Efficacy of Plant Growth–Promoting Rhizobacteria, Acibenzolar-*S*-Methyl, and Soil Amendment for Integrated Management of Bacterial Wilt on Tomato

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ABSTRACT

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Greenhouse experiments were conducted to study the effect of plant growth promoting rhizobacteria (PGPR; *Bacillus pumilus* SE 34, *Pseudomonas putida* 89B61, BioYield, and Equity), acibenzolar-*S*-methyl (Actigard), and a soil amendment with S-H mixture (contains agricultural and industrial wastes such as bagasse, rice husk, oyster shell powder, urea, potassium nitrate, calcium super phosphate, and mineral ash) on bacterial wilt incidence caused by *Ralstonia solanacearum* (race 1, biovar 1) in susceptible tomato (*Lycopersicon esculentum* cv. Solar Set). In experiments with PGPR, *Pseudomonas putida* 89B61 significantly reduced bacterial wilt incidence when applied to the transplants at the time of seeding and 1 week prior to inoculation with *Ralstonia solanacearum*. BioYield, a formulated PGPR that contained two *Bacillus* strains, decreased disease significantly in three experiments. Equity, a formulation containing more than 40 different microbial strains, did not reduced wilt incidence compared with the untreated control. With inoculum at low pathogen densities of 1×10^5 and 1×10^6 CFU/ml, disease incidence of Actigard-treated plants was significantly less than with nontreated plants. This is the first report of Actigard-mediated reduction of bacterial wilt incidence in a susceptible tomato cultivar. When PGPR and Actigard applications were combined, Actigard plus *P. putida* 89B61 or BioYield reduced bacterial wilt incidence compared with the untreated control. Incorporation of S-H mixture into infested soil 2 weeks before transplanting reduced bacterial wilt incidence in one experiment. Combination of Actigard with the S-H mixture significantly reduced bacterial wilt incidence in tomato in two experiments.

Bacterial wilt disease caused by the soilborne plant pathogen *Ralstonia solanacearum* (race 1) is a major limiting factor in field-grown tomato (*Lycopersicon esculentum*) production in the southeastern United States and in tropical and subtropical areas of the world. This disease affects many solanaceous species and also several other plant families (7,12). In tomato, symptoms are characterized by wilting of upper leaves for a few days followed by complete wilting of the plants. Brown discoloration of the vascular tissues in the lower stem can also be observed in the wilted plants. Bacteria will ooze from fresh cut stems placed in clear water.

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Management of bacterial wilt in tomato and in other crops has been difficult. Even though integrated management, including cultural practices, crop rotation, and use of resistant cultivars, provides some limited success, the disease still threatens commercial tomato production in the southeastern United States and elsewhere (12). Plant essential oils such as thymol or palmarosa are effective biofumigants against *R. solanacearum* (20) but require development of a practical and economical application method for field use.

Biological control has emerged as one of the important methods in the management of soilborne plant pathogens. Biological control reduces the dependence on high-risk chemicals for disease management and is ecologically sound and environmentally friendly (1,28). Plant growth promoting rhizobacteria (PGPR) are potential agents for biological control of plant pathogens (10). PGPR bring about disease suppression by various modes of action such as antagonism, competition for space and nutrients, and induction of systemic resistance (10,13,27,29). Combining multiple PGPR has been found to suppress disease development in many crop plants against a broad range of soilborne plant pathogens (6,21). Attempts have also been made to use bacterial antagonists for management of bacterial wilt of tomato (3,8,9,17).

Amendment of soils with organic and inorganic substances has been practiced for managing many soilborne plant pathogens (4). S-H mixture is a formulated soil amendment from Taiwan, which contains agricultural and industrial wastes such as bagasse, rice husk, oyster shell powder, urea, potassium nitrate, calcium super phosphate, and mineral ash as components (25). Addition of S-H mixture to soil has been shown to reduce the pathogen population and disease severity in many crop plants (2,25). The major components of S-H mixture that contribute to the disease suppression, especially in the case of tomato bacterial wilt, are urea and mineral ash. Incorporation of the mixture into soil has been reported to reduce the population of *R. solanacearum* and suppress bacterial wilt in tomato (5,25).

Systemic acquired resistance (SAR) is the phenomenon by which defense mechanisms in plants are activated by contact with a pathogen or their metabolites or by a diverse group of structurally unrelated organic and inorganic compounds (11). Acibenzolar-*S*-methyl (Actigard, Syngenta, Basel, Switzerland) is a chemical compound that triggers SAR when applied to plants (16). SAR inducers are potential candidates for controlling bacterial diseases of many crops. Actigard has been reported to reduce bacterial spot and speck diseases on tomato (14) and fire blight on apple (15).

The objectives of the study were to discover the efficiency of PGPR (as single strain or formulation), S-H mixture, and Actigard in reducing the bacterial wilt incidence in tomato under greenhouse conditions. Also, combined effect of Actigard with PGPR or S-H mixture was evaluated against bacterial wilt in tomato.

MATERIALS AND METHODS

Bacterial cultures, inoculum preparation, and plants. *R. solanacearum* (race 1, biovar 1) tomato strain Rs5 (19), isolated in Quincy, FL, was used as the pathogen in this study. Pathogenicity of the strain Rs5 on tomato was confirmed by inoculating the susceptible tomato cultivar Solar Set.

The bacterium was grown on casaminoacid peptone glucose medium (CPG) (7) for 48 h or overnight (18 h) in CPG broth in a shaker at 200 rpm at 28°C. Sterile deionized water was used for suspending the bacterial cells, and the concentration of the inoculum was determined spectrophotometrically at 600 nm. Except for soil infestation in soil amendment/Actigard experiments, inoculation with the pathogen was performed by drenching 5 ml of bacterial suspension containing a desirable number of CFU per milliliter into the individual cells of the transplant flats. Inoculated plants were transplanted into pots containing plant growth medium 3 days after challenge inoculation. Plant growth medium, Terra-lite agricultural mix (Scott Sierra Horticultural Products Co., Marysville, OH), was used for growing plants. Seeds of the cultivar Solar Set were sown in expanded polystyrene transplant flats with 2.5 × 2.5 cm cells. Pots were placed in a saucer containing water to maintain high moisture content in the soil. Transplants and plants were fertilized with Peter's peat lite special (15:16:17 N-P-K) solution prepared in water (7.5 g/liter) at 10-day intervals. Plants were maintained in a greenhouse with a night temperature of 23 to 28°C and a day temperature of 30 to 35°C. All experiments were repeated twice.

PGPR experiments. PGPR strains *Bacillus pumilus* SE 34 and *Pseudomonas putida* 89B61 were grown on tripticase soy agar (TSA) (Becton Dickinson and Co., Cockeysville, MD) and Pseudomonas Agar F (Difco Laboratories, Detroit, MI), respectively, for 48 h at 28°C. Cells were harvested by scraping them from the agar surface with a glass spreader after drenching the plates with 10 ml of sterile deionized water. The concentration of bacterial cells in the suspension was adjusted by diluting with sterile deionized water, and the concentration in CFU per milliliter was determined spectrophotometrically at 600 nm. PGPR strains *Bacillus pumilus* SE 34 and *Pseudomonas putida* 89B61 are known to induce systemic resistance against fungal, bacterial, and viral plant pathogens in tomato and cucumber (27,29).

Two products containing PGPR were also tested. BioYield flowable (Gustafson, LLC, Dallas, TX) is a formulation that contains two *Bacillus* strains, namely *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a. Equity (Naturize Inc., Jacksonville, FL) is a formulated product containing more than 40 different microbial strains. These two products induce systemic resistance (22,23) on tomatoes (J. W. Kloepper, *personal communication*). Treatments are listed in Table 1.

For evaluating the effect of PGPR strains and formulated PGPR products on bacterial wilt incidence, seeds were sown in separate flats having 32 cells each. Seeds were rinsed three times with deionized water before sowing. For first PGPR application, 5 ml of each PGPR suspension containing approximately 5×10^8 CFU/ml was applied to the seeds in each cell as drench. The suspensions of BioYield (10 ml/liter) and Equity (2.5 ml/liter) in deionized water were used for treating seeds as drench. Some treatments had a second application to further enhance the possibility of induced resistance (Table 1). The second application of PGPR was applied 7 days before inoculation with the pathogen. The pathogen was introduced by drenching each transplant flat cell (2.5 × 2.5 cm) with a 5-ml *R. solanacearum* suspension containing 6×10^7 CFU/ml when the transplants were 4 weeks old with four to five true leaves. Plants were transplanted 3 days after challenge inoculation into 10-cm pots containing moistened soil.

Actigard experiments. Acibenzolar-*S*-methyl (Actigard 50 WG, Novartis Crop Protection Inc., Greensboro, NC) was applied as a drench to the base of the plants as well as foliar spray. Initial foliar treatment was applied 14 days after seed germination and was followed by a second application, both of foliar spray and soil drench, 5 days prior to inoculation with the pathogen. For foliar application, a concentration of 56 mg of Actigard per liter of water was used. Leaves were sprayed with a hand-held sprayer till runoff. Tomato seedlings were drenched with 5 ml of Actigard solution (28 mg/liter) per cell of transplant flat. Concentrations were given as formulated product. Based on preliminary experiments, Actigard was more effective against *R. solanacearum* on the susceptible tomato cultivar if inoculum concentration is low (2×10^5 CFU/ml) and applied as foliar and drench solutions before inoculation (data not shown).

Development of bacterial wilt in the susceptible tomato cultivar Sun Set was studied by inoculating the seedlings with varying concentrations of *R. solanacearum*. Treatments are listed in Table 2. Seedlings were inoculated with *R. solanacearum* as previously described under "PGPR experiments". Inoculated plants were transplanted to Cone-tainers (Ray Leach "Cone-tainers", Stuewe and Sons, Inc., Corvallis, OR; 21 cm long, 3.8 cm diameter, and 165 ml capacity) filled with soil 3 days after inoculation. Four-week-old seedlings of tomato having four to five fully expanded leaves were transplanted to them and maintained on support trays.

Table 1. Final bacterial wilt incidence in tomato plants (cv. Solar Set) treated with plant growth promoting rhizobacteria (PGPR) strains and PGPR formulated products

Treatment[y]	PGPR applications	% Bacterial wilt[x]	
		Experiment 1	Experiment 2
SE 34	Seed	100.0 a	96.8 ab
SE 34	Seed and 2nd[z]	96.8 ab	87.5 bcd
89B61	Seed	81.2 b	78.1 ed
89B61	Seed and 2nd	43.7 c	53.1 f
BY	Seed	84.3 ab	81.2 cd
BY	Seed and 2nd	84.3 ab	68.7 e
EQTY	Seed	96.8 ab	96.8 ab
EQTY	Seed and 2nd	93.7 ab	90.6 abc
Untreated control	NA	100.0 a	100.0 a

[x] Final bacterial wilt incidence 30 days after inoculation of plants, mean of four replications having eight plants each, values followed by same letters in a column do not differ significantly according to Duncan's multiple range test ($P = 0.05$).
[y] PGPR strains: SE 34 = *Bacillus pumilus*, 89B61 = *Pseudomonas putida*, BY = BioYield, EQTY = Equity.
[z] Second PGPR applications were 7 days prior to inoculations with *Ralstonia solanacearum.*

Table 2. Final bacterial wilt incidence in tomato plants (cv. Solar Set) inoculated with different inoculum concentration of *Ralstonia solanacearum* as affected by Actigard applications

Treatment	% Bacterial wilt[x]			
	Inoculum concentration (CFU/ml)[y]			
	1×10^8	1×10^7	1×10^6	1×10^5
Actigard[z]	100.0 a	95.0 a	50.0 a	11.6 a
Untreated	100.0 a	96.6 a	73.3 b	30.0 b
Contrast	df	MS	*F*	*P* > *F*
Actigard vs. untreated	1	4,860	23.27	<0.001

[x] Final bacterial wilt incidence 30 days after inoculation of plants, three replicates with 10 plants per replicate for each experiment. Experiments were conducted twice (no statistical difference was found between experiments), and data from both experiments were combined for this analysis. Values followed by same letter in a column do not differ significantly according to *t* test ($P = 0.05$).
[y] Five milliliters of *R. solanacearum* applied to the base of each plant, transplanted after 3 days.
[z] Initial foliar treatment with Actigard was applied 14 days after seed germination and was followed by a second application, both of foliar spray and soil drench, 5 days prior to inoculation with *R. solanacearum*.

Water was provided from the bottom of the Cone-tainers as needed, and plants were fertilized as described earlier.

PGPR and Actigard experiments. Two experiments were conducted to evaluate the combined effect of PGPR and Actigard on bacterial wilt incidence. Seedlings were inoculated with *R. solanacearum* as previously described under "PGPR experiments". PGPR were applied twice in all treatments. Earlier PGPR experiments showed increased protection against the pathogen with two PGPR applications. First and second PGPR and Actigard applications were performed, and water and fertilizer were provided as described earlier. Treatments are listed in Table 3.

Soil amendment (S-H mixture) and Actigard experiments. S-H mixture was obtained from Wells Industrial Co., Ltd., Tainan, Taiwan. Incorporation of S-H mixture in the soil was done at a rate of 0.5% (vol/vol) by adding the required quantity of S-H mixture to polythene bags (90 × 50 × 20 cm) and mixing well. A suspension of *R. solanacearum* containing 5×10^8 CFU/ml was added to the soil to make the bacterial population 5×10^7 CFU/ml. The soil was again thoroughly mixed, and bags were maintained at room temperature. S-H mixture and *R. solanacearum* were added to the soil at three different time intervals: 14 days, 7 days, and 0 days prior to transplanting. Bags containing *R. solanacearum* alone were also prepared and kept as untreated controls for each time interval. "Cone-tainers" were filled with infested soil. Four-week-old tomato transplants, having four to five fully expanded leaves, were transplanted to them. Actigard was applied, and water and fertilizer were provided as described earlier. Treatments are listed in Table 4.

Disease assessment and statistical analysis. Disease incidence (percent bacterial wilt) was observed by counting the number of wilted plants in each experiment at weekly intervals as the proportion of wilted plants based on the initial number of plants. In all experiments, last disease assessment was made 30 days after inoculation. All experiments were designed as randomized complete block designs with four replicates and eight plants per replicate, except Actigard, for which inoculum density experiments were designed with three replicates and 10 plants per replicate. All experiments were repeated twice. Analysis of variance (ANOVA) was used to determine the effect of the treatments on bacterial wilt incidence. Duncan's multiple range test and orthogonal contrasts (only for data analysis in Table 2) were used for comparing the means, using the statistical package SAS version 8.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Effect of PGPR. Treatment of tomato plants with *P. putida* 89B61, *B. pumilus* SE 34, and BioYield resulted in reduction in bacterial wilt incidence compared with the untreated control. Two applications of *P. putida* 89B61 achieved the most reduction (approximately 50%) in disease incidence (Table 1). Two applications of *P. putida* 89B61 or BioYield were better than one application of the same treatment. Two applications of *B. pumilus* SE 34 reduced the disease incidence in PGPR experiment 2. Applications of Equity did not reduce wilt incidence compared with the untreated control. When applied twice, *P. putida* 89B61 or BioYield reduced disease incidence significantly in three out of four experiments (Tables 1 and 3).

Effect of Actigard. Lowering the bacterial inoculum concentration reduced bacterial wilt incidence in both Actigard and non-Actigard treatments (Table 2). Actigard experiments were repeated twice, and data were combined and analyzed, as there was no significant difference between the means of disease incidence at respective inoculum densities in both experiments. The incidence of disease was high (95 to 100%) for both treatments at higher inoculum concentrations of 1×10^7 and 1×10^8 CFU/ml. At lower inoculum concentrations of 1×10^6 and 1×10^5 CFU/ml, Actigard applied plants had significantly lower disease incidence (11.6 to 50%) compared with the non-Actigard plants (30 to 73.3%). When the data were analyzed with orthogonal contrasts with respect to Actigard treatment among the treated and untreated means, a significant difference was observed (Table 2).

Effect of PGPR and Actigard. In these experiments, PGPR were applied twice. Combination of Actigard with *P. putida* 89B61 or BioYield caused significant reduction in disease incidence compared with the untreated control (Table 3). Actigard enhanced this reduction significantly in experiment 1 (Table 3) when combined with BioYield. Equity combined with Actigard significantly reduced wilt incidence in experiment 2 (Table 3) compared with the untreated control, but no significant reduction was observed when Equity or Actigard was applied alone. The application of Actigard alone was not effective in reducing bacterial wilt incidence under high inoculum (10^7 CFU/ml) conditions (Table 3).

Effect of S-H mixture and Actigard. Planting tomato seedlings into soil infested with *R. solanacearum* (untreated controls) at 14, 7, and 0 days before transplanting caused similar disease incidence (Table 4). According to the wilt incidence results, when the pathogen was exposed for 14 days to S-H mixture before transplanting, disease was reduced significantly compared with the untreated control in experiment 1 (Table 4). However, the combination of 14-day S-H treatment with Actigard further enhanced the efficacy of 14-day S-H treatment in both experiments (Table 4). When the pathogen was exposed for 7 or 0 days to S-H mixture alone or in combination with Actigard before transplanting, no significant wilt reduction was observed compared with untreated controls (Table 4).

DISCUSSION

In this study, application of PGPR, *P. putida* 89B61, and *B. pumilus* SE 34 reduced bacterial wilt incidence significantly in tomato under greenhouse conditions, but the *P. putida* 89B61 was more consistent and effective against tomato bacterial wilt. Both biological agents have been shown to be effective in managing various diseases in several crops (6,27,29). Results of our studies showed that two applications of *P. putida* 89B61 or BioYield are an important factor that could enhance biological control activities. Increased colonization of the emerging roots by the biocontrol agents, which also serve as the major port of entry of the bacterial pathogen, might have prevented *R. solanacearum* from entering the host. *R. solanacearum* being a soilborne plant pathogen, effective colonization of the root system by the biocontrol agents would prevent the pathogen from attaching to the point of entry and proceeding further into the vascular tissue. Besides this, antagonistic action of the biocontrol agents may also play an important role when increased numbers of bacteria are present in the soil or rhizosphere. Applying these agents after transplanting may further increase the effectiveness of biological control. These bacterial agents might be inducing systemic resistance (ISR) or an-

Table 3. Percent bacterial wilt incidence in tomato plants (cv. Solar Set) treated with plant growth promoting rhizobacteria (PGPR) strains, formulated products, and Actigard

	% Bacterial wilt[x]	
Treatment[y]	Exp. 1	Exp. 2
SE 34	93.7 a	81.2 ab
SE 34 + Actigard[z]	84.3 ab	75.0 abc
89B61	84.3 ab	53.1 cd
89B61 + Actigard	68.7 b	46.8 d
BY	65.6 b	59.3 bcd
BY + Actigard	40.6 c	59.3 bcd
EQTY	100.0 a	78.1 abc
EQTY + Actigard	81.2 ab	75.0 bc
Actigard	96.8 a	96.8 a
Untreated control	100.0 a	96.8 a

[x] Final bacterial wilt incidence 30 days after inoculation of plants, mean of four replications having eight plants each, values followed by same letters in a column do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

[y] PGPR were applied twice (seed treatment and 7 days prior to inoculation with *Ralstonia solanacearum*): SE34 = *Bacillus pumilus*, 89B61 = *Pseudomonas putida*, BY = BioYield, EQTY = Equity.

[z] Initial foliar treatment with Actigard was applied 14 days after seed germination and was followed by a second application, both of foliar spray and soil drench, 5 days prior to inoculation with *R. solanacearum*.

tagonism against *R. solanacearum*; however, specifically designed experiments must be conducted to identify the mode(s) of action (26).

Multiple strains of PGPR as formulated product are thought to have increased efficiency in biological control compared with application of a single strain (18). BioYield contained spores of two bacterial strains. A combination of these bacteria with a chitosan carrier was previously found to influence plant growth in tomato, cucumber, tobacco, and pepper transplants and provided protection against bacterial spot and late blight in tomato, angular leaf spot of cucumber, and blue mold of tobacco (24). In this study, *P. putida* 89B61 performed better than Equity but similarly to BioYield. The bacterial components in BioYield provide both growth promotion and induced resistance (22,23); similar effects may be seen in the *R. solanacearum*–tomato pathosystem. Involvement of ISR by BioYield against bacterial wilt disease also needs to be investigated further with split-root-system assay, as *R. solanacearum* is a soilborne pathogen. Equity, a bacterial mixture formulated in a complex liquid food base, was not effective when applied alone. The bacterial strains in Equity are mainly strains of bacilli that were selected for various beneficial effects such as production of plant growth regulators and polysaccharides. One important reason for the lack of biological control with Equity could be that it does not contain bacteria that are specifically antagonistic to *R. solanacearum*. However, both Bio Yield and Equity had positive effects on the growth of tomato transplants in preliminary experiments without inoculation (data not shown).

Table 4. Final bacterial wilt incidence in tomato (cv. Solar Set) transplanted to soil infested with *Ralstonia solanacearum* in S-H mixture and Actigard experiments

	% Bacterial wilt[x]	
Treatment[y]	Exp. 1	Exp. 2
0d S-H + Act[z]	85.0 a	70.0 cd
0d S-H	100.0 a	85.0 abc
0d Act	100.0 a	95.0 a
0d UT Control	100.0 a	97.5 a
7d S-H + Act	80.0 a	72.5 bcd
7d S-H	97.5 a	70.0 cd
7d Act	90.0 a	92.5 ab
7d UT Control	100.0 a	90.0 abc
14d S-H + Act	30.0 b	55.0 d
14d S-H	47.5 b	77.5 abc
14d Act	100.0 a	72.5 bcd
14d UT Control	100.0 a	82.5 abc

[x] Final bacterial wilt incidence 30 days after inoculation of plants, mean of four replications having eight plants each, values followed by same letters in a column do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

[y] Identifications of treatments: 0d = soil infested with *R. solanacearum* on the day of transplanting, 7d = soil infested with *R. solanacearum* 7 days before transplanting, 14d = soil infested with *R. solanacearum* 14 days before transplanting. Act = plants treated with Actigard, S-H = soil amended with S-H, UT = untreated.

[z] Initial foliar treatment with Actigard was applied 14 days after seed germination and was followed by a second application, both of foliar spray and soil drench, 5 days prior to inoculation with *R. solanacearum*.

In this study, Actigard has been found to reduce the bacterial wilt incidence only when the low inoculum density (10^5 or 10^6 CFU/ml) was used for inoculations (Table 2). When Actigard combined with PGPR or S-H mixture, high pathogen density (10^7 CFU/ml) was used for inoculations. Only in two experiments Actigard significantly enhanced disease control: when it was combined with BioYield (Table 3) or S-H mixture (Table 4). In experiment 2 (Table 3), Equity plus Actigard reduced wilt incidence significantly compared with the untreated control. Even though the PGPR mode of action is not clear, they may be reducing pathogen populations. This possibility might explain why Actigard provided significant control when combined with PGPR treatments, but no control when it was used alone.

The mechanism by which S-H mixture reduces the population of *R. solanacearum* has been suggested by Hsu and Chang (5) as microbiological in nature. In our study, S-H mixture was not autoclaved, because the effectiveness of autoclaved S-H mixtures against *R. solanacearum* was reduced substantially in a previous study (5). Incubation of *R. solanacearum* with S-H mixture for 2 weeks reduced bacterial wilt incidence when compared with 1-week incubation, and with planting in soil without incubating with the S-H mixture. An increased incubation period of the pathogen in soil with S-H mixture would help in reducing the population of the bacteria to a level where application of Actigard to transplants could reduce wilt incidence. However, the effect of S-H mixture on the population dynamics of biocontrol PGPR strains has to be investigated before combining the soil amendment with PGPR strains for management of bacterial wilt disease.

Some level of host resistance to a pathogen could be enhanced by plant activators (26). In all the experiments conducted throughout this study, only a bacterial wilt–susceptible cultivar of tomato was used. In the *R. solanacearum*–tomato pathosystem, under high inoculum conditions, the enhancement of resistance by Actigard is effective in only a moderately resistant cultivar (M. T. Momol and J. B. Jones, *unpublished data*). Therefore, in a moderately resistant cultivar, activation of resistance by Actigard combined with PGPR or S-H mixture would be expected to give better results than the susceptible cultivar in reducing bacterial wilt incidence. Our results indicated that PGPR and S-H mixture alone or in combination with Actigard could be used to reduce bacterial wilt incidence in tomato. The most effective agents or their combinations from our study need to be tested further under field conditions.

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LITERATURE CITED

1. Bowen, G. D., and Rovira, A. D. 1999. The Rhizosphere and its management to improve plant growth. Adv. Agron. 66:1-102.
2. Chang, M. L., and Hsu, S. T. 1988. Suppression of bacterial wilt of tomato by soil amendments. Plant Prot. Bull. 30:349-359.
3. Ciampi-Panno, L., Fernandez, C., Bustamante, P., Andrade, N., Ojeda, S., and Contreras, A. 1989. Biological control of bacterial wilt of potatoes caused by *Pseudomonas solanacearum*. Am. Potato J. 66:315-332.
4. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN.
5. Hsu, S. T., and Chang, M. L. 1989. Effect of soil amendments on survival of *Pseudomonas solanacearum*. Plant Prot. Bull. 31:21-33.
6. Jetyanon, K., and Kloepper, J. W. 2002. Mixtures of plant growth promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biol. Control 24:285-291.
7. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
8. Kempe, J., and Sequeira, L. 1983. Biological control of bacterial wilt of potatoes: Attempts to induce resistance by treating tubers with bacteria. Plant Dis. 67:499-503.
9. Kim, D. H., and Misaghi, I. J. 1996. Biocontrol performance of two isolates of *Pseudomonas fluorescens* in modified soil atmospheres. Phytopathology 86:1238-1241.
10. Kloepper, J. W., Rodriguez-Kabana, R., Zehnder, G. W., Murphy, J. F., Sikora, E., and Fernandez, C. 1999. Plant root-bacterial interactions in biological control of soil-borne diseases and potential extension to systemic and foliar diseases. Aust. Plant Pathol. 28:21-26.
11. Kuc, J. 2001. Concepts and directions of induced systemic resistance in plants and its application. Eur. J. Plant Pathol. 107:7-12.
12. Kucharek, T. 1998. Bacterial Wilt of Row Crops in Florida. Circ-1207. University of Florida, IFAS, Cooperative Extension Service.
13. Kumari, V., and Srivastava, J. S. 1999. Molecular and biochemical aspects of rhizobacterial ecology with special emphasis on biological control. World J. Microbiol. Biotechnol. 15:535-543.
14. Louws, F. J., Wilson, M., Campbell, H. L., Cuppels, D. A., Jones, J. B., Shoemaker, P. B., Sahin, F., and Miller, S. A. 2001. Field control of bacterial spot and bacterial speck of tomato using a plant activator. Plant Dis. 85:481-488.
15. Momol, M. T., Norelli, J. L., and Aldwinckle, H. S. 1999. Evaluation of biological control agents, systemic acquired resistance inducers and bactericides for the control of fire blight on apple blossom. Acta Hortic. 489:533-557.
16. Oostendrop, M., Kunz, W., Dietrich, B., and Staub, T. 2001. Induced disease resistance in plants by chemicals. Eur. J. Plant Pathol.

107:19-28.
17. Phae, C. G., Shoda, M., Kita, N., Nakano, M., and Ushiyama, K. 1992. Biological control of crown and root rot and bacterial wilt of tomato by *Bacillus subtilis* NB22. Ann. Phytopathol. Soc. Jpn. 58:329-339.
18. Pierson, E. A., and Weller, D. M. 1994. Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. Phytopathology 84:940-947.
19. Pradhanang, P. M., and Momol, M. T. 2001. Survival of *Ralstonia solanacearum* in soil under irrigated rice culture and aquatic weeds. J. Phytopathol. 149:707-711.
20. Pradhanang, P. M., Momol, M. T., Olson, S. M., and Jones J. B. 2003. Effects of plant essential oils on *Ralstonia solanacearum* population density and bacterial wilt incidence in tomato. Plant Dis. 87:423-427.
21. Ramamoorthy, V., Raguchander, T., and Samiyappan, R. 2002. Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads. Eur. J. Plant Pathol. 108:429-441.
22. Raupach, G. S., and Kloepper, J. W. 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88:1158-1164.
23. Raupach, G. S., and Kloepper, J. W. 2000. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. Plant Dis. 84:1073-1075.
24. Reddy, M. S., Rodriguez-Kabana, R., Kenney, D. S., Ryu, C. M., Zhang, S., Yan, Z., Martinz-Ochoa, N., and Kloepper, J. W. 1999. Growth promotion and induced systemic resistance (ISR) mediated by a biological preparation. (Abstr.) Phytopathology 89:S65.
25. Sun, S.-K., and Huang, J.-W. 1985. Formulated soil amendment for controlling Fusarium wilt and other soilborne diseases. Plant Dis. 69:917-920.
26. Van Loon, L. C., Bakker, P. A. H. M., and Pieterse, C. M. J. 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36:453-483.
27. Wei, G., Kloepper, J. W., and Tuzun, S. 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology 86:221-224.
28. Whipps, J. M. 1997. Developments in biological control of soil borne plant pathogens. Adv. Bot. Res. 26:1-134.
29. Zehnder, G. W., Murphy, J. F., Sikora, E., and Kloepper, J. W. 2001. Application of rhizobacteria for induced resistance. Eur. J. Plant Pathol. 107:39-50.