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Biological Control of *Phytophthora* Root Rots on Alfalfa and Soybean with *Streptomyces*

Kun Xiao,* Linda L. Kinkel,* and Deborah A. Samac*,†

**Department of Plant Pathology and †USDA-ARS Plant Science Research, University of Minnesota, St. Paul, Minnesota 55108*

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A collection of 53 antibiotic-producing *Streptomyces* isolated from soils from Minnesota, Nebraska, and Washington were evaluated for their ability to inhibit plant pathogenic *Phytophthora medicaginis* and *Phytophthora sojae in vitro*. Eight isolates having the greatest pathogen-inhibitory capabilities were subsequently tested for their ability to control *Phytophthora* root rots on alfalfa and soybean in sterilized vermiculite and naturally infested field soil. The *Streptomyces* isolates tested significantly reduced root rot severity in alfalfa and soybean caused by *P. medicaginis* and *P. sojae*, respectively ($P < 0.05$). On alfalfa, isolates varied in their effect on plant disease severity, percentage dead plants, and plant biomass in the presence of the pathogen. The same eight isolates of *Streptomyces* were also tested for inhibitory activities against each other and against three strains of *Bradyrhizobium japonicum* and two strains of *Sinorhizobium meliloti* isolated from soybean and alfalfa, respectively. *Streptomyces* isolates clustered into two major compatibility groups: isolates within the same group were noninhibitory toward one another *in vitro*. The compatibility groups corresponded with groupings obtained based upon inhibition of *B. japonicum* and *S. meliloti* strains.

Key Words: **antibiosis; biological control; Glysine max; Medicago sativa.**

INTRODUCTION

Root rots of alfalfa (*Medicago sativa* L.) and soybean (*Glycine max* (L.) Merrill) caused by *Phytophthora medicaginis* Hansen & Maxwell and *Phytophthora sojae* Kauf. & Gerd., respectively, are highly destructive on these two crops throughout the world (Hansen and Maxwell, 1991; Erwin and Ribeiro, 1996). *P. medicaginis* and *P. sojae* can cause pre- and postemergence damping-off diseases. They are especially damaging when conditions are wet during the early stages of plant development and can significantly reduce both establishment and yield (Erwin and Ribeiro, 1996; Teutsch and Sulc, 1997). Between 1989 and 1991, *P. sojae* was the second-most important yield-suppressing disease in the north-central region of the United States. In 1994, an estimated 560,300 metric tons of soybean were lost to *P. sojae* in the United States, making it third among yield-suppressing diseases (Doupnik, 1993; Wrather *et al.*, 1997).

Phytophthora root rots are difficult to control effectively. A variety of methods have been studied for use in control of *Phytophthora* root rots of alfalfa and soybean. Plant resistance is currently the major control method for *Phytophthora* root rots on both crops (Erwin and Ribeiro, 1996). Unfortunately, none of the currently available resistant alfalfa cultivars is immune to the disease, especially at high pathogen inoculum densities or under wet conditions (Faris and Sabo, 1981). Under conditions that are favorable to the pathogen, even resistant cultivars can become severely infected by *P. medicaginis* (Havey and Grau, 1985; Erwin and Robeiro, 1996), indicating that other methods must be integrated with resistance to control the disease effectively. Race-specific resistance of soybean cultivars has been the most important method to control *P. sojae*. However, race-specific resistance can promote the buildup of new pathogen races and result in the failure of resistant soybean cultivars (Tooley and Bergstrom, 1984; Anderson and Buzzell, 1992; Ryley and Obst, 1992; Yang *et al.*, 1996; Abney *et al.*, 1997). Also, growers must know what races are present in their fields. This suggests that alternative or complementary methods can contribute significantly to effective and consistent disease control.

Cultural approaches, including enhancing soil drainage, reducing mechanical compaction of soil, and crop rotation, also contribute to successful disease management (Pulli and Tesar, 1975; Schmitthenner, 1985, 1988; Schmitthenner and Van Doren, 1985; Erwin and Ribeiro, 1996). However, *Phytophthora* root rot pathogens of alfalfa and soybean overwinter as oospores, which can survive in the soil for long periods of time in the absence of their hosts. Consequently, crop rotation may have only a minor effect on *Phytophthora* root rots (Schmitthenner and Van Doren, 1985).

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Fungicides, including ETMT [5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole] (Papavizas *et al.*, 1979), metalaxyl [*N*-(2,6-dimethyl phenyl)-*N*-(methylacetyl)alanine methyl ester] (Guy *et al.*, 1989; Rhodes and Myers, 1989), and pyroxyfur [2-chloro-6-(2-fruanylmethoxy)-4-(trichloromethyl)pyridine] (Diatloff *et al.*, 1983; Lazarovits, 1985) have been used as seed treatments or in-furrow applications to control *Phytophthora* root rots. Although metalaxyl, which is the major systemic fungicide used to control the *Phytophthora* root rots, is effective as a seed treatment (Apron) and an in-furrow treatment (Ridomil), it may not be cost effective for field crops (Schmitthenner and Van Doren, 1985; Schmitthenner, 1989). Also, research indicates that metalaxyl has reduced effectiveness in some cases due to tolerance developed by *Phytophthora* root rot pathogens (Davidse, 1981; Hunger *et al.*, 1982; Stack and Millar, 1985; Lamboy and Paxton, 1992; Bhat *et al.*, 1993).

In total, effective control of *Phytophthora* root rots will require integrated strategies (Schmitthenner, 1985, 1988; Schmitthenner and Van Doren, 1985; Rehm and Steienstra, 1993). One component of an integrated strategy may be biological control with antagonistic microorganisms. To date, biocontrol agents against *P. sojae* that have shown effectiveness in improving the establishment and yield of soybean include several *Actinomycetes* (Filonow and Lockwood, 1985), *Hyphochytrium catenoides* (Hsu and Lockwood, 1984), and *Bacillus cereus* UW85 (Osburn *et al.*, 1995), which also was used as a biocontrol agent against *P. medicaginis* on alfalfa (Handelsman *et al.*, 1990). In addition, research has shown that *Sinorhizobium meliloti* and *Bradyrhizobium japonicum* might play a role in protecting alfalfa and soybean roots from *P. medicaginis* and *P. sojae*, respectively (Tu, 1978, 1979, 1980). An antibiotic-producing *Streptomyces* isolate has also been used in research to control *P. medicaginis* on alfalfa (Jones and Samac, 1996). Because of their inhibitory abilities, *Streptomyces* spp. have been actively studied and utilized as biocontrol agents against various plant pathogens (Merriman *et al.*, 1974; Liu, 1992; El-Abyad *et al.*, 1993; Hiltunen *et al.*, 1995; Jones and Samac, 1996; Paulsrud, 1996; Gyenis *et al.*, 1999).

The goals of this research were to (1) evaluate the biocontrol potential of *Streptomyces* antagonists against *Phytophthora* root rots of alfalfa and soybean, (2) investigate the effect of *Streptomyces* antagonists on the growth of alfalfa seedlings in the absence of *P. medicaginis* and other microorganisms, (3) examine the potential for *Streptomyces* isolates to provide complementary and non-mutually inhibitory control of plant pathogens, and (4) determine the effect of antibiotic-producing *Streptomyces* antagonists on common symbiotic *Rhizobium* species isolated from alfalfa and soybean crops.

MATERIALS AND METHODS

In Vitro Inhibitory Potential of Streptomyces

Fifty-three antibiotic-producing *Streptomyces* isolates from Minnesota, Nebraska, and Washington were selected based on previous work (Liu, 1992; Paulsrud, 1996; L. L. Kinkel, unpublished data) and tested for their ability to inhibit the growth of *P. medicaginis* M2019 (Jones, 1994) and *P. sojae* (provided by the University of Minnesota Plant Disease Clinic). The double-layer agar method (Vidaver *et al.*, 1972) was used with modifications to evaluate inhibitory activity of each *Streptomyces* isolate toward the pathogens. Briefly, oatmeal agar medium (OA; 20 g/L oatmeal, 1 g/L casamino acid, and 15 g/L bacto-agar) was used as the bottom layer for the incubation of *Streptomyces* isolates (Liu, 1992). *Streptomyces* isolates were spotted onto each OA plate and grown for 3 days at 28°C. Plates then were overlaid with molten corn meal agar (CMA; about 15 ml/plate) after colonies of *Streptomyces* were killed by inversion of plates over 3 ml chloroform for 1 h. When the overlaid agar became firm, a 5-mm-diameter disk of the agar from the center of the top layer was removed and replaced with a 5-mm CMA disk from the actively growing margin of a 7- to 14-day-old pathogen culture. Plates then were incubated at room temperature for 7 to 14 days. The diameters of clear inhibition zones were measured 7 days after inoculation for *P. medicaginis* isolate M2019 and 14 days later for *P. sojae*.

Biocontrol Activity of Antibiotic-Producing Streptomyces Isolates in Sterilized Vermiculite

The 15 *Streptomyces* isolates having the largest average *in vitro* inhibition against *P. medicaginis* M2019 and *P. sojae* (Table 1) were selected for further study. The test tube assay described by Handelsman *et al.* (1990) was adapted as described below for evaluation and screening of these antibiotic-producing *Streptomyces* isolates against *Phytophthora* root rot pathogens on soybean and alfalfa.

Surface-sterilized (10% bleach) pregerminated alfalfa seeds (cv. Vernal) and surface-sterilized soybean seeds (cv. McCall) that had been soaked in sterile distilled water for 24 h were planted in glass test tubes (15 × 115 or 20 × 250 mm for alfalfa and soybean, respectively) filled with sterilized moistened vermiculite. Two seeds were planted into each tube for alfalfa and one seed per tube for soybean. Immediately following planting, 2 ml of spore inoculum of a single *Streptomyces* isolate (10^8 cfu/ml) was inoculated into each test tube. Inoculum for each *Streptomyces* isolate was defrosted from frozen storage (−12°C) in 20% glycerol and then suspended and diluted with sterile oatmeal broth (20 g/L oatmeal, 1 g/L casamino acid) to the

TABLE 1

Inhibitory Activity of *Streptomyces* Isolates *in Vitro* against *Phytophthora medicaginis* (Pm) M2019 and *P. sojae* (Ps)

Streptomyces isolate	Inhibition zone[a] (mm)		Average (mm)	Tukey[b] $\alpha = 0.05$
	Pm M2019	Ps		
GS-8-22	35	30	32.5	a
32	35	30	32.5	a
93	35	30	32.5	ab
GS-10-16	30	30	30	ab
15	30	30	30	ab
GS-93-96	30	30	30	ab
GS-8-1	35	20	27.5	bc
GS-8-16	35	20	27.5	bc
GS-93-23	30	20	25	cd
GS-6-17	19.5	28.5	24	de
GS-4-21	19.5	26.5	23	def
GS-43-5	18	26	22	defg
GST4-14	15	28	21.5	efg
GS-2-21	17.5	25.5	21.5	efg
PonSSII	30	10	20	fgh

[a] Inhibition zone diameter for each pathogen was averaged over two replicates.

[b] Tukey multiple-paired comparison was used. Average inhibition zones with the same letter are not significantly different.

desired concentration before inoculation. Test tubes were maintained at room temperature (24–26°C).

Two days after planting, 1 ml zoospore inoculum of the respective pathogen was added into each tube. Pathogen inoculum was prepared as described elsewhere (Handelsman *et al.*, 1990; Eye *et al.*, 1978). A completely randomized design was used for both alfalfa and soybean assays. Each treatment (*Streptomyces*–pathogen combination) was replicated in 10 test tubes with 20 alfalfa or 10 soybean seedlings. Tubes were subsequently flooded with sterile water and kept water-saturated for 7 days to encourage infection. Seedlings were incubated in a growth chamber with 12 h light, 24–25°C during the day and 20°C during the night. They were watered as needed.

Seedlings were harvested by carefully removing them from vermiculite, and disease incidence and severity were evaluated for all seedlings. Harvest was 21 days after inoculation of *P. medicaginis* M2019 for alfalfa and 18 days after inoculation of *P. sojae* for soybean. Disease severity for both alfalfa and soybean seedlings was rated using a 5-class scale: 0, healthy or no apparent discoloration; 1, less than 25% discoloration of the root; 2, 25–50% discoloration of the root; 3, 50–75% discoloration of the root; and 4, more than 75% discoloration of the root or dead plants. Average disease severity index (DSI) was evaluated as Σ[number of plants × disease index rate]/[total number of plants × 4]. Percentage disease control was estimated as [(treatment − control)/control] × 100.

Three experiments were performed evaluating biocontrol of *P. medicaginis* M2019 on alfalfa in sterilized vermiculite. In the first experiment, a total of 17 treatments (the 15 best isolates of *Streptomyces* plus the pathogen, the pathogen alone, and a no-inoculum treatment) were evaluated. Inoculum concentrations of 1×10^7 of *Streptomyces* spores/seedling and 1×10^3 of the pathogen zoospores/seedling were used in Experiment 1. The six best-performing isolates of *Streptomyces* (15, 32, PonSSII, GS-93-23, GS-6-17, and GS-8-22) were tested further (1×10^8 spores/seedling) in two subsequent experiments. Pathogen inoculum concentration varied in the experiments (1×10^3 and 5×10^2 zoospores/seedling for Experiments 2 and 3, respectively).

An additional experiment was performed to determine the effect of antibiotic-producing *Streptomyces* isolates on alfalfa seedling growth in the absence of any pathogen. Six isolates of *Streptomyces* (15, 32, PonSSII, GS-93-23, GS-6-17, and GS-8-22) were tested in this experiment. Methods used for the experiment were similar to the test tube method described by Handelsman *et al.* (1990), except that no pathogen inoculum was added to the tubes, and one dose (about 10^8 cfu/seed) spore inoculum of a single *Streptomyces* isolate was inoculated onto the vermiculite surface immediately following planting. The seedlings were maintained as described above. A completely randomized design was used for the experiment; each tube was planted with 2 seedlings, with a total of 40 seedlings for each treatment. The experiment was repeated twice. Seedlings were harvested by carefully removing them from vermiculite, and percentage emergence (E%), weight, height, and taproot length of each alfalfa seedling were evaluated 23 days after planting.

Two experiments were conducted to evaluate the potential for biocontrol of *P. sojae* on soybean in sterilized vermiculite. The 15 best isolates of *Streptomyces* (Table 1) as described above plus the pathogen, the pathogen only, and no-inoculum treatments were used in both experiments. The same concentration (2×10^8 spores/seed) of each *Streptomyces* isolate was used in both experiments, but pathogen inoculum density varied (2×10^3 and 1×10^4 zoospores/seed of *P. sojae* in Experiments 1 and 2, respectively).

Biocontrol of Phytophthora Root Rots of Alfalfa and Soybean with Streptomyces Isolates in Naturally Infested Soil under Controlled Conditions

Based on the results of *in vitro* and vermiculite assays, five isolates of *Streptomyces* were selected for further study of biocontrol of *Phytophthora* root rots of alfalfa (*Streptomyces* isolates 15, 32, PonSSII, GS93-23, GS8-22) and soybean (*Streptomyces* isolates 15, 93, PonSSII, GS2-21, GS43-5) using naturally infested field soil under controlled conditions. The soil for alfalfa experiments was obtained from an experimental field at the University of Minnesota Experiment Station, St. Paul, Minnesota. This field is a sandy loam soil

and has been used as an alfalfa disease nursery for evaluating resistance to *Phytophthora* root rot in alfalfa for over 30 years. The naturally infested soil for soybean studies was obtained from a soybean field in Minnesota having a history of *Phytophthora* infection (Dr. Jim Kurle, personal communication).

Seeds of alfalfa and soybean and spore suspensions of *Streptomyces* isolates were prepared as described above. The soil used for each experiment was mixed thoroughly and put into 8- and 10-cm-diameter pots for alfalfa and soybean, respectively. Pots were watered 1 day before sowing. A total of six treatments were evaluated for each crop (five isolates of *Streptomyces* and one no-antagonist control). Ten or 8 pots per treatment were used for alfalfa and soybean, respectively. Pots of the same treatment were put into a large tray with no drainage holes to restrict contamination among treatments and to maintain water-saturated conditions.

Alfalfa and soybean seeds were prepared for sowing as described above. Nine alfalfa or five soybean seeds were placed into each pot. One milliliter of spore suspension (10^8 cfu/ml) of *Streptomyces* was inoculated directly over each alfalfa seed, and 2 ml of spore inoculum of *Streptomyces* (10^8 cfu/ml) were inoculated over each soybean seed immediately after sowing. Controls (no antagonist) for each crop were treated with the corresponding volume of sterile oatmeal broth only. Seeds were covered with soil following inoculation of *Streptomyces*.

Alfalfa seedlings were maintained in a growth chamber for 4 weeks with 12 h light, 23°C during the day and 19°C during the night. Soybeans were kept in a growth chamber under 12 h light for 30 days with temperature at 25°C during the day and 20°C during the night. Water-saturated conditions were kept during the first 10 days for both alfalfa and soybean seedlings to encourage infection and then followed by 4 to 5 days of dry conditions for alfalfa plants to simulate drought stress in the field. All pots were subsequently watered when the soil surface was dry.

Seedlings were harvested by carefully removing them from the soil, washing gently with tap water, and blotting dry with paper towels. Disease incidence, disease severity, seedling height, fresh weight, and root length were measured for every seedling in each pot as described previously.

Evaluation of in Vitro Combination Potential among Antibiotic-Producing Streptomyces Isolates and the Effects of Streptomyces on Symbiotic Rhizobium of Alfalfa and Soybean

Compatibility among antagonistic *Streptomyces* isolates was evaluated to determine the potential for developing complementary isolate combinations. Specifically, the eight *Streptomyces* isolates used in biocontrol experiments in soil (15, 32, 93, PonSSII, GS93-23, GS43-5, GS2-21, and GS8-22) were tested *in vitro* for their compatibility with one another using the double-layer agar method as described above. After the colonies were killed with chloroform, molten OA (about 15 ml/plate) was poured onto the plates. When the agar became solid, 100 μl of stock suspension of a single *Streptomyces* isolate (10^7–10^8 cfu/ml) was spread on the surface of the plates using a sterile glass hockey stick. All plates were incubated at 28°C in the dark, and the diameter of any clear inhibition zone or growth reduction of target isolates was recorded after 7 days.

In a similar manner, the eight *Streptomyces* isolates were tested for their effect on two *Sinorhizobium meliloti* (USDA1098 and TAL380) and three *Bradyrhizobium japonicum* (USDA110, 123, and 127) strains of alfalfa and soybean, respectively, provided by Dr. M. Sadowsky, University of Minnesota. Modified yeast extract mannitol medium (Danso and Alexander, 1974) was used as the overlay medium, and each rhizobial strain was inoculated at a concentration of 10^9–10^{10} cfu/ml. Plates were incubated at 28°C in the dark, and the diameter of any clear inhibition zone or growth reduction on the plates was recorded after 5 days.

Statistical Analyses

Data were analyzed using SAS 6.12 for Windows. Analyses include one-way and two-way ANOVA, multiple-paired comparisons, and correlation analyses. Percentage data were arcsine transformed before analyses, though the original nontransformed percentage data are presented in the tables and figures.

RESULTS

In Vitro Inhibitory Potential of Streptomyces

Thirty-five of 53 (66%) *Streptomyces* isolates were able to inhibit the growth of *P. medicaginis* M2019, and 39 of 53 (74%) *Streptomyces* isolates inhibited *P. sojae in vitro*. *Streptomyces* isolates varied significantly in their ability to inhibit *P. medicaginis* M2019 and *P. sojae* (data not shown). The maximum inhibition zones were 35 mm against *P. medicaginis* M2019 and 30 mm against *P. sojae*. Overall, the ability of individual *Streptomyces* isolates to inhibit both *P. medicaginis* and *P. sojae* was significantly positively correlated (Spearman rank correlation $r = 0.65139$, $P = 0.0001$). However, there were some *Streptomyces* isolates that were effective in inhibiting one pathogen but not the other. Fifteen isolates were selected for further study based on the mean size of the inhibition zones against the two pathogens (Table 1).

Biocontrol Activity of Antibiotic-Producing Streptomyces Isolates in Sterilized Vermiculite

Alfalfa. Among the 15 *Streptomyces* isolates evaluated in sterile vermiculite, the percentage of dead

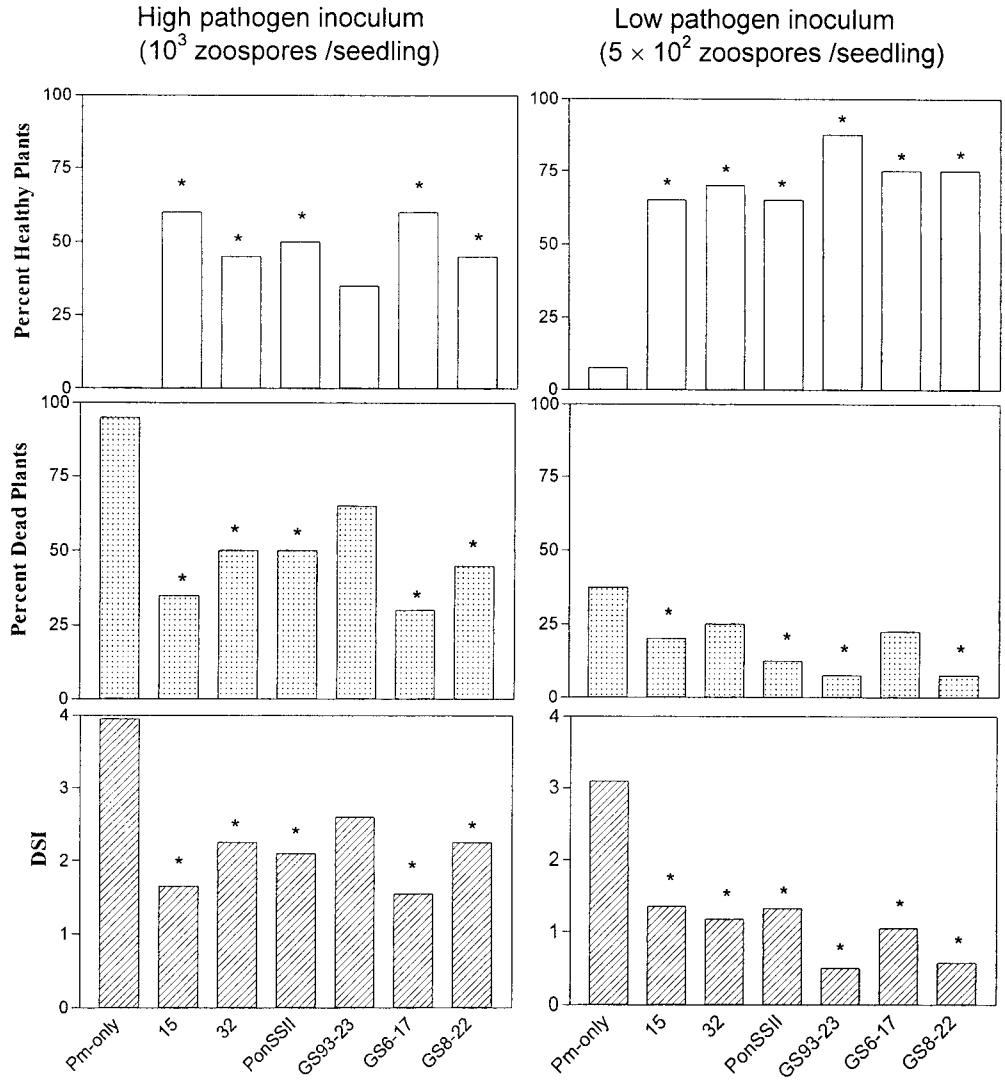

FIG. 1. Biocontrol of *P. medicaginis* M2019 with *Streptomyces* in sterilized vermiculite. Treatments labeled with "*" were significantly different from the pathogen-only treatment. Values represent the mean of $n = 20$ seedlings (LSD, $\alpha = 0.05$). *Streptomyces* isolates were inoculated (10^8 spores/seed) at sowing. The pathogen *P. medicaginis* M2019 was inoculated (10^3 and 5×10^2 zoospores/seed) 48 h after sowing. Percentage of dead seeds and seedlings, disease severity index (DSI), and percentage of healthy plants with a rating 0 or 1 were evaluated 21 days after inoculation of the pathogen.

plants and the DSI were reduced up to 35 and 26%, respectively, on inoculated plants as compared with the pathogen-only treatment (data not shown). Furthermore, *Streptomyces* treatments increased the percentage of healthy plants (disease rating 0 or 1) up to 30% compared with the pathogen-only treatment. The six *Streptomyces* isolates with the lowest percentage of dead plants (15, 32, PonSSII, GS93-23, GS6-17, and GS8-22) were tested further for their ability to reduce disease and enhance emergence of alfalfa seedlings in two subsequent experiments performed under two pathogen inoculum concentrations (10^3 and 5×10^2 zoospores/seedling). At the higher inoculum concentration, all *Streptomyces* isolates except GS93-23 significantly reduced the percentage of dead plants and the DSI and increased the percentage of healthy plants, as compared with the pathogen-only control (LSD, $\alpha = 0.05$; Fig. 1). Under the lower inoculum concentration (5×10^2 zoospores/seedling), all six *Streptomyces* isolates significantly decreased disease severity and significantly increased the percentage of healthy plants compared with the pathogen-only treatment (Fig. 1). Isolates 15, PonSSII, GS93-23, and GS8-22 also significantly reduced the percentage of dead plants. *Streptomyces* isolates 32 and GS6-17 also reduced the percentage of dead plants, though the reductions were not statistically significant (LSD, $\alpha = 0.05$).

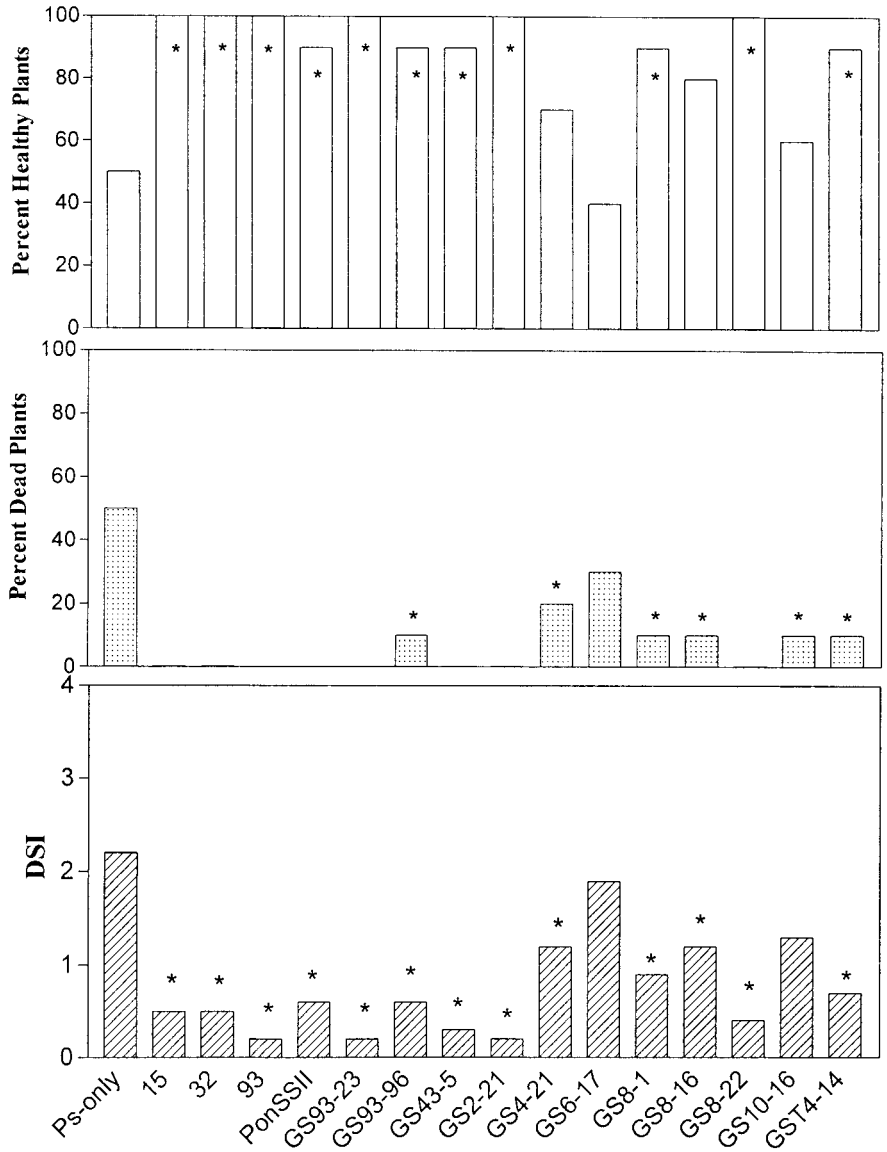

FIG. 2. Biocontrol of *P. sojae* (Ps) with *Streptomyces* in sterilized vermiculite under low pathogen inoculum (2×10^3 zoospores/seed). Treatments labeled with "*" were significantly different from the pathogen-only treatment. Values represent the mean of $n = 10$ seedlings (LSD, $\alpha = 0.05$). *Streptomyces* isolates were inoculated (10^8 cfu/seed) at sowing. The pathogen *P. sojae* (Ps) was inoculated (10^3 zoospores/seed) 48 h after sowing. Percentage of dead seeds and seedlings, disease severity index (DSI), and percentage of healthy plants with a rating of 0 or 1 were evaluated 18 days after inoculation of the pathogen.

Soybean. Among the 15 *Streptomyces* isolates evaluated, most were able to significantly reduce the percentage of dead plants and the disease severity index on soybeans inoculated with *P. sojae* as compared with the pathogen-only control (Fig. 2). Likewise, most of the 15 *Streptomyces* isolates increased the percentage of healthy plants compared with the *P. sojae* control. Some isolates (15, 32, 93, PonSSII, GS93-23, GS43-5, GS2-21, and GS8-22) reduced the percentage of dead plants to 0 under the low pathogen inoculum concentration (2×10^3 zoospores/seed) (Fig. 2).

In a subsequent experiment, zoospore inoculum of *P. sojae* was increased to 10^4 zoospores/seed. *Streptomyces* isolates 15, 93, PonSSII, GS43-5, GS2-21, and GS8-1 significantly reduced the percentage of dead plants and decreased disease severity (Fig. 3) as compared with the pathogen-only control. All treatments with *Streptomyces* isolate had a higher percentage of healthy plants than the pathogen-only control treatment (Fig. 3), though the increase was statistically significant only for PonSSII (LSD, $\alpha = 0.05$).

Overall, *Streptomyces* isolates provided better biocontrol against *P. sojae* under the low pathogen inoculum density (2×10^3 zoospore/seed) than under the high inoculum density (10^4 zoospores/seed). Spearman rank correlation analysis for the two different inocu-

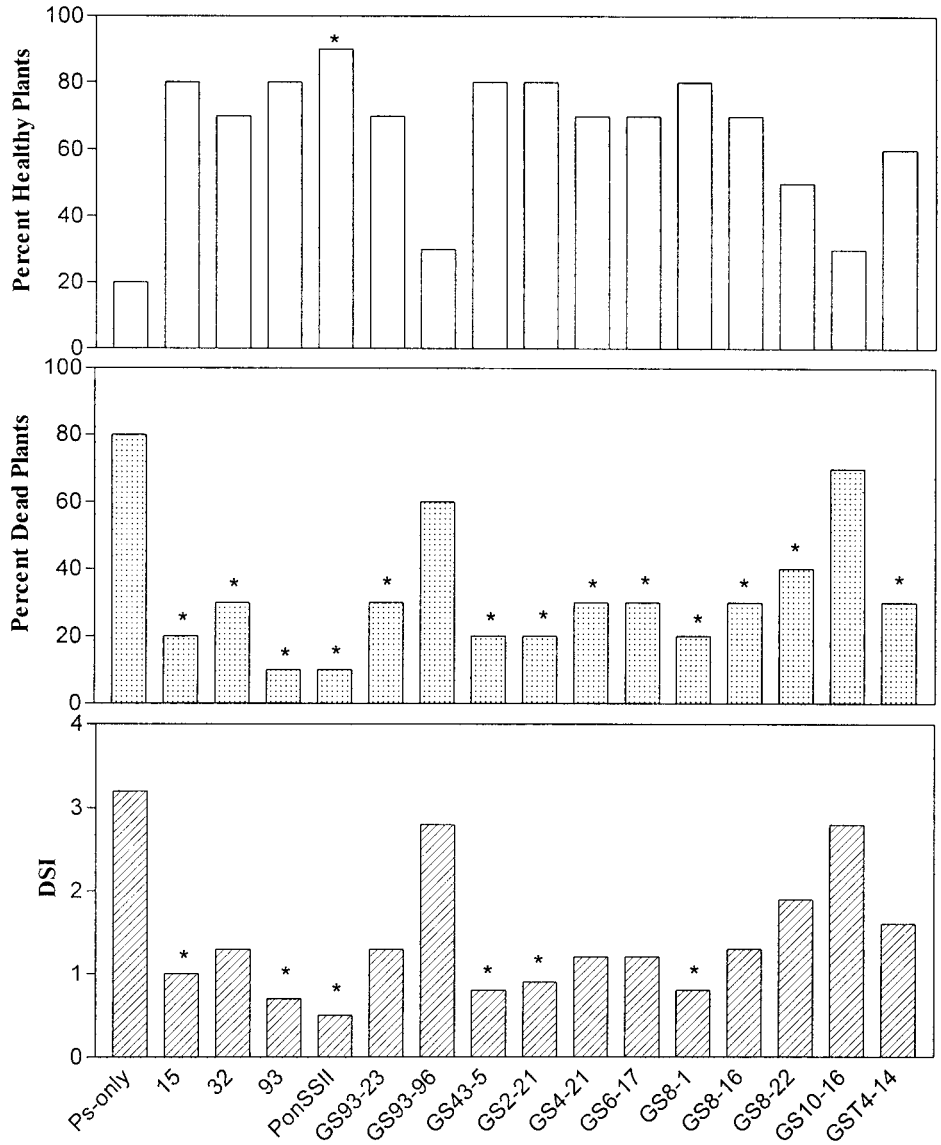

FIG. 3. Biocontrol of *P. sojae* (Ps) with *Streptomyces* in sterilized vermiculite under high pathogen inoculum (10^4 zoospores/seed). Treatments labeled with "*" were significantly different from the Ps-only control treatment. Values represent the mean of $n = 10$ seedlings (LSD, $\alpha = 0.05$). *Streptomyces* isolates were inoculated (10^8 cfu/seed) at planting. The pathogen *P. sojae* was inoculated (10^4 zoospores/seed) 48 h after planting. Percentage of dead seeds and seedlings, disease severity index (DSI), and percentage of healthy plants with a rating of 0 or 1 were evaluated 18 days after inoculation of the pathogen.

lum densities indicated that the percentage control [(treatment-control)/control × 100] provided by each *Streptomyces* isolate was only modestly positively correlated between the two inoculum densities ($r = 0.45959$, $P = 0.0848$). *Streptomyces* isolates 15, 93, PonSSII, GS2-21, and GS43-5 provided the best control of *P. sojae* in these two experiments.

Biocontrol of Phytophthora Root Rots of Alfalfa and Soybean with Streptomyces Isolates in Naturally infested Soil

Alfalfa. Streptomyces isolates 15, 32, PonSSII, GS93-23, and GS8-22 had no effect on the emergence of alfalfa seedlings compared with the control treatment in naturally infested soil in the growth chamber (Table 2). *Streptomyces* isolate GS93-23 significantly decreased the percentage of dead plants and the DSI and increased the percentage of healthy plants and yield (forage weight per pot) of alfalfa seedlings in Experiment 1 (Table 2) as compared with the noninoculated control. In the same experiment, *Streptomyces* isolates 15 and PonSSII significantly decreased disease severity, and isolate PonSSII significantly increased the mean plant fresh weight over that observed in the noninoculated control. *Streptomyces* isolates GS8-22 and 32 also reduced the percentage of dead plants and

TABLE 2

Biocontrol of *P. medicaginis* (Pm) on Alfalfa with *Streptomyces* Isolates from Naturally Infested Soil

	Mean[f]				
Treatment[a]	E%[b]	D%[c]	DSI	H%[d]	Weight[e] (g/pot)
Pm alone	87.79 ab	41.1 a	3.25 a	9.99 bc	1.47 c
GS-8-22	83.35 b	36.66 a	2.94 ab	7.77 c	1.59 c
32	95.56 a	37.75 a	2.96 a	9.99 bc	1.56 c
PonSSII	87.79 ab	31.1 a	2.52 bc	24.44 b	2.15 b
15	92.23 ab	27.76 ab	2.45 c	23.31 bc	1.83 bc
GS-93-23	86.68 ab	16.65 b	0.71 d	82.24 a	4.87 a

[a] 1ml 10^8 spores/seed inoculum of a single *Streptomyces* isolate was inoculated at sowing.
[b] Alfalfa seedling emergence (%).
[c] Percentage of seeds or seedlings that were dead.
[d] Percentage of healthy alfalfa plants (disease rating levels 0 and 1).
[e] Average weight/pot of above-ground alfalfa biomass (fresh weight).
[f] Means within a column with the same letter were not significantly different (LSD, $\alpha = 0.05$).

disease severity, though the differences were not statistically significant compared to the control. Similar results were obtained from a repeat of the experiment, though the disease pressure was lower in the second experiment (D% = 21.1%; DSI = 1.98) than in the first experiment (D% = 41.1%; DSI = 3.25).

Soybean. Five *Streptomyces* isolates (15, 93, PonSSII, GS2-21, and GS43-5) were tested for their ability to control *P. sojae* on soybean in field soil. The soil was from a field with a history of *Phytophthora* root rot and a susceptible variety (McCall) was planted under disease-conducive conditions. However, no disease was observed in any treatment, suggesting that pathogen inoculum was insufficient to cause disease.

Evaluation of the Effect of Antibiotic-Producing Streptomyces Isolates on Alfalfa Seedlings

Streptomyces isolates 32, PonSSII, and GS93-23 significantly increased the mean weight, height, and taproot length of alfalfa seedlings grown in sterilized vermiculite as compared with the noninoculated control (Table 3). Isolate 15 significantly increased the mean weight per plant and taproot length, while isolate GS8-22 significantly increased only mean weight per plant. Among the six *Streptomyces* isolates tested here, only GS6-17 did not provide any plant growth benefit as compared with the control. Similar results were obtained in a repeat of this experiment (data not shown).

In vitro inhibition zones against *P. medicaginis* or *P. sojae* were not significantly correlated with the biocontrol activity of antagonists against the pathogens in soil (e.g., Pearson correlations of inhibition zone size and percentage dead plants are $r = -0.7757$, $P = 0.1231$ for *P. medicaginis* and $r = -0.3487$, $P = 0.5653$ for *P. sojae*). However, *in vitro* weights per plant of alfalfa seedlings inoculated with *Streptomyces* in the absence of *P. medicaginis* were significant positively correlated with the forage weight per pot of alfalfa grown in pathogen-infested soil ($r = 0.9779$, $P = 0.0039$). Thus, enhancement of alfalfa growth by *Streptomyces* may be one of the mechanisms by which *Streptomyces* antagonists enhance plant health in field soil.

Evaluation of Combination Potential among Antibiotic-Producing Streptomyces Isolates and the Effects of Streptomyces on Symbiotic Rhizobium of Alfalfa and Soybean

Among eight *Streptomyces* isolates tested for their *in vitro* compatibility with one another (Table 4), only isolate GS93-23 was not compatible with any of the other seven *Streptomyces* isolates. Isolate GS93-23 produced inhibition zones averaging 25.8 mm against the other seven *Streptomyces* and was inhibited by all of the other isolates (average inhibition of 11.8 mm).

The seven remaining *Streptomyces* isolates can be divided into two compatibility groups. Isolates 15, GS2-21, and GS43-5 belong to one group, and isolates 32, 93, PonSSII, and GS8-22 belong to the other group. Isolates within each group are compatible with one another, while isolates belonging to different groups are incompatible.

Streptomyces isolates 93, GS93-23, 32, PonSSII, and GS8-22 were compatible with the *S. meliloti* and *B. japonicum* strains tested (Table 5), producing no *in vitro* inhibition. *Streptomyces* GS43-5 was least compatible with *Rhizobium*, as it inhibited the growth of

TABLE 3

Effect of *Streptomyces* Isolates on Alfalfa Seedling Growth in the Absence of Pathogens

	Means[b]		
Treatment[a]	Root length (cm)	Height (cm)	Weight (mg/plant)
GS93-23	13.76 ab	2.06 a	13.74 a
15	15.06 a	1.99 ab	12.66 ab
PonSSII	14.82 ab	2.06 a	11.93 b
32	14.55 ab	2.08 a	12.36 ab
GS8-22	13.65 bc	2.01 ab	11.51 b
GS6-17	11.62 d	1.81 b	11.2 bc
No *Streptomyces*	12.38 cd	1.81 b	9.66 c

[a] 10^8 cfu/seedling of *Streptomyces* was inoculated at sowing.
[b] Means of the taproot length, height, and weight per plant were measured 23 days after planting and averaged among 40 alfalfa seedlings. Means with the same letter are not significantly different (LSD, $\alpha = 0.05$).

TABLE 4

In Vitro Compatibility among Eight *Streptomyces* Isolates

	Inhibition zones (mm) lawn isolates							
Test isolate	15	93	GS93-23	GS2-21	32	PonSSII	GS43-5	GS8-22
15	—[a]	15	6	—	11.5	6.5	—	15
93	+[b]	—	6.5	—	—	—	+	—
GS93-23	23.5[c]	29	—	24.5	27.5	26.5	21	28.5
GS2-21	—	19	13.5	—	17	18.5	—	18.5
32	—	—	16	—	—	—	+	—
PonSSII	—	—	19.5	—	—	—	+	—
GS43-5	—	15	6.5	—	16	17	—	14
GS8-22	—	—	14.5	—	—	—	+	—

[a] No growth inhibition of lawn isolate.
[b] No clear zones, but with growth reduction of the lawn.
[c] Clear inhibition zones were measured after 7 days of incubation of the lawn, and values represent the mean of two replicates.

both *S. meliloti* strains and two *B. japonicum* strains. Isolates 15 and GS2-21 also inhibited four of five rhizobial strains, though the inhibition zone sizes were somewhat smaller than those for GS43-5. All of the eight tested *Streptomyces* isolates were compatible with *B. japonicum* USDA110, which is one of the most common strains of *B. japonicum* symbiotic on soybean.

The two compatibility groups of *Streptomyces* isolates described above were consistent with groupings obtained with rhizobia (Table 5). Specifically, isolates 15, GS2-21, and GS43-5 had similar antagonistic reactions with the rhizobial strains, while isolates 32, 93, PonSSII, and GS8-22 were not antagonistic to the symbionts.

DISCUSSION

Streptomyces isolates effectively reduced *Phytophthora* root rot on alfalfa, especially postemergence damping-off, and increased the proportions of both healthy plants and forage yield. GS 93-23 was the most effective *Streptomyces* isolate in biocontrol of *P. medicaginis* on alfalfa in naturally infested soil. In addition, this isolate produced larger average inhibition zones than other *Streptomyces* isolates against *P. medicaginis in vitro* and showed the ability to inhibit *in vitro* growth of nine additional alfalfa and soybean pathogen isolates, including *Aphanomyces euteiches, Pythium ultimum, Phoma medicaginis,* and *P. sojae* (data not shown). Furthermore, GS 93-23 was compatible with all rhizobial strains tested from alfalfa. In previous research, isolate GS 93-23 was one of the most effective antagonists in controlling potato scab in the field (Paulsrud, 1996). These data suggest that, in addition to its potential for controlling root rot on alfalfa, *Streptomyces* isolate GS 93-23 may be useful in integrated control against diverse soilborne plant pathogens.

The test tube method described by Handelsman *et al.* (1990) was adapted in this research and provided a fast, simple, and efficient procedure to screen *Strepto-*

TABLE 5

In Vitro Inhibition of *S. meliloti* and *B. japonicum* by *Streptomyces* Isolates

	Sinorhizobium meliloti		*Bradyrhizobium japonicum*		
Streptomyces isolate	USDA1098	TAL380	USDA123	USDA127	USDA110
15	+	11	+[b]	21[c]	—[a]
93	—	—	—	—	—
GS93-23	—	—	—	—	—
GS2-21	11.3	18	+	30.7	—
32	—	—	—	—	—
PonSSII	—	—	—	—	—
GS43-5	16	20	15	30.3	—
GS8-22	—	—	—	—	—

[a] No growth inhibition to the rhizobial strains.
[b] No clear zones, but with growth reduction of the rhizobial strains.
[c] Clear inhibition zones were measured after 5 days of incubation of rhizobial strains, and values were averaged over three replicates.

myces isolates for control of seedling diseases of alfalfa and soybean. Both antagonist and pathogen inoculum densities had a substantial effect on disease control. Generally, disease control was better at higher antagonist inoculum densities, though there were limits to the effectiveness of increasing *Streptomyces* densities (data not shown). Determining optimal antagonist inoculum densities will require further comparative data on the costs and benefits of particular antagonist inoculum doses in the presence of varying pathogen densities.

Although *Streptomyces* isolates selected based on *in vitro* antibiotic activity were effective in controlling *Phytophthora* root rots in sterilized vermiculite and infested field soil, the size of *in vitro* inhibition zones for individual *Streptomyces* isolates against specific pathogens was not significantly correlated with successful biocontrol of those pathogens. This result is consistent with the findings of others (e.g., Jones, 1994; Paulsrud, 1996; Schottel *et al.*, 2001), suggesting that *in vitro* antibiotic assays provide at best an imperfect means for identifying antagonists. As an alternative screening approach, we note the significant positive correlation between alfalfa seedling weights when inoculated with antagonists in sterile vermiculite in the absence of the pathogen and those when inoculated with antagonists in pathogen-infested field soil (r = 0.97794; P = 0.0039). Overall, the strongest predictor of alfalfa plant weight when inoculated with an antagonist and grown in pathogen-infested field soil was alfalfa biomass following inoculation of the same antagonist in sterile vermiculite. In this case, direct enhancement of alfalfa growth by *Streptomyces* may be one of the mechanisms by which *Streptomyces* antagonists enhance plant health in field soil. Thus, antagonist screening approaches that consider plant growth in the absence of any pathogens may provide an alternative or a complement to more traditional antibiotic screening assays. Further research is underway to determine the mechanisms of growth enhancement of alfalfa by *Streptomyces* antagonists.

Although *Streptomyces* isolate 15 was found to be incompatible with common isolates of *S. meliloti* both in previous research (Jones, 1994) and here, nodules were consistently observed on alfalfa roots in field soils inoculated with isolate 15. This implies that *in vitro* incompatibility between *S. meliloti* and *Streptomyces* antagonists may not be predictive of microbial interactions in the rhizosphere or that isolate 15 may be compatible with indigenous *S. meliloti* strains in field soil. Field trials are needed to provide more insight into the significance of *in vitro* inhibition of *S. meliloti* to *Streptomyces–S. meliloti* interactions in the rhizosphere.

In addition to the two *Phytophthora* root rot pathogens reported here, the 53 *Streptomyces* isolates were also evaluated for their *in vitro* inhibitory potential against seven other plant pathogens of alfalfa and soybean (data not shown). Plant pathogens varied significantly in their sensitivity to inhibition by different *Streptomyces* isolates. This suggests that a single *Streptomyces* isolate will be unlikely to provide broad-spectrum control of diverse soilborne plant pathogens. Furthermore, many factors are likely to affect biological control of root diseases, including environmental conditions such as soil temperature, morphology, water status, and nutrient availability, and pathogen population densities. Indigenous soil microbes can also interact with inoculated antagonists, thereby influencing their activities in soil. Consequently, it seems unlikely that a single antagonist will be capable of providing consistent and long-lasting control in different field locations under varying environmental conditions. Though the results presented here are promising, we note that this work studied single antagonist biological control on only one cultivar for each of the two plant hosts in field soil. Further work will consider multiple plant cultivars varying in their *Phytophthora* resistance and planted in multiple field soils varying in pathogen population densities and environmental conditions. Our long-term goal is the development of an integrated management scheme for soilborne plant pathogens that incorporates combinations of complementary *Streptomyces* isolates, resistant plant cultivars, and cultural practices including tillage and rotational schemes that integrate green manures optimal for the management of antagonist populations in soil.

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