Effects of Xylem-Colonizing *Bacillus* spp. on Verticillium Wilt in Maples

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ABSTRACT

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Bacillus spp. originally isolated from healthy maple stem tissue were introduced into stem wounds in silver or Norway maple seedlings. After 3 or 14 days, stems were inoculated with a conidial suspension of *Verticillium dahliae*. Several *Bacillus subtilis* isolates reduced the percentage of silver maple stems colonized by *V. dahliae*. One isolate reduced the percentage of Norway maple stems colonized by the fungus. Rifampicin-resistant isolates of *B. subtilis* were distributed upward from wounds in the lower stems of silver maples.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is destructive to maples in nurseries and landscape plantings. Fungicide applications to control this disease are costly and often ineffective; therefore, alternative methods of control are needed. The homeostatic properties of xylem tissue may provide an environment conducive to the growth and survival of xylophilic microorganisms antagonistic to this vascular wilt pathogen. Biological control of Dutch elm disease has been attempted by injecting antagonistic bacteria (8,17,18), *Trichoderma* spp. (21), or *Streptomyces* spp. (19) into stem tissues of American elm. Schreiber and Hock (23) found evidence of xylem-colonizing bacteria showing antagonism to vascular wilt pathogens. They demonstrated inhibition of growth, in vitro, of *Ceratocystis ulmi* (Buism.) C. Moreau and *V. dahliae* by bacteria from stem sections of silver maple seedlings. L. R. Schreiber (*unpublished*) found that silver maples from different seed sources contained bacteria that inhibited mycelial growth of both fungi in vitro.

Many bacteria, fungi, and yeasts are known to colonize healthy and diseased xylem in woody plants (1,6,11,15,16,20,24). Consequently, xylophilic and xylem-colonizing microorganisms of healthy plants deserve careful study. Our objectives were to isolate naturally occurring microbes from healthy maple stems, identify and test these microorganisms for antagonism to *V. dahliae* in vitro, and examine their effects on Verticillium wilt in silver and Norway maples.

MATERIALS AND METHODS

Isolations. Branches (two per tree, 2.5 cm in diameter or less) and attached twigs (1 cm in diameter or less) from *Acer saccharinum* L. (silver maple), *A. rubrum* L. (red maple), and *A. platanoides* L. (Norway maple) were collected in March, April, and May 1982 from 12- to 15-yr-old trees at the USDA-ARS Nursery Crops Research Laboratory near Delaware, OH. Debarked specimens were cut serially into 2-cm-long sections from the proximal to the distal end. Sections were placed serially in petri dishes, frozen, and stored at −20 C. Initial isolations were made within 2 wk of branch collections. Frozen sections were surface-sterilized by immersion in vigorously boiling water for 1–2 sec (15),

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split in half, and placed on silver maple diffusate agar (SMDA) (15), with the radial surface resting on the medium. Dishes were sealed with paraffin film and incubated in the dark at 24 C for 3–7 days. Bacteria growing from tissue were streaked on sucrose nutrient agar (SNA; Difco, Detroit, MI). After 3 days at 24 C, single colonies were restreaked on SNA. Stock cultures were prepared by sampling at least five single, similiar colonies with a sterile loop. The composite was restreaked on SNA and incubated at 24 C to verify growth habit, form, and color. Stock cultures were stored on yeast-dextrose-calcium carbonate agar (yeast extract, 10 g; dextrose, 20 g; calcium carbonate, 20 g; and agar, 20 g/L of distilled water) at 5 C and silica gel (26) at −20 C.

Antagonism in vitro. Bacteria isolated from the three maple species were compared in a dual-culture test for antagonism to *V. dahliae*. Bacteria were grown on SNA at 24 C for 1 wk, then assayed on SMDA, SNA, and Czapek-Dox agar (CDA; Difco, Detroit, MI) (three plates per medium). Conidial suspensions of the fungus were made from 2-wk-old cultures grown on CDA at 24 C by vigorously agitating four agar disks (8 mm in diameter) for 1 min in 10 ml of sterile distilled water. A 0.01-ml aliquot of bacterial cells was streaked across a petri dish containing 20 ml of medium, and 0.1 ml of a conidial suspension of the fungus was pipetted in a parallel line, 4 cm from the bacterial streak. A bacterial loop was drawn at right angles from the fungal inoculum through the bacterial inoculum and another from the bacterial inoculum through the fungal inoculum. Dishes were sealed with paraffin film, incubated for 7–10 days at 24 C, and examined. Interactions between bacteria and *Verticillium* were categorized as 1) no inhibition of mycelial growth, 2) slight inhibition of mycelial growth (1–3 mm between bacterium and fungus), or 3) pronounced inhibition of mycelial growth (more than 3 mm between bacterium and fungus).

From the procedure described, 11 isolates in inhibitory categories 1–3 were selected randomly for identification and retesting by the dual-culture technique on SNA and CDA. A 12th isolate, B-5, which showed antagonism to *Verticillium* in a previous study (23), was included.

Mutants resistant to rifampicin (Rf) (Sigma, St. Louis, MO) were made of two antagonistic bacterial isolates, B-5 and B-26, to facilitate their identification in later studies. Mutants were selected from gradient plates (2) amended with 50 or 100 μg/ml of Rf. Rf-resistant strains were denoted with the initials "rf" after the isolate numbers.

In vivo test A. The following experiment was conducted in 1982 and repeated in 1983. Eighty silver maple trees (5–9 mm in diameter × 26–77 cm high) were potted in a peat/perlite/soil mix (2:2:1) in 15-cm-diameter plastic pots and placed in a greenhouse for 2 wk. After budbreak, the maples were transferred to growth chambers at 24 C with a 16-hr photoperiod for 2 wk before treatments and inoculations.

Rf-resistant bacteria were grown in shake culture (78 rpm) in sucrose-nutrient broth (SNB; nutrient broth, 8 g/L and sucrose, 10 g/L) for 3 days at 24 C. Suspensions were centrifuged for 10 min at 2,000 × *g*, and the supernatant was discarded. The bacterial pellet was resuspended in sterile silver maple diffusate (SMD) (15) and stored at 5 C.

Plants were treated by introducing bacteria into the lower stems through wounds with a serum vial-cap technique (7). Five milliliters of a cell suspension containing $5–10 \times 10^5$/ml was placed in the cap. The stem below the surface of the suspension was wounded with a scalpel. As uptake occurred for about 24 hr, reservoirs were refilled as needed with sterile SMD. The bacterial treatments were B-5rf, B-26rf, B-5rf plus B-26rf, and sterile SMD (control). Twenty trees were used for each treatment, with five replicates per treatment placed in each of four growth chambers. Plants were inoculated 3 days after treatment with a conidial suspension made from three *V. dahliae* isolates from sugar and silver maples. Pathogenicity of the isolates had been verified previously by inoculation into silver and Norway maples. Five milliliters of fungal inoculum containing 1.5×10^5 conidia per milliliter of sterile distilled water was placed in the serum cap used for bacterial treatment, and trees were rewounded. Trees remained in the chambers for 2 wk and then were transferred to a lathhouse for 15 wk. A concurrent study was conducted in 1982 using the same procedure, except silver maple trees treated with each bacterial isolate were not inoculated with *Verticillium*.

After incubation in the lathhouse, trees were cut 1 cm below the inoculation point and their lengths measured. Leaves were removed serially from the stems and their petioles were placed in petri dishes, which were sealed with paraffin film, frozen, and stored at −20 C. Stems were debarked and cut serially, from the inoculation point upward, into 2-cm sections, frozen, and stored in serial order at −20 C. Lengths of columns of discolored wood from the inoculation point were recorded.

Data from the 1982 and 1983 experiments were analyzed separately. Contingency analysis was used to compare treatment effects on the occurrence of *V. dahliae* infection and wood discoloration. In addition, effects of treatments on the extent of stem colonization, based on recovery of the fungus and Rf-resistant bacteria, and lengths of columns of discolored wood were evaluated with the analysis of variance *F*-test.

In vivo test B. Nine bacterial isolates were tested in silver and Norway maples for their effects on Verticillium wilt. The procedures for conditioning and inoculating plants were as described before. Fungal inoculations were made 14 days after bacterial treatments, and trees were harvested 15 wk after inoculation. Sixteen trees were used for each treatment, with four trees from each treatment placed in each of four growth chambers. Fungal infection and discoloration data for silver and Norway maples were analyzed separately with contingency analysis and analysis of variance.

Isolations. To isolate the fungus from inoculated trees, frozen petioles were immersed momentarily in vigorously boiling water and placed on CDA with streptomycin (100 μg/ml) and novobiocin (100 μg/ml) (CDAA). Frozen stem sections (2 cm long) were cut at 8-cm intervals beginning at the inoculation wound and similarly sterilized for 1–2 sec. A section was split in half; to recover the fungus, one half was placed, split side down, on CDAA. To recover Rf-resistant bacteria, the other half was placed on SNA amended with Rf (50 μg/ml) plus 5 ml of a 1% solution of 2,3,5-triphenyltetrazolium chloride (TPT) (14) per liter.

Sections from stems treated with wild-type bacteria were placed on SNA plus TPT and on CDAA. These isolation procedures were repeated using adjacent stem sections to confirm initial isolation of *V. dahliae* and Rf-resistant and wild-type bacteria. Dishes were incubated at 18 C (27), and observations were made at weekly intervals for 4–6 wk. Bacterial colonies showing erumpent, crusty growth and a dull red color in the presence of Rf plus TPT (or TPT alone) were streaked and compared with stock cultures of Rf-resistant and wild-type isolates growing on these media. Trees were not evaluated for stem colonization by Rf-resistant bacteria.

Bacterial identification. Bacteria were identified using procedures outlined in *Bergey's Manual of Determinative Bacteriology* (4) and the *Manual of Methods for General Bacteriology* (2).

RESULTS

Isolations and antagonism in vitro. Three hundred fourteen bacterial isolates were obtained from wood of healthy silver, red, and Norway maples. These isolates varied in inhibitory effects on *Verticillium*, with 26, 38, and 36% causing no inhibition, slight inhibition, and pronounced inhibition, respectively. There was no correlation between tree species and classes of inhibition produced by the bacteria. Of the 12 bacterial isolates selected for further study, B-5, B-26, B-60, B-83, B-85, B-121, B-136, B-

150, and B-181 were *Bacillus subtilis* (Ehrenberg) Cohn, B-194 was *B. carotarum* Koch, and B-211 was *B. coagulans* Hammer. Isolates B-5, B-26, and B-60 caused pronounced inhibition of *V. dahliae* in vitro, whereas B-83, B-85, B-150, and B-181 were slightly inhibitory and B-121, B-135, B-136, B-194, and B-211 were noninhibitory. Isolates B-5, B-60, B-135, B-136, B-150 were recovered from silver maple; B-26, B-83, B-85, and B-121, from red maple; and B-181, B-194, and B-211, from Norway maple.

In vivo test A. Treatment of silver maples with isolate B-26rf, through stem wounds, significantly reduced the percentage of *Verticillium*-infected trees in both 1982 and 1983 (Table 1). Only bacterial treatment B-26rf in 1982 reduced the percentage of trees with vascular discoloration. Trees treated with bacteria but not inoculated with *Verticillium* did not show vascular discoloration. There was no evidence of natural infestation of *Verticillium* in the nursery stock used for these studies.

Rf-resistant bacteria resembling the introduced isolates were recovered from 28.3% of the treated silver maples in 1982. Bacterial colonization of the stem above the inoculation point averaged 15 cm (2–42 cm). In 1983, Rf-resistant bacteria were recovered from 76.6% of the treated trees, with an average colonization above the inoculation point of 15 cm (2–28 cm). *Bacillus*-like bacteria (colonies with spreading irregular form, wrinkled or textured surface, and cream-colored on SNA) were recovered on SNA without Rf from nearly all stem sections of most replicates in all treatments and the control. No Rf-resistant bacteria resembling the introduced isolates were recovered from untreated controls in either trial. The fungus and Rf-resistant bacteria were recovered infrequently from young, green stem tissue and leaf petioles.

In vivo test B. The effects of the two Rf-resistant and seven other *Bacillus* isolates on infection in Norway and silver maple are shown in Table 2. In Norway maple, isolate B-26rf significantly reduced the percentage of *V. dahliae* infections. *B. carotarum* (B-194) increased the percentage of infections by the fungus but not significantly. Bacterial treatments did not significantly influence the percentage of trees with, or the extent of vascular discoloration caused by, *Verticillium* in inoculated trees. In silver maple, the percentage of trees infected with *V. dahliae* was reduced significantly by *B. subtilis* isolates B-121, B-135, B-150, and B-181 but not by B-26rf and B-5rf. *B. subtilis* isolates B-121, B-136, B-150, and B-181 reduced the number of trees with vascular discoloration (Table 2).

Bacterial treatments did not reduce the lengths of columns of discolored wood or the extent of stem tissue colonization by the fungus in either in vivo test. Foliar symptoms of Verticillium wilt were not observed in the in vivo studies.

DISCUSSION

Attempts to control vascular wilt disease in trees with microbial antagonists have had limited success (8,17,19,22,28). *B. subtilis* has been used for the biological control of Nectria canker and crown rot in apple trees (29,32), Verticillium wilt in strawberry (9), charcoal rot of potato (30), root infections leading to stalk rot in corn (13), and white rot in onions (33). It may reduce soil populations of rhizosphere and nonrhizosphere bacteria (12) and be antagonistic to *V. albo-atrum* (5). Our results suggest that *B. subtilis* isolates that are natural colonizers of maple stem tissue can inhibit Verticillium wilt.

In general, antagonism in vitro was not correlated with activity in vivo. Isolates of three *Bacillus* spp. caused varying degrees of inhibition of mycelial growth in vitro, but only *B. subtilis* isolates reduced the frequency of stem infection by *V. dahliae* in vivo. Although the introduction of *V. dahliae* through stem wounds in juvenile trees is unlike the natural means of infection, which occurs predominantly through the roots (3,5), stem inoculations were used to maximize establishment of both the antagonist and pathogen (31). Root inoculations of maples were attempted in preliminary experiments, but infections were infrequent. With stem inoculations, we obtained infection frequencies in silver maple controls of 55 and 62% and 81% in Norway maples (Tables 1 and 2).

Bacterial isolates showed prophylactic activity by affecting fungal infection but not vascular discoloration. Vascular discoloration is associated with pathogenesis by vascular wilt fungi. Reductions in the frequency of infected trees were not associated with reductions in frequency of trees with vascular discoloration (Tables 1 and 2) or the length of discoloration columns. *B. subtilis* may influence disease development by preventing initial wound colonization or, after early infection, limit stem colonization. There may be a critical point in the infection process beyond which a bacterial treatment is not effective in controlling this disease. Theraputic

Table 1. Effects of rifampicin-resistant (rf) *Bacillus subtilis* treatments on percentage of infection and xylem discoloration by *Verticillium dahliae* in silver maple

Bacterial treatment	Percentage of trees[w]			
	Infected[x]		Discolored[y]	
	1982	1983	1982	1983
B-26rf	16 b[z]	11 b	50 b	94 a
B-5rf	35 a	42 a	100 a	95 a
B-5rf + B-26rf	50 a	44 a	95 a	100 a
Control (none)	55 a	62 a	90 a	100 a

[w] Twenty seedlings 26–77 cm high per treatment. Treatments applied through stem wounds; after 3 days, stems were inoculated at the same location with a spore suspension of *V. dahliae*. Plants were harvested after 17 wk.
[x] A tree was considered infected if *Verticillium* was recovered above the inoculation point.
[y] Trees showing xylem discoloration above the inoculation points.
[z] In each column, numbers followed by the same letter are not significantly different at $P = 0.05$ based on tables of exact probabilities for 2×2 contingency tests.

Table 2. Effects of *Bacillus* spp. on percentage of infection and xylem discoloration by *Verticillium dahliae* in Norway and silver maples

Bacterial treatment	Percentage of trees[v]			
	Norway		Silver	
	Infected[w]	Discolored[x]	Infected	Discolored
B. subtilis				
B-5rf[y]	69 a[z]	100 a	27 ab	60 ab
B-26rf	47 b	80 a	27 ab	66 ab
B-121	80 a	93 a	20 b	27 b
B-135	50 a	62 a	13 b	81 ab
B-136	69 a	87 a	38 ab	47 b
B-150	62 a	94 a	13 b	7 c
B-181	69 a	94 a	19 b	50 b
B. carotarum B-194	94 a	81 a	31 ab	100 a
B. coagulans B-211	75 a	94 a	50 ab	88 a
Control (none)	81 a	100 a	56 a	73 a

[v] Sixteen trees per treatment. Treatments applied through stem wounds; after 2 wk, stems were inoculated at the same location with a spore suspension of *V. dahliae*. Plants were harvested after 15 wk.
[w] Trees from which *Verticillium* was recovered above the inoculation points.
[x] Trees showing xylem discoloration above the inoculation points.
[y] Rifampicin-resistant mutants designated "rf."
[z] In each column, numbers followed by the same letter are not significantly different at $P = 0.05$ based on tables of exact probabilities for 2×2 contingency tests.

bacterial treatments were not attempted.

In test A (Table 1) for 1982 and 1983 trials with silver maple, infection frequencies of silver maples treated with B-26rf were significantly lower than those of the controls. In test B (Table 2) also, B-26rf reduced the infection frequency below that of the controls (27 vs. 56%), but the reduction was not significant. Analysis of results may have been influenced by the number of replicates. Replicate numbers for treatments in test A and test B were 20 and 16 trees, respectively. In test B, several trees were killed by the wounding. We found that 20 replicates per treatment were needed to ensure adequate survival for contingency analysis to be effective and that 15 replicates represented a minimum number below which analysis would be difficult to interpret. Thus, the loss of two replicates from stem wounding may account for the lack of significance in the reduction in infection frequency by B-26rf. In addition, trees in test B were inoculated 14 days after treatment rather than 3 days as in test A. This too may have influenced differences in control of *Verticillium* infection in the two tests.

An effective biocontrol agent of Verticillium wilt must colonize old and newly formed host tissue. We found that *B. subtilis* was translocated upward in the xylem of silver maples and could be recovered from stem tissue after 17 wk. More prolonged survival and invasion and colonization of new xylem should be studied. The ability of *Bacillus* spp. to produce antibiotics (10,25), grow over a wide temperature range (4), adapt to the vascular environment in maples, and form spores makes them particularly suitable as candidate biocontrol agents.

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LITERATURE CITED

1. Aho, P. E., Seidler, R. J., Evans, H. J., and Raju, P. N. 1974. Distribution, enumeration, and identification of nitrogen-fixing bacteria associated with decay in living white fir trees. Phytopathology 64:1413-1420.
2. American Society for Microbiology. 1981. Manual of Methods for General Bacteriology. The Society: Washington, DC. 524 pp.
3. Born, G. L. 1974. Root infection of woody hosts with *Verticillium albo-atrum*. Ill. Nat. Hist. Surv. Bull. 31:209-248.
4. Buchanan, R. E., and Gibbons, N. E. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. Williams & Wilkins, Baltimore. 1,268 pp.
5. Caroselli, N. E. 1957. Verticillium wilt of maples. R.I. Agric. Exp. Stn. Bull. 335. 84 pp.
6. Cosenza, B. J., McCreary, M., Buck, J. D., and Shigo, A. L. 1970. Bacteria associated with discolored and decayed tissues in beech, birch, and maple. Phytopathology 60:1547-1551.
7. Gregory, G. F. 1969. A technique for inoculating plants with vascular pathogens. Phytopathology 59:1014.
8. Harrison-Lavoie, K. J., Hoffman, M. A., Selfridge, S. S., and McFarland, G. L. 1984. Inability of three applied fluorescent pseudomonads to successfuly colonize elms. (Abstr.) Phytopathology 74:805.
9. Jordan, V. W. L., and Tarr, H. S. 1978. Inoculum suppression of *Verticillium dahliae*. Ann. Appl. Biol. 89:139-141.
10. Katz, E., and Demain, A. L. 1977. The peptide antibiotics of *Bacillus*: Chemistry, biogenesis and possible functions. Bacteriol. Rev. 41:449-474.
11. Knutson, D. M. 1973. The bacteria in sapwood, wetwood and heartwood of trembling aspen (*Populus tremuloides*). Can. J. Bot. 51:498-500.
12. Ko, W. H., and Chow, F. K. 1977. Characteristics of bacteriostasis in natural soils. J. Gen. Microbiol. 102:295-298.
13. Kommedahl, T., and Mew, I. C. 1975. Biocontrol of corn root infection in the field by seed treatment with antagonists. Phytopathology 65:296-300.
14. Leben, C. 1972. The development of a selective medium for *Pseudomonas glycinea*. Phytopathology 62:674-676.
15. Leben, C. 1978. Biological control of decay fungi: A wood disk evaluation method. For. Sci. 24:560-564.
16. Murdoch, C. W., and Campana, R. J. 1983. Bacterial species associated with wetwood of elm. Phytopathology 73:1270-1273.
17. Murdoch, C. W., Campana, R. J., and Hock, J. 1984. On the biological control of *Ceratocystis ulmi* with *Pseudomonas fluorescens*. (Abstr.) Phytopathology 74:805.
18. Myers, D. F., and Strobel, G. A. 1983. *Pseudomonas syringae* as a microbial antagonist of *Ceratocystis ulmi* in the apoplast of American elm. Trans. Br. Mycol. Soc. 80:389-394.
19. O'Brien, J. G., Blanchette, R. A., and Sutherland, J. B. 1984. Assessment of *Streptomyces* spp. from elms for biological control of Dutch elm disease. Plant Dis. 68:104-106.
20. Rayner, A. D. 1979. Population and community structure and dynamics of fungi in decaying wood. Adv. Bot. Res. 7:333-420.
21. Ricard, J. L. 1983. Field observations on the biocontrol of Dutch elm disease with *Trichoderma viride* pellets. Eur. J. For. Pathol. 13:60-62.
22. Scheffer, R. J. 1983. Biological control of Dutch elm disease by *Pseudomonas* species. Ann. Appl. Biol. 103-21-30.
23. Schreiber, L. R., and Hock, W. K. 1975. Effects of benomyl and thiabendazole on growth of several plant species. J. Am. Soc. Hortic. Sci. 100:309-313.
24. Shigo, A. L., and Hillis, W. E. 1973. Heartwood, discolored wood and microorganisms in living trees. Annu. Rev. Phytopathol. 11:197-221.
25. Shoji, J. 1980. Recent chemical studies on peptide antibiotics from the genus *Bacillus*. Adv. Appl. Microbiol. 24:187-214.
26. Sleesman, J. P., and Leben, C. 1978. Preserving phytopathogenic bacteria at −70 C or with silica gel. Plant Dis. Rep. 62:910-913.
27. Smith, L. D. 1983. Major nutrient influence on *Verticillium dahliae* infections of *Acer saccharum*. J. Arboric. 9:277-281.
28. Strobel, G. A., and Lanier, G. N. 1981. Dutch elm disease. Sci. Am. 245:56-55.
29. Swinburne, T. R. 1978. The potential value of bacterial antagonists for the control of apple canker. Ann. Appl. Biol. 89:94-96.
30. Thirumalachar, M. J., and O'Brien, M. J. 1977. Suppression of charcoal rot in potato with a bacterial antagonist. Plant Dis. Rep. 61:543-546.
31. Townsend, A. M., and Hock, W. K. 1973. Tolerance of half-sib families of red maple to Verticillium wilt. Phytopathology 63:673-676.
32. Utkhede, R. S. 1984. Effect of bacterial antagonists on *Phytophthora cactorum* and apple crown rot. Phytopathol. Z. 109:169-175.
33. Utkhede, R. S., and Rache, J. E. 1983. Chemical and biological control of onion white rot in muck and mineral soils. Plant Dis. 67:153-155.