Evaluation of Bacterial Antagonists for Reduction of Summer Patch Symptoms in Kentucky Bluegrass

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ABSTRACT

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Ten bacterial strains previously identified for their ability to control soilborne pathogens on agronomic crops were evaluated for their ability to suppress summer patch disease caused by *Magnaporthe poae* in Kentucky bluegrass (*Poa pratensis* L). Bacterial strains varied in the ability to inhibit the growth of *M. poae* in agar plate bioassays, although most strains inhibited the fungus to some degree. Three strains originally isolated from wheat, *Pseudomonas fluorescens* 2-79, *P. fluorescens* 13-79, and *Bacillus subtilis* D-39Sr, and two strains originally isolated from cotton, *Enterobacter cloacae* EcH-1 and EcCT-501, significantly reduced summer patch symptoms by between 29 and 46% compared to untreated control plants after a 5-week period in the growth chamber. The five strains did not reduce summer patch disease in field trials in 1990. However, in 1991, *B. subtilis* D-39Sr and *E. cloacae* EcH-1 reduced summer patch severity by 53 and 49%, respectively, over the entire season compared to untreated control plots. In 1994, both *B. subtilis* D-39Sr and *E. cloacae* EcH-1 also reduced summer patch severity over the entire season by up to 39 and 34%, respectively, compared to the untreated control in field plots. All five bacteria that reduced summer patch in growth chamber studies were present in the rhizosphere of greenhouse/growth chamber grown turfgrass at 10^4 to 10^6 CFU/g of fresh weight sample 2 weeks after application to plants. A single sampling of field plots during 1991 and four separate samplings of field plots during 1994 indicated that introduced bacteria were present within the turf at populations above 10^3 CFU/g of sample.

Additional keywords: biological control

Summer patch is one of the most destructive turfgrass diseases in the northeastern United States, affecting cool-season grass varieties such as annual bluegrass (*Poa annua* L.), Kentucky bluegrass (*Poa pratensis* L.), and fine fescues (*Festuca* spp.) (11,13). Symptoms of the disease develop under conditions of sustained high soil temperature and high soil moisture, and are observed as necrotic foliar areas that occur in circular patches (6,8). Under severe conditions, patches may coalesce and are often recolonized by less desirable grass species or weeds. The causal agent of the disease, *Magnaporthe poae* Landschoot & Jackson, characteristically grows ectotrophically as darkly pigmented runner hyphae on roots prior to infection. Under the appropriate conditions, infection occurs and leads to extensive colonization of vascular tissue, resulting in root dysfunction and the development of foliar symptoms (12).

Due to the demands for maintaining high-quality turfgrass, the control of summer patch as well as most other turfgrass diseases relies heavily on the use of fungicides. However, increasing pressures to reduce chemical inputs have resulted in the development of alternative methods to control turfgrass diseases such as summer patch. For example, management practices that reduce root stress resulting from soil compaction or low mowing heights were shown to alleviate symptoms of summer patch (11). Similarly, the use of acidifying sources of nitrogen fertilizer in field studies resulted in substantial disease control (24). One approach that has not been intensively investigated for summer patch is biological control. Although it seems unlikely that biocontrol would replace fungicides for turfgrass disease control, the development of biocontrol may be useful in an integrated approach to disease management and may ultimately contribute to a reduction in chemical inputs.

Encouraging results were previously obtained with biological control of turfgrass diseases. Several studies demonstrated the use of microbial antagonists for control of foliar diseases of turfgrasses, including dollar spot caused by *Sclerotinia homoeocarpa* F.T. Bennett (3,5,16,19), brown patch caused by *Rhizoctonia solani* Kühn (1,18,19,21,31), and gray snow mold caused by *Typhula phacorrhiza* (Reichard:-Fr.) Fr. (2,14). Fewer studies have investigated the use of biocontrol against root diseases of turfgrasses, although promising results were reported for biocontrol of Pythium blight caused by *Pythium* spp. (17,18,28) and take-all patch caused by *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *avenae* (E.M. Turner) Dennis (29,30). More recently, promising results were obtained with biocontrol to suppress summer patch in greenhouse studies (9,10,25); however, the efficacy of biocontrol agents in the field has yet to be determined.

The objective of this study was to evaluate strains of bacteria, which have functioned as biocontrol agents of soilborne diseases in agronomic crops, for their ability to reduce summer patch disease in Kentucky bluegrass in growth chamber and field studies. Preliminary results of this study have been published (23).

MATERIALS AND METHODS

Bacterial strains and fungal isolates. Bacterial strains, described in Table 1, were maintained on either nutrient broth yeast extract (NBY) agar or trypticase soy (TS) agar. Bacteria were obtained as strains resistant to rifampicin (Rif^+) or were selected for spontaneous resistance to the antibiotic by plating heavy suspensions of each strain (approximately 10^{10} CFU/ml) on NBY or TS agar supplemented with an antibiotic at 50 µg/ml. Single colonies were restreaked onto the same medium to confirm resistance. Isolates with antibiotic resistance and unaltered phenotypic characteristics were selected for further use. Prior to their use in 1994, Rif^+ strains were selected for resistance to nalidixic acid (Nal^+) on TS agar supplemented with nalidixic acid at 50 µg/ml. All bacteria were routinely stored at –20°C and –70°C in NBY or TS broth supplemented with 50% glycerol.

Isolates of *M. poae* were obtained in New Jersey from annual bluegrass exhibiting symptoms of summer patch. The pathogen was isolated from roots by surface-sterilizing infected tissue in 10% commercial bleach for 1 min, rinsing, and plating roots on potato-dextrose agar (PDA) supplemented with cycloheximide

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(100 µl/ml). Species identification was confirmed by mating unknown isolates with mating type test isolates ATCC 64411 and ATCC 64412 to initiate the production of perithecia, as described by Landschoot and Jackson (13). Virulence of isolates was confirmed by inoculating Kentucky bluegrass cv. Baron (KBG) in growth chamber assays as previously described (13). All isolates selected for further use were grown and maintained on half-strength PDA at 20°C.

Growth inhibition bioassays. For in vitro growth inhibition assays, 5-day-old cultures of *M. poae* grown on half-strength PDA were comminuted in a sterile blender, and 50 ml of the mixture was added to 650 ml of molten half-strength PDA cooled to 50°C. Approximately 20 ml of the suspension was poured into a 9-cm-diameter petri dish and allowed to solidify. Bacteria were prepared by growing cells on TS agar for 1 to 2 days before suspending in sterile H_2O to concentrations of approximately 10^8 CFU/ml (OD_{595} = 0.3). Each bacterial suspension was spotted (25 µl) at five locations around the perimeter of a single agar plate. After 7 days incubation at 25°C, zones of *M. poae* growth inhibition were measured from the edge of each bacterial colony to the nearest edge of mycelial growth.

Growth chamber studies. All experiments were conducted on KBG plants grown in 20.5-cm-long by 4-cm-diameter conical polyethylene containers. KBG was planted in containers plugged at the bottom with two absorbent cotton balls and filled with a dry-heat pasteurized mix of fine sand and peat (4:1 vol/vol) to 2 cm from the top. Twenty-five mg of KBG seed was evenly spread on the soil surface of each container and covered with 0.3 cm of the sand–peat mix. Planted containers were placed in a mist chamber for 2 weeks at 24°C to allow seed germination. Once germinated, plants were moved to a growth chamber with 14 h/day of 500 µE fluorescent light at 28°C. Each container received 25 ml of H_2O daily and, beginning 4 weeks after seeding, plants were clipped to a leaf length of 2.5 cm and fertilized with 20-20-20 N-P-K weekly.

Fungal inoculum was prepared by autoclaving 25 g of tall fescue seed (*Festuca arundinacea* Schreb.) in 23 ml of H_2O for 60 min on each of three successive days. Five *M. poae* isolates were grown separately on the sterilized seed for 2 to 3 weeks at room temperature before they were air-dried and mixed together for use as inoculum. All plants were inoculated after 4 weeks of growth by placing 0.1 g of inoculum on the soil surface in each container.

Bacterial treatments were prepared by growing strains in shake cultures of NBY or TS broth for 48 h at 30°C. Cells were collected by centrifugation at 1,000 × *g* for 5 min and were resuspended in 5 mM potassium phosphate buffer (pH 7) to approximately 10^8 CFU/ml (OD_{595} = 0.3). Bacterial suspensions were applied as soil drenches at a rate of 50 ml per container at 3 and 5 weeks after planting. Each treatment was replicated 10 times, and treatments were arranged in a randomized complete block design. Each bacterial strain was evaluated in two or more experiments.

Summer patch symptoms were evaluated 8 weeks after planting, based on the percent turf area with visible necrosis. A 1 to 9 disease rating scale was used, where 1 = 0 to 4%; 2 = 5 to 9%, 3 = 10 to 19%, 4 = 20 to 39%, 5 = 40 to 59%, 6 = 60 to 79%, 7 = 80 to 94%, 8 = 95 to 99%, and 9 = 100% necrosis. Ratings were changed to percentage values by assigning the mean of the percentage range for each division of the foliar rating scale. Percent disease control was calculated by subtracting the percentage of foliar necrosis in treated plants from the percentage of foliar necrosis in untreated controls, and dividing the difference by the percentage of foliar necrosis in the untreated control.

Field study: 1990 to 1991. The five bacterial strains that suppressed the development of summer patch symptoms in growth chamber studies were selected for application to pathogen-inoculated field plots. Field studies were conducted on a 1-year-old stand of Kentucky bluegrass cv. Fylking growing on a Sassafras sandy loam in North Brunswick, New Jersey. Turf was maintained at a mowing height of 3.8 cm and fertilized with nitrogen at 12 kg/ha every 6 weeks using Nutralene, a slow-release mix of methylene urea polymers (Agrevo Chemical Co., Wilmington, DE). Individual plots were 1.5 × 1.5 m. Prior to fungal inoculation in 1990, all plots were aerated with two passes of a hollow-tine aerator that produced 7.5-cm-deep × 0.6-cm-diameter holes every 26 cm^2 on each pass. Irrigation supplemented measured rainfall to provide a total of 2.5 cm of water per week.

M. poae inoculum was prepared as described for growth chamber studies, except that sterile oat seed (*Avena sativa* L.) was substituted for tall fescue seed and inoculated seeds were incubated for 4 weeks at room temperature. Plots were inoculated with *M. poae* once, on 6 June 1990, for the 2-year study. With the exception of uninoculated controls, all plots were inoculated at the four sites marked by corners of a 0.6 × 0.6 m square frame positioned in the center of each plot. At two of the four sites, a 2.5-cm-diameter hole was cut to a depth of 5 cm and filled with 40 cm^3 of infested oat seed. At the remaining two sites, holes were cut to a depth of 2.5 cm and filled with 20 cm^3 of infested oat seed. The specified hole depths placed the top of the inoculum 2.5 cm below the soil surface. Inoculum was covered with soil and a sod plug, and was irrigated to prevent drying. Since sterile oat seed had no effect on turf quality in previous summer patch field studies (B. Clarke, *unpublished*), holes in the control plots in this study were filled with soil instead of uninfested oat seed.

Bacteria were prepared for application to field plots by two methods. In the first method, a 1:3:1 mix of cornmeal, sand, and water (CSW) was prepared as described by Nelson and Craft (16). Fifty ml of bacterial suspension, at 10^8 CFU/ml (OD_{595} = 0.3), was added to 1,200 cm^3 aliquots of sterilized CSW, and cultures were incubated for 36 h at 20°C. In the second method, suspensions of bacteria were prepared for application as soil drenches by growing cells in TS broth for 48 h at 30°C. Cells were collected by centrifugation at 1,000 × *g* for 5 min and resuspended in sterile H_2O to a concentration of approximately 10^8 CFU/ml.

In 1990, bacteria were initially applied 2 weeks prior to fungal inoculations by uniformly distributing 1,200 cm^3 of bacteria-infested CSW over the surface of individual plots. Bacteria were then applied as

Table 1. Bacterial strains tested as biological control agents against *Magnaporthe poae* in laboratory, growth chamber, and field studies

Bacterial strain	Host origin and biocontrol use	Source (ref.)
Bacillus pumilus 147R	Isolated from wheat	M. Elliott (7)
B. subtilis D-39Sr	Isolated from wheat	M. Elliott (7)
B. subtilis D-60R	Isolated from wheat	M. Elliott (7)
Bacillus sp. 58R	Isolated from wheat	M. Elliott (7)
Enterobacter cloacae EcCT-501	Isolated from cotton hypocotyls; biocontrol agent for Pythium damping-off of cotton and dollar spot of bentgrass	E. Nelson (15)
E. cloacae EcH-1	Isolated from cotton hypocotyls; biocontrol agent for Pythium damping-off of cotton and dollar spot of bentgrass	E. Nelson (15)
E. cloacae E1	Isolated from cucumber seeds; biocontrol agent for Pythium rot	E. Nelson (4)
E. cloacae E6	Isolated from cucumber seeds; biocontrol agent for Pythium rot	E. Nelson (4)
Pseudomonas fluorescens 13-79	Isolated from wheat; biocontrol of wheat take-all	H. Wilkinson (27)
P. fluorescens 2-79	Isolated from wheat; biocontrol of wheat take-all	H. Wilkinson (27)

drenches on two additional dates of 12 June and 7 August by uniformly spreading 2 liters of cell suspensions over the surface of individual plots using a watering can. In 1991, bacteria were applied as in 1990. Bacteria were first applied to plots on 24 May as a CSW mix. Subsequent applications were made as cell suspensions on 14 June, 12 July, 16 August, and 3 September. On all application dates, control plots (pathogen inoculated and uninoculated) were treated with either sterile CSW or sterile H_2O. After each application, plots were irrigated overhead with 0.5 to 1 cm of water to prevent drying and to facilitate dispersal of bacterial treatments into the turf.

Summer patch was assessed in individual plots by rating foliar symptoms that developed at the four inoculation sites. In 1990, disease ratings were first recorded when symptoms of summer patch initially appeared at individual inoculation sites on 22 August. Additional disease ratings were recorded on 28 August and 9 September. In 1991, symptoms were evaluated on 9, 16, and 24 July, and 7 and 20 August. For each patch, diameters were determined as the mean of two perpendicular measurements. The area of each patch was then calculated using the formula for area of a circle. The percentage of necrotic foliage (PNF) within a patch was estimated using a scale from 0 to 3, where 0 = no observable symptoms, 1 = 1 to 20%, 1.5 = 21 to 40%, 2 = 41 to 60%, 2.5 = 61 to 80%, and 3 = 81 to 100% of leaves in patch necrotic. Disease severity was calculated by multiplying the area of a patch by the PNF value for that patch. The area under the disease progress curve (AUDPC) was determined for each treatment by averaging disease severity values for two consecutive observations and multiplying the results by the number of days between the observations. Values calculated between consecutive observations were then summed over the entire period of observation.

Field study: 1994. The site consisted of a 3-year-old stand of Kentucky bluegrass cv. Baron established on a Norton loam soil in North Brunswick. Turf plots were maintained throughout the year as described for the 1990 to 1991 study. Bacterial treatments were replicated six times and were arranged in a randomized complete block design, with each replicate consisting of a 0.9 × 0.9 m plot with a 0.3-m border.

The quantity of *M. poae* inoculum used to inoculate plots in the 1994 study was reduced due to the high level of disease that appeared in plots during the 1990 season. Inoculum was prepared on oat seeds as described for 1990. *M. poae* mycelium was also grown on filter paper overlaid onto half-strength PDA. Each of five fungal strains was inoculated on the paper surface and incubated at 28°C. Once the mycelium reached the edge of the filter paper, the colonized paper was removed, dried in a desiccator, and divided into 1 cm^2 pieces. The center of each field plot was inoculated with *M. poae* on 11 May at four sites as described for the 1990 to 1991 study. Two sites were inoculated with 20 cm^3 of infested oat seed, and the remaining two sites were inoculated with five 1 cm^2 pieces of mycelium-colonized filter paper, each containing one of the five different *M. poae* isolates. Since summer patch symptoms did not develop at any site inoculated with colonized filter paper, only data from the two sites inoculated with infested oat seeds were collected and analyzed.

For the 1994 study, bacteria were prepared and applied to plots as suspensions, as described for the 1990 to 1991 study. However, the frequency of bacterial applications was increased to 2-week intervals from 23 May until 16 August. Plots were evaluated for disease on 26 August and 9 and 20 September.

Estimation of bacterial populations in turf. For growth chamber studies, bacterial populations in the KBG rhizosphere were estimated 2 weeks after the final application of bacteria. Bacteria were recovered from the rhizosphere by gently shaking plants to remove loosely adhering soil from the roots. Roots were cut from the crown and washed in 50 ml of H_2O by shaking for 15 min, sonicating for 1 min, and shaking again for 15 min. Samples

Table 2. Effect of bacterial strains on growth of *Magnaporthe poae* in growth inhibition assays and suppression of summer patch symptoms in the growth chamber

Bacterial strain	In vitro growth inhibition[a]	Growth chamber assay[b]	
		Foliar necrosis (%)	Disease control (%)
Bacillus subtilis D-39Sr	0.1 to 0.3 cm inhibition zone	40.0*	46*
Bacillus sp. 58R	No inhibition zone	58.6	21
B. subtilis D-60R	>0.3 cm inhibition zone; <1.5 cm	62.9	15
B. pumilus 147R	>0.3 cm inhibition zone; <1.5 cm	62.9	15
Enterobacter cloacae E1	No inhibition zone	51.4*	31*
E. cloacae EcH-1	<0.1 cm inhibition zone	52.9*	29*
E. cloacae EcCT-501	<0.1 cm inhibition zone	60.0	19
E. cloacae E6	>0.3 cm inhibition zone; <1.5 cm	68.6	8
Pseudomonas fluorescens 13-79	>0.3 cm inhibition zone; <1.5 cm	41.4*	44*
P. fluorescens 2-79	>0.3 cm inhibition zone; <1.5 cm	51.4*	31*
Control	...	74.3	0
MSD ($P = 0.05$)[c]		20.9	28

[a] Values were the results of means of measurements from five replicates for growth inhibition assays against *M. poae*.

[b] Disease rating is the percentage of foliar necrosis. Values for disease ratings are means of 10 replicates. Means followed by * are significantly different from the untreated, pathogen-inoculated control. Data is from 1 of 2 trials.

[c] MSD is the minimum significant difference between two means according to Waller-Duncan *k*-ratio multiple comparison *t* test at $k = 100$.

Table 3. Summer patch size (area) and percent necrotic foliage within a patch (PNF) in bacteria-treated field plots of Kentucky bluegrass cv. Fylking during 1991

Treatment[a]	9 July		16 July		24 July		7 August		20 August	
	Area[b]	PNF[c]	Area	PNF	Area	PNF	Area	PNF	Area	PNF
Bacillus subtilis D-39Sr	210	18*	171	25	279	45	217*	40	384	45
Enterobacter cloacae EcH-1	146*	23*	215	22	250	39	290*	41	365*	45
E. cloacae E1	241	28	356	30	339	30	353	50	458	50
Pseudomonas fluorescens 2-79	258	35	272	28	328	48	294	50	432	53
P. fluorescens 13-79	217	37	281	32	340	53	389	53	536	53
Control	368	50	377	41	368	48	499	50	652	52
MSD ($P = 0.05$)[c]	174	24	257	20	301	22	204	13	285	12

[a] Bacterial strains applied to field plots in 1991. Control refers to pathogen-inoculated plots with no bacterial treatment.

[b] Mean values for areas, in cm^2, for individual patches.

[c] MSD is the minimum significant difference between two means within a column according to the Waller-Duncan multiple comparison *k*-ratio *t* test at $k = 100$. * = significantly different from untreated control.

were serially diluted and plated onto NBY agar supplemented with rifampicin (50 µg/ml) and cycloheximide (100 µg/ml).

Bacterial populations were estimated in field samples collected one time in 1991 on 26 August. In 1994, populations of bacteria were evaluated four times, on 25 May, 5 and 7 Jul, and 16 August. These dates corresponded to 1 day after the first application of bacteria, 2 weeks after the third application, 1 day after the fourth application, and 2 weeks after the sixth application. On each date, samples were taken from four replicate plots of each treatment. Samples consisted of three 2.5-cm-diameter × 7.5 cm-deep-turf cores recovered from arbitrarily selected sites within each plot. The three cores were combined, and bacterial populations were determined from the entire sample, which consisted of densely packed soil, roots, thatch, and foliage. The fresh weight of each sample was determined prior to washing in 50 ml of H_2O by shaking at 200 rpm for 15 min, sonicating for 30 s, and shaking again for 45 min. Samples were serially diluted and plated on TS agar supplemented with antibiotics.

Statistical analysis. Analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC). The General Linear Models procedure was used to determine treatment means and minimum significant differences (MSD) between means according to the Waller-Duncan *k*-ratio, multiple comparison *t* test (k = 100, which approximates P = 0.05). Tests were analyzed as randomized complete block designs. Since disease ratings for different inoculum levels in 1990 to 1991 were similar, data from individual inoculation sites were combined and analyzed as replicates within a plot. Only two sites per plot were analyzed in 1994. For disease data obtained using a rating scale, values were converted to percents (midpoint of ranges) prior to analysis. Values for disease severity and AUDPC were calculated for each disease patch prior to analysis.

RESULTS

Growth inhibition in bioassays. Most bacterial strains tested in this study inhibited the growth of *M. poae* in the agar plate bioassay (Table 2). The amount of growth suppression ranged from none to zones between 0.3 to 1 cm in size from the edge of colonies. Substantial inhibition of *M. poae* was observed with strains belonging to all three genera, *Bacillus*, *Enterobacter*, and *Pseudomonas* spp., represented in the test.

Growth chamber studies. Summer patch symptoms were significantly reduced (P = 0.05) in 7-week-old plants treated with five of the 10 bacteria in growth chamber studies (Table 2). The five strains included one *B. subtilis* isolate, D-39Sr, two *E. cloacae* isolates, EcH-1 and E1, and two *P. fluorescens* isolates, 13-79 and 2-79. Disease in plants treated with these bacteria was reduced by 29 to 46% compared with levels of disease in untreated control plants. However, treatments that were significantly different from untreated controls were not significantly different from each other (P = 0.05).

Summer patch suppression in the field: 1990 to 1991. In 1990, no significant differences (P = 0.05) were observed in patch area or the percentage of necrosis within patches that developed in bacteria-treated plots compared with untreated control plots. Furthermore, no significant differences were observed when treated plots were compared with untreated plots by disease severity (patch area × % necrotic area) on individual dates, or by total disease over the course of the observation period, as determined by AUDPC (data not shown).

New growth of turfgrass during the spring of 1991 resulted in complete recovery of disease symptoms that had developed at individual inoculation sites in field plots during 1990. Although plots were not reinoculated with *M. poae*, summer patch symptoms began to appear by late June, approximately 1 month after the first application of bacteria. In all cases, disease patches appeared within plots only at inoculation sites from the previous year. Symptoms developed much earlier in 1991 than in the previous year, which was attributed to the favorable environmental conditions for summer patch development that occurred unusually early in the season. As a result of the early appearance of disease, ratings were recorded on five observation dates that began on 9 July and ended on 20 August.

On three of the five observation dates, significant differences in patch area were observed in plots treated with either *B. subtilis* D-39Sr or *E. cloacae* EcH-1 when compared with untreated control plots (Table 3). Reduction of patch area in plots treated with these bacteria compared to untreated controls was especially evident on the last two observation dates. In contrast, differences in the percentage of necrotic area within patches were observed only on the first observation date.

Disease severity, which represents percentage of necrotic area over total patch area, increased over time for all treatments in 1991. However, compared with untreated controls, disease severity was significantly reduced (P = 0.05) in plots treated with *E. cloacae* EcH-1 on 9 July and 7 August, and with *B. subtilis* D-39Sr on 9 July and 7 and 20 August (Fig. 1). Significant differences (P = 0.05) in total disease, as determined by the AUDPC over the entire observation period, were also observed in plots treated with D-39Sr (AUDPC = 4,354) and EcH-1 (AUDPC = 4,779) compared to untreated control plots (AUDPC = 9,305). Calculated values for the AUDPC indicated that total disease was reduced by 53 and 49% in plots treated with D-39Sr and EcH-1, respectively. No significant differences were observed for *E. cloacae* E1, *P. fluorescens* 2-79, or *P. fluorescens* 13-79 in individual disease ratings (Table 3), or in total disease severity compared to untreated control plots (E1, AUDPC = 6,332; 2-79, AUDPC = 6,344; 13-79, AUDPC = 7,970).

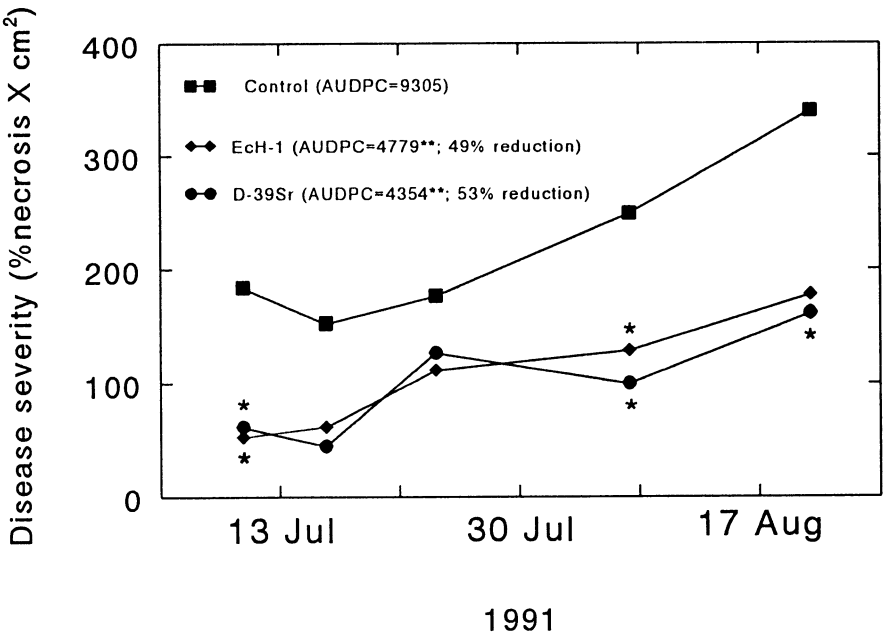

Fig. 1. Summer patch disease severity in field plots of Kentucky bluegrass cv. Fylking treated with *Enterobacter cloacae* EcH-1 and *Bacillus subtilis* D-39Sr during 1991. The control represents pathogen-inoculated plots that received no bacterial treatment. Area under disease progress curve (AUDPC) values are expressed as (cm² × % necrosis × days). * = disease severity values were significantly different from control; ** = AUDPC values were significantly different from control based on Waller-Duncan *k*-ratio *t* test at k = 100, which approximates P = 0.05.

Summer patch suppression in the field: 1994. In 1994, *B. subtilis* D-39Sr, *E. cloacae* EcH-1, and *P. fluorescens* 2-79 were applied to pathogen-inoculated field plots located at a separate site from the 1990 to 1991 plots. In this year, summer patch symptoms were first observed and evaluated on 26 August. Over all, less disease developed in field plots in 1994 compared to the 1990 to 1991 study.

Table 4. Summer patch size (area) and percent necrotic foliage within a patch (PNF) in bacteria-treated field plots of Kentucky bluegrass cv. Baron during 1994

	26 August		9 September		20 September	
Treatment[a]	**Area[b]**	**PNF**	**Area**	**PNF**	**Area**	**PNF**
Enterobacter cloacae EcH-1	37	13	111	88	68*	80
Bacillus subtilis D-39Sr	41	23	102*	57*	71	78
Pseudomonas fluorescens 2-79	84	26	99*	65*	84	74
Control	74	26	156	88	117	76
MSD ($P = 0.05$)[c]	46	23	47	21	46	17

[a] Bacterial strains applied to field plots in 1994. Control refers to inoculated plots with no bacterial treatment.
[b] Mean values for areas, in cm^2, for individual patches.
[c] MSD is the minimum significant difference between two means within a column according to the Waller-Duncan multiple comparison *k*-ratio *t* test at $k = 100$. * = significantly different from untreated control.

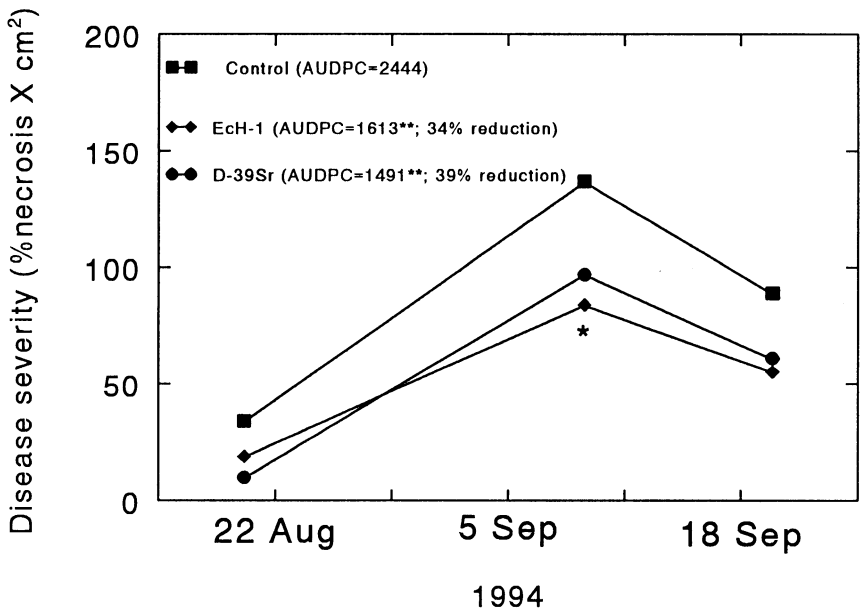

Fig. 2. Summer patch disease severity in field plots of Kentucky bluegrass cv. Baron treated with *Enterobacter cloacae* EcH-1 and *Bacillus subtilis* D-39Sr during 1994. Control is pathogen-inoculated plots with no bacterial treatment. Values for AUDPC are expressed as (cm^2 × % necrosis × days). * = disease severity values were significantly different from control; ** = AUDPC values were significantly different from control based on Waller-Duncan *k*-ratio *t* test at $k = 100$, which approximates $P = 0.05$.

Table 5. Bacterial populations in the rhizosphere of Kentucky bluegrass treated with bacterial antagonists

	Bacterial populations[a]	
Strain	**Growth chamber[b]**	**1991 field plots[c]**
Bacillus subtilis D-39Sr	5.8	3.9
Enterobacter cloacae E1	6.2	3.9
E. cloacae EcH-1	5.3	3.8
Pseudomonas fluorescens 2-79	4.3	3.6
P. fluorescens 13-79	5.0	3.5
MSD ($P = 0.05$)[d]	NS	NS

[a] Values represent log CFU/g of fresh weight of sample.
[b] Samples consisted of roots and rhizosphere soil from 7-week-old plants, 2 weeks after final applications of bacteria.
[c] Samples consisted of turf cores obtained from plots 10 days after the fourth application of bacteria in 1991.
[d] MSD is the minimum significant difference between means within a column according to the Waller-Duncan multiple comparison *k*-ratio *t* test, at $k = 100$. NS = not significant.

Similarly to 1991, significant disease reduction was observed in plots treated with bacteria when compared with untreated plots, but the disease reduction varied depending on the date of observation and the disease rating. For example, plots treated with *E. cloacae* EcH-1 were significantly reduced ($P = 0.05$) only in patch area on the final observation date of 20 September (Table 4). Significant reduction in both patch area and percentage of necrotic area within patches was observed in plots treated with *B. subtilis* D-39Sr, but only on the single observation date of 9 September. In contrast, no significant differences in either patch area or percentage of necrotic area within patches were observed on any observation date in plots treated with *P. fluorescens* 2-79 compared with untreated control plots. When disease severity values were compared, only D-39Sr was significantly reduced ($P = 0.05$) on 9 September (Fig. 2). However, significant reduction ($P = 0.05$) in total disease was observed in plots treated with D-39Sr (AUDPC = 1,491) or EcH-1 (AUDPC = 1,613); whereas no significant reduction in total disease was observed in plots treated with *P. fluorescens* 2-79 (AUDPC = 1,670) compared to untreated control plots (AUDPC = 2,444) (Fig. 2). Total disease was reduced by 39% in plots treated with D-39Sr and by 34% in plots treated with EcH-1 compared with untreated control plots.

Bacterial populations in turf. Turfgrass rhizosphere populations were evaluated for the five strains that significantly reduced summer patch development in the growth chamber. Estimates of bacterial populations in the rhizosphere of greenhouse-grown plants 2 weeks after the second application indicated that all five strains were present in the rhizosphere at populations ranging from 10^4 to 10^6 CFU/g of fresh weight sample (Table 5). Although populations of the five different bacteria were not significantly different, the isolates with two lowest mean values were both *P. fluorescens* strains.

Bacterial populations were also estimated in field plots during 1991 and 1994. On 26 August 1991, 10 days after the fourth application, all five strains were present in the turf at populations ranging between 10^3 and 10^4 CFU/g of turf sample (Table 5). Similarly to growth chamber studies, no significant differences were detected between populations of the five isolates tested in the field; however, the two isolates with the lowest mean population values were *P. fluorescens* strains 13-79 and 2-79.

In 1994, field plots were sampled at four different times to determine the populations of introduced bacteria throughout the course of the study. Mean population values for *E. cloacae* EcH-1 were higher than values for both *B. subtilis* D-39Sr and *P. fluorescens* 2-79 on all sampling dates (Fig. 3). On three of the four dates, the

population values for EcH-1 were significantly higher (P = 0.05) than values for either D-39Sr or 2-79. Populations of EcH-1 ranged between 10^4 and 10^5 CFU/g of sample over the course of the sampling period. In contrast, populations of D-39Sr ranged between 5×10^3 and 5×10^4 CFU/g of sample, and 2-79 ranged between 5×10^3 and 5×10^4 CFU/g of sample.

DISCUSSION

Five of 10 bacterial strains tested in this study significantly reduced disease symptoms in Kentucky bluegrass in growth chamber studies. Two of these bacteria, *B. subtilis* D-39Sr and *E. cloacae* EcH-1, consistently reduced summer patch symptoms in two different field studies, raising optimism for the use of biocontrol in the management of turfgrass diseases caused by root-infecting fungi.

Reduced disease was observed in bacteria-treated plots when compared to untreated controls on specific observation dates, as well as over the course of the season. According to disease progress curves, disease appeared to progress similarly over the course of the season, but at lower severity values in treated plots compared with untreated control plots. Although no models were fit to the data, these observations may be interpreted as substantial delays in the onset of the disease epidemic following application of bacteria. In previous studies conducted in growth chambers, significant reduction in summer patch by strains of *Serratia marcescens* and *Xanthomonas maltophilia* delayed summer patch symptom development; whereas rates of symptom progression were similar between treated and untreated plants (10). In other studies, reduced *M. poae* inoculum delayed summer patch symptom development but did not appear to affect the rate of disease progression (D. Kobayashi and D. Thompson, *unpublished*). Based on these observations, disease reduction in the field was interpreted to be the result of bacteria affecting early stages of the disease cycle, perhaps by affecting the pathogen directly or by preventing pathogen colonization of host roots prior to infection.

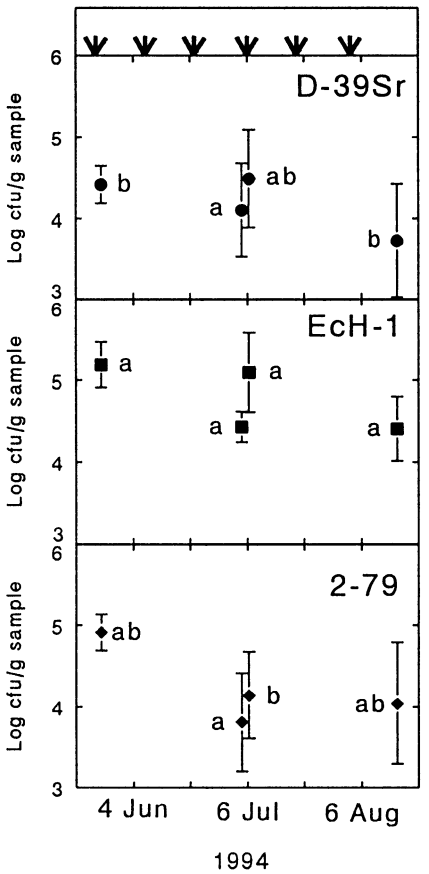

Fig. 3. Populations of *Bacillus subtilis* D-39Sr, *Enterobacter cloacae* EcH-1, and *Pseudomonas fluorescens* 2-79 in field plots of Kentucky bluegrass cv. Baron during 1994. Arrows depict dates of bacterial applications. Turf plots were sampled on 25 May, 5 and 7 July, and 16 August. Minimum significant differences between strains within an observation date were 0.34 (25 May), 1.04 (5 July), 0.86 (7 July), and 0.67 (16 August) according to Waller-Duncan k-ratio t test at k = 100, which approximates P = 0.05. Population means with the same letters were not significantly different on the date of evaluation.

Establishment and persistence of high populations of biocontrol agents within the rhizosphere is critical to achieving effective control of soilborne diseases (20,26). Nelson and Craft (16) previously demonstrated that *E. cloacae* EcH-1 colonized bentgrass turf and controlled dollar spot. Although the strains used in this study were originally isolated from other plant species, our results indicated that they colonized the rhizosphere of Kentucky bluegrass in the growth chamber and maintained high populations in the field.

Four of the six strains that suppressed summer patch symptoms in our growth chamber studies were not effective in the field in 1990 to 1991. Many factors, including adverse environmental conditions, may have affected the ability of these isolates to reduce disease. None of the strains in this study controlled summer patch in 1990, possibly from high disease pressures, low bacterial concentrations resulting from too few applications, or a combination of both. Likewise, *P. fluorescens* 2-79 did not reduce summer patch symptoms in any field study, although substantial populations of this strain were present in turf at all sampling dates during 1991 and 1994. In our studies, a detailed population study was not conducted; however, 2-79 may not have reached or maintained population levels required for disease control.

Although the mechanisms for suppression of summer patch by the bacterial strains used in this study are unknown, all field-tested strains expressed some antifungal activity against *M. poae* in agar plates. These observations support antibiosis as a mechanism for biocontrol by these strains (7,22,27).

In the two different field trials, summer patch was reduced by treatments of *E. cloacae* EcH-1 by 34 to 49%, and by treatments of *B. subtilis* D-39Sr by 39 to 53%. This level of control is not acceptable for turf disease management in most situations. However, the reduction indicates that biological components integrated with other management practices may provide high levels of disease suppression while reducing fungicide inputs.

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