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Characterization of *Streptomyces lydicus* WYEC108 as a Potential Biocontrol Agent against Fungal Root and Seed Rots†

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The actinomycete *Streptomyces lydicus* WYEC108 showed strong in vitro antagonism against various fungal plant pathogens in plate assays by producing extracellular antifungal metabolites. When *Pythium ultimum* or *Rhizoctonia solani* was grown in liquid medium with *S. lydicus* WYEC108, inhibition of growth of the fungi was observed. When WYEC108 spores or mycelia were used to coat pea seeds, the seeds were protected from invasion by *P. ultimum* in an oospore-enriched soil. While 100% of uncoated control seeds were infected by *P. ultimum* within 48 h after planting, less than 40% of coated seeds were infected. When the coated seeds were planted in soil 24 h prior to introduction of the pathogen, 96 h later, less than 30% of the germinating seeds were infected. Plant growth chamber studies were also carried out to test for plant growth effects and for suppression by *S. lydicus* WYEC108 of *Pythium* seed rot and root rot. When WYEC108 was applied as a spore-peat moss-sand formulation (10^8 CFU/g) to *P. ultimum*-infested sterile or nonsterile soil planted with pea and cotton seeds, significant increases in average plant stand, plant length, and plant weight were observed in both cases compared with untreated control plants grown in similar soils. WYEC108 hyphae colonized and were able to migrate downward with the root as it elongated. Over a period of 30 days, the population of WYEC108 colonized emerging roots of germinating seeds and remained stable (10^5 CFU/g) in the rhizosphere, whereas the nonrhizosphere population of WYEC108 declined at least 100-fold (from 10^5 to 10^3 or fewer CFU/g). The stability of the WYEC108 population incubated at 25°C in the formulation, in sterile soil, and in nonsterile soil was also evaluated. In all three environments, the population of WYEC108 maintained its size for 90 days or more. When pea, cotton, and sweet corn seeds were placed into sterile and nonsterile soils containing 10^6 or more CFU of WYEC108 per g, it colonized the emerging roots. After a 1-week growing period, WYEC108 populations of 10^5 CFU/g (wet weight) of root were found on pea roots in the amended sterile soil environment versus 10^4 CFU/g in amended nonsterile soil. To further study the in vitro interaction between the streptomycete and *P. ultimum*, mycelia of WYEC108 were mixed with oospores of *P. ultimum* in agar, which was then used as a film to coat slide coverslips. After 6 h of incubation of these preparations in staining jars at 25°C, direct interactions between the microorganisms were visualized by scanning electron microscopy. Results showed that WYEC108 was capable not only of destroying germinating oospores of *P. ultimum* but also of damaging the cell walls of the fungal hyphae. These results show that *S. lydicus* WYEC108 is potentially a potent biocontrol agent for use in controlling *Pythium* seed and root rot.

Ideally, an agent of biological control of fungal root pathogens should exert a sufficient amount of antagonistic activity in the rhizosphere to significantly reduce root disease symptoms (9, 37). Attempts have been made to develop *Streptomyces* species as fungal root disease control agents, since *Streptomyces* spp. are capable of producing a remarkably wide spectrum of antibiotics as secondary metabolites (14, 18, 19), as well as a variety of fungal cell wall-degrading enzymes, such as cellulases, hemicellulases, chitinases, amylases, glucanases, etc. (5, 15; unpublished data). An extensive study using antagonistic *Streptomyces* culture filtrates to control foliage diseases was carried out by Pridham et al. (28, 29) in the 1950s. Results showed that 9 of the 10 products tested suppressed at least one disease in a greenhouse study. Reddi and Rao (30) reported that isolates of *Streptomyces ambofaciens* were able to control *Pythium* damping-off in tomato plants and *Fusarium* wilt in cotton plants in an artificially infested soil. Similar root disease suppression has been achieved by others by applying the streptomycetes in the form of spores, mycelia, or combinations of the two in growth chambers and greenhouses (1a, 3, 4, 6, 12, 13, 21, 34–36). Notably, Rothrock and Gottlieb (31) reported direct evidence which showed that the control of *Rhizoctonia* root rot in pea plants by *S. hygroscopicus* var. *geldanus* in an artificially infested sterile soil depended upon the in situ concentration of geldanamycin (≅20 μg/g of soil), an antibiotic produced by this strain in the soil. Although many studies have indicated that streptomycetes are quantitatively and qualitatively important in the rhizosphere (22, 23), where they may act as plant growth stimulants, as well as root colonizers, Milus and Rothrock (24) are among the very few groups who have monitored the fate of specific *Streptomyces* strains in the rhizosphere over a relatively long period of time (1 year). However, their *Streptomyces* strain had the smallest population size (10^2 to 10^5 CFU/g) among the bacterial strains tested in a wheat rhizosphere under the conditions tested.

Streptomyces species have been studied extensively for their antifungal antagonism, generally with different plate assays (3, 12, 15, 25, 28–30, 38). Surprisingly, little effort has been devoted to monitoring the populations of potential biocontrol *Streptomyces* species in soil or the rhizosphere. No studies have determined their distribution along the rhizoplane or their interactions with specific fungal pathogens. Most studies have, instead, emphasized fungal and bacterial agents such as *Glio-*

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TABLE 1. In vitro antagonism of *S. lydicus* WYEC108

Pathogen	Source	Medium	Antagonism[a] at:		Viability[b] at day 5
			Day 2	Day 5	
Pythium ultimum Wheat 1	W. Chun[c]	PDA	+++	+++	−
Pythium ultimum P8	J. Kraft[d]	PDA	+++	+++	−
Pythium ultimum P9	J. Kraft	PDA	+++	+++	−
Aphanomyces euteiches A15	J. Kraft	CMA[e]	+++	+++	−
Aphanomyces euteiches Bob-F1	J. Kraft	CMA	+	+++	−
Aphanomyces euteiches A6	J. Kraft	CMA	++	+++	−
Aphanomyces euteiches Aph4	Laboratory stock	CMA	+	+++	−
Fusarium oxysporum	Laboratory stock	PDA	−	++	+
Fusarium solani f. sp. pisi	G. Knudsen[c]	PDA	−	+	+
Fusarium solani f. sp. pisi F6	J. Kraft	PDA	−	++	+
Fusarium solani f. sp. pisi F46	J. Kraft	PDA	+	++	+
Fusarium solani f. sp. pisi F9	J. Kraft	PDA	−	+	+
Rhizoctonia solani	Laboratory stock	PDA	+	++	+
Rhizoctonia solani R4	J. Kraft	V-8A[f]	+	++	+
Rhizoctonia solani W 1	Laboratory stock	V-8A	+	++	+
Rhizoctonia solani X5FS	Laboratory stock	V-8A	+	++	+
Phymatotrichum omnivorum	S. Lyda[d]	PDA	+	++	+

[a] Ratings: +++, $\Delta\gamma > 2.0$ cm; ++, 2.0 cm $> \Delta\gamma > 1$ cm; +, 1 cm $> \Delta\gamma > 0.5$ cm; −, $\Delta\gamma < 0.5$ cm. The results shown are averages of five replications of each pathogen.

[b] Viability was evaluated as recovery of fungal mycelial plugs on fresh PDA or CMA plates (−, no growth; +, growth).

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[d] Irrigated Agricultural Research and Extension Center, Prosser, Wash., and Plant Pathology Department, Texas A&M University.

[e] CMA, half-strength corn meal agar.

[f] V-8A, V-8 juice agar (250 ml of V-8 tomato juice–2.5 g of $CaCO_3$ centrifuged at 13,200 × *g* for 15 min; the supernatant was used to move 1 liter of V-8A medium).

cladium virens, *Trichoderma* spp., and fluorescent *Pseudomonas* species. Previously, Crawford et al. (12) tested the in vitro antifungal antagonism of 83 actinomycetes isolated from four rhizosphere and four nonrhizosphere soils. One *Streptomyces* strain, WYEC108, later shown to be a strain of *Streptomyces lydicus* (34), was among the several strains that demonstrated strong activity against *Pythium ultimum* in plate assays. *Pythium* species are major soil-borne pathogens that cause seed rot and pre- and postemergence damping-off of a broad range of host plants.

An objective of this study was to further characterize the antifungal activities of *S. lydicus* WYEC108 against fungal root pathogens in vitro. We also monitored the stability of WYEC 108 spore populations in a peat moss-sand formulation and in sterile and nonsterile soils, and we observed the colonization of germinating roots by WYEC108. To evaluate the biocontrol ability of *S. lydicus* WYEC108 against *Pythium* plant diseases, we used a spore-peat moss-sand formulation in growth chamber studies. Lastly, we examined the interactions between WYEC108 and *Pythium* oospores, one of the primary *Pythium* survival structures in soil, by using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Media and cultures. *S. lydicus* WYEC108 cultures (12) were stored as 1-ml spore suspensions in 10% glycerol frozen in 1.5-ml microcentrifuge tubes at −70°C. Working cultures were obtained by streaking a loopful of spore stock onto sporulation agar (SPA) plates (1) or suspending the spores in Casamino Acids-yeast extract-glucose broth (12). SPA plates were incubated at 30°C for 3 to 4 weeks, until the confluent growth was well sporulated (colonies turned gray), and then stored at 4°C until used. For production of mycelia, Casamino Acids-yeast extract-glucose broth was inoculated with spore suspensions and incubated with shaking (30°C and 200 rpm) for 48 h, by which time the culture had entered the logarithmic growth phase.

Isolates of *P. ultimum* designated P8 and P9 were used throughout this study. These virulent strains were kindly provided by J. Kraft (Irrigated Agriculture Research and Extension Center, Prosser, Wash.) The cultures were maintained on potato dextrose agar (PDA; Difco) slants at 4°C. Mycelial agar plugs were transferred to PDA plates and incubated at 25°C for 48 h to prepare an oospore inoculum.

In vitro antagonism test. Many of the pathogenic fungi used in this study were provided by plant pathologists at the University of Idaho and Washington State University. The names and sources of the fungal strains are listed in Table 1. A modified procedure of Crawford et al. (12) was used to test the in vitro antagonism of WYEC108 against these plant pathogens. Briefly, strains of *P. ultimum*, *Aphanomyces euteiches*, *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, and *Phymatotrichum omnivorum* were grown on the applicable agar medium (Table 1) for 48 h before the test. A loopful of WYEC108 spores was streaked onto one side of each PDA plate (pH 7.0). The plates were incubated at 30°C for 72 h, at which time the colonies had just become visible. Then, a 0.45-cm-diameter agar plug of fungal mycelium was transferred onto the center of the other side of each plate. Fungal plugs were also placed on uninoculated PDA plates separately as uninhibited controls. Cultures were incubated at 30°C, and the plates were examined for inhibition of growth after 2 and 5 days. The level of inhibition was defined as the subtraction of the fungal growth radius (γ_0 [in centimeters]) of a control culture from the distance of the growth in the direction of WYEC108 (γ [in centimeters]), where $\Delta\gamma = \gamma_0 - \gamma$. After 5 days of paired incubation, mycelial plugs from the fungal colony edge in the vicinity of WYEC108 colonies were transferred to fresh medium to check for the viability of the mycelia in that area.

Fungal growth inhibition in liquid medium. A modified procedure of Nelson et al. (26) was used to examine the inhibition of fungal growth in liquid medium by *S. lydicus* WYEC108. Briefly, agar disks (diameter, 0.55 cm) of *P. ultimum* P8 and P9 and *R. solani* X5FS were each placed in 15 ml of sugar-Casamino Acids-mineral salts broth in petri dishes. Three sugars were tested in this study, glucose, L-sorbose, and sucrose (all at 20 mM). The plates were incubated at 25°C for 48 h, and 0.5 ml of a WYEC108 mycelium suspension (10^5 CFU/ml) in 0.25 mM phosphate buffer (pH 7.2) was added to the fungal cultures. The paired cultures were incubated at 25°C for 48 h. The cultures were then vacuum filtered through preweighed Whatman no. 2 filter paper. The retained mycelia and the filter papers were dried overnight in an oven at 60°C and reweighed.

Formulation of *S. lydicus* WYEC108. A spore suspension of strain WYEC108 in a peat moss-sand formulation (the WYEC108 formulation [unpublished procedure]) was used in the growth chamber studies. Briefly, spores from 25 to 30 heavily sporulated SPA plates were scraped off of the surfaces with a sterile spatula and added to 90 g of sterile sand. This sand was mixed well into a sterile sand-peat moss carrier (1,600 g-310 g), and the formulation was stored at room temperature. Three 1-g samples from the formulation were taken immediately after spores were mixed into the carrier and diluted into a 9-ml sterile distilled water (SDW) blank. Further serial dilutions were made and plated onto SPA plates to determine the initial density of the viable spores in the formulation. For monitoring of the shelf life of the formulation, samples were taken periodically and subjected to the same plate count procedure.

Stability of WYEC108 in soil. Soil from the University of Idaho Agricultural Experimental Station, Parma, was used for the stability study and growth chamber assays. The soil, a silt loam, had a pH of 7.4, an organic matter content of 1.3%, and the following inorganic matter characteristics: nitrate, 23 ppm; sulfate, 18 ppm; phosphorus, 17 ppm; calcium, 3,666 ppm; sodium, 378 ppm; potassium,

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532 ppm; iron, 13.3 ppm (data from John P. Taberna, Soil Scientist, Western Laboratories, Parma, Idaho). The soil was sieved through a 2-mm sieve, air dried, and kept at room temperature until used. Sterile soil was obtained by steam autoclaving the air-dried soil at 121°C three consecutive times over 3 days. A formulation of WYEC108 (10^7 CFU/g) that had been stored at room temperature was mixed with sterile or nonsterile soil at a 1:10 (wt/wt) ratio. The mixtures were then incubated in a growth chamber (CMP-3244; Conviron, Pembina, N. Dak.) at 30°C for 16 h and 20°C for 8 h with a 16-h photoperiod. This temperature range simulated the daily temperature fluctuations observed in Idaho during the summer of 1994. At least three random 1-g samples were taken periodically from both the sterile and nonsterile soil mixtures. Serial dilutions were made, and the proper dilutions were plated onto both SPA and antibiotic-amended SPA (A-SPA; containing carbenicillin and cycloheximide, each at 100 μg/ml) (Sigma). Colonies from a pure culture of WYEC108 grown on SPA were used as a positive colony morphological control to help to ascertain the identity of the WYEC108 colonies retrieved from the soil. WYEC108 colonies on both SPA and A-SPA plates were distinctive actinomycete colonies whose aerial mycelia turned gray-green when they sporulated. They were counted after 5 days of incubation at 30°C. The efficiency of A-SPA in the recovery of WYEC108 from the native soil was also evaluated.

Root colonization of WYEC108. Untreated cotton, pea (Puget), and sweet corn (variety 8701; Abbott & Cobb, Inc., Feasterville, Pa.) seeds and seeds treated with fungicides (Captan, Thiram, Imazaul, and Metalaxyl) were planted in Conetainers (Stuewei & Sons Inc., Corvallis, Oreg.) containing either WYEC108-amended sterile or nonsterile soil (as described above). After planting, the tubes were saturated with SDW and incubated in the same growth chamber. To determine if WYEC108 had quickly colonized the plant rhizospheres, five plants of each type were removed from the Conetainers after 7 days of growth. Root samples were shaken to remove loosely retained soil particles, and the weight of each root sample was measured. The roots were then placed in test tubes with 5 ml of SDW and shaken vigorously for 15 s. Dilution platings of these suspensions were then done on SPA amended with Benomyl (50 μg/ml). The population of WYEC108 that colonized the root was defined as CFU per gram (wet weight) of the root.

***P. ultimum* oospore production.** *P. ultimum* P8 was grown on PDA plates at 25°C for 48 h. Four-millimeter-diameter mycelial agar plugs were then transferred onto petri plates containing 15 ml of V8-cholesterol (Sigma) liquid medium (2). A procedure of Ayers and Lumsden (2) for oospore harvesting was used in this study with only minor modifications. The homogenized mycelial and oospore mixture was filtered through two layers of cheesecloth. Samples were taken to monitor the purity of the oospores and to measure their density with a hemocytometer. The filtrate was centrifuged at 5,000 × *g* (JS-7.5 rotor, Beckman J2-MC centrifuge) for 10 min. The supernatant was discarded, and the pellet was resuspended in 50 ml of 0.25 mM phosphate buffer to obtain an approximate concentration of 10^5 oospores per ml. Oospore suspensions produced by this method at different times were used for infection delay tests, growth chamber assays, and SEM examinations.

Infection delay test. *P. ultimum* P8 oospore-enriched soil was obtained by spraying 400 ml of an oospore suspension (10^3 oospores per ml) into 8 kg of sterile soil in a plastic container (8). The soil was then incubated at 28°C for 2 days before planting. Pea seeds (Puget) were surface disinfected in a 10% NaOCl–10% ethanol solution for 1.5 min. After being rinsed thoroughly with SDW twice, the seeds were mixed thoroughly with either a WYEC108 mycelium (10^6 CFU/g) or spore (10^9 CFU/g) suspension in 0.25 mM phosphate buffer, pH 7.0, in a glass beaker and then incubated for 20 min. Ten treated seeds were buried in a glass petri dish (9-cm diameter) containing 45 g of oospore-enriched sterile soil (50 propagules per g). Ten milliliters of SDW was added to each plate. The average height of soil in the petri dishes was 1 cm; thus, the oospore density in the dishes was about 35 propagules per cm^3. Seeds from three plates of each treatment (total, 30 seeds per treatment per sampling time) were removed from the plates at 6, 12, 24, 48, 72, and 96 h and surface disinfected with 10% NaOCl–10% ethanol solution for 1 min. The disinfected seeds were placed on water-agar plates to check for invasion of *P. ultimum*. Alternatively, coated seeds were planted in sterile soil for 24 h and subsequently transferred into the petri dishes containing oospore-enriched soil. The infection process was then examined periodically as described above.

Growth chamber assay. Growth chamber experiments were used to examine the ability of WYEC108 to control *Pythium* damping-off in vivo under conditions optimal for *Pythium* growth. Conetainers were used in this study to grow plants in the growth chamber. The tubes were half filled with either sterile or nonsterile soil and then saturated with water. One milliliter of a *Pythium* oospore suspension (500 or 100 oospores per ml) was dripped onto the surface of the soil. A thin layer (0.5 cm) of a WYEC108 formulation or a peat moss-sand carrier without WYEC108 (control) was then placed on top of the soil. Pea and cotton seeds were surface disinfected by soaking in 10% NaOCl–10% ethanol solution for 1.5 min and then rinsed with SDW three times. Disinfected seeds were planted on the top of the formulation or carrier and covered with another 2 cm of soil. Finally, the tubes were saturated with SDW again. The tubes were placed in racks in a completely randomized block design and incubated in a growth chamber (Conviron) at 28°C with a 16-h photoperiod. After 30 days, percentages of standing plants and the shoot weight and length and root weight and length of each of the plants were measured. At least three root samples were collected for each of the WYEC108 treatments. Adhering soil was gently scraped off with forceps, and the seminal roots were cut into three sections, 0 to 3, 3 to 6, and 6 to 9 cm, from the subcrown internode and the seed. The root sections were placed in test tubes containing 5 ml of SDW. Dilution platings of these preparations were done on SPA amended with Benomyl (50 μg/ml) for the roots that were retrieved from sterile soil and on A-SPA for those from nonsterile soil. The size of the population of WYEC108 associated with the root was determined as defined previously (CFU per g of wet weight). Discolored or lesion-containing root samples were surface sterilized with 10% NaOCl–10% ethanol solution, rinsed twice with SDW, and then placed on a 1.5% water–agar plate to reisolate the fungal pathogen.

Statistical analysis. Treatment effects and changes over time were determined by analysis of variance with a GLM module and SAS (version 6.08 for UNIX environments, 1993; SAS Institute Inc., Cary, N.C.). Bacterial population numbers were log transformed and tested for normality prior to analyses. All other data were also examined for distribution normality before analysis of variance. Mean comparisons were made after analysis of variance with Fisher's least significant difference test ($\alpha = 0.05$).

SEM study of the interaction between *S. lydicus* WYEC108 and *Pythium* oospores. Ten milliliters of *Pythium* oospores (10^5 oospores per ml), 10 ml of a 48-h-old Casamino Acids-yeast extract-glucose broth culture of WYEC108, and 40 ml of molten 45°C agar (2%) were mixed together. Slide coverslips were coated on one side with this oospore-WYEC108-agar suspension (10, 16). The coated coverslips were then placed vertically into staining jars with Casamino Acids-mineral salts liquid medium (26) supplemented with a homogenized *Pythium* cell wall preparation as the carbon source (0.2% [wt/vol]). The *Pythium* cell wall material was prepared by growing *P. ultimum* in Casamino Acids-mineral salts (26) broth (125 ml) in 250-ml flasks at 30°C. After incubation for 3 days, mycelial mats were rinsed three times with SDW, resuspended in 100 ml of SDW, and then homogenized with a tissue homogenizer set at speed 7 for 4 min. A sample was taken from the suspension to check microscopically for the purity of the cell wall material. If necessary, the flask was set on a bench at 4°C for 5 min and the supernatant was transferred into another sterile flask for a second homogenization. When no intact hyphal cells were observed, the suspension was centrifuged for 6 min at 1,500 rpm. The pellet was resuspended with SDW and deep-frozen for lyophilization.

The jars were incubated at room temperature on a shaker at 200 rpm for 6 h. The coverslips were then carefully removed and rinsed several times gently with SDW. The samples were then fixed overnight in 1.5% glutaraldehyde in 0.2 M sodium cacodylate buffer; this step was followed by two thorough washes with 0.2 M sodium cacodylate buffer and dehydration in a graded series of ethanol washes. The washed coverslips were air dried and affixed to SEM stubs with colloidal silver conducting paint. Finally, the coverslip samples were thinly coated with 60/40 gold-palladium and examined by SEM (Amary 1830).

RESULTS

In vitro antagonism test. The in vitro antagonism assays (Table 1) showed that WYEC108 is an antagonist of a variety of fungal plant pathogens. The levels of inhibition were based on the values of $\Delta\gamma$. Since streptomycetes have a relatively lower growth rate on agar plates than most of the fungi, preinoculation (48 h) was used to establish WYEC108 colonies on the agar surface first. WYEC108 has broad antifungal activities. The antagonistic effects were more pronounced after 5 days of paired incubation than after 2 days. All tested fungi, except for two *F. solani* isolates, were inhibited by WYEC108 at the ++ or +++ level (see Table 1, footnote *a*, for definitions) after 5 days of incubation. The insignificant inhibition of *F. solani* after 2 days was probably due to the naturally low growth rate of this fungus. The morphology of the fungal mycelium along the edges of the colonies facing WYEC108 was examined under both a dissecting microscope at a magnification of ×4 and a compound microscope at magnifications of ×10 and ×40. However, no abnormal morphology (swollen hyphal tips and abnormal branching) or lysis of hyphal tips was observed for any of the fungal strains at these magnifications. Most likely, the inhibition of fungal growth by WYEC108 was due mainly to excreted, diffusible antifungal compounds. Viability tests showed that after 5 days of incubation, hyphae of *P. ultimum* and *A. euteiches* taken from peripheral areas of the inhibited colonies facing WYEC108 could no longer be recovered on fresh agar medium, indicating either local death or nonculturability of the hyphae caused by the antifungal metabolites released by WYEC108. In contrast, other fungi

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TABLE 2. Ability of *S. lydicus* WYEC108 to inhibit mycelial growth of fungi in liquid medium with different sugars as carbon sources

Sugar	Avg dry wt[a] (mg) ± SD					
	P. ultimum P8		*P. ultimum* P9		*R. solani* X5FS	
	Control	WYEC108	Control	WYEC108	Control	WYEC108
None (control)	29.0 ± 4.55	19.5 ± 4.16[b]	40.1 ± 4.08	22.0 ± 3.46[b]	34.5 ± 7.96	30.4 ± 1.37[b]
Glucose	40.6 ± 6.05	18.0 ± 2.41[b]	28.3 ± 3.56	19.9 ± 5.44[b]	27.6 ± 5.87	19.5 ± 1.72[b]
L-Sorbose	26.4 ± 4.56	16.3 ± 3.47[b]	39.6 ± 6.93	11.8 ± 1.41[b]	44.1 ± 3.61	24.9 ± 2.75[b]
Sucrose	28.4 ± 1.97	28.2 ± 1.70	22.6 ± 5.56	31.6 ± 11.8	38.1 ± 2.83	40.5 ± 2.90

[a] The value for each treatment is the average dry weight of three mycelial mats.
[b] Significant reduction ($P < 0.05$) in dry weight of fungal mycelia between the control and *S. lydicus* WYEC108 treatment.

(*Fusarium*, *Rhizoctonia*, and *Phymatotrichum* spp.) were more resistant in that peripheral hyphae were still culturable. The presence of the living antagonist was required for full inhibition.

Inhibition of fungal growth in liquid medium. Since the roles of cell surface carbohydrates in hyphal interaction between *S. lydicus* WYEC108 and *P. ultimum* are still not known, we tested the influences of three sugars, glucose, L-sorbose, and sucrose, on the inhibition of *Pythium* growth by WYEC108 (Table 2). In no-sugar control medium, the dry weight of mycelia was significantly reduced in all three of the fungi tested ($P < 0.05$). *S. lydicus* WYEC108 significantly inhibited the growth of the fungi in the broth containing glucose and L-sorbose ($P < 0.05$) but not in the medium containing sucrose.

Shelf life of the *S. lydicus* WYEC108 formulation. The initial density of spores in the well-mixed formulation was 10^9 CFU/g. After 2.5 months of storage at room temperature (20°C), the density of WYEC108 in the formulation had declined to 10^8 CFU/g. Over an additional 3 months, this level of viability was maintained (Fig. 1). On the basis of our preliminary growth chamber studies, we could still dilute this 3.5-month-old, shelf-stored formulation up to 100-fold (down to 10^6 CFU/g) prior to its use as a seed treatment.

WYEC108 population dynamics in sterile and nonsterile soil. A formulation (5×10^7 CFU/g) of WYEC108 was mixed with both sterile and nonsterile soils at a 1:10 (wt/wt) ratio. The stability of the WYEC108 population in soil was monitored for 3.5 months (Fig. 1). The initial densities of WYEC108 in both sterile and nonsterile soils were about the same, 10^6 CFU/g.

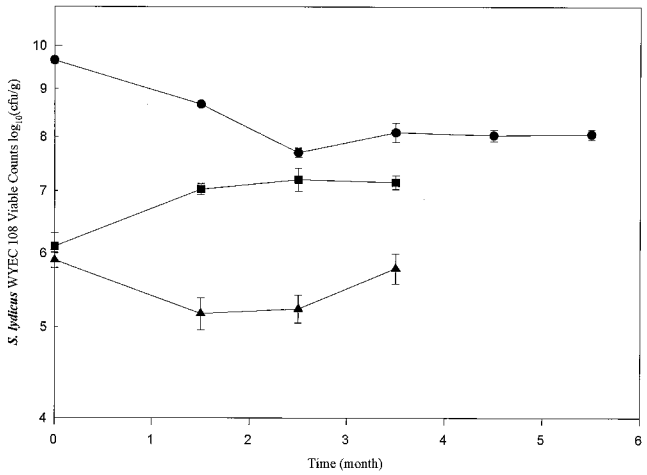

FIG. 1. Population dynamics of *S. lydicus* WYEC108 in a peat moss-sand formulation and sterile and nonsterile soils. Symbols: ●, WYEC108 in formulation; ■, WYEC108 in sterile soil; ▲, WYEC108 in nonsterile soil.

The population in sterile soil then increased to 10^7 CFU/g and maintained this level over the test period. In nonsterile soil, the WYEC108 population declined to 1.6×10^5 CFU/g initially, but it went back up to 10^6 CFU/g over the following 2.5 months. Competition with the indigenous microflora in the nonsterile soil was probably responsible for the initial decrease in the population of WYEC108 in the native soil.

Recovery of WYEC108 from nonsterile soil. To recover WYEC108 and distinguish it from the indigenous microflora in the native soil, A-SPA selective medium was developed. WYEC108 is naturally resistant to cycloheximide and carbenicillin at the 100-μg/ml level (11a). WYEC108-specific colonies were detected at dilution levels of 10^{-2} to 10^{-3} (10^2 to 10^3 CFU/ml) on A-SPA plates. On A-SPA agar, 99% of the total soil bacterial count and almost all of the fungi were eliminated by the combination of these two antibiotics. In contrast, on regular SPA, confluent growth of other bacteria and fungi hampered the establishment of WYEC108 colonies on the agar surface until the dilution level reached 10^{-5} to 10^{-6}.

Root colonization by WYEC108. To examine the ability of WYEC108 to colonize the newly emerging roots of germinating seedlings, pea, cotton, and sweet corn seeds were planted in WYEC108-amended sterile and nonsterile soils and incubated for 7 days at 28°C in a plant growth chamber with a 16-h photoperiod. Five germinated plants of each type were then removed from the Conetainers, and WYEC108 populations associated with the roots were monitored. In sterile soil, populations of WYEC108 were at levels of about 3×10^5 CFU/g on pea and cotton roots and 10^3 CFU/g on sweet corn roots. There were three- and fivefold decreases of the WYEC108 populations on pea and cotton roots, respectively, but only about a twofold decline on sweet corn roots in nonsterile soil compared with sterile soil (Table 3). WYEC108 was capable of colonizing not only untreated pea and cotton roots but also sweet corn roots germinating from fungicide-treated seeds. Thus, there was some compatibility of WYEC108 with the four fungicides (Captan, Thiram, Imazaul, and Metalaxyl).

Infection delay test. Seeds from three plates (a total of 30 seeds) in each treatment were retrieved at each sampling time to check for the presence of *Pythium* organisms in the seeds and roots. The percentage of infection (Fig. 2) at each sampling time was the average of three duplicate counts. Coating of seeds with WYEC108 spores and mycelia greatly reduced the amount of the *Pythium* invasion of the germinating seeds in oospore-enriched soil. While 70 and 100%, respectively, of uncoated seeds were *Pythium* infected 24 and 48 h after planting, fewer than 40 and 70% of the seeds coated with either spores or mycelia were infected (Fig. 2). Even better protection was achieved by planting coated seeds 24 h prior to their encounter with *Pythium* oospores. In this case, 96 h later, fewer than 30% of the germinating seeds were *Pythium* infected.

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TABLE 3. Initial WYEC108 colonization of pea, cotton, and sweet corn roots in sterile and nonsterile soils

Soil type	Avg[a] no. of CFU/g (wet wt) of root ± SD		
	Pea	Cotton	Sweet corn
Sterile	$3.01 \times 10^5 \pm 2.22 \times 10^5$	$2.47 \times 10^5 \pm 2.74 \times 10^4$	$7.66 \times 10^3 \pm 3.75 \times 10^3$
Nonsterile	$9.78 \times 10^4 \pm 6.29 \times 10^4$	$5.20 \times 10^4 \pm 3.99 \times 10^3$	$3.00 \times 10^3 \pm 1.5 \times 10^3$

[a] Averages of five samples are shown. When planted, the populations of WYEC108 were 1×10^7 and 1.6×10^5 CFU/g in sterile and nonsterile soils, respectively. Root samples were taken 7 days after planting.

Coating with the mycelial form of WYEC108 resulted in slightly better protection than coating of the seeds with spores. For example, in the preincubation groups after 72 and 96 h (Fig. 2), the percentages of seed infection for WYEC108 mycelium-treated seeds were 20 and 30%, respectively, compared with 36.7 and 40% with spore treatments.

Growth chamber assays of biological control. (i) Biological control of *Pythium* disease in cotton and pea in artificially infested sterile and nonsterile soils. *S. lydicus* WYEC108 spores were applied in a peat moss-sand formulation containing a spore count of 10^8 CFU/g. In control soil, at either pathogen concentration (500 or 100 oospores per ml), severe seed rot and pre- and postemergence damping-off of untreated pea and cotton plants were observed (Table 4). Root sections with lesions and discolorations were surface sterilized and placed on water-agar plates. Fungal mycelia that grew out of the root samples were identified as *P. ultimum*. We also examined some randomly selected root samples from untreated control and WYEC108-only-treated control soils. Two of the 11 samples examined were infected in these soils. The fungus from one of these two positive samples did not show a typical *Pythium* morphology. In comparison, 13 of 14 roots examined from the *Pythium*-only-infected soils were positive for the pathogen. In sterile soil treated with the WYEC108 formulation, at either pathogen level, the percentages of healthy, standing pea plants were significantly increased from fewer than 14.3% for pathogen-only-treated plants to over 64.2% for the WYEC108-treated groups (Fig. 3A). Similar increases in the number of standing plants were observed with cotton plants as well, in soil treated with the WYEC108 formulation (from less than 7.1% to over 83.6%) (Fig. 3B). With pea plants in sterile soil, treatment with WYEC108 greatly reduced the amount of seed rot and damping-off at both low and high *Pythium* levels, respectively (Table 4). With cotton plants, a 46.5% or greater disease reduction was observed at the low and high pathogen levels after treatment of seeds with WYEC108 (Table 4). Weights and lengths of treated pea and cotton plants were also significantly enhanced compared with those of the untreated groups (Table 4).

When seeds were planted in nonsterile soil, disease levels were also greatly reduced, from 67.8 and 78.6% in untreated control pea seeds and 84.7 and 96.4% in untreated cotton seeds to 32.1 and 46.6% in WYEC108-treated pea plants and 32.1 and 39.2% in treated cotton plants, at the low and high pathogen levels, respectively (Table 4). Disease control with WYEC108 formulation treatment of pea and cotton plants in nonsterile soil was not as efficient as it was in sterile soil (Table 4). This result was probably due to competition for colonization of the roots between WYEC108 and the indigenous microflora.

(ii) Population density and distribution of WYEC108 in sterile soil. After gentle removal of the soil columns with pea plants from Conetainers, the soil that did not adhere to the root (bulk soil) at different depths, as well as root sections (0 to 3, 3 to 6, and 6 to 9 cm from the top), were collected, vortexed, diluted, and plated to determine the population of *S. lydicus* WYEC108 in the bulk soil and rhizosphere. In nonrhizosphere soil, the WYEC108 population declined from about 5×10^6 CFU/g at less than 2 cm from the surface (where the formulation was applied) to about 10^4 CFU/g at 3 to 6 cm and 2.0×10^3 CFU/g at 6 to 9 cm from the soil surface. In the rhizosphere, the populations of WYEC108 were 3.2×10^5, 1.7×10^5, and 2.7×10^4 CFU/g of root wet weight at the three depths (0 to 3, 3 to 6, and 6 to 9 cm), respectively (Table 5). Thus, the change in the population of WYEC108 along the root fell less than 1 log unit between the highest and lowest points sampled. In contrast, in nonrhizosphere soil the population declined 3 log unit over the same distance (Table 5).

(iii) Population density and distribution of WYEC108 in nonsterile soil. The population distribution of WYEC108 in nonsterile soil differed from that in sterile soil (Table 5). In WYEC108-only-amended soil, the WYEC108 population remained fairly stable along the root over the 9-cm distance (10^5 CFU/g). In contrast, the population declined sharply from 1.1×10^5 CFU/g in the first 3 cm from the top, where the formulation was applied, to a nondetectable level in deeper soil (3 to 9 cm from the surface). In *Pythium* (500 oospores per ml)- and WYEC108-amended soil, the density of WYEC108 gradually increased downward along the roots (from not de-

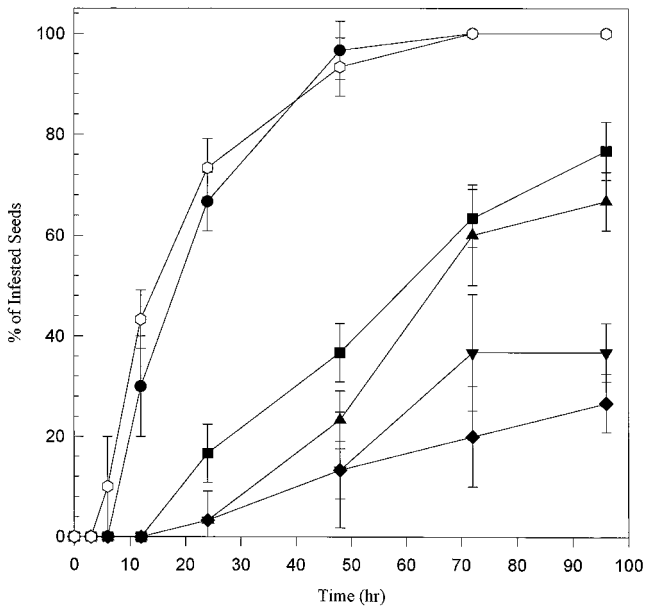

FIG. 2. Delaying of *P. ultimum* infection of pea seeds by WYEC108. Symbols: ●, untreated control seeds; ■, seeds coated with a WYEC108 spore suspension; ▲, seeds coated with a WYEC108 mycelial suspension; ▼, preincubated, WYEC108 spore-coated seeds; ◆, preincubated, WYEC108 mycelium-coated seeds; ○, untreated control preincubated seeds.

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TABLE 4. Influence of seed treatment with *S. lydicus* WYEC108 on seed rot, damping-off, height, and fresh weight of pea plants grown in soil artificially infested with *P. ultimum* P8

Treatment[a]	Pea (Puget)			Cotton		
	Mean no. of diseased plants (%)[b] ± SD	Plant length (cm)[c]	Plant wt (g)	Mean no. of diseased plants (%) ± SD	Plant length (cm)[c]	Plant wt (g)
Sterile soil						
None (control)	14.3 ± 5.68cd	18.1a	0.89b	7.15 ± 3.58c	18.8a	1.33a
P. ultimum (low)	85.7 ± 11.8a	1.14c	0.09c	92.9 ± 8.25a	3.57c	0.19c
P. ultimum (high)	96.4 ± 7.15a	0.29c	0.03c	96.4 ± 7.15a	0.30d	0.01d
P. ultimum (low) + WYEC108	28.5 ± 16.5bc	11.6b	0.98b	46.4 ± 21.4b	13.8b	0.71b
P. ultimum (high) + WYEC108	35.7 ± 14.3b	13.3b	1.29ab	35.7 ± 18.4b	16.4ab	0.78b
WYEC108 control	8.26 ± 7.15d	19.0a	1.50a	7.15 ± 3.58c	17.3a	0.79b
Nonsterile soil						
None (control)	21.4 ± 18.4cd	16.0a	0.73ab	8.25 ± 7.15d	19.7a	0.96a
P. ultimum (low)	67.8 ± 13.7ab	3.58c	0.26bc	84.7 ± 11.8a	3.87c	0.17c
P. ultimum (high)	78.6 ± 18.5a	0.74d	0.12c	96.4 ± 7.15a	1.82cd	0.12c
P. ultimum (low) + WYEC108	32.1 ± 7.11bcd	10.1b	0.65b	32.1 ± 13.6cb	12.8b	0.64ab
P. ultimum (high) + WYEC108	46.6 ± 18.0abc	7.65bc	0.44bc	39.2 ± 17.9b	8.37bc	0.41b
WYEC108 control	8.26 ± 7.15d	18.9a	0.92a	21.4 ± 8.25cd	16.6ab	0.62ab

[a] All experiments were repeated two or three times. Conetainers were set up in racks in a completely random design. Each treatment contained at least three replications, and each replication contained 14 plants. *P. ultimum* (low), soil treated with 100 oospores per ml; *P. ultimum* (high), soil treated with 500 oospores per ml.
[b] Seed rot and pre- and postemergence damping-off were counted as diseased. Means with the same letter within a column are not significantly different ($P < 0.05$).
[c] Means within a column were separated by using Fisher's least significant difference test ($P < 0.05$).

tectable to 2.12×10^5 CFU/g). In contrast, in the bulk soil it dropped at least 100-fold.

SEM studies of the interaction between WYEC108 and *P. ultimum*. Hyphal interactions between germinating *P. ultimum* oospores and WYEC108 hyphae were seen as early as 6 h of incubation. When the germ tube of one oospore reached the colony of WYEC108 (Fig. 4B), it responded to the presence of the antagonist by changing its direction of elongation by 180°. However, the hyphal structure at the turn point was ruptured (Fig. 4B) and the intracellular components of the hypha in the vicinity of the WYEC108 colony had leaked out, resulting in a collapsed hyphal and oospore structure compared with a healthy germinating oospore (Fig. 4A). Another examination showed that when the *P. ultimum* hypha grew across the top of a WYEC108 colony (Fig. 4C), the colony was capable of causing multiple damaged sites on the fungal hypha.

DISCUSSION

Potential uses of root-colonizing streptomycetes as replacements or supplements for agricultural chemical fungicides have been addressed in many reports (9, 14, 18, 37). Our previous work (12, 34) showed that *S. lydicus* WYEC108 is a strong in vitro antagonist against a variety of fungal plant root rot and white-rot pathogens and various wood decay fungi. When chickpea seeds were coated with a WYEC108 mycelium suspension in alginate solution (3%), significant suppression of *Pythium* damping-off was observed (34). The present study further demonstrates the biocontrol potential of this strain by showing its population dynamics in a peat moss-sand formulation and soil, population density and distribution in the rhizosphere, and specific interactions with the pathogen *P. ultimum* (in vitro).

Although it has been argued that there is no general relationship between in vitro antagonism of a biocontrol candidate and disease suppression in vivo, potential biocontrol *Streptomyces* species seem to be exceptions to this conclusion in many studies (28–30, 35, 36). In the present study, in vitro plate assays showed that all of the fungal root pathogens tested were moderately to very sensitive to the antifungal metabolites released by WYEC108 in an agar matrix. WYEC108 also caused local death or at least prolonged inhibition of the growth of the fungal hyphae of *P. ultimum* and *A. euteiches* at the peripheral areas of hyphae adjacent to WYEC108. However, despite the inhibition of fungal growth, neither lysed hyphal tips nor swollen or abnormal branching of hyphae due to the presence of WYEC108 was observed upon light microscopic examination after 72 h of paired incubation on agar medium.

When three sugars common in the rhizosphere, glucose, L-sorbose, and sucrose, were used as carbon sources in liquid medium, differential inhibitions of *P. ultimum* and *R. solani* by WYEC108 were observed. In general, there was no inhibition in sucrose medium compared with a no-sugar control and other sugar media. It is well established that carbohydrates have an important impact on both the production of antibiotics by streptomycetes (15, 16) and the interactions of bacterial cells and the fungal hyphal cell surface (26). *S. lydicus* WYEC108 does not seem to require direct contact with a target fungal structure for antagonism, since culture filtrates of WYEC108 have shown strong antifungal activities (unpublished data). Interestingly, however, this strain also has the capability of parasitizing *F. solani* hyphae on an agar surface (unpublished data). Whether there is any correlation between mycoparasitism and antibiosis by WYEC108 in vitro and what specific roles the sugars tested may play in inhibition of fungal growth are still not known.

According to R. J. Cook (11), mass production of stable and inexpensive formulations is one of the most critical steps in the development of a microbial biocontrol agent. Here, a WYEC 108 formulation (10^9 CFU/g) was obtained by scraping spores of WYEC108 from 25 SPA plates into 2 kg of a peat moss-sand carrier. Stability tests showed that the density of the viable spores in the formulation could be maintained at 10^8 CFU/g over a 6.5-month period of storage at room temperature. This result is consistent with the statement by Tahvonen (35) that peat moss seems to be a natural organic substrate for *Streptomyces* spp. Studies of population dynamics of WYEC108 in soil demonstrated that, as delivered in this formulation, WYEC108 maintained its viability in the soil environment tested. The population in sterile soil was about 1.3 log units higher than it

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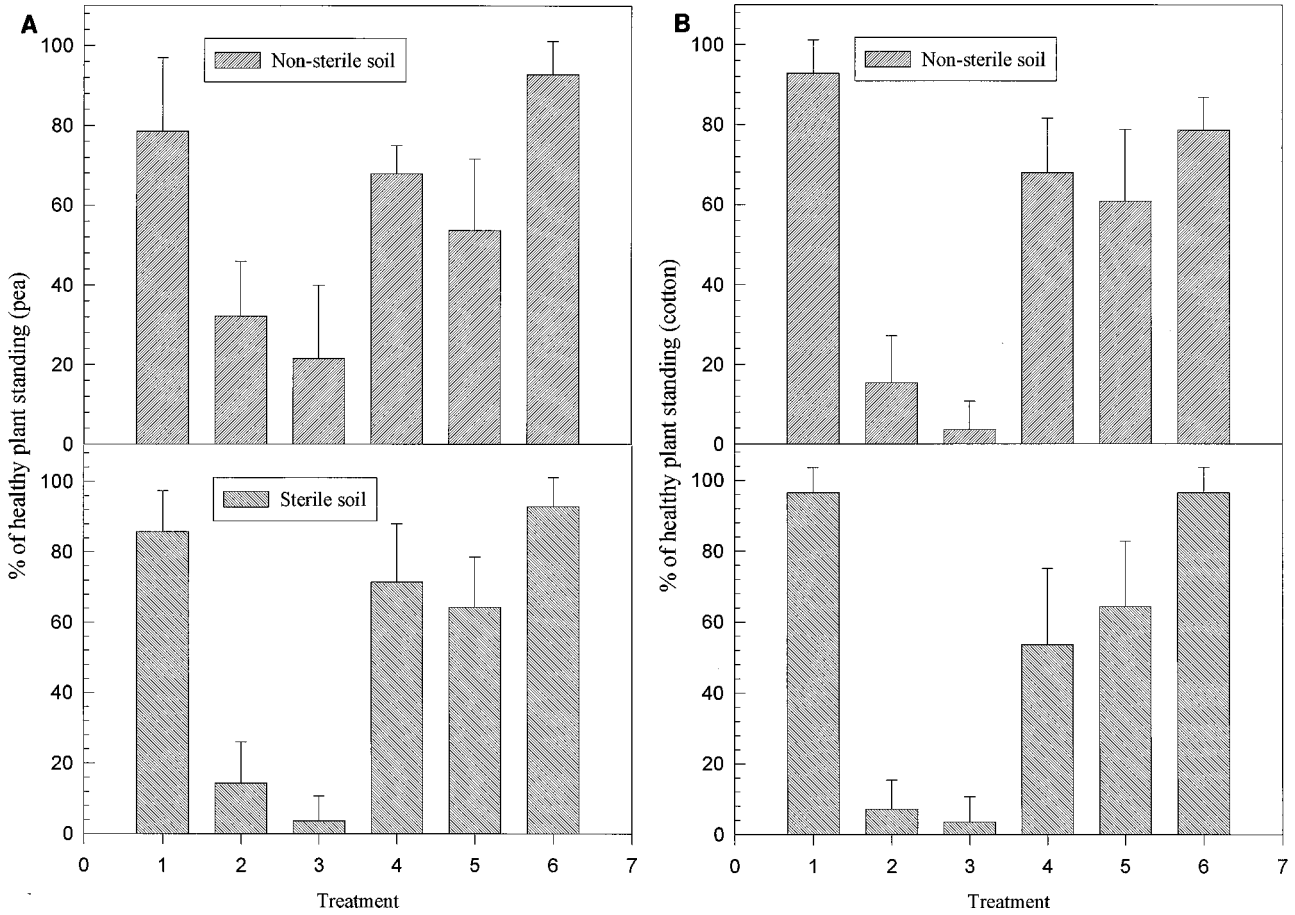

FIG. 3. Biological control of *Pythium* damping-off diseases by WYEC108. (A) Percentages of healthy standing pea plants. 1, untreated control; 2, low level of *P. ultimum* (100 oospores per ml); 3, high level of *P. ultimum* (500 oospores per ml); 4, low *P. ultimum* level plus WYEC108 formulation; 5, high *P. ultimum* level plus WYEC108 formulation; 6, *S. lydicus* control treatment. (B) Percentages of healthy standing cotton plants. Treatments were the same as for panel A. The measurements were taken after a 30-day incubation time. Each treatment contained at least three replications, and each replication had at least 14 plants.

was in nonsterile soil after a 3.5-month incubation period; this was probably due to the lack of competition and the abundance of readily utilizable lysed cell biomass in the former environment. In nonsterile soil, even though the population of WYEC108 declined 0.73 U in the first month of incubation, it then steadily increased over the next 2 months and finally returned to a level which was statistically insignificantly different from the initial population (least significant difference test, $P < 0.05$). This indicates that the indigenous microorganisms did not inhibit the population of WYEC108.

When pea, cotton, and sweet corn seeds were planted in the formulation-amended soil, WYEC108 rapidly colonized the roots of the germinating seeds. The abilities of *Streptomyces* spp. to colonize the rhizosphere and maintain their population

TABLE 5. Population of WYEC108 in bulk soil and rhizosphere of pea plants

Location	Avg no. of CFU[a] ± SD at depths of:		
	0–3 cm	3–6 cm	6–9 cm
Sterile soil treated with WYEC108			
Rhizosphere[b]	$3.2 \times 10^5 \pm 1.0 \times 10^5$	$1.7 \times 10^5 \pm 1.5 \times 10^4$	$2.7 \times 10^5 \pm 2.3 \times 10^4$
Bulk soil[b]	$4.5 \times 10^6 \pm 1.3 \times 10^6$	$1.0 \times 10^4 \pm 3.5 \times 10^3$	$2.0 \times 10^3 \pm 1.5 \times 10^3$
Nonsterile soil treated with WYEC108			
Rhizosphere	$9.3 \times 10^4 \pm 1.3 \times 10^4$	$5.9 \times 10^4 \pm 1.3 \times 10^4$	$2.0 \times 10^5 \pm 2.8 \times 10^4$
Bulk soil	$1.1 \times 10^5 \pm 1.7 \times 10^4$	ND[c]	ND
Rhizosphere[b]	ND	$9.8 \times 10^4 \pm 9.8 \times 10^3$	$2.1 \times 10^5 \pm 3.0 \times 10^4$
Bulk soil[b]	$4.7 \times 10^5 \pm 4.4 \times 10^4$	ND	$3.8 \times 10^3 \pm 2.2 \times 10^3$

[a] WYEC108 populations were defined as CFU per gram (wet weight) of root or CFU per gram of soil (for nonrhizosphere [bulk soil] samples). The values shown are averages of at least five replicates.
[b] Soil treated with both WYEC108 formulation and 500 *P. ultimum* oospores per ml.
[c] ND, population of WYEC108 in that space was not detectable ($\leq 10^2$ CFU/g).

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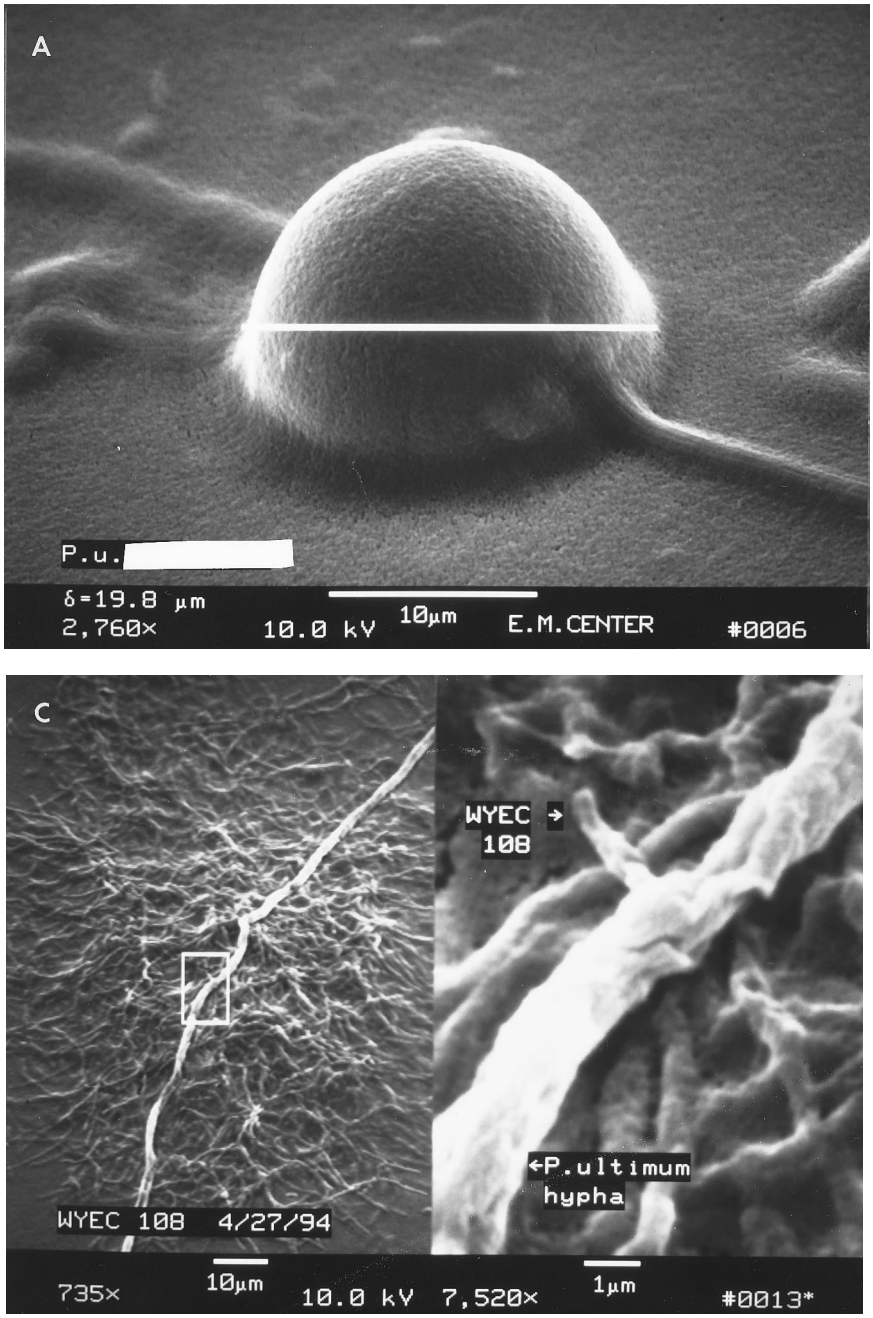

FIG. 4. SEM of *S. lydicus* WYEC108 interacting with hyphae of *P. ultimum*. (A) germinating oospore of *P. ultimum* P8; (B) collapsed oospore and germ tube 6 h after paired incubation; (C) *P. ultimum* P8 hyphal cell wall damaged by WYEC108.

sizes were also documented by Miller et al. (22, 23). The WYEC108 population in the rhizospheres ($\cong 10^5$ CFU/g) of pea and cotton plants was 0.5 to 1.0 log units less than WYEC108 inocula ($\cong 10^6$ CFU/g) in soil; this could be due to the spatial limitation of the root surface area, differential distribution of the population on the root (the average of the population along the entire root was measured), and rhizosphere competition with the soil microflora (17, 37). In addition, traditional serial dilution may lack sensitivity when mycelial propagules of actinomycetes in the rhizosphere are measured. There was, in general, less colonization of the germinating roots of sweet corn, perhaps because of the treatment of this seed with the chemical fungicides. Still, about 3×10^3 to 7×10^3 CFU of WYEC108 per g were detected in the corn rhizosphere, suggesting colonization and some tolerance of the fungicides by the actinomycete.

Most of the damage from *Pythium* diseases occurs at the very early stage of seed germination, when seedlings release a large amount of exudates and have not yet developed mature cell walls. Thus, they are highly susceptible to attack by seed and seedling pathogens, such as *P. ultimum*. In the present study, a closed system was used to examine the potential of early protection of seedlings by WYEC108 against *Pythium* infection. In soil with an oospore density of 35 propagules per cm^3 48 h after

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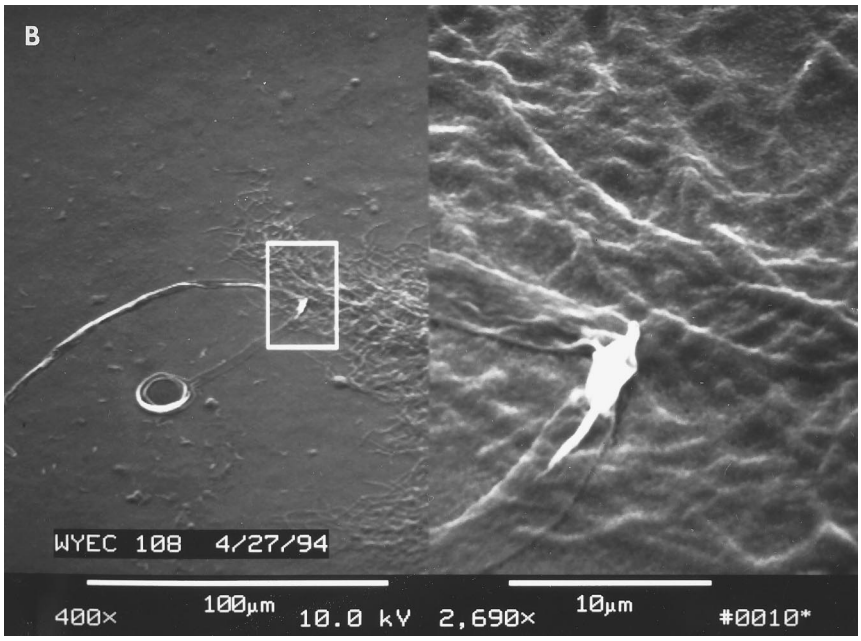

FIG. 4—*Continued.*

planting, WYEC108-coated seeds were 2.5- or 8-fold less infected than untreated controls, without or with preincubation, respectively (Fig. 2). This finding strongly agrees with the statement that preemptive colonization of the rhizosphere by a potential biocontrol agent is important for effective disease suppression (37).

The growth chamber studies demonstrated that, when applied as a spore formulation, WYEC108 controlled *Pythium* damping-off in both sterile and nonsterile soils. Interestingly, the WYEC108 population distribution along the roots differed strongly in these two soil environments. In sterile soil, without microbial rhizosphere competition, WYEC108 colonized along the roots uniformly over a 30-day incubation period. However, in nonsterile soil, the population of WYEC108 on the root tips was about 1,000-fold higher than in the subcrown root section. This differential colonization is probably partially responsible for the smaller overall rhizosphere population in WYEC108-amended nonsterile soil (Table 3). Unfortunately, we did not monitor population dynamics during further elongation of the root. Although the effect of water percolation, compared with the migration rate in bulk soil, was not excluded in this study, it is strongly suggested that passive carriage by the elongating root, chemotatic attraction by the root exudates, and/or local proliferation of WYEC108 are important facilitators of downward microbial migration. Accumulation of a WYEC108 population in the root tips might also be important for long-term root disease control, since the young cells in root tips are very vulnerable to attacks of soil-borne fungal pathogens.

The potential of WYEC108 to mycoparasitize the target pathogen was investigated by SEM. Although no direct evidence of parasitization of the host (7) was observed during the time course tested, WYEC108 was capable of damaging multiple sites on *Pythium* hyphae. It is well known that *Streptomyces* species are capable of mycoparasitizing fungal spores and hyphal structures (27, 32) and producing extracellular cell wall-degrading enzymes, such as chitinases, cellulases, amylases, and 1,3-β-glucanases (5, 15). Disintegration of fungal cell walls would subsequently lead to cell death and to more efficient penetration by other antifungal compounds (20). The results obtained from this study do not exclude the potential of this strain to mycoparasitize pathogenic fungi. In a preliminary study, we observed that after longer incubation times (14 days), WYEC108 was able to coil around the hyphae of *F. solani* and lyse the hyphal tips.

The demonstration that *Pythium* hyphae altered their direction of growth in response to the presence of *S. lydicus* WYEC108 is significant. As observed by SEM (Fig. 4), when a *Pythium* hypha turned away, it was likely because a germinating oospore germ tube was in the same dimensional space as *S. lydicus* cells. In contrast, in the SEM showing a hypha overlying an *S. lydicus* colony, the fungal hypha was probably growing in a different plane sufficiently above the streptomycete colony so that it continued to grow. It is noteworthy that even though the samples were handled with extreme care during preparation for SEM, there was still the possibility of artificial damage to the fungal structures. We tried to avoid this false-positive result by examining as many view fields as possible before the pictures were taken.

Despite all of the in vitro fungal antagonisms of WYEC108 demonstrated in this study, no hard evidence indicated the exact mechanisms responsible for disease suppression by this strain under growth chamber conditions. Separation of antagonistic phenotypes, if possible, and examination of each individual phenotype and possible combinations of these traits in vivo is the next step. Detailed studies of the biochemistry of the antifungal metabolites (antibiotics, extracellular hydrolytic enzymes, etc.) and the regulation of their production by WYEC 108 are in process.

Plating of serially diluted samples on a selective medium and colony morphology were used to recover and identify WYEC 108 colonies in the presence of the native soil microflora. These techniques are rapid and simple but not extremely accurate or sensitive. To date, the only way we have to monitor WYEC108 populations in soils is to use a selective medium and count the distinctive actinomycete colonies that form a gray-green aerial mycelium upon sporulation. In soils

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inoculated with WYEC108, these colonies were the dominant actinomycete colonies on the plates. Uninoculated control soils yielded only a few such colonies that might be mistaken for WYEC108. Also, to increase the accuracy of selection of WYEC108 colonies on selective media, we had at least two people do the enumeration separately. Thus, we are confident in our data. Several molecular genetic techniques are being developed which might be used to identify *Streptomyces* species to the strain level. These techniques detect highly conserved 16S RNA sequences or produce DNA fingerprints by randomly amplified polymorphic DNA analysis (unpublished data; 17, 33). We hope to use these procedures in the future as alternative tools to study biocontrol agent population dynamics in the rhizosphere.

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