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Abstract

The soilborne pathogens *Rhizoctonia solani*, *Pythium ultimum,* and *Meloidogyne incognita* can cause severe economic losses to field- and greenhouse-grown cucumber. A collection of bacterial isolates and isolates GL3 and GL21 of *Trichoderma virens* were screened for suppression of diseases caused by these pathogens. *T. virens* isolates GL3 and GL21 provided the most effective suppression of damping-off caused by *R. solani* in greenhouse bioassays. *Burkholderia ambifaria* BC-F, *B. cepacia* BC-1, and *Serratia marcescens* N1-14 also provided significant suppression of *R. solani* relative to the pathogen check in some experiments. *T. virens* isolates GL3 and GL21 and *S. marcescens* isolates N1-6, N1-14, and N2-4 provided the most consistent and effective suppression of damping-off of cucumber caused by *P. ultimum* in growth chamber experiments. No microbial treatment containing individual or combined microbes significantly suppressed populations of *M. incognita* on cucumber or improved plant vigor in greenhouse bioassays. *T. virens* GL21 applied as a granular formulation, in combination with *B. cepacia* BC-1 or *B. ambifaria* BC-F applied as a seed treatment, significantly improved suppression of damping-off caused by *R. solani* over individual applications of these microbes in at least one experiment. Treatments combining *B. cepacia* BC-1, *B. ambifaria* BC-F, or *S. marcescens* isolates N1-14 or N2-4 with *T. virens* GL21 in *R. solani* biocontrol assays always resulted in plant stands that were similar or greater than treatments containing individual applications of these microbes. *B. ambifaria* BC-F combined with *T. virens* GL21 in seed treatments resulted in significantly improved suppression of damping-off caused by *P. ultimum* in two of three experiments. Populations of *T. virens* GL3 and GL21 were both substantially reduced after coincubation with *B. cepacia* BC-1, or *S. marcescens* isolates N1-14 or N2-4 for 10 to 12 d in cucumber rhizospheres. Populations of *T. virens* GL21 were slightly reduced after coincubation with *B. ambifaria* BC-F. Results presented here substantiate other studies reporting enhanced biocontrol performance

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with certain combinations of biocontrol agents. These results also indicate that antagonism among combinations of biocontrol agents can vary with the assay system employed.

Keywords: Biological control; Combinations; *Meloidogyne incognita*; *Pythium ultimum*; *Rhizoctonia solani*; *Serratia marcescens*; *Trichoderma virens*

1. Introduction

Due to environmental concerns there is considerable interest in finding alternatives to chemical pesticides for suppression of soilborne plant pathogens and plant-parasitic nematodes (Larkin et al., 1998; Raupach and Kloepper, 1998). Numerous microbes are antagonistic to soilborne plant pathogens and plant-parasitic nematodes, with some microbes suppressing disease or pathogen populations. Relatively few of these antagonistic microbes have been commercialized as biocontrol agents due to problems such as inconsistent performance in the field, lack of broad-spectrum disease suppression activity, or slower or less complete suppression when compared with chemical pesticides (Larkin et al., 1998; Meyer and Roberts, 2002).

Inconsistent performance by microbial antagonists has been attributed to biotic and abiotic factors. Biotic factors include interactions with non-target organisms, varying rhizosphere or soil colonization by the biocontrol agent, varying initial population levels and genetic diversity of the target pathogens, and host plant species and cultivar effects (Stirling, 1991; Boeger et al., 1993; Sikora and Hoffmann-Hergarten, 1993; Pierson and Weller, 1994; Kerry and Bourne, 1996; Raupach and Kloepper, 1998; Meyer and Roberts, 2002). Abiotic factors include climate and varying physical and chemical composition of the soil or rhizosphere (Stirling, 1991; Ownley et al., 1992; Sikora and Hoffmann-Hergarten, 1993).

The majority of strategies for biocontrol of soilborne plant pathogens and plant-parasitic nematodes rely on a single microbial biocontrol agent for pathogen or nematode suppression (Larkin et al., 1998). Unfortunately, biocontrol agents applied individually are not likely to perform consistently against all pathogens of the crop or under diverse rhizosphere and soil environmental conditions. An approach to overcoming this inconsistent performance is to include a combination of biocontrol agents in a single preparation. A combination of biocontrol agents is more likely to have a greater variety of traits responsible for suppression of one or more pathogens and also is likely to have these traits expressed over a wide range of environmental conditions (Lemanceau and Alabouvette, 1991; Lemanceau et al., 1993; Pierson and Weller, 1994; Crump, 1998).

Numerous studies (Raupach and Kloepper, 1998; Meyer and Roberts, 2002) have reported increased performance in suppression of pathogens or disease by combinations of biocontrol agents. However, there are several studies of combinations of microbial antagonists that resulted in decreased performance relative to individual applications of these biocontrol agents (Meyer and Roberts, 2002). Incompatibility amongst microbes combined in a biocontrol preparation is possible since biocontrol agents are typically selected based on their antagonistic behavior toward other microbes (Leeman et al., 1996; Meyer and Roberts, 2002). Several researchers have indicated that strains combined in biocontrol preparations must be compatible for increased disease suppression to occur (Baker, 1990; Janisiewicz and Bors, 1995; Janisiewicz, 1996; Raupach and Kloepper, 1998).

Soilborne microbes causing significant economic loss to cucumber include the fungi *Pythium ultimum* and *Rhizoctonia solani* and the root-knot nematode *Meloidogyne incognita* (Zitter et al., 1996; Koenning et al., 1999). The long-term goal of our research is to develop combinations of biocontrol organisms effective under diverse environmental conditions for management of these soilborne cucumber diseases. The first objective was to identify microbes with broad-spectrum suppression of cucumber pathogens. We report here bacterial and fungal isolates capable of suppressing damping-off caused by the fungal pathogens *P. ultimum* and *R. solani* and capable of suppressing egg hatch of the nematode *M. incognita* in vitro. We also study compatibility among these isolates in disease suppression assays and in rhizosphere coexistence assays. For the purposes of this report, compatible microbes are defined as microbes that, when combined, do not have diminished disease suppression or reduced persistence in the rhizosphere relative to the same strains applied individually.

2. Materials and methods

2.1. Bacterial, fungal, and nematode isolates

Bacteria used in this study are listed in Table 1. Bacteria from roots of wheat or cucumber plants grown in natural soil were isolated by sonicating roots for 5 min in sterile distilled water (SDW) and dilution-plating onto Nutrient Broth (NB) agar. All bacterial isolates are from the Sustainable Agricultural Systems Laboratory (SASL; USDA-ARS, Beltsville, MD) culture collection. Spontaneous rifampicin-resistant mutants of *Burkholderia cepacia* isolates BC-1 and BC-2

Table 1
Bacterial strains used in this study

Strain[a]	% Match	Plant[b]	Biocontrol assay[c]	Comments/Source
Acinetobacter calcoaceticus 0018	0.292	Wheat	PU	This study
Acinetobacter calcoaceticus 0035	0.641	Wheat	PU	This study
Acinetobacter johnsonii 009	0.286	Wheat	PU	This study
Acinetobacter radioresistens C0032	0.690	Cucumber	PU	This study
Acinetobacter radioresistens 0055	0.659	Wheat	MI, PU	This study
Agrobacterium radiobacter 99-42	0.231	Wheat	PU	This study
Arthrobacter aurescens 99-28	0.645	Wheat	PU	This study
Arthrobacter globiformis 99-58	0.676	Wheat	PU	Stromberg et al. (2002)
Arthrobacter ilicis 99-41	0.669	Wheat	PU	Stromberg et al. (2002)
Arthrobacter pascens 99-73	0.512	Wheat	PU	Stromberg et al., (2002)
Bacillus cereus C0053	0.673	Cucumber	PU	This study
Bacillus cereus 0019	0.292	Wheat	PU	This study
Bacillus cereus 99-46	0.211	Wheat	PU	Stromberg et al. (2002)
Bacillus circulans 99-10	0.167	Wheat	MI, PU	This study
Bacillus dipsosauri C0027	0.723	Cucumber	PU	This study
Bacillus lentimorbus 99-16	0.650	Wheat	PU	Stromberg et al. (2002)
Bacillus megaterium C0024	0.829	Cucumber	PU	This study
Bacillus marinus 99-75	0.293	Wheat	PU	Stromberg et al. (2002)
Bacillus megaterium 99-3	0.904	Wheat	PU	Stromberg et al. (2002)
Bacillus pasteurii 0022	0.652	Wheat	MI, PU	This study
Bacillus pumilus 99-23	0.804	Wheat	PU	Stromberg et al. (2002)
Bacillus sphaericus 0040	0.749	Wheat	PU	This study
Bacillus thuringiensis kurstakii C0057	0.772	Cucumber	PU	This study
Brevibacillus laterosporus 99-2	0.831	Wheat	PU	Stromberg et al. (2002)
Burkholderia ambifaria BC-F[d]		Corn	MI, PU, RS	Mao et al. (1998)
B. ambifaria BC-FR8				Rifampicin-resistant derivative of BC-F; Li et al. (2002)
Burkholderia cepacia BC-1	0.833	Corn	MI, PU, RS	Obtained from W. Mao
B. cepacia BC-1R1				Rifampicin-resistant derivative of BC-1
Burkholderia cepacia BC-2	0.671	Corn	MI, PU	Obtained from W. Mao
B. cepacia BC-2R2				Rifampicin-resistant derivative of BC-2
Chryseobacterium indologenes C0063	0.900	Cucumber	PU	This study
Enterobacter asburiae C0015	0.787	Cucumber	PU	This study
Enterobacter asburiae 501R3[e]		Cotton	MI, PU, RS	Roberts et al. (1992)
Klebsiella planticola C0014	0.759	Cucumber	PU	This study
Kluyvera cryocrescens C0016	0.535	Cucumber	PU	This study
Kocuria kristinae 99-33	0.595	Wheat	PU	Stromberg et al. (2002)
Kocuria rosea 99-81	0.540	Wheat	PU	This study
Micrococcus luteus 0042	0.532	Wheat	PU	This study
Paenibacillus polymyxa 99-32	0.700	Wheat	PU	This study
Pantoea agglomerans 0020	0.870	Wheat	MI, PU	This study
Pseudomonas chlororaphis 0050	0.761	Wheat	MI, PU	This study
Serratia marcescens N1-6	0.532	Soil	PU, RS	Obtained from D. Kobayashi
Serratia marcescens N1-8	0.560	Soil	PU, RS	Obtained from D. Kobayashi
Serratia marcescens N1-14	0.526	Soil	MI, PU, RS	Obtained from D. Kobayashi
S. marcescens N1-14R5				Rifampicin-resistant derivative of N1-14
Serratia marcescens N2-4[f]		Soil	MI, PU, RS	Obtained from D. Kobayashi
S. marcescens N2-4R1				Rifampicin-resistant derivative of N2-4
Serratia marcescens N2-7	0.533	Soil	PU, RS	Obtained from D. Kobayashi
Serratia marcescens N4-1	0.590	Soil	PU, RS	Obtained from D. Kobayashi
Serratia marcescens N4-13	0.495	Soil	PU, RS	Obtained from D. Kobayashi
Serratia marcescens N4-19	0.613	Soil	PU, RS	Obtained from D. Kobayashi
Stenotrophomonas maltophilia C001	0.796	Cucumber	PU	This study
Stenotrophomonas maltophilia C0058	0.784	Cucumber	PU	This study
Streptomyces fulvissimus 99-60	0.758	Wheat	PU	Stromberg et al. (2002)
Rhodococcus fascians 99-39	0.832	Wheat	PU	This study
Unknown 0031	0.000	Wheat	PU	This study
Unknown 0034	0.000	Wheat	PU	This study

Table 1 (*continued*)

Strain[a]	% Match	Plant[b]	Biocontrol assay[c]	Comments/Source
Xanthomonas arboricola 99-37	0.915	Wheat	PU	Stromberg et al. (2002)
Xanthomonas axonopodis 001	0.105	Wheat	PU	This study

[a]Except where noted otherwise, bacterial strains were identified from gas chromatographic profiles of cellular fatty acids using MIDI software. A match of 0.500 or greater is considered valid at the species level while a match of 0.200 or greater is valid at the genus level.

[b]Corn, isolated from corn rhizosphere; Cotton, isolated from cotton hypocotyl; Cucumber, isolated from cucumber rhizosphere; Soil, isolated from soil using chitin enrichment; Wheat, isolated from wheat rhizosphere.

[c]Biocontrol assays were performed with this isolate for suppression of MI, *Meloidogyne incognita*; PU, *Pythium ultimum*; RS, *Rhizoctonia solani*.

[d]Identified as *B. ambifaria* by Coenye et al. (2001).

[e]Identified as *E. asburiae* by Hoffman and Roggenkamp (2003).

[f]Identified as *S. marcescens* by analysis of sequence of the 16S rDNA gene. Identified as *Cedecia davisae* by gas chromatographic profiles of cellular fatty acids, percent match was 0.258.

and *Serratia marcescens* isolates N1-14 and N2-4 were isolated as described previously (Miller, 1972). Rifampicin-resistant strains BC-1R1, BC-2R2, BCF-R8, N1-14R5, and N2-4R1 were similar to respective parental strains in colony morphology and growth characteristics. *Trichoderma virens* GL3 and GL21 were from the Alternate Crops and Systems Laboratory (USDA-ARS, Beltsville, MD) culture collection. *P. ultimum* Puzc and *R. solani* R-23A (AG-4) were from the SASL culture collection. This isolate of *R. solani* was chosen because AG-4 is most closely associated with vegetable seedling damping-off (Farr et al., 1989). *M. incognita* race 1 was from the Nematology Laboratory (USDA-ARS, Beltsville, MD) culture collection.

2.2. Identification of bacterial isolates

Bacteria were identified from gas chromatographic profiles of cellular fatty acids using the MIDI system (Microbial ID, Inc., Newark, DE) except where indicated otherwise in Table 1. *S. marcescens* isolates N1-6, N1-8, N2-4, and N4-1 were further characterized by sequencing the 16S rDNA gene. For this, genomic DNA was extracted as described previously (de Souza et al., 2003). The primers used for PCR amplification of the 16S rDNA gene were 8fn (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1429r (5′-ACGGCTACCDTTGTTACGACTT-3′) (Esikova et al., 2002). The PCR amplification cycle included an initial 2 min denaturation at 95 °C followed by 10 cycles of denaturation at 95 °C for 1 min, 1 min primer annealing at 65 °C with the annealing temperature decreased by 1 °C with each succeeding cycle, and 1 min elongation at 72 °C. PCR products were purified by electrophoresis in agarose gels using standard methods (Sambrook and Russell, 2001) and sequenced using PCR-mediated Taq DyeDeoxy terminator cycle sequencing. The LASERGENE (DNAStar Inc., Madison, WI) sequence analysis software package and BLAST software package (Altschul et al., 1997) were used for all DNA sequence analysis. Bacteria were identified from nucleotide sequence resulting from sequencing a portion of the 16S rDNA gene. All sequences had 99% identity with sequence from the *S. marcescens* DNA sequence in the database. Nucleotide sequences have been deposited in GenBank under accession numbers AY514431, AY514432, AY514433, AY514434, and AY514435 for *S. marcescens* N1-6, N1-8, N1-14, N2-4, and N4-1, respectively.

2.3. Preparation of formulations containing beneficial microbes

Bacterial isolates were grown 48 h in NB at 22 °C and 250 rpm. Bacterial cultures were washed and resuspended in SDW. *T. virens* isolates were grown 7 d on Potato Dextrose Agar (PDA). The mycelium and agar were blended, added to 25 ml Molasses Yeast (MY) broth (Papavizas et al., 1984), incubated 7 d and used to inoculate 500 ml MY broth. The freshly inoculated MY broth was incubated 14 d at 25 °C and 230 rpm, centrifuged at $9000 \times g$, the pellet resuspended in 100 ml SDW, and fungal biomass dispersed with a tissue mizer (IKA Works, Inc., Wilmington, NC). Bacterial or fungal suspensions (2.1 ml) were mixed with 0.7 ml 12% gelatin (w/v; Sigma Chemical Company, St. Louis, MO) and applied to 140 cucumber (*Cucumis sativum* cv. Marketmore 76) seeds. The gelatin had been previously autoclaved and cooled to 40 °C. Treated seeds were dried under a laminar flow hood for approximately 2 h prior to planting. For seed treatments containing combinations of microorganisms, equal volumes of bacterial and fungal suspensions were mixed prior to addition to the gelatin solution. Seed treatments containing combinations of microorganisms did not have significantly more CFU per seed than individual treatments. Bacteria also were applied to cucumber seed in a peat-bond formulation as described previously (Roberts et al., 1997). For preparation of granular formulations containing *T. virens* GL3 or GL21, 68 g rice flour, 23 g pyrax, and 9 g vermiculite were mixed,

autoclaved, and combined with 13 g gluten and 7 g GL3 or GL21 biomass. The check was prepared similarly except 7 g pyrax was added in place of fungal biomass. Canola oil (13 ml) was added, the mixture kneaded to a dough-like consistency, granulated, dried overnight, and blended (Hebbar et al., 1999). Population levels in seed treatments were determined by sonicating treated seeds for 5 min followed by dilution-plating onto NB agar for bacteria and TME agar for *T. virens* (Papavizas and Lumsden, 1982). Populations levels in the granular formulation were determined by dilution-plating onto TME agar.

2.4. Suppression of damping-off of cucumber caused by *R. solani*

Redi-Earth (Scott's Horticultural Products, Marysville, OH) infested with *R. solani* R-23A was prepared as described previously (Lewis and Larkin, 1997; Lewis and Lumsden, 2001). Redi-Earth infested with *R. solani* R-23A and granular pellets containing *T. virens* (approximately 6.0 $\log_{10}$ CFU per gram granular pellet) were mixed with non-infested Redi-Earth planting medium at rates of 3% (w/w) and 1% (w/w), respectively, where indicated. Cucumber seeds coated with the peat-bond formulation with bacteria (approximately 8.0 $\log_{10}$ CFU per seed) and without bacteria, were sown into the infested Redi-Earth in $18 \times 12 \times 6.6$-cm flats at a rate of 21 seeds per flat in some experiments and 30 seeds per flat in other experiments. There were four replicate flats per treatment. Flats were arranged in a completely randomized design and incubated in the greenhouse at 27 °C. Mean percent plant stand per flat was determined at 28 d and differences between means determined by Tukey's Studentized Range Test in SAS (SAS Institute, Cary, NC). Experiments were analyzed independently.

2.5. Suppression of damping-off of cucumber caused by *P. ultimum*

To produce sporangia, Corn Meal Agar plates were inoculated with *P. ultimum* Puzc, incubated at 22 °C for 3 d, flooded with sterile soil extract (Ayers and Lumsden, 1975), and subsequently incubated at 22 °C for 14–28 d. Sporangia were incorporated into Redi-Earth as described previously at rates ranging between 0 and 600 sporangia per cm^3 (Roberts et al., 1997). Bacteria and *T. virens* isolates were applied to cucumber seed in the gelatin formulation. Seed treatments contained approximately 8.0 $\log_{10}$ CFU bacterial isolate per seed or 5.0 $\log_{10}$ CFU *T. virens* isolate per seed. Redi-Earth, Redi-Earth amended with sporangia of *P. ultimum* or SDW, treated seeds, and Redi-Earth amended with sporangia or SDW were added as sequential layers to 6-cm diam. cups. Eight replicate cups for each treatment were sown with five seeds each and incubated in a growth chamber at 22 °C with a 12 h photoperiod. Treatments were arranged in a completely randomized design. Mean percent plant stand per cup was determined after 14 d and means separated by Tukey's Studentized Range Test. Experiments were performed at least twice for treatments with some evidence of disease suppression and analyzed independently.

2.6. In vitro inhibition of *M. incognita*

Culture filtrates from bacterial isolates were prepared by growing bacteria in NB for 2 d at 22 °C and 200 rpm, centrifuging the cultures at $10{,}000 \times g$ for 20 min, and passing the supernatant through a 0.2 μm filter. Culture filtrates of *T. virens* GL21 were prepared by growing GL21 in Potato Dextrose Broth (PDB) for 8 d at 22 °C and 50 rpm.The culture was clarified by centrifugation at 10, $000 \times g$ and filtered through a 0.2 μm filter. Culture filtrates were tested in 24-well tissue culture plates for effects on *M. incognita* egg hatch as described previously (Nitao et al., 1999). Number of eggs applied to wells was consistent within an experiment and varied between 126 and 203 in the different experiments. Counts were made of total second stage juveniles (J2) in each well after approximately 14 d. Mean percent egg hatch for each treatment was determined and compared using Tukey's Studentized Range Test. The experiment was performed twice with six replicates and experiments were analyzed independently.

2.7. Suppression of *M. incognita* on cucumber

Four pot experiments were conducted in the greenhouse. In all experiments, bacteria and fungi were applied to cucumber seed in the gelatin formulation and the treated seeds were planted in pasteurized soil in 10-cm diam. pots in the greenhouse. In the first experiment, seed treatments consisted of non-treated seed, gelatin formulation without bacteria, and gelatin formulation with *S. marcescens* N2-4, *S. marcescens* N1-14, *Bacillus circulans* 99-10, *E. asburiae* 501R3, *Pantoea agglomerans* 0020, *B. pasteurii* 0022, *Pseudomonas chlororaphis* 0050, or *Acinetobacter radioresistens* 0055. In the second experiment, the treatments in the first experiment were used as well as treatments consisting of dead cells of *B. cepacia* BC-2 or dead cells of *B. ambifaria* BC-F. Treatments were applied as a seed treatment (approximately 7.0 $\log_{10}$ CFU per seed) in the gelatin formulation and a 10 ml drench (approximately 8.0 $\log_{10}$ CFU per ml) per plant 5 weeks after planting. In the third experiment, treatments consisted of *T. virens* GL3 and of *T. virens* GL21 applied as a seed treatment in the gelatin formulation (approximately 4.0 $\log_{10}$ CFU per seed) and a 10 ml drench in SDW (2.0 $\log_{10}$ CFU per ml)

per plant 5 weeks after planting. In the first three experiments *M. incognita* (approx. 10,000 eggs in water) was applied to each pot at planting time. In the fourth experiment, treatments consisted of *T. virens* GL3, *T. virens* GL21, dead cells of *B. cepacia* BC-1 or BC-2, *S. marcescens* N1-14, *S. marcescens* N2-4, *P. agglomerans* 0020, GL3 plus dead cells of *B. cepacia* BC-1, GL3 plus dead cells of *B. cepacia* BC-2, GL3 plus *S. marcescens* N2-4, and GL3 plus *P. agglomerans* 0020. These treatments were applied as a seed treatment in the gelatin formulation (approximately 7.0 $\log_{10}$ CFU per seed for bacteria and 4.0 $\log_{10}$ CFU per seed for fungi), a root dip at transplant (approximately 8.0 $\log_{10}$ CFU per ml of bacteria and 3.0 $\log_{10}$ CFU per ml *T. virens*), and a 10 ml drench (approximately 8.0 $\log_{10}$ CFU per ml of bacteria and 5.0 $\log_{10}$ CFU per ml *T. virens*) per plant 3 weeks after transplant. At transplant the roots were dipped in bacterial or fungal suspensions in SDW and the plants were inoculated with *M. incognita*. Pots were arranged in a completely randomized design. In all experiments plants were harvested 8 weeks after application of *M. incognita* (2 life cycles for *M. incognita*). Roots and soil from the pots were processed for nematode eggs and J2 as described previously (Meyer et al., 2000, 2001). Mean shoot fresh weight, shoot dry weight, root fresh weight, plant height, number of fruit, fruit fresh weight, fruit dry weight, nematode eggs on roots, nematode eggs in soil, J2 on roots, and J2 in soil for each treatment were determined and compared by ANOVA. Each treatment was replicated six times with one plant per pot. Experiments were analyzed independently.

2.8. In situ compatibility assays

Bacterial isolates were grown in NB while rifampicin-resistant derivative strains were grown in NB plus 100 μg rifampicin per ml for 48 h at 22 °C and 200 rpm. Cultures were centrifuged, washed, and resuspended in SDW. *T. virens* GL3 and GL21 biomass was prepared as described above and resuspended in SDW. Suspensions (40 μl) of bacteria or fungi were combined with a 40 μl suspension containing another isolate or 40 μl SDW, mixed, and applied to individual cucumber seeds in 2 ml Redi-Earth in 14 ml sterile snap-capped tubes (Roberts et al., 1992). Bacterial isolates applied to cucumber seed ranged from 7.82 to 7.63 $\log_{10}$ CFU per seed, while *T. virens* isolates ranged from 5.77 to 5.33 $\log_{10}$ CFU per seed. Experiments were performed with sterile Redi-Earth when populations of *T. virens* were monitored. Controls were cucumber seeds in Redi-Earth without added bacteria or fungi. Tubes were incubated at 22 °C with a 12 h photoperiod in the growth chamber for 10 to 12 d. Populations of monitored bacterial strains were determined by dilution-plating onto NB agar containing 100 μg rifampicin per ml. Populations of *T. virens* isolates were determined by dilution-plating onto TME agar. Experiments were performed twice for each strain pair with six replicates arranged in a completely randomized design. Means with standard deviation were determined from independently analyzed experiments.

3. Results

3.1. Identity of Serratia isolates

Isolates N1-6, N1-8, N1-14, N2-4, N2-7, N4-1, N4-13, and N4-19 were identified as *S. marcescens* by analysis of cellular fatty acids with the exception of N2-4 (Table 1). Isolate N2-4 was identified as *C. davisae* with a percent match of 0.258. Isolates N1-6, N1-8, N1-14, N2-4, and N4-1 were further characterized by sequencing approximately 1425 bp of the 16S rDNA gene from each of these strains. All five isolates had $\geqslant$99% DNA sequence identity with the *S. marcescens* 16S rDNA gene in the database. Isolate N2-4 was determined to be *S. marcescens* due to the low, and therefore inconclusive, percent match obtained when identification was based on analysis of cellular fatty acids.

3.2. Suppression of damping-off of cucumber caused by R. solani

S. marcescens isolates N1-6, N1-8, N1-14, N2-4, N3-12, N4-1, N4-13, N4-19, *B. cepacia* BC-1, *B. ambifaria* BC-F, and *Enterobacter asburiae* 501R3 were screened for suppression of damping-off caused by *R. solani* in greenhouse bioassays (Table 2). These bacteria were applied as a seed treatment in the peat-bond formulation in these bioassays. *B. cepacia* BC-1 provided significant suppression ($P \leqslant 0.05$) relative to the pathogen check in two of two assays while *B. ambifaria* BC-F and *S. marcescens* isolates N1-6, N1-14, N2-4, N4-1, and N4-13 provided significant suppression in one of two assays. There was no evidence of phytotoxicity with any of the bacterial isolates applied as a seed treatment in the peat-bond formulation. Percent plant stand with these bacterial seed treatments in the absence of *R. solani* was always similar ($P > 0.05$) to that of the healthy check (data not shown).

B. ambifaria BC-F, *B. cepacia* BC-1, and *S. marcescens* isolates N1-6, N1-14, and N2-4 were selected for a second series of bioassays where these bacteria, applied as seed treatments in the peat-bond formulation, were compared with each other (Table 3). In the first experiment, treatments containing *B. cepacia* BC-1 and *S. marcescens* N1-14 both resulted in mean percent plant stands that were similar to the healthy check and significantly greater ($P \leqslant 0.05$) than that of the peat-bond-only check and the pathogen check. Seeds treated

Table 2
Experimental trials with bacterial isolates for suppression of damping-off of cucumber caused by *R. solani*

Treatment[a]	*R. solani*[b]	Mean percent plant stand per flat[c]							
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8
Healthy Check	–	96.7 A	96.7 A	96.7 A	97.8 A	96.7 A	82.2 A	87.8 A	95.6 A
B. cepacia BC-1	+	70.0 AB	47.8 B						
S. marcescens N4-19	+	54.4 BC	18.9 CD						
S. marcescens N4-13	+	50.0 BC	28.9 BC						
S. marcescens N1-6	+			55.6 B	36.7 BC				
S. marcescens N1-14	+			52.2 B	48.9 B				
S. marcescens N2-4	+			43.3 BC	41.1 B				
S. marcescens N1-8	+			26.7 C	22.2 CD				
S. marcescens N3-12	+					61.1 B	8.9 CD		
S. marcescens N4-1	+					30.0 C	31.1 BC		
B. ambifaria BC-F	+							36.7 B	52.2 B
E. asburiae 501R3	+							26.7 BC	35.6 BC
Pathogen Check	+	27.8 C	1.1 D	34.4 BC	15.6 D	37.8 BC	3.3 D	11.1 C	38.9 BC

[a]Healthy check and pathogen check, naked seed in the absence and presence of inoculum of *R. solani*, respectively. Bacteria were applied at approximately 8.0 $\log_{10}$ CFU per seed in a peat-bond formulation.
[b]Presence (+) or absence (−) of *R. solani* R-23A. *R. solani* infested Redi-Earth was applied to Redi-Earth planting medium at a rate of 3% (w/w).
[c]Mean percent stand per flat. Plant stand was determined 4 weeks after sowing cucumber seed. Treatments within an experimental trial followed by the same letter are not significantly different ($P \leqslant 0.05$) as determined by Tukey's Studentized Range Test. A blank entry in a trial indicates that this strain was not tested in that particular trial.

Table 3
Comparison of bacterial isolates with each other for suppression of damping-off of cucumber caused by *R. solani*

Treatment[a]	*R. solani*[b]	Mean percent plant stand per flat[c]	
		Experiment 1	Experiment 2
Healthy check	–	98.9 A	100.0 A
B. ambifaria BC-F	+	64.4 BC	100.0 A
B. cepacia BC-1	+	76.7 AB	84.1 A
S. marcescens N1-6	+	55.6 BCD	ND[d]
S. marcescens N1-14	+	71.1 AB	41.2 B
S. marcescens N2-4	+	47.8 BCD	ND
Peat-bond only check	+	38.9 CD	30.2 B
Pathogen check	+	31.1 D	ND

[a]Healthy check and pathogen check, naked cucumber seed in the absence and presence, respectively, of *R. solani* inoculum; peat-bond only check, cucumber seed treated with the sterile peat-bond formulation. Bacteria were applied at approximately 8.0 $\log_{10}$ CFU per seed in the peat-bond formulation.
[b]Presence (+) or absence (−) of *R. solani* R-23A. *R. solani* infested Redi-Earth was applied to Redi-Earth planting medium at a rate of 3% (w/w).
[c]Mean percent stand per flat. Plant stand was determined 4 weeks after sowing cucumber seed. Treatments within an experiment followed by the same letter are not significantly different ($P \leqslant 0.05$) as determined by Tukey's Studentized Range Test.
[d]ND, not determined in this experiment.

with *B. ambifaria* BC-F had a mean percent plant stand that was significantly greater ($P \leqslant 0.05$) than the pathogen check. Treatments containing *S. marcescens* N1-6 or N2-4 did not provide disease suppression. In the second experiment, treatments containing *B. ambifaria* BC-F and *B. cepacia* BC-1 provided mean percent plant stands that were similar ($P > 0.05$) to each other and to the healthy check, and significantly greater ($P \leqslant 0.05$) than the peat-bond-only check. The treatment containing *S. marcescens* N1-14 had a mean percent plant stand that was similar to that of the peat-bond-only check. *B. cepacia* BC-1 was the best performing bacterial isolate providing significant disease suppression in four of four bioassays.

3.3. Suppression of damping-off of cucumber caused by *P. ultimum*

All bacterial isolates listed in Table 1, with the exception of the rifampicin-resistant derivative strains, were screened for suppression of damping-off of cucumber caused by *P. ultimum* (using the assay system depicted in Table 4). All isolates of *S. marcescens* (N1-6, N1-8, N1-14, N2-4, N2-7, N4-1, N4-13, N4-19) provided superior control when applied to cucumber seed in a gelatin formulation in two of two screening trials (data not shown). In all cases mean percent plant stand per cup was statistically similar ($P > 0.05$) to that of the healthy check when these isolates of *S. marcescens* were applied as a seed treatment. A subset of these *S. marcescens* isolates (N1-6, N1-14, N2-4, N4-1) and other bacterial isolates that provided significant suppression of damping-off in two of two screening trials (data not shown) were tested together in biocontrol assays with different levels of inoculum of *P. ultimum*

Table 4
Suppression of damping-off of cucumber caused by *Pythium ultimum* with beneficial bacteria applied individually in seed treatments

Treatment[b]	Mean percent plant stand per cup[a]					
	Experiment 1			Experiment 2		
Level of *P. ultimum*[c]:	0	40	60	0	40	300
Gelatin only	97.5 A	15.0 CDE	5.0 DE	97.5 A	0.0 E	0.0 E
Serratia marcescens N4-1	ND[d]	87.5 A	82.5 A	ND	52.5 ABCDE	0.0 E
Serratia marcescens N1-6	ND	92.5 A	52.5 ABCD	ND	60.0 AB	2.5 DE
Serratia marcescens N1-14	ND	87.5 A	55.0 ABC	ND	57.5 ABC	12.5 BCDE
Serratia marcescens N2-4	ND	85.0 A	70.0 AB	ND	55.0 ABCD	27.5 BCDE
Burkholderia ambifaria BC-F	ND	90.0 A	57.1 ABC	ND	32.5 BCDE	2.5 DE
Klebsiella planticola C0014	ND	28.6 BCDE	2.5 E	ND	ND	ND
Enterobacter asburiae 501R3	ND	55.0 ABC	0.0 E	ND	15.0 BCDE	0.0 E
Pantoea agglomerans 0020	ND	15.0 CDE	2.5 E	ND	ND	ND
Bacillus pasteurii 0022	ND	17.5 CDE	15.0 CDE	ND	ND	ND
Pseudomonas chlororaphis 0050	ND	17.5 CDE	2.5 E	ND	ND	ND
Acinetobacter radioresistens 0055	ND	0.0 E	0.0 E	ND	ND	ND
Streptomyces spp. 99-60	ND	12.5 CDE	10.0 CDE	ND	0.0 E	0.0 E

[a]Values are the mean of eight replicates each containing five seeds expressed as percent plant stand per cup. Numbers followed by the same letter are not significantly different ($P \leqslant 0.05$) within an experiment as determined by the Tukey's Studentized Range Test.
[b]Bacteria were applied in a gelatin formulation at approximately 8.0 $\log_{10}$ CFU per seed. Gelatin only indicates seeds were treated with sterile gelatin without bacteria. No isolates were found to by phytotoxic in previous experiments.
[c]Quantity of sporangia of *P. ultimum* per cm^3 of Redi-Earth inoculum.
[d]ND, not determined.

(Table 4). In the first experiment, *B. ambifaria* BC-F and the *S. marcescens* isolates N1-14, N2-4, and N4-1 were superior to the other strains providing significant suppression of damping-off relative to the gelatin-only check at both levels of *P. ultimum* inoculum tested (Table 4). *S. marcescens* N1-6 provided significant suppression of damping-off at the lower inoculum level. In the second experiment, only *S. marcescens* isolates N1-6, N1-14, and N2-4 provided significant suppression of damping-off relative to the gelatin-only check at the lower inoculum level. No seed treatments provided significant disease suppression at the high pathogen inoculum level (Table 4). No bacterial isolates listed in Table 4 were found to be phytotoxic in previous bioassays (data not shown). *S. marcescens* N1-6, N1-14, and N2-4 were the best performing isolates providing significant suppression of damping-off caused by *P. ultimum* in four of four bioassays.

S. marcescens N1-6 was selected for a third series of bioassays where this bacterium, as well as *B. cepacia* BC-1, and *T. virens* isolates GL3 and GL21, which had shown promise for suppression of *R. solani* (Lewis and Lumsden, 2001), were compared with regard to suppression of *P. ultimum* on cucumber when applied as a seed treatment in the gelatin formulation (Table 5). *B. cepacia* BC-2 was included because it was effective in suppression of *M. incognita* on another crop species (Meyer et al., 2001). All isolates tested provided suppression of damping-off that was significantly greater ($P \leqslant 0.05$) than the gelatin-only check at one or more pathogen inoculum levels in both experiments.

3.4. Suppression of *M. incognita*

In vitro inhibition experiments were conducted with isolates shown to be effective in suppressing damping-off of cucumber caused by *P. ultimum* and/or *R. solani*. Culture filtrates from *B. cepacia* BC-1 and *S. marcescens* isolates N1-6, N1-14, and N2-4 grown in NB significantly suppressed ($P \leqslant 0.05$) egg hatch of *M. incognita* in vitro. Egg hatch was 11%, 6%, 4%, and 6%, respectively, while egg hatch in the sterile NB control was 30%. Culture filtrate from *T. virens* GL21 grown in PDB also significantly suppressed ($P \leqslant 0.05$) egg hatch of *M. incognita*. Egg hatch was 13% with culture filtrates from *T. virens* GL21 and 75% with the sterile PDB control. Similar results were obtained in a second experiment except that culture filtrates from *B. cepacia* BC-1 grown in NB did not significantly suppress egg hatch.

Treatments containing individual applications of several bacterial isolates (Table 1), *T. virens* GL3, *T. virens* GL21, or autoclaved cells of *B. ambifaria* BC-F or *B. cepacia* isolates BC-1 or BC-2 were tested for suppression of populations of *M. incognita* on cucumber in greenhouse bioassays. No microbial treatment significantly suppressed populations of *M. incognita* or improved plant vigor relative to the pathogen check in any experiment (data not shown).

3.5. In situ compatibility

Most combinations of isolates effective in suppression of damping-off caused by *P. ultimum* and/or *R. solani*

Table 5
Suppression of damping-off of cucumber caused by *P. ultimum* with beneficial microorganisms applied individually

Treatment[b]	Mean percent plant stand per cup[a]					
	Experiment 1			Experiment 2		
Level of *P. ultimum*[c]:	0	15	70	0	70	300
Gelatin only	97.5 AB	40.0 CD	2.5 D	95.0 A	7.5 C	2.5 C
B. cepacia BC-1	97.5 AB	82.5 AB	60.0 BC	100.0 A	60.0 AB	37.5 BC
B. cepacia BC-2	100.0 A	80.0 AB	75.0 ABC	97.5 A	75.0 AB	55.0 AB
S. marscesens N1-6	100.0 A	97.5 AB	95.0 AB	97.5 A	92.5 A	77.5 AB
T. virens GL3	97.5 AB	97.5 AB	97.5 AB	100.0 A	82.5 A	67.5 AB
T. virens GL21	100.0 A	67.5 ABC	90.0AB	95.5 A	97.5 A	97.5 A

[a]Values are the mean of eight replicates each containing five seeds expressed as percent plant stand per cup. Numbers followed by the same letter are not significantly different ($P \leqslant 0.05$) within a particular experiment as determined by the Tukey's Studentized Range Test.
[b]Microbes were applied in a gelatin formulation at approximately 8.0 $\log_{10}$ CFU per seed for bacteria and 5.0 $\log_{10}$ CFU per seed for *T. virens* isolates. Gelatin only indicates seeds were treated with sterile gelatin without bacteria.
[c]Quantity of sporangia of *P. ultimum* per cm^3 of Redi-Earth inoculum.

were compatible when coincubated in cucumber rhizosphere (Table 6). Populations of monitored strains (*B. cepacia* isolates BC-1R1 and BC-2R2, *B. ambifaria* BCF-R8, *S. marcescens* isolates N1-14R5 and N2-4R1, *T. virens* isolates GL3 and GL21) were compared among treatments. These strains were incubated alone or coincubated with a second strain for 10–12 d in cucumber rhizospheres. Only populations of *B. cepacia* BC-2R2 coincubated with *B. ambifaria* BC-F, *S. marcescens* N1-14R5 coincubated with *B. ambifaria* BC-F, and *T. virens* GL21 coincubated with *B. ambifaria* BC-F were slightly reduced relative to treatments containing *B. cepacia* BC-2R2, *S. marcescens* N1-14R5, or *T. virens* GL21 applied alone, respectively. In addition, populations of *T. virens* isolates GL3 and GL21 were both substantially reduced after coincubation with *B. cepacia* BC-1 and *S. marcescens* isolates N1-14 and N2-4. No *T. virens*-like colonies were detected after 10 d in both runs of this experiment. Populations of monitored strains were unaffected by coincubation with paired strains in all other combinations (Table 6).

3.6. Strain combinations for suppression of damping-off and M. incognita on cucumber

B. cepacia BC-1, *B. ambifaria* BC-F, and *S. marcescens* isolates N1-14 and N2-4, applied as seed treatments in the peat-bond formulation, were tested alone and in combination with *T. virens* GL21 applied as a granular formulation, with regard to suppression of damping-off caused by *R. solani*. Combining BC-1 with GL21 resulted in improved suppression of damping-off caused by *R. solani* ($P \leqslant 0.05$) over treatments containing individual applications of these microbes in two of two experiments. In the first experiment application of *B. cepacia* BC-1 in combination with *T. virens* GL21 resulted in a mean percent plant stand that was similar

Table 6
In situ compatibility of paired isolates in cucumber rhizosphere[a]

Treatment[b]		Log_{10} CFU monitored strain[c]		Log_{10} CFU paired strain
Monitored strain	Paired strain	Initial	Final	Initial
	SDW		8.04	–
	BC-2		7.96	7.76
	BC-F		8.04	7.68
BC-1R1	N1-14	7.87	7.97	7.63
	N2-4		8.15	7.75
	GL3		8.18	5.68
	GL21		8.19	5.33
	SDW		8.02	–
	BC-1		7.71	7.82
	BC-F		7.38**	7.68
BC-2R2	N1-14	7.74	8.09	7.63
	N2-4		7.82	7.75
	GL3		8.10	5.68
	GL21		8.04	5.33
	SDW		8.04	–
	BC-1		7.62	7.82
	BC-2		8.02	7.76
BC-FR8	N1-14	7.70	8.25	7.63
	N2-4		8.08	7.75
	GL3		8.12	5.68
	GL21		8.29	5.33
	SDW		7.51	–
	BC-1		7.62	7.72
	BC-2		7.34	7.70
N1-14R5	BC-F	7.82	7.11**	7.65
	GL3		7.26	5.77
	GL21		7.40	5.60
	SDW		7.13	–
	BC-1		7.16	7.72
	BC-2		7.12	7.70

Table 6 (*continued*)

Treatment[b]		Log_{10} CFU monitored strain[c]		Log_{10} CFU paired strain
Monitored strain	Paired strain	Initial	Final	Initial
N2-4R1	BC-F	7.22	7.26	7.65
	GL3		7.40	5.77
	GL21		7.54	5.60
	SDW		6.35	–
	BC-1		BDT[d]**	7.01
	BC-2		6.50	7.80
GL3	BC-F	6.41	6.35	7.76
	N1-14		BDT**	7.82
	N2-4		BDT**	7.79
	SDW		6.51	–
	BC-1		BDT**	7.91
	BC-2		6.46	7.80
GL21	BC-F	6.09	6.17**	7.76
	N1-14		BDT**	7.82
	N2-4		BDT**	7.79

[a]Results of a single experiment with these treatments. All treatments were replicated six times and were tested in two independent experiments. Asterisks indicate the mean of the monitored strain in the presence of this paired strain is greater than one standard deviation lower than the mean of the monitored strain added to cucumber seed with SDW only.

[b]BC-1R1, *B. cepacia* BC-1R1; BC-2R2, *B. cepacia* BC-2R2; BC-FR8, *B. ambifaria* BC-FR8; N1-14R5, *S. marcescens* N1-14R5; N2-4R1, *S. marcescens* N2-4R1; GL3, *T. virens* GL3; GL21, *T. virens* GL21; SDW, sterile distilled water; BC-1, *B. cepacia* BC-1; BC-2, *B. cepacia* BC-2; BC-F, *B. ambifaria* BC-F; N1-14, *S. marcescens* N1-14; and N2-4, *S. marcescens* N2-4.

[c]Populations were determined by dilution-plating onto NA plus 100 ug per ml rifampicin for bacterial isolates and TME for *T. virens* GL3 and GL21. Final populations of the monitored strain were incubated 10 to 12 d in cucumber rhizosphere after application with SDW or a second strain.

[d]BDT, below detectable threshold; <2.00 log_{10} CFU per plant.

to the healthy check (92.2 mean percent stand; $P>0.05$) and significantly greater ($P\leqslant0.05$) than the no seed treatment + no pellet check in the presence of *R. solani* (Table 7). Mean percent plant stand per flat with this combination treatment was also significantly greater ($P\leqslant0.05$) than the peat-bond-only check and the sterile pellet check in the presence of *R. solani*. Individual application of *B. cepacia* BC-1 or *T. virens* GL21 did not provide effective disease suppression. Mean percent plant stand per flat with these two treatments was similar ($P>0.05$) to the no seed treatment + pellet check in the presence of *R. solani*. In the second experiment, the combined application of *B. cepacia* BC-1 with *T. virens* GL21 showed slight improvement over individual application of these microbes. Mean percent plant stand per flat was 98.4%, 98.4%, 87.3%, 73.0%, and 47.6% for the healthy check, the combination of BC-1 with GL21, the individual application of GL21, the individual application of BC-1, and the no pellet + no seed treatment check in the presence of *R. solani*, respectively. All treatments were significantly greater ($P\leqslant0.05$) than the no seed treatment + no pellet check in the presence of *R. solani*.

Combining *B. ambifaria* BC-F with *T. virens* GL21 improved suppression of damping-off caused by *R. solani* over individual application of these microbes in the first experiment (Table 8). This combined treatment was the only treatment significantly greater ($P\leqslant0.05$) than the no seed treatment + no pellet check in the presence of *R. solani*. Mean percent plant stand per flat in the second experiment was 97.8%, 88.9%, 87.3%, 85.7%, and 47.6% for the healthy check, the combination of BC-F with GL21, the individual application of GL21, the individual application of BC-F and the no pellet + no seed treatment check in the presence of *R. solani*, respectively. All microbial treatments were similar ($P>0.05$) and significantly greater than the no pellet + no seed treatment check in the presence of *R. solani*.

In no case did combining isolates *B. cepacia* BC-1, *B. ambifaria* BC-F, or *S. marcescens* isolates N1-14 or N2-4 (data not shown) with *T. virens* GL21 result in decreased suppression of damping-off caused by *R. solani* relative to individual application of these microbes. There was a negative interaction in certain experiments with the sterile granular pellet formulation where disease was enhanced when these pellets were applied (Tables 7 and 8). It is possible that nutrients in this granular formulation stimulated activity by *R. solani* and enhanced disease.

Combinations of bacterial isolates with *T. virens* GL3 or GL21 were tested for suppression of damping-off caused by *P. ultimum* under conditions of high pathogen inoculum (Table 9). All beneficial microbes were applied together in seed treatments in the gelatin formulation. Combinations of *T. virens* GL3 with these bacterial isolates performed inconsistently. *T. virens* GL3 performed very well in the first experiment, resulting in 95% mean percent plant stand per cup. Application of bacterial isolates in combination with *T. virens* GL3 did not decrease effectiveness of this isolate in this experiment. *T. virens* GL3 applied individually did not provide biological control in the second experiment. Combining bacterial isolates with GL3 improved disease suppression in all cases in this experiment. In the third experiment, combining *T. virens* GL3 with bacterial isolates resulted in slight to substantial decreases in disease suppression relative to individual application of GL3.

Combining *T. virens* GL21 with *B. ambifaria* BC-F resulted in improved suppression of damping-off caused by *P. ultimum* relative to individual application of these microbes in experiments 2 and 3 (Table 9). In these two

Table 7
Suppression of damping-off of cucumber caused by *R. solani* with *B. cepacia* BC-1 or *T. virens* GL21 applied individually and in combination[a]

Seed treatment	Mean percent stand per flat[b]		
	No pellet	Pellet without *T. virens* GL21	Pellet with *T. virens* GL21
No seed treatment	10.0 CDE	0.0 E	53.3 ABC
Peat-bond without bacteria	24.4 CDE	3.3 DE	46.7 BCD
Peat-bond with *B. cepacia* BC-1	52.2 ABC	0.0 E	92.2 A
Peat-bond with *S. marcescens* N1-14	35.6 BCDE	11.1 CDE	70.0 AB

[a]All treatments were tested in the presence of *R. solani* isolate R-23A infested Redi-Earth inoculum applied at a rate of 3% (w/w). Bacteria were applied at approximately 8.0 $\log_{10}$ CFU per seed in a peat-bond formulation. Granular pellets containing *T. virens* GL21 (6.0 $\log_{10}$ CFU per gram granular pellet) were applied to Redi-Earth planting medium at a rate of 1% (w/w). Results are from a single experiment.

[b]Mean percent plant stand per flat was determined 4 weeks after sowing cucumber seed. Treatments followed by the same letter are not significantly different ($P \leqslant 0.05$) as determined by the Tukey's Studentized Range Test. Mean percent plant stand in the Healthy Check (no seed treatment, no pellet, no *R. solani* inoculum) was 92.2%.

Table 8
Suppression of damping-off of cucumber caused by *R. solani* with *B. ambifaria* BC-F or *T. virens* GL21 applied individually and in combination[a]

Seed treatment	Mean percent stand per flat[b]		
	No pellet	Pellet without *T. virens* GL21	Pellet with *T. virens* GL21
No seed treatment	35.6 BC	0.0 C	74.4 AB
Peat-bond without bacteria	52.2 B	0.0 C	73.3 AB
Peat-bond with *B. ambifaria* BC-F	54.4 B	0.0 C	96.7 A

[a]All treatments were tested in the presence of *R. solani* isolate R-23A infested Redi-Earth inoculum applied at a rate of 3% (w/w). Bacteria were applied at approximately 8.0 $\log_{10}$ CFU per seed in a peat-bond formulation. Granular pellets containing *T. virens* GL21 (6.0 $\log_{10}$ CFU per gram granular pellet) were applied to Redi-Earth planting medium at a rate of 1% (w/w). Results are from a single experiment.

[b]Mean percent plant stand per flat was determined 4 weeks after sowing cucumber seed. Treatments followed by the same letter are not significantly different ($P \leqslant 0.05$) as determined by the Tukey's Studentized Range Test. Mean percent plant stand in the Healthy Check (no seed treatment, no pellet, no *R. solani* inoculum) was 97.8%.

Table 9
Biocontrol agents applied alone and in combination for suppression of damping-off of cucumber caused by *P. ultimum*

Treatment	PU[b]	Mean percent plant stand per cup[a]		
		Experiment 1	Experiment 2	Experiment 3
Gelatin only	−	100.0 A	92.5 A	75.0 AB
Gelatin only	+	5.0 D	12.5 C	7.5 D
T. virens GL3	+	95.0 A	70.0 ABC	82.5 A
T. virens GL3 + *B. cepacia* BC-1	+	100.0 A	85.7 AB	65.0 ABC
T. virens GL3 + *B. cepacia* BC-2	+	87.5 A	85.0 AB	67.5 AB
T. virens GL3 + *B. ambifaria* BC-F	+	100.0 A	92.5 A	50.0 ABCD
T. virens GL3 + *S. marcescens* N1-14	+	ND[c]	85.0 AB	65.0 ABC
T. virens GL21	+	80.0 AB	70.0 ABC	20.0 CD
T. virens GL21 + *B. cepacia* BC-1	+	52.5 ABCD	80.0 AB	42.5 ABCD
T. virens GL21 + *B. cepacia* BC-2	+	77.5 AB	60.0 ABC	20.0 CD
T. virens GL21 + *B. ambifaria* BC-F	+	72.5 ABC	80.0 AB	62.5 ABC
T. virens GL21 + *S. marcescens* N1-14	+	ND	77.5 AB	50.0 ABCD
B. cepacia BC-1	+	22.5 CD	37.5 ABC	22.5 BCD
B. cepacia BC-2	+	35.0 BCD	32.5 BC	22.5 BCD
B. ambifaria BC-F	+	72.5 ABC	67.5 ABC	20.0 CD
S. marcescens N1-14	+	ND	75.0 AB	50.0 ABCD

[a]Values are the mean of eight replicates each containing five seeds expressed as percent plant stand per cup. Numbers followed by the same letter are not significantly different ($P \leqslant 0.05$) within a particular experiment as determined by the Tukey's Studentized Range Test.

[b]PU, *P. ultimum*; −, no *P. ultimum* inoculum added; +, *P. ultimum* inoculum added. Levels of inoculum used were: 600 sporangia per cm^3 in experiment 1; 250 sporangia per cm^3 in experiment 2; and 600 sporangia per cm^3 in experiment 3.

[c]ND, not determined.

experiments individual application of *T. virens* GL21 or *B. ambifaria* BC-F did not provide significant disease suppression while the combination treatment did. There was no significant effect with the combined treatment relative to individual application of GL21 and BC-F in the first experiment. Combining *T. virens* GL21 with the bacterial isolates *B. cepacia* BC-1, *B. cepacia* BC-2, or *S. marcescens* N1-14 did not consistently alter disease suppression relative to individual application of these microbes.

Treatments containing GL3 combined with bacterial isolates or dead cells of *B. cepacia* BC-1, *B. cepacia* BC-2, or *B. ambifaria* BC-F were applied as a seed treatment, a root dip at transplant, and a drench and screened for suppression of *M. incognita* on cucumber. None of these combination treatments suppressed populations of *M. incognita* or improved plant vigor (data not shown).

4. Discussion

B. ambifaria BC-F, *S. marcescens* isolates N1-6, N1-14, and N2-4 and *T. virens* isolates GL3 and GL21 had broad-spectrum activity against soilborne pathogens of cucumber. In addition to suppressing damping-off caused by the fungal pathogens *R. solani* and/or *P. ultimum*, culture filtrates from BC-F, N1-6, N1-14, N2-4, and GL3 and GL21 inhibited in vitro egg hatch by the nematode *M. incognita* in experiments reported here or elsewhere (Meyer et al., 2000; Li et al., 2002). This broad-spectrum activity is likely due, in part, to inhibitory metabolites produced by these organisms. Inhibitory metabolites produced by isolates of *Serratia* include pyrrolnitrin, oocydin A, carbapenem, prodigiosin, and serrawettin as well as chitinase and other cell-wall and cell-membrane degrading enzymes (Lindum et al., 1998; Asano et al., 1999; McGowan et al., 1999; Strobel et al., 1999; Kamensky et al., 2003). Isolates of *Burkholderia* have been shown to produce pyrrolnitrin, altericidins, and other compounds with anti-biotic activity (Kirinuki et al., 1984; Roitman et al., 1990; Burkhead et al., 1994; Kang et al., 1998). *T. virens* GL3 and GL21 produce the antibiotics glioviren and gliotoxin, respectively, as well as a number of other inhibitory metabolites (Lumsden et al., 1992; Howell et al., 1993). Certain of these compounds have broad-spectrum activity against microorganisms (Jones and Hancock, 1988; Burkhead et al., 1994; McGowan et al., 1999; Bennet and Bentley, 2000).

Combining certain microorganisms with broad-spectrum activity showed promise for increased consistency of suppression of damping-off caused by *R. solani* and *P. ultimum*. For example, in experiments directed at comparing application of individual versus combined microbial treatments, the combination of *B. ambifaria* BC-F with *T. virens* GL21 always provided significant biocontrol of these pathogens with these two assay systems. *T. virens* GL21 applied alone provided significant biocontrol of *R. solani* in one of two experiments and significant biocontrol of *P. ultimum* in one of three experiments. Likewise, *B. ambifaria* BC-F provided significant biocontrol of *R. solani* in one of two experiments and significant biocontrol of *P. ultimum* in one of three experiments. Combining microorganisms with broad-spectrum activity also showed promise for increasing the level of suppression of damping-off of cucumber caused by *R. solani*. Combining *B. cepacia* BC-1, applied as a seed treatment, with *T. virens* GL21, applied as a granular formulation, increased disease suppression in two of two experiments relative to individual application of these microbes. Combining *B. ambifaria* BC-F with *T. virens* GL21 increased the level of disease suppression in one of two assays relative to individual application of these microbes.

Further testing against a genetically diverse collection of *P. ultimum* and *R. solani* isolates under a wide range of environmental conditions is required before the full potential of these strain combinations for improved disease suppression is known. Tests reported here were conducted with a single isolate of each pathogen under a single set of environmental conditions. It is possible that treatments containing combinations of microbes will provide more consistent disease suppression due to the increased likelihood of expression of important traits by strain combinations under broader environmental conditions. Further tests also are required to completely rule out the possibility that improved disease suppression with certain combinations is due just to increased numbers of biocontrol agents applied in disease assays.

Combinations of microbes that resulted in enhanced suppression, or had no effect on suppression, of damping-off of cucumber were incompatible when coincubated for 10–12 d in cucumber rhizosphere (*T. virens* GL21 combined with *B. cepacia* BC-1, *S. marcescens* N1-14, or *S. marcescens* N2-4). It is possible that the short window of vulnerability of cucumber to damping-off in the *P. ultimum* biocontrol assays (Roberts et al., 1997) and/or the spatial separation (bacteria applied to the seed and *T. virens* mixed in the Redi-Earth) of biocontrol agents in the *R. solani* biocontrol assays allowed disease suppression despite antagonism among isolates. Antagonism among these strains may be more important when attempting to suppress pathogens, such as *M. incognita*, where long-term coexistence of the biocontrol agents in the rhizosphere is likely necessary.

The isolates studied here should be a valuable resource for investigations regarding mechanisms leading to incompatibility among biocontrol strains used in strain mixtures. Incompatibility between *T. virens* isolates and bacteria varied among related strains. The related strains *B. cepacia* BC-1, *B. ambifaria* BC-F, and

B. cepacia BC-2 strongly inhibited, slightly inhibited, and had no effect, respectively, on populations of *T. virens* GL21 in cucumber rhizosphere. There is considerable genotypic diversity among certain related biocontrol agents and these microbes have been shown to vary with regard to antibiotic production and other phenotypes (Keel et al., 1996; Sharifi-Tehrani et al., 1998; McSpadden-Gardener et al., 2000; Raaijmakers and Weller, 2001). It is possible that differences such as these are responsible for differences in compatibility among closely related strains seen here. We are initiating genetic studies to determine the basis of incompatibility among these isolates.

Results reported here substantiate reports of increased biocontrol effectiveness with certain strain combinations (Raupach and Kloepper, 1998; Meyer and Roberts, 2002). However, these results also indicate that compatibility among strains needs to be carefully analyzed. Compatibility between particular strain pairs was determined with regard to suppression of damping-off caused by *R. solani* and by *P. ultimum* and with regard to coexistence in the cucumber rhizosphere. Compatibility between particular isolate pairs varied with the assay and possibly the method of application. Compatibility also varied among related microbes. Results presented here suggest that compatibility between particular isolates in strain combinations needs to be carefully determined for all applications of these combination treatments.

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