Pathogenicity of *Xanthomonas translucens* from Annual Bluegrass on Golf Course Putting Greens

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ABSTRACT

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Bacterial wilt of *Poa annua* has been seen increasingly in the Northeast and mid-Atlantic United States in the past few years. The disease causes severe injury to putting greens and can kill large stands of turfgrass. For some time, however, both the bacterial origin of the disease and the causal agent were in doubt. In order to investigate the identity of the causal agent, isolation of the pathogen was undertaken and pathogenicity was confirmed using Koch's postulates on *P. annua*. Additional pathogenicity trials then were undertaken to determine the host range of the causal bacterium. Ability of the bacterium to cause disease was restricted to *P. annua* var. *annua* and *P. attenuata*. However, the bacterium was able to survive asymptotically in vascular systems of *P. annua* var. *reptans* and *P. trivialis*. Experiments to determine the optimal growth temperature of the organism demonstrated that the bacterial growth peaked between 30 and 35°C. Fatty acid analysis suggested that the bacterium might be a species of *Xanthomonas* but was inconclusive. Ribosomal RNA analysis demonstrated significant similarity to the American Type Culture Collection isolate of *Xanthomonas translucens* pv. *poae* at 99.8%. Comparison of the host range to previously reported data agrees with our molecular findings and indicates that the likely causal organism of bacterial wilt of annual bluegrass is *X. translucens* pv. *poae*.

In much of the northern United States, golf course putting greens are composed of a mixture of bentgrasses, usually creeping bentgrass (*Agrostis palustris* Huds., also known as *A. stolonifera*) and the invasive annual bluegrass (*Poa annua* L.). Because of the expense and difficulty of preventing annual bluegrass encroachment, many golf courses focus on suppressing the production of annual bluegrass seed heads with growth regulators, as opposed to outright *P. annua* removal. Although some *P. annua* reduction may be achieved using this tactic, the immediate result is a more consistent and aesthetically appealing playing surface. Thus, mitigation of *P. annua* impact is currently more practical than control and putting greens significantly composed of *P. annua* are commonly encountered.

Although *P. annua* can provide an excellent putting green playing surface, it is more susceptible to diseases and environmental stresses than *A. palustris*. In the past several years, a bacterial wilt of an-

nual bluegrass has been observed with increasing frequency in the northeastern United States (3). In 2002, the University of Rhode Island Turfgrass Disease Diagnostic Laboratory identified bacterial wilt on *P. annua* for approximately 8% of the disease samples received, primarily from Connecticut but also from Rhode Island, Massachusetts, and Pennsylvania. On putting greens composed of a high percentage of annual bluegrass, this intractable disease was the cause of substantial turf loss.

Symptoms of bacterial wilt begin as lesions formed at the site of infection, typically the ends of the clipped leaves. The bacteria move down into the sheaths to the plant crowns, causing wilting of entire leaves. Eventually, crowns are killed and small patches of dead and dying grass are left behind. On closely cut grasses, leaf lesions are not readily observed, because the bacteria quickly move the short distance into the sheaths and crowns. Symptoms may be confused with anthracnose (*Colletotrichum graminicola* (Ces.) G. W. Wils.), which also can produce small yellow dispersed patches of dead grass. Bacterial wilt symptoms can be highly variable in color, however, and diagnosis often is difficult because of the lack of organismal signs. Damage usually is most severe on the peripheral or clean-up areas and walk-on and walk-off areas of putting greens.

Bacterial wilt of amenity turfgrasses originally was identified in the 1970s on *A. palustris* cv. Toronto in the Chicago area.

The causal organism was identified as *Xanthomonas campestris* pv. *graminis* and the only host identified was Toronto C-15 creeping bentgrass (15). Successful control was achieved by overseeding with an alternate creeping bentgrass cultivar (14). In the 1995 revision of the genus *Xanthomonas* by Vauterin et al., molecular analysis and other factors placed the pathovars of *X. campestris* that infect grasses into the species *X. translucens* (20).

Bacterial wilt was first reported on *P. annua* by Roberts et al. (16). The causal organism of bacterial wilt of *P. annua* originally was identified as *X. campestris* pv. *poaannua* and a number of researchers have evaluated its potential as a biological control in the United States (8,11,18,21). In Japan, a similar disease has been observed and the causal organism has been identified as *X. campestris* pv. *poae* (13). Researchers in Japan also have experimented with the Japanese isolates for the biological control of *P. annua* (7,12,13).

Beyond the work that originally was undertaken to study *X. campestris* pv. *graminis* on creeping bentgrass, very little research has been conducted on this emerging disease, particularly as it affects *P. annua* (2). The purpose of our research was to identify potential causal agents of bacterial wilt of annual bluegrass in the northeastern United States.

MATERIALS AND METHODS

Isolation. Cup-cutter plugs of *P. annua* plants suspected of being affected by bacterial wilt were dissected and placed under a compound microscope in order to observe bacterial streaming emanating from vascular tissue. Central shoots were removed from infected *P. annua* plants, cut into 1- to 2-mm sections, surface disinfested with 0.6% sodium hypochlorite solution for 3 min, and plated on nutrient agar (NA) according to the labeled recipe (BD Difco, Sparks, MD). All of the resulting bacteria were streaked onto new NA plates in order to obtain single colonies. From the first round of isolations that occurred in July 2001, a total of 15 bacterial isolates (obtained from the original 15 different colony types) were obtained from diseased grass samples originating in Rhode Island, Massachusetts, Connecticut, New York, and Canada. Pathogenicity testing then was undertaken to satisfy Koch's postulates on 5-month-old *P. annua* plants (seed obtained locally from wild

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material) grown in conetainers in the greenhouse. A single colony of rod-shaped, gram-negative bacteria that produced a yellow pigment on YDC agar (recipe according to N. W. Schaad; 19) was identified as pathogenic and designated M-1. Colonies of this isolate did not fluoresce on King's B medium and were unable to ferment glucose anaerobically. Nonpathogenic bacteria were not classified further and it is possible that the same nonpathogenic species was present in multiple isolates. The second isolation was from a cup-cutter plug from Alpine Country Club in Cranston, RI taken in August 2003 (AP-LINE isolate), and the third isolation was from a cup-cutter plug from Pawtucket Country Club in Pawtucket, RI in October 2003 (PAWT isolate). The bacteria originating from these isolations shared the same physiological characteristics as the M-1 isolate.

rDNA sequence analysis. A partial 500-bp 16S rDNA sequence was obtained from the M-1, ALPINE, and PAWT isolates, produced by Microbial ID (Newark, DE) according to their published procedures. Full rDNA sequencing was performed on three bacterial isolates: the M-1 isolate, *X. translucens* pv. *graminis* (American Type Culture Collection [ATCC] no. 29087), and *X. translucens* pv. *poae* (ATCC no. 33804). Polymerase chain reaction (PCR) amplification of the partial 16S rDNA, complete intergenic spacer region, and partial 23S rDNA was accomplished using three separate primer sets. The first primer set, XAN1 (5'-GGA-GAGTTAGATCTTGGCTCAG-3')/XAN2 (5'-CCGGGTTTCCCCATTCGG-3'), was taken from Jones et al. (9). These two primers were used to sequence the outer 1,000 bp, the set XAN3 (5'-CCC-GGTGTAGCAGTGAAATG-3')/XAN4 (5'-ATGCAGGTTACTTGTGAGGACG-3') was used to sequence the next 1,000 bp (derived from the PCR product of the XAN1/XAN2 amplification), and the set XAN5 (5'-TACTAGCGATTCCGACTTCATGG-3')/XAN7 (5'-TCCTTAGTTGCCAGCAGTCAT-3') was used to sequence the internal most sequence (derived from the PCR product of the XAN3/XAN4 amplification). Each individual sequence then was assembled to form the contiguous sequence. For DNA extraction, nutrient broth tubes were seeded with bacterial colonies and grown for 5 days at 25°C. DNA was extracted per Sambrook et al. (17). Ribosomal sequences were amplified using a PTC-100 thermal cycler (Waltham, MA) and each reaction tube consisted of a total of 14 µl of sterile distilled water, 1 µl of template DNA, and PCR reactants supplied by Promega Corporation (Madison, WI), including: 2.5 µl of 25 mM MgCl₂, 2.5 µl of magnesium-free buffer, 1.3 µl of 200 µM dNTPs, and 0.5 µl each of 10 µM primer. Light mineral oil was added to each tube to cover the reaction mixture.

Tubes were placed into the thermal cycler at 94°C and 0.5 U of *Taq* DNA polymerase (Promega Corp.) was then added to each tube through the mineral oil. The program for the cycler was as follows: 1 min at 94°C, 1 min at 51°C, and 2 min at 72°C repeated 25 times, followed by 5 min of extension at 72°C. After amplification, reaction products were run on a 1% agarose gel and the amplified DNA was extracted using a Qiagen Gel Extraction kit and an IEC microcentrifuge. Purified DNA from the combined reactions was quantified against a ΦX174 HaeIII-restricted plasmid standard and sequenced at the Yale University Sequencing Facility using an ABI 3700 automated sequencer.

Pathogenicity testing. For each experiment, five germinated seedlings of each individual grass species were transplanted into each conetainer (measuring 4 by 20.5 cm) in a mixture of 80:20 sand:peat. Plants were allowed to grow for 5 months to reach maturity. Fertilizer was applied every 2 weeks and pyrethroid insecticides were sprayed as needed for control of *Thrips* spp. Plants were clipped at regular intervals to a height of approximately 2 cm. Each experiment was repeated once and used five conetainers containing treated grass and five conetainers containing water controls per grass.

For inoculation, bacteria were streaked on YDC medium and were grown for 72 h at 22°C. To harvest, plates were flooded with 1 ml of sterile 0.9% NaCl (aq) and colonies were scraped into a test tube with a Nalgene policeman. The bacterial cell suspension was vortexed, and turbidity measured at 640 nm. Concentrations were adjusted to range from 10⁸ to 10⁹ cells ml⁻¹.

Before plants were inoculated, grass blades were clipped to approximately 1 cm in length and gently misted. Then, inoculation was performed by submerging leaf tips in a bacterial cell suspension for approximately 15 s. Control plants were treated in the same manner, but dipped in 0.9% NaCl. Conetainers then were transferred to a greenhouse humidity chamber constructed of thick translucent plastic draped over a wooden frame and lined with a moistened capillary mat using natural lighting. After 1 week, plants were removed from the humidity chamber and transferred to a greenhouse bench for 1 week prior to evaluation. Plants were rated for foliar disease symptoms on a 0-to-10 scale, where 0 = unaffected and 10 = dead. The rating system corresponded approximately to the percentage of affected grass blades, where 5 = 50% necrotic. Additional observations were made by examining five symptomatic leaf tips per replicate, excised 2 to 3 mm below tip lesions, and the base of five central shoots per conetainer for bacterial streaming at ×200 under a compound microscope. In the first experiment, three leaf tips and three central shoot bases from three conetainers

were surface disinfested and plated on YDC to confirm the presence of *Xanthomonas* spp. in inoculated plants. For root inoculations, plant roots were washed free of soil, wounded by clipping, and dipped in the previously mentioned bacterial cell suspension. Plants were maintained in the greenhouse at an average daytime temperature of 32°C and 60% relative humidity for all experiments except for the fourth, in which the average daytime temperature was 18°C and the relative humidity was approximately 40% (as a result of seasonal differences effecting greenhouse conditions). When all three isolates (M-1, ALPINE, and PAWT) were tested simultaneously in a repeated experiment, plants were maintained in the laboratory under florescent lights on a 12-h illumination cycle at approximately 30% relative humidity and 21°C.

Four separate experiments to test pathogenicity were performed and each was repeated once. In the first experiment, all bacterial isolates were screened for virulence on *P. annua*. This process identified the M-1 isolate in the first round of screening. This isolate was used to confirm pathogenicity of the other two isolates eventually identified in subsequent repetitions of this experiment. In addition to leaf inoculations, the preliminary screen utilizing the M-1 isolate also incorporated the root inoculation. The second experiment was undertaken to determine the pathogenicity of the M-1 isolate on the perennial biotype of *P. annua*, *P. annua* var. *reptans* (Hausskn.) T. Koyama (obtained as mature plugs from a local golf course). The third experiment was similar to the second, but included *A. palustris* cv. Providence, *A. capillaris* L. cv. Exeter, *P. pratensis* L. cv. Lofts 1757, *Festuca rubra* L. (unspecified cultivar), and *Lolium perenne* L. cv. Night Hawk. The fourth pathogenicity experiment was undertaken on a collection of *Poa* spp. that included *P. annua* var. *reptans*, *P. compressa* cv. Harmony (PI 595588), *P. compressa* L. (PI 618804), *P. trivialis* L. cv. Sabre (PI 594396), *P. attenuata* Trin. (PI 619560), and *P. nemoralis* L. (PI 628725). In all experiments, *P. annua* var. *annua* was used as a standard for comparison. All seed in the fourth experiment, other than *P. annua*, were obtained from the National Germplasm Repository.

Temperature optima. For this experiment, cultures of all three locally collected isolates were incubated on a temperature gradient plate (5,10). A serial dilution of standards ranging from 10⁻⁴ to 10⁻³ CFU/ml grown in nutrient broth (BD Difco, Sparks, MD) culture was used to produce a linear equation relating absorbance to cell density at 640 nm, measured on a Shimadzu UV-160A UV/VIS spectrophotometer. Absorbance measurements and number of colonies counted were undertaken following 3 days of incubation at

25°C. For the experiment, petri dishes (5 cm) were filled with 5 ml of sterile nutrient broth and inoculated with 100 µl of a 3-day-old nutrient broth culture. Six replicates per temperature (6, 10, 15, 20, 25, 30, 35, and 40°C) were placed on the thermal gradient plate in the dark. Following 24 and 48 h of incubation, three plates were removed from each temperature series, vortexed, transferred to a cuvette using a pipette, and absorbance measured. Statistical analysis of pathogenicity and temperature data was undertaken using the general linear model procedure of SAS (Cary, NC).

Fatty acid analysis. The M-1 isolate was submitted for cellular fatty acid analysis for species identification. A commercial service, Microbial ID, was used to perform the analysis and isolates were submitted per the preparation instructions of the company.

RESULTS

Isolation. Three pathogenic bacterial isolates were isolated and designated M-1 (Torrington, CT), ALPINE (Cranston, RI), and PAWT (Pawtucket, RI). Although 15 other unidentified bacteria were isolated at the same time as the M-1 isolate, only three other bacterial colony types (yellow but not mucoid) were cultured on YDC with the ALPINE and PAWT isolates and included one *Frateuria* sp., one *Caulobacter* sp., and one *Curtobacterium* sp. based on Microbial ID rDNA sequencing. None of these bacteria have been reported as being pathogenic and it is likely that these other species were surviving epiphytically on grass shoots or in root tips when isolated. Work by Behrendt et al. (1) demonstrated numerous bacteria existing on the phyllosphere of grassland swards of *Poa*, *Festuca*, and *Agropyron* spp., commonly including those identified in this study (1).

rDNA sequence analysis. A BLAST (NCBI BLAST Web Server) comparison of the rDNA sequences of the M-1 isolate indicated considerable similarity to three GenBank accessions: AF209764 (*X. translucens* pv. unspecified), X99299 (*X. translucens* pv. *translucens*), and X99298 (*X. translucens* pv. *graminis*). However, only a limited number of nucleotides were submitted under these accessions, preventing a complete comparison from being made. A comparison of the M-1 isolate (GenBank accession AY253329) and the *X. translucens* pv. *graminis* sequence (ATCC no. 29087; GenBank accession AY247064) was performed and demonstrated a nucleotide similarity of 99.1% (data not shown). Of the 2,190 nucleotides sequenced, three transitions, 11 transversions, and five indels were identified. A comparison of the M-1 isolate and the *X. translucens* pv. *poae* sequence (ATCC no. 33804; GenBank accession AY572961) demonstrated a nucleotide similarity of 99.8% (differences were identified as one transition, two

transversions, and two indels). The majority of total polymorphisms occurred between base pairs 1,701 to 1,712, located in the internal transcribed spacer (ITS)-2 region, which is highly polymorphic among *Xanthomonas* spp. (6). Comparison of the 500-bp 16S rDNA region of the M-1, ALPINE, and PAWT sequenced by Microbial ID demonstrated 100% similarity between the M-1 and ALPINE isolate and a single A to G transversion between the M-1 and PAWT isolate at base pair 481.

Pathogenicity testing. In the first experiment, of the 15 isolates tested, only the M-1 isolate was found to be pathogenic on *P. annua* var. *annua*. Bacterial streaming from the bases of symptomatic central shoots, confirming infection, was observed only in the inoculated *P. annua* var. *annua* plants in 88% of the shoots examined ($n = 25$). Infected plants had a disease rating of 8.2 ± 2.1 and 8.1 ± 0.9 (initial and repeated trial) compared with the control plants, which had a disease rating of 1.2 ± 0.8 and 0.8 ± 0.5 . Koch's postulates were satisfied through reisolation. Root inoculations did not result in disease symptoms and no bacterial streaming from excised roots or central shoots was evident. This experiment was repeated for the M-1, ALPINE, and PAWT isolates in the laboratory (Table 1).

In the second experiment, pathogenicity of the M-1 isolate was examined on *P. annua* var. *annua* and *P. annua* var. *reptans*. Disease symptoms were evident only on inoculated *P. annua* var. *annua*, with bacterial streaming observed from the central shoots of 100 and 92% (initial and repeated trial, respectively) of the inocu-

lated *P. annua* var. *annua* plants examined ($n = 25$). Inoculated *P. annua* var. *annua* plants had an average disease rating of 8.5 ± 0.6 and 8.0 ± 0.9 compared with control plants, which had a disease rating of 0.9 ± 0.7 and 0.6 ± 0.3 .

In the third experiment, disease was observed only on inoculated *P. annua* var. *annua*. Bacterial streaming from bases of the central shoots was observed in 100% (initial and repeated trial) of the *P. annua* var. *annua* plants examined ($n = 25$). The M-1 isolate was unable to infect *Agrostis palustris*, *A. capillaris*, *Poa pratensis*, *Festuca rubra*, and *Lolium perenne*. All of these plants were symptomless after 2 weeks and bacterial streaming was not observed in excised central shoots. Disease also was not observed on untreated *P. annua* control plants. A quantitative measure of disease was not recorded for the first trial of this experiment; however, diseased plants were similar in appearance to those in both the second and third experiment. In the second trial of this experiment, infected *P. annua* var. *annua* plants had an average disease rating of 8.1 ± 0.4 and control plants had a disease rating of 0.7 ± 0.2 . All other species had a disease rating of 0, regardless of whether they were inoculated or control plants.

In the fourth experiment, comparing susceptibility of a number of *Poa* spp., *P. annua* var. *annua* and *P. attenuata* were the only grasses demonstrating symptoms of bacterial wilt (Table 2); specifically, wilting and tip dieback. Bacterial streaming from bases of central shoots was observed in 82 and 94% (initial and repeated trial, respectively) of the shoots examined ($n =$

Table 1. Pathogenicity of three isolates of *Xanthomonas translucens* pv. *poae* on *Poa annua* var. *annua*^x

Isolate (source)	Symptomatic shoots (%) ^y	Streaming from shoot bases (%) ^z
Control	1.7 a	0.0 a
ALPINE (Cranston, RI)	57.5 c	85.0 c
M-1 (Torrington, CT)	27.5 b	45.0 b
PAWT (Pawtucket, RI)	8.75 ab	55.0 b

^x Values in a column with the same letter are not statistically different according to Tukey's standardized range test ($P < 0.01$).

^y Five symptomatic shoot bases were examined from each container (pooled $n = 40$) to determine the proportion with bacterial streaming.

^z Data were pooled from the initial and repeated trial of the experiment.

Table 2. Mean disease rating for *Poa* spp. inoculated with the M-1 isolate of *Xanthomonas translucens* pv. *poae*

Species	Disease rating ^z	
	Inoculated	Control
<i>Poa annua</i> var. <i>annua</i>	7.7 a	1.6 b
<i>P. annua</i> var. <i>reptans</i>	1.0 b	1.0 b
<i>P. compressa</i> (PI 618804)	1.1 b	1.8 b
<i>P. compressa</i> cv. Harmony	0.9 b	0.8 b
<i>P. attenuata</i> (PI 619560)	5.6 a	0.9 b
<i>P. nemoralis</i> (PI 628725)	2.5 b	1.0 b
<i>P. trivialis</i> cv. Sabre	1.9 b	2.2 b

^z Disease symptoms were assessed on a 0-to-10 scale, where 0 = unaffected and 10 = dead. Data were pooled from the initial and repeated trial of the experiment. Values with the same letter are not statistically different according to Tukey's standardized range test ($P < 0.01$) within columns or rows.

25) from *P. annua* var. *annua* and 44 and 62% (initial and repeated trial, respectively) from *P. attenuata*. Although exhibiting no symptoms of wilt, bacterial streaming was seen from the examined stem bases of 16 and 7% of inoculated *P. trivialis* plants, 16 and 12% of inoculated *P. annua* var. *reptans* plants, and 12 and 0% of *Poa annua* var. *annua* control plants in the first and second trials, respectively.

Temperature optima. The optimal temperature for growth of the M-1 isolate occurred between 30 and 35°C (Fig. 1). In laboratory experiments, growth peaked around 31°C when cultures were examined at 24 h and 34°C when cultures were examined at 48 h. Very little growth occurred below 20 or above 40°C.

Fatty acid analysis. Fatty acid analysis using the Microbial Identification System employed by Microbial ID determined that the M-1 isolate had a similarity index to *X. axonopodis* pv. *vesicatoria* of 0.124 (with a similarity index of 0.6 or higher representing the same species). There was no similarity to the profile for *X. translucens* pv. *graminis* stored in the database and only a 0.086 similarity index to *X. translucens* pv. *translucens*. The isolate was retested by Microbial ID 6 months after the initial submission to ensure that only a pure culture was present and similar results were obtained. The Microbial ID database does not contain a profile for *X. translucens* pv. *poae*.

DISCUSSION

Based on sequencing of rDNA, this study has demonstrated that the isolated bacteria were *X. translucens* pv. *poae*. Pathogenicity testing of the three isolates and comparison of the results to the published host range determined by Nishino and Fujimori (12) provide further evidence

that the isolates are *X. translucens* pv. *poae*, suggesting that *X. translucens* pv. *poae* is the most likely causal agent of bacterial wilt of annual bluegrass. The work of Nishino and Fujimori (12) demonstrated that both the ATCC isolate of *X. translucens* pv. *poae* (ATCC no. 33804) and Japanese isolates of *X. translucens* pv. *poae* can cause disease on *P. annua*, *P. trivialis*, *P. supina*, and *P. ampla* (12). They also determined that, although the bacteria did not cause disease on *P. pratensis*, *P. compressa*, *P. secunda*, and *P. bublosa*, the organism did multiply in leaf tips, never moving into sheaths or crowns. This study did not incorporate experiments on *P. ampla*, *P. bublosa*, *P. secunda*, or *P. supina*, because they could not be obtained at the time. In the current study, the Connecticut isolate (M-1) caused disease on *P. annua* var. *annua* and *P. attenuata* (which was not included in previous studies by other researchers), infecting both sheaths and crowns. It did not cause disease on *P. compressa*, *P. nemoralis*, or *P. pratensis*. Although disease was not observed in *P. trivialis* or *P. annua* var. *reptans*, limited bacterial streaming was observed in a small percentage of their central shoots. Although it is unclear why disease did not develop on *P. trivialis*, it is possibly that the M-1 isolate is a strain distinct from the Japanese isolates with altered virulence. Nishino and Fujimori (12) observed significant differences in virulence between at least two native Japanese isolates of *X. translucens* pv. *poae*.

Savage et al. (18) indicated that differences between the host range of their American isolate and *X. translucens* pv. *poae* were significant enough to establish the pathovar *X. translucens* pv. *poaannua*, but Nishino et al. (13) was unable to confirm significant differences using the iso-

late of Savage et al. (XCPA; 13,18). Unfortunately, Savage et al. (17) did not indicate exactly which species or cultivars their isolate was tested on in the published report. Both our rDNA and pathogenicity results agree with Nishino et al. that the correct pathovar name is *X. translucens* pv. *poae* (13).

De Cleene et al. (4) published a detailed description of the host range of *X. translucens* pv. *graminis*. Their results indicate that its primary hosts are *L. perenne*, *L. multiflorum* L., *Phleum pratense* L., and *F. pratensis* Huds. In the present study, *L. perenne* was the only grass species examined that appears on the list of De Cleene et al. (4), but it was determined not to be a host of the New England isolates. This single piece of evidence does not exclude the isolates identified in New England from being *X. translucens* pv. *graminis*, but does suggest that it is unlikely in the context of the additional information presented here. The only report of *X. translucens* pv. *graminis* in the United States is from *A. palustris* cv. Toronto and indicates that the bacteria would not reproduce on any other hosts, including *Lolium* spp. (14). This would suggest that these authors misidentified this pathogen; thus, *X. translucens* pv. *graminis* has not yet been identified in the United States.

Fatty acid analysis was inconclusive. Although it did suggest that the M-1 isolate was a Xanthomonad, the similarity to existing profiles in the Microbial ID database was very low, in contrast to the rDNA analysis. This suggests that fatty acid analysis may not be a useful tool for identification of *X. translucens* pv. *poae* or that the Microbial ID database does not contain a representative sample of Xanthomonad profiles.

Bacterial wilt typically has been considered a stress-related disease on golf course putting greens (exacerbated by low cutting heights and excessive compaction); however, we were able to induce dramatic disease in the greenhouse on extremely vigorous plants (N. Jackson, *personal observation*). *P. annua* plants grown at a 2- to 3-cm height of cut in a sand-based media were extremely susceptible to leaf wilting and crown rotting caused by *X. translucens* pv. *poae*. In addition, allowing inoculated grass to grow to 10 cm did not mitigate disease symptoms after infection (*data not shown*). Symptoms were most dramatic under conditions of high humidity and average temperatures of 30°C, when artificially inoculated with high levels of bacteria. These observations correspond to the in vitro temperature optima data reported in this article and coincide with the highest incidence of disease in the field. Even under suboptimal conditions of low humidity and temperatures of approximately 18°C (as experienced in the first trial of the fourth experiment), leaf wilting did occur. Under suboptimal conditions,

Fig. 1. Temperature-dependent growth curve for the M-1 isolate of *Xanthomonas translucens* pv. *poae*.

however, plants remained asymptomatic for a longer period of time than under optimal conditions (2 to 4 weeks longer), though bacterial streaming could be identified in the majority of leaves, sheaths, and plant crowns within 2 weeks of inoculation.

Isolation of the pathogen initially was difficult because the medium used for isolation, NA, allowed many nonpathogenic bacteria present to grow. Additionally, the causal *Xanthomonas* grew much more slowly than the epiphytic and soil-borne bacteria, quickly becoming overwhelmed and unrecoverable. When the semiselective medium YDC was employed for further isolations, the process became less difficult. Unfortunately, isolation timing appears to play a role in isolation success. When isolations are made early in the infection, *Xanthomonas* spp. are the predominant bacteria recovered. When a sample is considerably degraded by the effects of wilting, many more epiphytic or saprobic bacteria are recovered. Difficulty isolating this bacterium has not been reported by other researchers.

The ability of this organism to fully colonize the *P. annua* vascular system yet fail to produce dramatic disease symptoms under suboptimal conditions suggests that it may be widely prevalent on golf courses and remain undetected. It also suggests that *P. annua* plants in natural surroundings may harbor the pathogen asymptotically in a highly evolved parasitic relationship. In addition, low levels of infection (in the absence of disease) also occurred in *P. trivialis* and *P. annua* var. *reptans* in the last experiment, suggesting that tolerant hosts may regularly harbor the organism. Nishino and Fujimori demonstrated bacterial multiplication in leaf tips of numerous nonhost grasses in the absence of expressed disease (12). Although only a few species of *Poa* were examined for susceptibility in this experiment, it is quite probable that other hosts do exist in natural surroundings, providing an additional reservoir for the pathogen. *P. attenuata* was severely affected by the pathogen but is not native to the United States.

Although mowers probably provide the most efficient means of pathogen transmission, as well as producing entry wounds, it appears that insects can transfer the bacterium. In the first trial of the last experiment, disease symptoms started to develop on a small percentage of *P. annua* control plants 4 weeks after inoculation (inoculated plants developed disease symptoms within 2 weeks of inoculation). Disease was limited to minimal tip die-back and streaming could not be detected outside of diseased tissue. The most probable explanation for this disease transmission was an infestation of thrips present at the time.

The amount of inoculum transmitted by these insects would have been minimal compared with the dipping procedure used on the treated plants, providing an explanation for the slow spread of the disease within the control plants. It is unlikely that splash transmission occurred because plants were separated by a distance of a meter and were grown in containers a half-meter off the greenhouse bench surface.

Molecular differences between the M-1 isolate of *X. translucens* pv. *poae* and the ATCC isolate of *X. translucens* pv. *graminis* were significant enough to indicate that it may be possible to develop molecular markers to separate *X. translucens* pathovars. The pathovar distinction is not considered a taxonomic level of classification; however, the fact that some ITS variation exists between at least two *X. translucens* pathovars suggests that the taxonomic applicability of the pathovar distinction may need further investigation.

Our work provides an initial basis for the use of rDNA markers to separate *X. translucens* pathovars. Isolation of these bacteria can be difficult because multiple species of yellow bacteria can be isolated from grass leaves and pathogenicity testing of each isolate is extremely tedious. Development of reliable molecular markers could greatly increase the speed and ease of identification. Additional testing of *X. translucens* pathovars will need to be undertaken to determine the utility of rDNA-targeted PCR primers.

Additional research is needed to confirm that *X. translucens* pv. *poae* is the proper pathovar designation for the bacterium isolated and it is the only bacterial species involved in causing bacterial wilt of annual bluegrass in the northeastern United States. Additional isolates should be collected and tested for pathogenicity using a full range of host species, focusing on those used in previous studies. To date, the only plant pathogen identified from our isolations was a *Xanthomonas* sp.; however, this does not rule out the possibility of the involvement of additional bacterial species. In order to conclusively determine the identity of all three *Xanthomonas* isolates, the full 2,190-bp rDNA sequence will be generated from the ALPINE and PAWT isolates.

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