A Miniaturized and Rapid Bioassay for the Selection of Soil Bacteria Suppressive to Pythium Blight of Turfgrasses

Eric B. Nelson and Cheryl M. Craft

Assistant professor and research support specialist, respectively, Department of Plant Pathology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca 14853.
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ABSTRACT

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A miniaturized plant assay was developed to screen bacterial strains for their ability to suppress Pythium blight of turfgrasses caused by *Pythium aphanidermatum*. Bacteria were recovered from soil and thatch from both high-maintenance and low-maintenance turfgrass sites and tested on creeping bentgrass plants grown in wells of tissue culture plates (TCP) and then in pots of perennial ryegrass in the growth chamber (GC). Of the 200 strains screened in TCP assays, 86 significantly suppressed Pythium blight (disease rating < 3.0 after 7 days) as compared with untreated plants. The highest frequency of antagonist recovery (54.5%) was from the thatch of both low- and high-maintenance turfgrass sites, while the lowest frequency of antagonist recovery (27.3%) was from rhizosphere soil in both types of maintenance sites. Of the strains from thatch that were suppressive in TCP assays, 45% also were effective in GC assays. The lowest frequency of suppressive bacterial strains in both TCP assays and GC assays (24.1%) was with strains recovered from rhizosphere soil under high-maintenance (i.e., golf course) turf. Bacterial strains recovered from thatch were more suppressive than those recovered from rhizosphere soil in TCP assays but not in GC assays. Antagonistic bacteria recovered from media selective for enteric bacteria and *Pseudomonas* spp. were as suppressive to Pythium blight of creeping bentgrass as those recovered from a nonselective medium. However, enteric bacteria were more suppressive to Pythium blight of perennial ryegrass than were general heterotrophic bacteria or *Pseudomonas* spp. Only two strains of *Enterobacter cloacae* tested were effective in TCP assays and only one of these was effective in GC assays. All bacterial strains that were ineffective in TCP assays also were ineffective in GC assays. The TCP assay developed in this study proved to be an effective means of rapidly screening large numbers of bacterial strains in plant assays for the suppression of Pythium blight.

Additional keyword: biological control.

Pythium blight, caused by *Pythium aphanidermatum* (Edson) Fitzp., is one of the fastest developing and more destructive diseases affecting turfgrasses worldwide (23,25). Under the appropriate conditions of temperature and moisture, asymptomatic turf may develop advanced symptoms in as little as 24 h, resulting in heavy losses of both seedling and established turfgrass stands. Because of this threat, turfgrass managers have relied on preventive fungicide applications for effective control. This has led, in many cases, to unnecessary and excessive applications of *Pythium*-selective fungicides. As a consequence of the heavy reliance on chemical fungicides, control has become problematic due to adverse nontarget effects (22) and the selection of fungicide-resistant populations of *P. aphanidermatum* (18–20).

Biological control is an attractive strategy to reduce preventive applications of fungicides on turfgrasses. However, despite the development of biological control strategies for diseases of other crop species (5), this approach for controlling turfgrass diseases rarely has been explored. Antagonists suppressive to Pythium blight have been isolated (17,27) in laboratory and greenhouse

tests, but no detailed reports of the efficacy of these antagonists are published. Antagonists suppressive to take-all patch caused by *Gaeumannomyces graminis* var. *avenae* (28,29), brown patch caused by *Rhizoctonia solani* (3,26), dollar spot caused by *Sclerotinia homoeocarpa* (8,16), and gray snow mold caused by *Typhula incarnata* and *T. ishikariensis* (4,10) have been described from both greenhouse and field studies. Additionally, complex mixtures of antagonists found in composts and organic fertilizers have been shown to be suppressive to dollar spot (15) and brown patch (14) in field trials.

A major limiting factor in the development of a biological control strategy for different plant diseases is the formulation of efficient screening procedures to rapidly screen large numbers of organisms for biological control activity. While field screening should theoretically provide the best selection of efficient biocontrol strains, limitations of space, labor, cost, and optimum environmental conditions preclude the use of this type of screening strategy. On the other hand, laboratory assays based on the in vitro inhibition of pathogens or production of particular metabolites by biological control agents offer a rapid and relatively inexpensive means of screening organisms but may not be a good indicator of biocontrol potential. Not surprisingly, biocontrol strains selected in vitro on the basis of phenotypes with unknown links to biological control activity in plant systems do not always perform as expected under greenhouse or field conditions (9,11,27). It is logical that plant assays should provide the most direct assessment of biological control potential among individual organisms. Therefore, the challenge in screening biological control agents is to develop an assay with the simplicity of an in vitro assay combined with the sensitivity of a plant assay. This selection also should reasonably predict expression of biological control traits in more complex ecosystems.

The purpose of this investigation was to develop a rapid and miniaturized laboratory plant assay for the screening and identification of soil bacteria suppressive to Pythium blight of creeping bentgrass.

MATERIALS AND METHODS

Isolation and maintenance of bacterial strains. Bacterial strains were isolated from turfgrasses grown in areas that differed in their management intensities. High-maintenance sites were characterized by heavy pesticide and fertilizer applications accompanied by adequate irrigation and intensive grooming procedures. Low-maintenance sites were characterized by the limited use of pesticides, fertilizers, irrigation, and mowing. A total of three high-maintenance (three golf courses) and four low-maintenance (two parks, two cemeteries) sites were sampled. For low-maintenance sites, 20–25 cores (2-cm-diameter) were randomly taken to a depth of approximately 5 cm from areas with a high plant density. Foliar tissues were excised and the remaining portions of the cores (thatch, soil, and roots) were separated into thatch and rhizosphere soil samples. In all of the samples, there was a clear boundary between the thatch layer and soil, allowing easy separation into the respective fractions. Core samples were pooled to create composite rhizosphere soil and thatch samples for each site. These composite samples then were used to prepare dilution series. For golf course sites, 10-cm-diameter cores were removed from putting greens to a depth of approximately 5 cm with the aid of a cup cutter. Subsamples were taken from cores and separated into soil and thatch samples as described above and used to prepare dilution series.

Ten grams of thatch or soil was suspended in 90 ml of sterile distilled water and placed on a shaker for 10 min. A 1-ml aliquot was removed from each sample and a series of tenfold dilutions was prepared in phosphate-buffered saline (PBS). The saline was a 0.01 M potassium phosphate buffer (pH 7.2) containing 0.15% NaCl. A 0.1-ml aliquot from appropriate dilutions was plated onto one-third strength trypticase soy agar (TSA) for general heterotrophic bacteria, Difco MacConkey agar for enteric bacteria, and a medium selective for *Pseudomonas* spp. (6). After 48 h of incubation at 30 C, colonies with differing morphologies, growth rates, and pigmentation were selected from plates containing 80–100 colonies and streaked onto TSA slants. After colony purification on TSA, cultures were stored on silica gel at −20 C before screening in plant assays (21).

In one experiment, strains of *Enterobacter cloacae* isolated from other crop plants were tested for their ability to suppress Pythium blight on creeping bentgrass and perennial ryegrass. These strains have been described previously and are effective antagonists of *P. ultimum* (7,12,13).

Bacteria used in biocontrol assays were grown on trypticase soy broth (TSB) for 24 h at 30 C. Cells from 30 ml of a TSB culture were removed from suspension by centrifugation (10,000 *g* for 10 min) and resuspended in 10 mM potassium phosphate buffer (pH 7.0). For laboratory and growth chamber experiments, cells were resuspended in 2 and 20 ml of phosphate buffer, respectively. Cell densities varied among bacterial strains, but suspensions ranged from 10^8 to 10^{11} cells per milliliter.

Tissue culture plate assays. Bacteria were screened in the laboratory for their ability to suppress Pythium blight of creeping

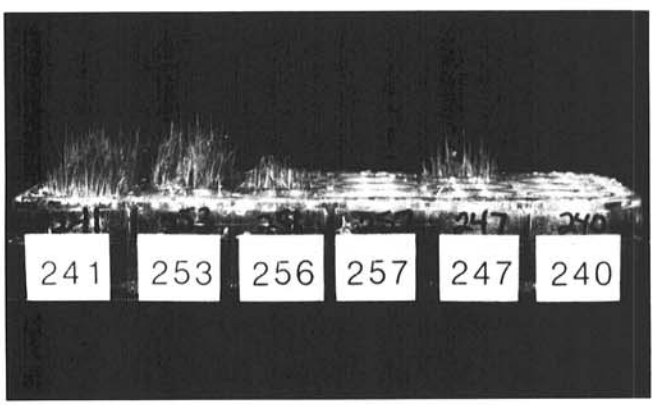

Fig. 1. Effect of various soil bacteria on the suppression of Pythium blight of creeping bentgrass in tissue culture plate assays. Each row of four wells contains the same test bacterium. Wells with healthy turf indicate an effective bacterial antagonist. Strain numbers are indicated below each row of wells.

TABLE 1. Suppression of Pythium blight by bacterial antagonists isolated from low and high maintenance turfgrass areas and nonturfgrass soils

Source	TCP antagonists		GC antagonists	
	Number[t]	Mean disease rating[u]	Number[v]	Mean disease rating[w]
Low maintenance turf[x]				
Thatch	31/57	1.29 a[y]	9/25	2.50 a
Soil	5/22	2.15 b	2/4	2.50 a
High maintenance turf[z]				
Thatch	15/41	1.67 a	9/15	2.25 a
Soil	35/80	2.14 b	7/29	2.50 a
Total	86/200	1.97	27/73	2.45

[t] Number of strains suppressing Pythium blight (disease rating < 3.00 after 7 days) in tissue culture plate (TCP) assays out of the total number of strains tested.

[u] Only those strains giving rise to plants with significantly ($P = 0.05$) lower ratings than those in untreated wells were included in calculating the mean disease rating.

[v] Number of strains suppressing Pythium blight (disease rating < 3.75 after 4 days) in growth chamber (GC) assays out of the total number of TCP-effective strains tested.

[w] Only those strains giving rise to plants with significantly ($P = 0.05$) lower ratings than those in untreated wells were included in calculating the mean disease rating.

[x] Parks and cemeteries.

[y] Mean disease ratings followed by the same letter are not significantly ($P = 0.05$) different according to the LSD test.

[z] Golf courses.

bentgrass in tissue culture plate (TCP) assays. Wells of 24-well tissue culture plates were first filled with 0.5 g of air-dried sterile fine sand. A colonized 4-mm-diameter agar disk taken from a 48-h culture of *P. aphanidermatum* (P32Pa) growing on Difco cornmeal agar (CMA) then was placed on the sand surface and covered with an additional 4 g of sand. Each of four replicate wells was drenched with 0.75 ml of a bacterial cell suspension. Wells then were seeded with creeping bentgrass (*Agrostis palustris* Hudson 'Penncross') by sprinkling seed over the surface of the plates with the aid of a salt shaker until each well was completely covered with a layer of seed. Seeds then were covered with an additional 0.5-g layer of sand. Controls consisted of noninfested wells and wells infested with *P. aphanidermatum* and drenched with phosphate buffer only. Plates were covered, sealed, and incubated for 3 days at 28 C under a 24-h photoperiod. Covers were removed from plates after 3 days and all wells were misted lightly with distilled water. Plates then were placed into plastic containers (25 × 30 cm) that were sealed and incubated at 28 C. Seedlings emerged in 3 days and plants were rated daily for disease development on a scale of 1–5, for which 1 = no disease, 2 = up to 25%, 3 = up to 50%, 4 = up to 75%, and 5 = up to 100% of the seedlings nonemerged or necrotic.

Experiments were performed twice with similar results. Data were analyzed by analysis of variance as factorial experiments with maintenance level, source, and isolation medium as the main effect treatments. Means were separated using the LSD test or the Waller-Duncan Bayesian least significant difference test. Bacterial strains that were suppressive to Pythium blight (mean disease rating < 3.75 after 7 days) in tissue culture plate assays then were tested in growth chamber experiments.

Growth chamber experiments. Perennial ryegrass (*Lolium perenne* L. 'All Star') was used in growth chamber evaluations because it was more susceptible to *P. aphanidermatum* and more readily maintained in the greenhouse than creeping bentgrass. Perennial ryegrass was seeded in 7.5-cm-diameter pots containing sterilized (121 C for 4 h) fine quarry sand at approximately 10 mg/cm^2 and maintained in the greenhouse for 7–10 wk before inoculation. Turf was fertilized weekly with a 20-20-20 soluble fertilizer and clipped as needed to maintain a height of approximately 1 cm. Turf was inoculated with *P. aphanidermatum* by placing a 4-mm-diameter disk, removed from the edge of a 24-h CMA culture, just below the sand surface in the center of the pot. Inoculated pots were immediately drenched with appropriate bacterial suspensions at the rate of 5 ml of suspension per pot. Pots (four replications per strain) were placed in plastic bags to maintain high relative humidity and to prevent moisture loss during the experiment and then placed in a growth chamber maintained at 30 C with a 12-h photoperiod. Controls consisted of uninoculated perennial ryegrass as well as perennial ryegrass inoculated with *P. aphanidermatum* and drenched with phosphate buffer alone. As a fungicide standard, metalaxyl was applied as a drench (750 μg a.i./ml). Turf was evaluated after 4 days for disease severity and rated on a scale of 1–5 as outlined above.

Experiments were performed at least twice with similar results. Data were analyzed as factorials by analysis of variance and means were separated by the Duncan-Waller Bayesian least significant difference test.

RESULTS

Suppression of Pythium blight by strains of soil bacteria was apparent in the TCP assay 4 days after seedling emergence (Fig. 1) and could readily be distinguished from the controls. Of the 200 strains screened in TCP assays, 86 (43.0%) were suppressive to Pythium blight (mean disease rating < 3.0) (Table 1). The highest frequency of suppressive bacteria was recovered from the thatch layer of low-maintenance turfgrass sites (54.4%), whereas the lowest frequency of antagonist recovery was from the soil of low-maintenance areas (22.7%). There was no significant difference in recovery of suppressive bacteria between low- and high-maintenance turfgrass sites. Antagonistic strains recovered from thatch of both low- and high-maintenance areas were more suppressive in TCP assays than those recovered from soil.

Of the effective strains from TCP assays, only 37% were effective in growth chamber (GC) assays (disease rating < 3.75 after 7 days). The highest frequency of suppressive bacteria in GC assays was from those strains recovered from the thatch in golf course putting greens (60.0%). The lowest frequency of strains suppressive in GC assays (24.1%) was from those recovered from the soil under golf course putting greens. Of the 10 TCP-effective strains tested from samples collected at golf course A, five were effective in GC tests (Table 2). Of the six TCP-effective strains tested from samples collected at golf course B, three were effective in GC tests. Of the 15 TCP-effective strains tested from samples collected from park A, eight were effective in GC tests. In total, 13.8% of the strains screened were effective in both TCP and GC assays. In contrast, bacterial strains that were ineffective in TCP assays also were ineffective in growth chamber (GC) assays (data not shown).

TABLE 2. Suppression of Pythium blight by bacterial strains from different sources in tissue culture plate (TCP) tests and growth chamber (GC) tests

Golf course A			Golf course B			Park A		
Strain number	TCP rating[w]	GC rating[x]	Strain number	TCP rating[w]	GC rating[x]	Strain number	TCP rating[w]	GC rating[x]
Untreated[y]	5.0 a[z]	5.0 a	Untreated	5.0 a	5.0 a	Untreated	5.0 a	5.0 a
93	1.5 cd	3.5 bc	191	2.5 b	4.8 a	21	2.0 bc	3.5 cd
122	1.8 b–d	2.8 c	194	2.3 b	4.0 a	22	1.3 bc	1.5 f
123	2.0 bc	1.5 d	200	2.8 b	4.0 a	23	2.8 b	3.5 cd
126	2.3 bc	4.5 ab	241	1.0 c	1.0 b	25	1.3 bc	1.8 ef
127	1.8 b–d	4.8 a	253	2.0 bc	2.0 b	26	2.8 b	5.0 a
128	1.5 cd	5.0 a	259	1.0 c	1.5 b	28	1.8 bc	4.3 a–c
132	2.0 bc	5.0 a	Uninoculated	1.0 c	1.3 b	34	1.3 bc	2.8 de
134	2.0 bc	3.0 c				35	1.0 c	1.5 f
136	2.3 bc	5.0 a				37	2.0 bc	3.8 b–d
138	2.5 b	3.0 c				38	2.0 bc	4.5 a–c
Uninoculated	1.0 d	1.3 d				42	1.0 c	1.8 ef
						57	1.0 c	5.0 a
						58	2.3 bc	4.8 ab
						61	1.0 c	4.8 ab
						62	2.3 bc	5.0 a
						Uninoculated	1.0 c	1.0 f

[w] Rated on a scale of 1–5, for which 1 = no disease and 5 = 100% of the seedlings nonemerged or necrotic. Determined 7 days after inoculation.
[x] Rated on a scale of 1–5, where 1 = no disease and 5 = 100% of the seedlings nonemerged or necrotic. Determined 4 days after inoculation.
[y] Inoculated with *Pythium aphanidermatum* but not treated with bacterial suspension.
[z] Numbers in each column followed by the same letter are not significantly ($P = 0.05$) different according to the Duncan-Waller Bayesian least significant difference test.

A higher frequency of antagonistic strains was found among the general heterotrophic bacteria than within the selected groups of enteric bacteria and *Pseudomonas* spp. (Table 3). Over 59% of the general heterotrophic bacterial strains were effective in suppressing Pythium blight (mean disease rating < 3.00 after 7 days) in TCP assays as compared with 34.2 and 33.0% of the enteric bacterial strains and *Pseudomonas* spp., respectively. Similarly, nearly 56% of the general heterotrophic bacteria effective in TCP assays also were effective in GC assays. Only 21.7 and 30.8% of the TCP-effective enteric bacteria and *Pseudomonas* spp., respectively, were effective in GC assays. There was no significant effect of maintenance level or source (soil or thatch) on recovery of antagonists on each of the three media.

Although isolations of general heterotrophic bacteria yielded higher frequencies of effective antagonists, strains of enteric bacteria were more suppressive to *P. aphanidermatum* on perennial ryegrass than general heterotrophic bacteria or *Pseudomonas* spp. (Table 3). Disease ratings on creeping bentgrass did not differ ($P = 0.05$) among bacterial groups.

Only strains EcH-1 and EcCT-501 of *E. cloacae* were effective in suppressing Pythium blight in TCP assays, and only strain EcCT-501 also was effective in GC assays (Table 4, Fig. 2). The level of control provided by strain EcCT-501 in GC assays did not differ ($P = 0.05$) from that provided by the fungicide metalaxyl.

DISCUSSION

The TCP assay used in this study proved to be an effective means of screening potential biological control agents for the suppression of Pythium blight on turfgrasses. This assay was a relatively rapid screening procedure compared with standard growth chamber or greenhouse assays. For example, results could be obtained within 7 days after setting up the entire TCP assay, as compared with 8–11 wk for the GC assay. Although results could be obtained within 4–7 days after inoculation in both the TCP and GC assays, the major limiting factor in the GC assay is the time required to establish a dense turf in pots, which prolongs the setup time.

In our study, elimination of bacterial strains from further testing based on their performance in TCP assays did not result in the rejection of strains that would otherwise be effective in GC assays, because all strains that were ineffective in TCP tests were also ineffective in GC tests. This contrasts with a study by Kommedahl and Windels (9) where a majority of bacterial strains were rejected for not providing effective biological control of Pythium seed rot of pea on the basis of in vitro pathogen inhibition. However, these same strains were quite effective biological control agents when tested in greenhouse and field experiments. In our study, 24–60% of the strains initially screened in TCP assays were effective in GC assays. However, in another similar survey (2), 40% of 3,500 bacterial strains were effective in suppressing *R. solani* and several other soilborne pathogens on agar plates, but only 4% were effective in suppressing Rhizoctonia damping-off in greenhouse tests in steamed soil. It is clear that the selection of effective turfgrass strains from TCP assays insures a higher rate of success among these strains in subsequent assays than those selected from other types of in vitro screenings.

Although we used *P. aphanidermatum* and creeping bentgrass as a model in this investigation, the assay should be suitable for use with other pathogens and turfgrass species. We have used the assay effectively to screen antagonists against other species of *Pythium* and *Rhizoctonia solani* on both creeping bentgrass and Kentucky bluegrass (E. B. Nelson, *unpublished data*). However, the use of larger seeded turfgrass species, such as perennial ryegrass, in TCP assays may not be suitable for the selection of antagonists. The large seed size of perennial ryegrass restricts the number of seeds per well and thus limits the density of turf in each well, resulting in increases in well-to-well variability. This can be overcome by planting known numbers of perennial ryegrass seed and determining seedling stands after 7 days (E. B. Nelson and C. M. Craft, *unpublished data*).

A high frequency of effective antagonists of *P. aphanidermatum* could be found in rhizosphere soil and thatch at all turfgrass sites. However, turfgrass thatch samples were a richer source of effective antagonists than were rhizosphere soil samples. This is not surprising because most heterotrophic soil bacteria are effective saprophytes well-suited for the degradation of organic matter (1). Although the sample size of golf course thatch strains was somewhat small in this study (41 strains), isolations from thatch of golf course putting greens may provide the greatest frequency of strains suitable for field testing (i.e., effective in both TCP and GC assays). Since golf course putting greens receive relatively heavy applications of pesticides, this, at first glance, seems contrary to what one would expect. However, Smiley and

TABLE 3. Suppression of Pythium blight by major bacterial groups recovered from soil and thatch

Bacterial group	TCP antagonists[u]		GC antagonists[v]	
	Number	Mean disease rating	Number	Mean disease rating
General heterotrophs[w]	42/71	2.01 a[x]	19/34	2.45 b
Enteric bacteria[y]	25/73	1.88 a	5/23	1.85 a
Pseudomonas spp.[z]	29/88	2.06 a	8/26	2.47 b

[u] Number of strains suppressing Pythium blight of creeping bentgrass (disease rating < 3.00 after 7 days) in tissue culture plate (TCP) assays out of the total number of strains tested.
[v] Number of strains suppressing Pythium blight of perennial ryegrass (disease rating < 3.75 after 4 days) in growth chamber (GC) assays out of the total number of TCP-effective strains tested.
[w] Strains recovered on 1/3-strength trypticase soy agar.
[x] Numbers followed by the same letter are not significantly ($P = 0.05$) different according to Duncan-Waller Bayesian least significant difference test.
[y] Strains recovered on Difco MacConkey agar.
[z] Strains recovered on a medium selective for *Pseudomonas* spp. (6).

TABLE 4. Suppression of Pythium blight of perennial ryegrass in tissue culture plate (TCP) and growth chamber (GC) asays by strains of *Enterobacter cloacae*

Treatment	Disease rating[w]	
	TCP assay	GC assay
Untreated	5.0 a[x]	5.0 a
Metalaxyl[y]	NT[z]	2.3 bc
E6	4.5 a	4.3 ab
EcCT-501	1.0 b	2.8 bc
EcH-1	1.0 b	3.0 a–c
E1	5.0 a	3.5 ab
Uninoculated	1.0 b	1.0 c

[w] Based on a scale of 1–5, for which 1 = no foliar blight and 5 = 100% of the seedlings nonemerged or blighted.
[x] Numbers in each column followed by the same letter are not significantly ($P = 0.05$) different according to the LSD test.
[y] Pots drenched with metalaxyl at the rate of 705 μg a.i./ml.
[z] Not tested.

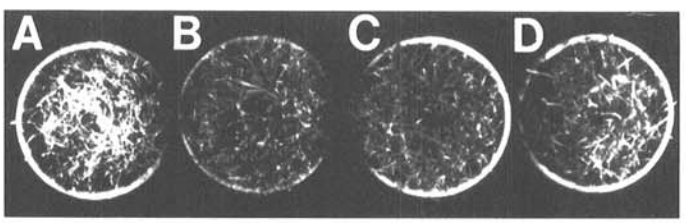

Fig. 2. Effect of various strains of *Enterobacter cloacae* and the fungicide metalaxyl on the suppression of Pythium blight of perennial ryegrass in growth chamber experiments. Disease severity was rated on a scale of 1–5, for which 1 = no foliar blight and 5 = 100% foliar blight. **A,** Untreated; **B,** drenched with metalaxyl (750 μg a.i./ml); **C,** treated with *E. cloacae* strain EcCT-501; and **D,** treated with *E. cloacae* strain E6.

Craven (24) found that bacterial populations increased after continuous application of many commonly used turfgrass fungicides. This, coupled with the observation that many of these fungicides also promote thatch accumulation (22), suggests that prolonged fungicide applications to turfgrasses may select for thatch populations of effective antagonists. This hypothesis must be tested before solid conclusions about this phenomenon can be made.

Although enteric bacteria and species within the genus *Pseudomonas* are known to be effective antagonists of *Pythium* species (5), fewer strains within these groups were effective in suppressing Pythium blight than were other heterotrophic bacterial strains. However, the strains of enteric bacteria recovered were more highly suppressive to Pythium blight than were *Pseudomonas* species or other general heterotrophic bacteria. Although antagonists can be recovered more frequently if isolated on a nonselective culture medium compared with media selective for enteric bacteria or *Pseudomonas* spp., the selection of highly suppressive antagonists may be improved by looking within Enterobacteriaceae.

Strains of *E. cloacae* used in this study have been effective antagonists of *P. ultimum* in other studies (7,12,13) and also have been effective against *P. ultimum* in TCP assays (A. P. Maloney and E. B. Nelson, *unpublished data*). However, only strains EcH-1 and EcCT-501 of *E. cloacae* were effective in suppressing *P. aphanidermatum* in TCP assays, while in GC assays only strain EcCT-501 gave rise to plant stands equivalent to those in uninoculated pots or pots treated with metalaxyl. The reasons for the differential suppression of *Pythium* species by strains of *E. cloacae* are not readily apparent but may be suggestive of suppression specificity among enterobacterial antagonists of *Pythium* species.

The miniaturized TCP assay developed in this study was an effective means of rapidly screening bacterial strains in plant assays so that those lacking biocontrol potential could be eliminated from further screening. This assay was simple, and it provided the sensitivity normally found in more cumbersome plant assays because disease suppression and, by implication, only those traits directly involved in disease suppression were evaluated. Another advantage of this assay was that small amounts of bacterial growth media, seeds, pathogen inoculum, sand, and space were needed, thus reducing the costs normally associated with large screening programs. By screening strains initially on plants, as opposed to pathogen-inhibition assays in petri plates, we hope to minimize the erroneous selection of strains on the basis of biological control traits that would not be expressed in more complex ecosystems. Further refinements of this assay, including the use of unsterilized soils and other turfgrass growing media, should improve the capacity to predict antagonist activity in the field. Finally, we conclude that thatch-inhabiting enteric bacteria are likely to be the most effective antagonists of *P. aphanidermatum* in turfgrass ecosystems.

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