | + | + | - |
| J7A1 | +++ | + | ++++ | + | - |
| **Group III (white colored isolates)** | | | | | |
| Rhizosphere | | | | | |
| P4A | - | ++ | ++ | ++++ | + |
| Rhizoplane | | | | | |
| R1U-1 | - | ++ | ++++ | + | ++++ |
| R1I-2 | - | ++ | ++++ | - | - |
| Rhizoplane plus internal | | | | | |
| G3 | - | + | + | ++ | - |
| Internal | | | | | |
| RF2 | - | + | + | ++ | - |
| RF3 | - | + | - | ++ | - |
| Seed | | | | | |
| J1A8 | - | ++++ | ++++ | ++ | + |
| J2B3 | - | +++ | +++ | ++++ | ++++ |
| J6B1 | - | + | +++ | ++ | ++++ |
| **Group III (yellow colored mucoid isolates)** | | | | | |
| S2A | - | - | - | - | + |
| S4A | - | - | - | - | - |
| S4B | - | - | - | - | - |
| S4C | - | - | - | - | - |
| S6A | - | - | - | - | + |
| S8A | - | - | - | - | + |
| S10B | - | - | - | - | + |
| S10C | - | - | - | - | + |
| S12B | - | - | - | - | + |
| S15B | - | - | - | - | + |
| SH2 | - | - | ++ | NA | NA |
| ST6 | - | - | - | - | + |
| J1C7 | - | - | + | - | - |
| J4B9 | - | ++ | + | - | - |
| J4C9 | - | + | - | - | - |

[†]Bacteria were isolated from seed, from the root surface, or from internal tissues of seedlings as described in Materials and Methods, and classified into three Groups (I, II and III) by their appearance.
[‡]HCN production and inhibition of growth of *Phoma betae* or *Rhizoctonia solani* AG-2 were assayed on two solid media, King's medium B (KB) and potato dextrose agar (PDA). HCN production assays were evaluated on a scale of + + + for high production to - for no production. NA = not available.
[§]Growth inhibition was measured on a scale: - no inhibition (fungus grew to and over the bacteria); + slight inhibition (fungus stopped growing 1 to 3 mm from bacteria); + + moderate inhibition (fungus stopped growing 4 to 7 mm from the edge of bacteria); and + + + strong inhibition (fungal growth stopped at least 8 mm from bacteria).

R1-U-2 and R1-I-2 from the rhizoplane strongly inhibited growth of *P. betae.* Certain of these white-colored isolates (R1-U-1, J2B3 and J6B1) also inhibited growth of *R. solani.* In addition, two Group III rhizosphere isolates, P3D and P4A, with lesser effects on *P. betae* were inhibitory to *R. solani.*

**Disease suppression.** Four of the Group II fluorescent pseudomonads (P1E, P2E, P5A, and P9D), one Group I pseudomonad (R1-I-1), and two bacteria from Group III (J2B3 and R3L2) which strongly inhibited growth *in vitro* of *P. betae* were tested for effects on *P. betae* infection in seedlings. Each bacterium increased the survival of healthy seedlings compared to the *P. betae*-treated controls. The surviving seedlings had no lesions on the hypocotyls. Little effect was observed on emergence (Table 3). Isolates R1-I-1 and J2B3 provided less protection than P1E, P2E, P5A, P9D and R3L-2.

**Table 3.** Effect of bacterial seed treatment of *Phoma betae* infested seed on emergence and disease in greenhouse-grown seedlings.

| Treatment[†] | % Emergence[‡] | % Healthy[‡] seedlings |
|---|---|---|
| *Phoma* alone | 75 a | 0 a |
| Group I | | |
| *Phoma* + R 1-I-1 | 60 b | 50 b |
| Group II | | |
| *Phoma* + P1E | 84 a | 83 d |
| *Phoma* + P2E | 82 a | 73 c |
| *Phoma* + P5A | 64 b | 87 d |
| *Phoma* + P9D | 82 a | 81 d |
| Group III | | |
| *Phoma* + R3L-2 | 75 a | 76 c |
| *Phoma* + J2B3 | 75 a | 25 b |

[†]Bacteria and *Phoma betae* were vacuum infiltrated into sugarbeet seeds before planting.

[‡]Germination and seedling health were determined after 7 and 10 days of incubation. Data are expressed as the percent germinated seeds relative to the number of seeds planted, and the percentage of those germinated seeds that grew to healthy plants. Data are the means of three trials with 50 seeds each; within columns, data followed by the same letter column are not statistically different according to Duncan's multiple range test, $P = 0.05$.

Isolates that protected against *P. betae* were also examined for efficacy against *R. solani* (Table 4). Additional isolates (J1A8, J6B1, P8E and R6) were included because they displayed strong growth inhibition of *R. solani in vitro.* The bacteria did not improve germination but increased the numbers of healthy seedlings. Protection against *R. solani* was displayed by each of the Group I, II and III isolates (Table 4). These isolates included P9D, P1E, P5A, R1-I-1, and R3L-2 used in the *P. betae* trials, and isolates J6B1 and P8E.

**Table 4.** Effect of bacterial seed treatments on disease caused by *Rhizoctonia solani.*

| Treatment[†] | Germinated seeds | Healthy seedlings | Survival |
|---|---|---|---|
| | | | % |
| Control | 33a | 32a | 96 |
| *Rhizoctonia* | 17b | 0 | 0 |
| Group I | | | |
| *Rhizoctonia* + R1-I-1 | 19b | 10b | 53 |
| *Rhizoctonia* + P8E | 19b | 16c | 84 |
| *Rhizoctonia* + R6 | 21b | 8b | 38 |
| Group II | | | |
| *Rhizoctonia* + P1E | 18b | 11b | 71 |
| *Rhizoctonia* + P5A | 21b | 16c | 76 |
| *Rhizoctonia* + P9D | 15b | 9b | 55 |
| Group III | | | |
| *Rhizoctonia* + R3L-2 | 20b | 12b | 60 |
| *Rhizoctonia* + J1A8 | 15b | 10b | 66 |
| *Rhizoctonia* + J6B1 | 20b | 18c | 90 |

[†]Seed was treated with water, as a control, or with *R. solani* or *R. solani* plus bacteria (see Materials and Methods). Data are the results after 10 days of three trials of 40 seeds each; within columns, values followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

[‡]Percent survival = $\frac{\text{healthy seedling X 100}}{\text{germinated seeds}}$

Five of the Group II isolates (PIE, P2E, P5A, P9A, P9D) were identified as *Pseudomonas tolaasii* (Table 3). Both P5A and PIE produced components that copurified through benzene extractions and TLC with an antibiotic, phenazine-1-carboxylic acid, that is also produced by *P. fluorescens* 2-79 (Thomashaw et al. 1990). The yellow-green phenazine from sugarbeet isolates P5A and PIE displayed absorbance peaks at 251 and 370 nm and mobility on TLC characteristic of phenazine-1-carboxylic acid (Brisbane et al. 1987).

Three group I isolates, R1-I-1, R6 and P8E, were identified as *P. aureofaciens*. Group III isolates R3-L-2 and J2B3 were identified as *Serratia* and *Enterobacter*, respectively, and J1A8 and J6B1 as *Bacillus subtilis*.

**Beneficial traits: HCN production.** HCN production occurred in all three groups of bacteria (Tables 1 and 2). The lowest incidence of HCN production occurred in isolates obtained from seed and the internal colonizers from 2 mo-old roots. The highest incidence of isolates that produced HCN occurred among the root-surface colonizers, especially the rhizosphere fluorescent pseudomonads, where 8 of 22 bacterial isolates produced HCN.

## DISCUSSION

Growth inhibition of the sugarbeet seedling pathogens *P. betae* and *R. solani* was detected *in vitro* with several bacterial isolates obtained from sugarbeet seed grown in Oregon and from the roots of sugarbeet plants from fields in Idaho. Certain isolates demonstrating strong *in vitro* growth inhibition of the fungal pathogens also protected sugarbeet seedlings in the greenhouse from *P. betae* and *R. solani*. Each of the isolates (PIE, P5A, P9D, and R1-I-1) used in studies of seedling protection were effective in the greenhouse in inhibiting disease caused by both pathogens. The most effective fluorescent pseudomonads (PIE, P5A and P9D) were designated by fatty acid analysis as *P. tolaasii* and R1-I-1 as *P. aureofaciens*. Previously, pseudomonads have been demonstrated to protect sugarbeet seedlings against *Pythium ultimum* (Osburn et al., 1989). Isolates R3L-2 and J2B3 which displayed protection were classified in the genera *Serratia* and *Enterobacter*, respectively. Species of *Enterobacter* and *Serratia*, as well as *P. aureofaciens* isolates, are described as root colonizers for other plant species (Kloepper et al., 1988; Scher et al. 1988; Thomashow et al., 1990). Isolates of *P. tolaasii* are pathogens on mushrooms (Wong and Preece, 1979).

HCN and fluorescent siderophore production, traits associated with growth inhibition in other systems (Kloepper et al. 1988; Voisard et al. 1989), were not essential for the growth inhibition and disease suppression of *P. betae* or *R. solani.* Certain isolates that displayed *in vitro* and *in planta* inhibition in our studies were *Serratia* and *Enterobacter* species that do not produce fluorescent pyoverdine siderophores characteristic of the pseudomonads. In addition, the growth inhibition *in vitro* of *P. betae* and *R. solani* by fluorescent pseudomonads on both PDA (iron-sufficient) and KB (iron-deficient) media suggests to us that pyoverdine siderophore production is not involved. Not all of the 27 isolates that inhibited *P. betae* or the 16 isolates that inhibited *R. solani* produced HCN. Fewer bacteria isolated from seed and internal root tissues produced HCN than the root-surface colonizers.

The yellow-pigmented bacteria detected from the seed displayed little *in vitro* growth inhibition and a high proportion of inhibitory bacteria were fluorescent pseudomonads. Whether antibiotics account for growth inhibition observed on plate medium is being investigated. Antibiotic production is proposed as a primary mechanism for fungal suppression by pseudomonads in other systems (Thomashow et al., 1990). Certain of the Group II pseudomonads produce a component that copurifies with phenazine-l-carboxylic acid from the fluorescent *P. fluorescens* isolate 2-79 (Thomashow et al. 1990). This phenazine has a broad spectrum of activity against several fungal species and is implicated in the field suppression of *Gaeumannomyces graminis* var. *triticii* by *P. fluorescens* 2-79 (Thomashow et al., 1990).

Additional studies (Zdor and Anderson 1992) with isolates P5A, R1-I-1, and P5A show that these pseudomonads differ in abilities to elicit defense responses in bean. Thus, enhanced plant defenses may be another mechanism involved in the suppression of disease in the seedlings.

Because some of the isolates with *in planta* and *in vitro* inhibitory potential were obtained from roots of field-grown sugarbeet, these isolates probably have traits that enable them to be competitive root colonizers in the field. The presence of suppressive isolates in seeds indicates that successful inhibitors also may colonize the aerial portions of field plants as the seed matures. Thus, it may be possible to manipulate, by inoculation, the bacterial flora of seed and roots of developing seedlings to increase the proportions of bacteria antagonistic to potential pathogens. From our data, we suggest that a single bacterial isolate may be effective against more than one pathogen.

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