Interaction of Common Bacterial Blight Bacteria with Disease Resistance Quantitative Trait Loci in Common Bean

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ABSTRACT

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Common bacterial blight (CBB) of common bean (*Phaseolus vulgaris* L.) is caused by *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans*, and is the most important bacterial disease of this crop in many regions of the world. In 2005 and 2006, dark red kidney bean fields in a major bean-growing region in central Wisconsin were surveyed for CBB incidence and representative symptomatic leaves collected. Xanthomonad-like bacteria were isolated from these leaves and characterized based upon phenotypic (colony) characteristics, pathogenicity on common bean, polymerase chain reaction (PCR) with *X. campestris* pv. *phaseoli*- and *X. fuscans* subsp. *fuscans*-specific primers, and repetitive-element PCR (rep-PCR) and 16S-28S ribosomal RNA spacer region sequence analyses. Of 348 isolates that were characterized, 293 were identified as common blight bacteria (i.e., pathogenic on common bean and positive in PCR tests with the *X. campestris* pv. *phaseoli*- and *X. fuscans* subsp. *fuscans*-specific primers), whereas the other isolates were nonpathogenic xanthomonads. Most (98%) of the pathogenic xanthomonads were *X. campestris* pv. *phaseoli*, consistent with the association of this bacterium with CBB in large-seeded bean cultivars of the Andean gene pool. Two types of *X. campestris* pv. *phaseoli* were involved with CBB in this region: typical *X. campestris* pv. *phaseoli* (P) isolates with

yellow mucoid colonies, no brown pigment production, and a typical *X. campestris* pv. *phaseoli* rep-PCR fingerprint (60% of strains); and a new phenotype and genotype (Px) with an *X. campestris* pv. *phaseoli*-type fingerprint and less mucoid colonies that produced brown pigment (40% of strains). In addition, a small number of *X. fuscans* subsp. *fuscans* strains, representing a new genotype (FH), were isolated from two fields in 2005. Representative P and Px *X. campestris* pv. *phaseoli* strains, an FH *X. fuscans* subsp. *fuscans* strain, plus five previously characterized *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* genotypes were inoculated onto 28 common bean genotypes having various combinations of known CBB resistance quantitative trait loci (QTL) and associated sequence-characterized amplified region markers. Different levels of virulence were observed for *X. campestris* pv. *phaseoli* strains, whereas *X. fuscans* subsp. *fuscans* strains were similar in virulence. The typical *X. campestris* pv. *phaseoli* strain from Wisconsin was most virulent, whereas *X. campestris* pv. *phaseoli* genotypes from East Africa were the least virulent. Host genotypes having the SU91 marker-associated resistance and one or more other QTL (i.e., pyramided resistance), such as the VAX lines, were highly resistant to all genotypes of common blight bacteria tested. This information will help in the development of CBB resistance-breeding strategies for different common bean market classes in different geographical regions, as well as the identification of appropriate pathogen genotypes for screening for resistance.

Common bacterial blight (CBB) is the most important bacterial disease of common bean (*Phaseolus vulgaris* L.) in many common bean-growing regions of the world. The disease is caused by *Xanthomonas campestris* pv. *phaseoli* Smith (Dye) (synonym: *X. axonopodis* pv. *phaseoli* (Smith) Vauterin et al.) and *X. fuscans* subsp. *fuscans* sp. nov. (10,45). Symptoms induced by *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* are indistinguishable, and both bacteria can cause significant negative impact on yield and seed quality, especially in highly susceptible cultivars (45,49). Common blight bacteria infect the foliage, pods, and seed and disease development is favored by warm, humid conditions (45,48). The bacteria survive and spread with infected or contaminated seed but can also survive in association with plant debris and weeds (10,12,38,45,46).

Results of genetic, phenotypic, and physiological studies have revealed that *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* are distinct species (1,5,6,10,11,23,25,34,47). Host-pathogen interaction studies have revealed that these bacteria

differ in their prevalence and virulence on genotypes of the two major common bean gene pools, with *X. campestris* pv. *phaseoli* most commonly associated with large-seeded Andean bean cultivars and *X. fuscans* subsp. *fuscans* associated with CBB in Andean and Middle-American bean cultivars. In addition, numerous studies have revealed that *X. campestris* pv. *phaseoli* strains show more genetic and pathogenic variability compared with the more homogeneous *X. fuscans* subsp. *fuscans* strains (1,23,25,34,36). Three *X. campestris* pv. *phaseoli* genotypes were described by Mkandawire et al. (34) based upon repetitive-element polymerase chain reaction (rep-PCR) and pathogenic variability. One of these genotypes is represented by a large homogeneous group of *X. campestris* pv. *phaseoli* strains from New World (e.g., North and South America, Mexico, and the Caribbean) and Old World (e.g., Spain and Australia) locations (hereafter referred to as typical *X. campestris* pv. *phaseoli* strains), whereas the other genotypes are represented by strains from East Africa (23,34). The East African strains were significantly more virulent on Andean than Middle-American genotypes, whereas no such differential virulence was observed for typical *X. campestris* pv. *phaseoli* strains. Two *X. fuscans* subsp. *fuscans* genotypes, one from the New World and one from the Old World, have been identified based on minor differences in the rep-PCR fingerprints;

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however, strains of these genotypes are similar in virulence on Andean and Middle-American genotypes.

Effective management of CBB requires an integrated pest management strategy beginning with pathogen-free seed, which is commonly produced in arid regions such as the western United States. Crop rotation and deep tillage are also important management strategies for CBB inoculum associated with bean debris and weeds in or around fields. However, the most effective and sustainable management strategy is host resistance. Unfortunately, little or no CBB resistance is present in most commercial common bean cultivars. Low to moderate levels of CBB resistance have been identified in a few common bean genotypes from the Middle-American gene pool, such as Great Northern (GN) Montana No. 5, whereas no resistance has been identified in the large-seeded Andean gene pool (49). The resistance quantitative trait loci (QTL) from GN Montana no. 5 and GN Nebraska no. 1, selection 27 has been associated with the SAP6 molecular marker (28). Moderate to high levels of CBB resistance have been introgressed into common bean breeding lines from scarlet runner bean (*P. coccineus* L.) (33,39) and tepary bean (*P. acutifolius* A. Gray) (27,40), respectively. This resistance is quantitative, and resistance QTL introgressed from *P. acutifolius* have been associated with the molecular markers SU91 (42) and BC420 (53). The presence of these markers in common bean breeding lines, such as XAN 159, has been associated with high levels of CBB resistance (51).

Despite the availability of CBB resistance QTL and associated markers, only low levels of CBB resistance have been introgressed into common bean cultivars of any market class (31,32). This may be due, in part, to the fact these markers are linked with undesirable traits, including low yield and undesirable seed size or color (9,35,37). Thus, a major challenge for common bean breeding is to introgress high levels of CBB resistance while

maintaining or improving the seed yield and quality and other agronomic characteristics.

In this study, we further investigated aspects of this pathosystem. First, we tested the hypothesis that *X. campestris* pv. *phaseoli* is typically associated with CBB in large-seeded Andean genotypes by characterizing xanthomonads associated with CBB of dark red kidney (DRK) bean cultivars in central Wisconsin, a region where these genotypes predominate. Second, we assessed whether common bean genotypes with introgressed CBB resistance QTL, especially those with pyramided resistance, are resistant to a diversity of common blight bacteria by determining the response of common bean genotypes having various combinations of CBB resistance QTL to *X. campestris* pv. *phaseoli* or *X. fuscans* subsp. *fuscans* strains representing the different genotypes identified by rep-PCR. This information will help in the selection of germplasm or breeding lines and strains of common blight bacteria for CBB resistance-breeding programs.

MATERIALS AND METHODS

CBB surveys and isolation of xanthomonad-like bacteria.

Surveys for CBB were performed in 2005 and 2006 in a major DRK bean production area in central Wisconsin (Table 1). In all, 26 fields were surveyed over this 2-year period: 11 in 2005 and 15 in 2006. Fields were sampled in mid-August, when plants were in the early to late flowering stage (R1 to R3). Disease incidence was determined by the estimation of the proportion of plants with CBB symptoms in each field: low (<1% CBB), moderate (1 to 10% CBB) and high (>10% CBB). Trifoliolate leaves with CBB-like symptoms (irregular necrotic lesions with yellow borders and water-soaked spots) were collected, and isolations were performed as previously described (34). Briefly, sections from the margin of healthy and diseased leaf tissue were macerated in

TABLE 1. Relative incidence of common bacterial blight (CBB) in dark red kidney bean fields surveyed in central Wisconsin in 2005 and 2006 and the recovery of xanthomonads from leaves with CBB symptoms collected from these fields

Year, location	CBB incidence ^a	No. of xanthomonad-like strains recovered	P1 ^b	P3 ^b	P2 ^c	P4 ^d
2005						
D. South	L	9	2	3	1	3
Field no. 5	H	21	21	0	0	0
Road 1	M	18	0	15	0	3
Field no. 1	M	12	0	12	0	0
Field no. 2	M	18	15	3	0	0
Field no. 4	M	18	13	0	5	0
Claveland North	M	15	15	0	0	0
Niece	M	18	0	18	0	0
Field no. 2, Rogers	L	21	0	0	0	21
Kohler	L	18	4	0	0	14
Field no. 4, location 2	M	14	13	0	0	1
2006						
Field no. 3	L	9	3	0	0	6
Field no. 5	M	15	15	0	0	0
Abbot North	L	6	6	0	0	0
Arkansas North 1	L	4	0	3	0	1
Arkansas North 2	L	12	0	9	0	3
Claveland North	L	6	0	6	0	0
Webster South	L	6	0	6	0	0
D. South	H	32	0	32	0	0
Field no. 4	H	25	25	0	0	0
Flush Shower South	L	9	6	0	0	3
Knudson	L	3	3	0	0	0
Quiling 85	M	17	8	9	0	0
Quiling 126	M	10	10	0	0	0
Rock Falls East	L	6	6	0	0	0
Rock Falls North	L	6	3	3	0	0
Total	...	348	168	119	6	55

^a L = low, <1% CBB incidence; M = medium, 1 to 10% CBB incidence; and H = high >10% CBB incidence.

^b P1 = phenotype 1, typical (*P*) *Xanthomonas campestris* pv. *phaseoli* strains, and P3 = phenotype 3, intermediate (*Px*) *X. campestris* pv. *phaseoli* strains.

^c P2 = phenotype 2, *X. fuscans* subsp. *fuscans*.

^d P4 = phenotype 4, nonpathogenic xanthomonads. Pathogenicity was determined by inoculation of the susceptible cv. Topcrop and by polymerase chain reaction with *X. campestris* pv. *phaseoli*- and *X. fuscans* subsp. *fuscans*-specific primers.

sterile distilled water, and the macerate was diluted and streaked onto plates of MXP, a semiselective media for common blight bacteria (7). Plates were incubated for 3 to 5 days at 28°C. From each plate, three representative xanthomonads (yellow mucoid colonies surrounded by zones of starch hydrolysis) were subcultured onto 523 media (19). After 3 days of growth at room temperature, the colony characteristics of each isolate, including shape, size, elevation, and extracellular brown pigment and polysaccharide (mucosity) production, were recorded.

Identification of common blight bacteria: pathogenicity in common bean and PCR with *X. campestris* pv. *phaseoli*- and *X. fuscans* subsp. *fuscans*-specific primers. Common blight bacteria (*X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains) were identified based on a general pathogenicity test with the susceptible cv. Topcrop and PCR with primers specific for *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains (3). For the pathogenicity test, a suspension of bacterial cells was prepared in sterile distilled water, adjusted to a concentration of 1×10^8 CFU/ml, and rub inoculated onto the first trifoliolate leaf dusted with celite (12). The inoculum was kept on ice and used within 3 h. Plants were maintained in a greenhouse (day and night temperatures of 26 and 18°C, respectively, and relative humidity of 45 to 55%). Inoculated leaves were evaluated 14 days post-inoculation (dpi). Isolates were rated as pathogenic (presence of water-soaked spots and chlorosis and necrosis around the inoculated area) or nonpathogenic (wound damage but no chlorosis or water-soaking). Controls were sterile distilled water and a known *X. campestris* pv. *phaseoli* strain (Xcp25). Each isolate was tested twice with two replicates for each experiment.

DNA was extracted from each isolate using a cetyltrimethylammonium bromide DNA extraction protocol (20). PCR with the *X. campestris* pv. *phaseoli*- and *X. fuscans* subsp. *fuscans*-specific primer pair (X4c and X4e) was performed as previously described (3). This primer pair directs the amplification of an ≈ 700 -bp fragment from total genomic DNA of common blight bacteria. All isolates were tested twice.

Rep-PCR. Fingerprints were generated using rep-PCR primers targeting conserved repetitive sequences as previously described (24,43,44). For each isolate, fingerprints were generated with repetitive extragenic palindromic (REP), enterobacterial repetitive intergenic consensus (ERIC), and BOX 1A primers, as previously described (34). Two reactions were performed for each isolate and each primer pair. The rep-PCR fingerprints were examined visually and compared with fingerprints of the control strains. In addition, fingerprints were also analyzed with GelCompar II V. 4.011 (Applied Maths, Belgium) to produce a dendrogram. Results were standardized by comparing the fingerprint of each isolate to the fingerprint of each control.

Sequence analysis of 16S-28S ribosomal RNA spacer region. The 16S-28S ribosomal RNA (rRNA) spacer region was amplified by PCR with the G1/L1 primer pair (16), and sequences were determined as previously described (4). The sequences were compared with those in the GenBank using the BLAST program available at the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/BLAST/>), and assembled using the BioEdit program version 7.05 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequences were aligned using Clustal W (50). Phylogenetic trees were constructed from the aligned sequences using MEGA 4 (21), with a neighbor-joining method and 1,000 bootstrap replicates.

Inoculation of *P. vulgaris* genotypes with strains representative of the genotypic diversity of common blight bacteria. Eight strains were selected to represent the genotypic diversity of *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* as revealed by rep-PCR analysis. These included the new *X. campestris* pv. *phaseoli* Px (strain Px100) and *X. fuscans* subsp. *fuscans* FH (strain FH61) genotypes identified in this study. Two typical *X. campestris* pv. *phaseoli* strains were used: PR1, a strain

from Puerto Rico, and Xcp25, a strain isolated from DRK bean in Wisconsin. The two East African *X. campestris* pv. *phaseoli* genotypes were represented by strains M11 and T21, respectively (34). The two previously identified *X. fuscans* subsp. *fuscans* genotypes were represented by PR8F, a New World strain from Puerto Rico, and M12F, an Old World strain from East Africa (34).

In all, 28 common bean genotypes having different combinations of CBB resistance QTL and markers (SAP6, SU91, and BC420) and 2 genotypes lacking known resistance QTL and markers (cvs. Topcrop [Andean] and Sutter Pink [Middle American]) were individually inoculated with each of the eight bacterial strains to assess the host-pathogen interaction. Of the common bean genotypes with resistance QTL, 1 was from the Andean gene pool, 7 were from the Middle-American gene pool, 4 had *P. coccineus*-derived resistance, 5 had *P. acutifolius*-derived resistance, and 11 had pyramided resistance.

Common bean plants were grown in a temperature-controlled greenhouse, as described above. Each plant was grown in a 4-liter pot containing University of California (UC) potting mix. An automated watering system (Argus, White Rock, BC, Canada) was used to apply water daily to the soil for 5 min each at 7:00 a.m., 10:00 a.m., and 1:00 p.m.. Plants were fertilized every other watering with modified Hoagland's solution. At 17 days post-planting, plants were inoculated with the *X. campestris* pv. *phaseoli* or *X. fuscans* subsp. *fuscans* strains. The three leaflets of three-quarters-expanded first trifoliolate leaves were inoculated with a 1×10^8 CFU/ml cell suspension with the razor blade method (41). Disease symptoms on each leaflet were evaluated 21 dpi on a 1-to-9 rating scale (disease severity index [DSI] 1 < 4 = resistant, 4 < 7 = intermediate, and 7 to 9 = susceptible) (22). Scores for the three leaflets were averaged to generate a mean DSI for each plant.

Each strain was inoculated onto each host genotype, and plants were arranged in a randomized complete block design (RCBD). Each strain-host combination represented a treatment, and treatments were replicated four times within the RCBD. Two independent experiments were performed in the summer of 2007, with planting dates of 5 and 12 June. Although these two experiments were performed in the same season with similar planting dates, we would expect that results would be consistent over seasons because the experiments were performed under controlled conditions in the greenhouse.

Molecular marker analysis. Samples were taken from primary leaves for DNA extraction and molecular marker analysis. Total genomic DNA was extracted from ≈ 20 mg of primary leaf tissue with the Dellaporta extraction method (8). The marker composition of each plant was determined with a multiplex PCR reaction that allows for the simultaneous detection of the three CBB resistance markers (30). Primers were mixed in equimolar (2 μ M) concentrations (from a 50- μ M stock solution) with 20 ng of total genomic DNA, and added to the Qiagen Multiplex Mastermix (Qiagen, Valencia, CA). The amplification conditions included an initial denaturation step at 95°C for 15 min; followed by 35 cycles of 94°C for 30 s (denaturation), 58°C for 1.5 min (annealing), and 72°C for 1.5 min (extension); and a final extension step at 72°C for 10 min. All PCR reactions were performed in a PTC-100 thermocycler (MJ Research Inc., Waltham, MA). PCR products were analyzed in 1.5% agarose gels in Tris-acetate-EDTA buffer. Gels were stained with ethidium bromide and DNA was visualized with UV light. The presence or absence of different-sized fragments was determined visually and recorded. All reactions were performed twice. In the few cases where different results were obtained for the two tests, a third test was performed and the consensus result was recorded.

Data analysis. Analysis of variance, mean DSI, standard error, and Fisher's least significant difference values were calculated using the Analyst function in SAS 9.1 (SAS Institute Inc., Cary,

NC). The CBB DSI values from the host–pathogen interaction analyses met all statistical assumptions and, thus, the data from the two independent experiments were combined. GelCompar II was used to analyze the rep-PCR fingerprints and produce an unweighted pair-group method with arithmetic means (UPGMA) dendrogram using the composite data from all three rep-PCR primer sets. The similarity coefficient was calculated using the Pearson correlation coefficient.

RESULTS

CBB surveys and isolation of xanthomonad-like bacteria. Surveys of DRK bean fields in central Wisconsin in 2005 and 2006 revealed CBB symptoms (irregular necrotic lesions with yellow borders and water-soaked spots) in all fields of this major production area (≈1,200 ha). In most fields, CBB incidence was low (<1%) or moderate (1 to 10%) (Table 1). Representative symptomatic leaves were collected from each field. In some fields with low disease incidence, this was complicated by “pseudo-CBB” symptoms, which are irregular necrotic lesions with yellow

borders (but lacking water-soaking) due to an abiotic disease induced by heat and water stress. Xanthomonad-like bacteria (yellow mucoid colonies surrounded by zones of starch hydrolysis on MXP medium) were isolated from all symptomatic leaves collected and, in total, 348 isolates (individual colonies) representing all the surveyed fields were selected for further characterization (Table 1).

Phenotypic properties of xanthomonad-like bacteria. Each of the 348 xanthomonad-like isolates was subcultured onto 523 medium. Based on colony morphology and brown pigment production, four phenotypes were identified (Table 2). Phenotype 1 colonies were large, yellow, mucoid, convex, and did not produce brown pigment (Fig. 1A). This was the most common phenotype (48% of the isolates), and isolates were recovered from 17 of the 26 fields. Phenotype 2 colonies were yellow but less mucoid and flatter compared with phenotype 1 colonies. These colonies produced a large quantity of brown pigment (Fig. 1B). Only six phenotype 2 isolates were isolated, and these came from two fields surveyed in 2005. Phenotype 3 colonies were yellow, less mucoid, and convex to flat, more similar to phenotype 2 than

TABLE 2. Phenotypes of xanthomonad-like bacteria isolated from leaves with common bacterial blight symptoms collected from dark red kidney bean plants in central Wisconsin in 2005 and 2006

Phenotype	Colony morphology	Brown pigment production	No. of strains (%) ^a	No. of fields
Xcp25 ^b	Yellow, mucoid, convex	None	NA ^c	NA
PR8F ^d	Yellow, less mucoid, convex-flat	High	NA	NA
1	Yellow, mucoid, convex	None	168 (48)	17
2	Yellow, less mucoid, convex-flat	High	6 (2)	2
3	Yellow, less mucoid, convex-flat	Low	119 (34)	12
4	Light to dark yellow, mucoid to nonmucoid, convex-flat	None	55 (16)	9

^a Indicates the number of strains of each phenotype that were recovered from a total of 348 strains. Numbers in parentheses are percentages of the total.

^b Known strain of *Xanthomonas campestris* pv. *phaseoli*.

^c NA = not applicable.

^d Known strain of *X. fuscans* subsp. *fuscans*.

Fig. 1. Colony morphology and brown pigment production of the four phenotypes of xanthomonad-like bacteria isolated from dark red kidney bean leaves with common blight symptoms collected in surveys conducted in central Wisconsin in 2005 and 2006. **A**, Phenotype 1, typical *Xanthomonas campestris* pv. *phaseoli* strain; **B**, phenotype 2, *X. fuscans* subsp. *fuscans* strain; **C**, phenotype 3, intermediate *X. campestris* pv. *phaseoli* strain; and **D**, phenotype 4, nonpathogenic xanthomonad strain.

phenotype 1 colonies (Fig. 1C). Phenotype 3 colonies also produced brown pigment but substantially less than phenotype 2 colonies (compare Fig. 1B and C; Table 2). This phenotype was also commonly isolated (34% of the isolates), and isolates were recovered from 12 of the 26 fields. Phenotype 4 isolates were more heterogeneous, with colonies that were light to dark yellow, mucoid to nonmucoid, and convex to flat; these isolates did not produce brown pigment. This phenotype comprised 16% of the isolates and was recovered from nine fields, most of which had low CBB incidence and pseudo-CBB symptoms.

Identification of *X. campestris* pv. *phaseoli* or *X. fuscans* subsp. *fuscans* strains: pathogenicity on common bean and detection by PCR with *X. campestris* pv. *phaseoli*- and *X. fuscans* subsp. *fuscans*-specific primers. All 348 isolates were tested for pathogenicity on the susceptible cv. Topcrop. Phenotype 1, 2, and 3 isolates were pathogenic, producing necrotic lesions with yellow borders and water-soaked spots along the inoculated area, whereas phenotype 4 isolates were nonpathogenic, with only mechanical wound damage in the inoculated area. Thus, the phenotype 4 isolates likely represent nonpathogenic xanthomonads, similar to those previously described by Gilbertson et al. (12). Based upon comparison with phenotypes of known *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains (Table 2), phenotype 1 isolates were similar to *X. campestris* pv. *phaseoli* (i.e., yellow mucoid colonies with no brown pigment production), whereas phenotype 2 isolates were similar to *X. fuscans* subsp. *fuscans* (i.e., yellow, less mucoid colonies with copious brown pigment production). Phenotype 3 colonies were yellow and moderately mucoid but produced brown pigment like *X. fuscans* subsp. *fuscans*; thus, these were referred to as intermediate strains.

Phenotype 1, 2, and 3 isolates were positive in the PCR test with the *X. campestris* pv. *phaseoli*- and *X. fuscans* subsp. *fuscans*-specific primers (i.e., the expected-size 700-bp fragment was amplified), whereas phenotype 4 isolates were negative (no fragment was amplified). These results were consistent with results of the pathogenicity tests and, together, indicate that phenotype 1, 2, and 3 isolates are common blight bacteria. Finally, it is interesting to note that, although these phenotypes were occasionally isolated from the same field, it was more common that a single phenotype was predominant in a given field (Table 1).

Rep-PCR analysis. In preliminary experiments performed with the REP primers, known *X. campestris* pv. *phaseoli* strains were distinguished from *X. fuscans* subsp. *fuscans* strains, and the three previously reported *X. campestris* pv. *phaseoli* genotypes were also differentiated (Fig. 2A to C). The two *X. fuscans* subsp. *fuscans* genotypes were differentiated with the ERIC but not the REP primers (compare Fig. 2A and B). The BOX primers differentiated *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains but not the *X. campestris* pv. *phaseoli* or *X. fuscans* subsp. *fuscans* genotypes (Fig. 2C). These results were in agreement with previous reports (23,34).

Analysis of the 293 isolates of common blight bacteria isolated from DRK bean fields in Wisconsin with rep-PCR revealed three distinct types of fingerprints. All 168 phenotype 1 isolates, including representative strain Xcp25, had identical rep-PCR fingerprints (Fig. 3A), and these fingerprints were identical to those of the typical *X. campestris* pv. *phaseoli* strain PR1 (Fig. 2A to C, compare lanes 5 and 6). Thus, these results support the notion that phenotype 1 isolates are typical *X. campestris* pv. *phaseoli* strains.

All six phenotype 2 isolates, including representative strain FH61 had identical rep-PCR fingerprints (Fig. 3B), which were similar to those of the known *X. fuscans* subsp. *fuscans* strains (Fig. 2A to C, compare lanes 8 to 10). Taken together with the production of brown pigment, these results indicated that phenotype 2 isolates are *X. fuscans* subsp. *fuscans*. Furthermore, the Wisconsin *X. fuscans* subsp. *fuscans* strains were differentiated from the two known *X. fuscans* subsp. *fuscans* genotypes based on fingerprints generated with the ERIC primers. Here, the

Fig. 2. Agarose gel showing DNA fingerprint patterns generated from total genomic DNA of *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) and *X. fuscans* subsp. *fuscans* (*Xff*) strains used in the host-pathogen study. **A**, Repetitive extragenic palindromic primers; **B**, enterobacterial repetitive intergenic consensus primers; and **C**, BOX primers. Lane 1, negative control (sterile H₂O); lane 2, nonpathogenic xanthomonad (NP); lane 3, *X. campestris* pv. *phaseoli* strain from East Africa (M11); lane 4, *X. campestris* pv. *phaseoli* strain from Puerto Rico (T21); lane 5, typical *X. campestris* pv. *phaseoli* strain from Wisconsin (Xcp25); lane 6, typical *X. campestris* pv. *phaseoli* strain from Wisconsin (Px100); lane 7, intermediate *X. campestris* pv. *phaseoli* strain from East Africa (M12F); lane 8, *X. fuscans* subsp. *fuscans* strain from Puerto Rico (PR8F); and lane 9, *X. fuscans* subsp. *fuscans* strain from Wisconsin (FH61). Size markers are indicated (kb) (1-kb ladder, Invitrogen).

fingerprints of the Wisconsin *X. fuscans* subsp. *fuscans* strains had ≈ 2.4 - and ≈ 0.9 -kb fragments, whereas those of the other *X. fuscans* subsp. *fuscans* genotypes had only one of these fragments (Fig. 2B, compare lanes 8 to 10). This new *X. fuscans* subsp. *fuscans* genotype is referred to as FH.

All 119 phenotype 3 (intermediate) isolates, including representative strain Px100, had identical rep-PCR fingerprints (Fig. 3C), and these were nearly identical to those of the typical *X. campestris* pv. *phaseoli* strains (Fig. 2A to C, compare lanes 5 to 7). However, the phenotype 3 isolates were differentiated from the typical *X. campestris* pv. *phaseoli* strains (Xcp25 and PR1) based on fingerprints generated with the ERIC primers. Here, the typical *X. campestris* pv. *phaseoli* strains had an ≈ 1.5 -kb fragment that the phenotype 3 isolates lacked, whereas the phenotype 3 isolates had ≈ 2.1 - and ≈ 2.4 -kb fragments that the typical *X. campestris* pv. *phaseoli* strains lacked (Fig. 2B, compare lanes 5 to 7). Thus, although the phenotype 3 isolates had an intermediate phenotype, they were nearly identical, genotypically, to typical *X. campestris* pv. *phaseoli* strains. This new *X. campestris* pv. *phaseoli* phenotype and genotype is referred to as Px.

Finally, the 55 nonpathogenic phenotype 4 isolates, including representative strain NP, had a diversity of rep-PCR fingerprints (Fig. 3D), all of which were distinct from those of *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains (Fig. 2A to C). These results established that these isolates compose a genetically diverse group of nonpathogenic xanthomonads.

An UPGMA cluster analysis was performed with data generated from the three rep-PCR analyses of the eight strains repre-

senting the genotypic diversity of *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* and one nonpathogenic xanthomonad strain (Table 3; Fig. 4). The three *X. campestris* pv. *phaseoli* strains with New World origins (PR1, Xcp25, and Px100) clustered together with $\geq 94\%$ similarity, with the new *X. campestris* pv. *phaseoli* Px phenotype and genotype most divergent. The three *X. fuscans* subsp. *fuscans* strains also clustered together, with $\approx 90\%$ similarity. The two *X. fuscans* subsp. *fuscans* strains with New World origins (PR8F and new genotype FH61) were more similar than the strain from the Old World (M12F). The two East African *X. campestris* pv. *phaseoli* strains (M11 and T21) were distinct from the other *X. campestris* pv. *phaseoli* strains and from the *X. fuscans* subsp. *fuscans* strains. Interestingly, on the basis of this analysis, these East African *X. campestris* pv. *phaseoli* genotypes were more similar to the *X. fuscans* subsp. *fuscans* strains than to the other *X. campestris* pv. *phaseoli* strains (Fig. 4). The nonpathogenic xanthomonad strain (NP) was genetically distinct from all *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains, with $< 45\%$ similarity.

16S-28S rRNA spacer region sequence analysis. The 16S-28S rRNA spacer region sequences (493 nucleotides [nt]) of the eight common blight bacteria strains had a high level ($> 95\%$) of sequence identity. The sequences of the three *X. campestris* pv. *phaseoli* strains with New World origins (PR1, Px100, and Xcp25) were identical, as were those of the three *X. fuscans* subsp. *fuscans* strains (M12F, PR8F, and FH61). These *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* sequences were 98% identical, with most of the 9 nt differences located in one

Fig. 3. Agarose gel showing DNA fingerprint patterns generated from total genomic DNA (with enterobacterial repetitive intergenic consensus primers) for the four phenotypes of xanthomonad-like bacteria isolated from dark red kidney bean leaves with common bacterial blight symptoms collected in surveys conducted in central Wisconsin in 2005 and 2006. **A**, Phenotype 1, typical *Xanthomonas campestris* pv. *phaseoli* strains; **B**, phenotype 2, *X. fuscans* subsp. *fuscans* strains; **C**, phenotype 3, intermediate *X. campestris* pv. *phaseoli* strains; and **D**, phenotype 4, nonpathogenic xanthomonad strains. Lane M, size markers are indicated (kb) (1-kb ladder, Invitrogen); lane 1, negative control (sterile H_2O); and lanes 2 to 9, representative strains of each phenotype.

region. The sequences of the East African *X. campestris* pv. *phaseoli* genotypes (M11 and T21) were identical to each other and nearly identical to those of *X. fuscans* subsp. *fuscans* strains, differing in only a single nucleotide. Consistent with these results, the *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* spacer region sequences in the GenBank, which are from African strains, were nearly identical to those of the East African *X. campestris* pv. *phaseoli* genotypes and *X. fuscans* subsp. *fuscans* strains. These results suggest that the East African *X. campestris* pv. *phaseoli* genotypes may be more closely related to *X. fuscans* subsp. *fuscans* strains than to typical *X. campestris* pv. *phaseoli* strains, which is in agreement with results of the rep-PCR analyses (Fig. 4).

To gain further insight into the significance of the sequence divergence in the spacer region sequences of common blight bacteria, comparisons were performed with sequences of other xanthomonad pathovars or species. The *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* sequences were 99% identical with that of *X. axonopodis* pv. *dieffenbachiae* and 98% identical with those of *X. axonopodis* pv. *citri*, *X. axonopodis* pv. *malvacearum*, and *X. campestris* pv. *vesicatoria*. Slightly lower identities were obtained in comparisons with sequences of *X. campestris* pv. *vitians* (97%), *X. campestris* pv. *carotae* (96%), and *X. campestris* pv. *campestris* (95%). These results revealed that the 16S-28S spacer region sequence is conserved among these xanthomonads, and that the level of divergence in the

sequences of typical *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains, and possibly in the East African *X. campestris* pv. *phaseoli* strains, was consistent with these genotypes representing different species. Our results also revealed a region of sequence diversity in the spacer sequence, located between nucleotides 465 and 477.

Host-pathogen interaction. All of the selected common blight bacteria strains (Table 4), except *X. fuscans* subsp. *fuscans* PR8F, were significantly more virulent on the susceptible Andean cv. Topcrop than on the Middle-American cv. Sutter Pink (note that neither cultivar possesses known CBB resistance genes). The typical *X. campestris* pv. *phaseoli* strain from Wisconsin (Xcp25) was most virulent on cv. Topcrop; whereas the new *X. fuscans* subsp. *fuscans* genotype from Wisconsin (FH61) was most virulent on cv. Sutter Pink.

When inoculated onto the series of common bean genotypes with CBB resistance QTL, the genotypes of common blight bacteria showed a range of virulence variability. The typical *X. campestris* pv. *phaseoli* strain from Wisconsin (Xcp25) was most virulent on most genotypes, followed by Px100 (the new brown-pigmented *X. campestris* pv. *phaseoli* phenotype and genotype) and PR1, respectively. Strains Xcp25 and Px100 showed similar virulence on most genotypes, whereas strain PR1 (typical *X. campestris* pv. *phaseoli* from Puerto Rico) was significantly less virulent on a number of genotypes, particularly those with the SAP6 marker (e.g., Chase, Montcalm, TARS VCI-4B, ICB 3, ICB

TABLE 3. Characteristics of *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains selected to represent pathogen diversity in the host-pathogen interaction study

Strain	Species	Geographical origin	Gene pool source ^a	Phenotype ^b	Genotype ^c	Pathogenicity ^d	PCR ID ^e
PR1	<i>X. campestris</i> pv. <i>phaseoli</i>	Puerto Rico	Andean	1	P	+	+
Xcp25	<i>X. campestris</i> pv. <i>phaseoli</i>	Wisconsin	Andean	1	P	+	+
Px100	<i>X. campestris</i> pv. <i>phaseoli</i>	Wisconsin	Andean	3	Px	+	+
M11	<i>X. campestris</i> pv. <i>phaseoli</i>	Malawi	Andean	1	EA1	+	+
T21	<i>X. campestris</i> pv. <i>phaseoli</i>	Tanzania	Andean	1	EA2	+	+
PR8F	<i>X. fuscans</i> subsp. <i>fuscans</i>	Puerto Rico	Middle American	2	NWF	+	+
M12F	<i>X. fuscans</i> subsp. <i>fuscans</i>	Malawi	Andean	2	EAF	+	+
FH61	<i>X. fuscans</i> subsp. <i>fuscans</i>	Wisconsin	Andean	2	FH	+	+

^a Indicates the gene pool of the common bean cultivar from which the strain was isolated.

^b Indicates the phenotypic group of the strain. Phenotype 1, yellow, mucoid growth with no pigment production; phenotype 2, yellow, mucoid to nonmucoid growth with copious brown pigment production; and phenotype 3, yellow, mucoid to nonmucoid growth with a low level of brown pigment production.

^c Repetitive-element polymerase chain reaction (PCR) genotype: P = typical *X. campestris* pv. *phaseoli*; Px = intermediate *X. campestris* pv. *phaseoli*; M11 and T21 represent East African (EA) *X. campestris* pv. *phaseoli* genotypes 1 and 2, respectively; NWF = New World *X. fuscans* subsp. *fuscans*; EAF = East African *X. fuscans* subsp. *fuscans*; and FH = the new Wisconsin *X. fuscans* subsp. *fuscans* genotype.

^d Symbol (+) indicates strain was pathogenic on the susceptible cv. Topcrop.

^e Symbol (+) indicates strain was positive in the PCR with the *X. campestris* pv. *phaseoli*- and *X. fuscans* subsp. *fuscans*-specific primers.

Fig. 4. Unweighted pair-group method with arithmetic means cluster analysis showing relationships among a nonpathogenic xanthomonad strain and the eight strains selected to represent the genetic diversity of *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) and *X. fuscans* subsp. *fuscans* (*Xff*) strains based upon results of repetitive-element polymerase chain reaction analyses with repetitive extragenic palindromic, enterobacterial repetitive intergenic consensus, and BOX primer pairs. Strains are as follows: PR1, typical *X. campestris* pv. *phaseoli* strain from Puerto Rico; Xcp25, typical *X. campestris* pv. *phaseoli* strain from Wisconsin; Px100, intermediate *X. campestris* pv. *phaseoli* strain from Wisconsin; M11, *X. campestris* pv. *phaseoli* strain from East Africa; T21, *X. campestris* pv. *phaseoli* strain from East Africa; PR8F, *X. fuscans* subsp. *fuscans* strain from Puerto Rico; FH61, *X. fuscans* subsp. *fuscans* strain from Wisconsin; M12F, *X. fuscans* subsp. *fuscans* strain from East Africa; and NP, nonpathogenic xanthomonad from Wisconsin.

11, ICB 67, VAX 1, VAX 2, G 17341, and G 17344) or with the SAP6 and SU91 markers (e.g., USPT-CBB-5) (Table 4). However, the most striking variability was observed for the East African strains (M11 and T21), which were significantly less virulent than the other *X. campestris* pv. *phaseoli* strains and the *X. fuscans* subsp. *fuscans* strains on most host genotypes (Table 4). An exception was on genotype OAC Rex, where the East African *X. campestris* pv. *phaseoli* strain T21 was the most virulent strain. The East African strains were similar in virulence on most genotypes but T21 was significantly more virulent on a number of genotypes, including ICB 11, 53, and 67 (with *P. coccineus*-derived resistance) and OAC Rex and OAC 88-1 (with *P. acutifolius*-derived resistance). The East African *X. campestris* pv. *phaseoli* strains and the typical *X. campestris* pv. *phaseoli* strain PR1 were also significantly less virulent than other *X. campestris* pv. *phaseoli* strains (Xcp25 and Px) on most of the bean genotypes with the SAP6 marker (Table 4).

In contrast to the virulence variability observed for *X. campestris* pv. *phaseoli* strains, *X. fuscans* subsp. *fuscans* strains were similar in virulence, with only minor differences observed on a few genotypes (e.g., ICB 67, VAX 1, and G 17341). Overall, the *X. fuscans* subsp. *fuscans* FH61 strain was most virulent followed by PR8F and M12F, respectively (Table 4); and *X. fuscans* subsp. *fuscans* strains were slightly less virulent compared with the most virulent *X. campestris* pv. *phaseoli* strain (Table 4).

Eight host genotypes were resistant to all eight pathogen genotypes, and most of these had pyramided resistance associated with the SU91 marker and one or both of the other markers (SAP6 and BC420) (Table 4). The highest levels of resistance were observed in the VAX breeding lines (especially lines VAX 3, VAX 4, VAX 5, and VAX 6), which have pyramided resistance associated with the SAP6 and SU91 markers. The one genotype with only the SU91 marker, OAC Rex, also had a high level of resistance, though not as high as the genotypes with pyramided resistance. Other host genotypes with pyramided resistance that also showed high levels of resistance included HR 45, Wilkinson 2, XAN 159, and XAN 309. These genotypes had SAP6 and SU91 (XAN 309), SU91 and BC420 (XAN 159 and Wilkinson 2), or all three markers (HR 45). Thus, the SU91 marker was consistently associated with high levels of resistance. Most of these highly resistant host genotypes were small seeded, although Wilkinson 2 and XAN 159 have a medium seed size.

In addition to the highly CBB-resistant genotypes, several other genotypes showed good levels of CBB resistance (Table 4). These included ICB 3, VAX 1, VAX 2, OAC Rex, OAC 88-1, and USPT-CBB-5. These host genotypes had various combinations of markers: ICB 3, VAX 1, and VAX 2 had SAP6; OAC Rex had SU91; and OAC 88-1 and USPT-CBB-5 had SAP6 and SU91. A number of other genotypes showed resistant or low intermediate responses (DSI values <5.0) to some pathogen genotypes; these

TABLE 4. Response of common bean genotypes with different combinations of common bacterial blight (CBB) disease resistance quantitative trait loci and markers to inoculation with genotypes of *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* representing pathogen diversity as revealed by repetitive-element polymerase chain reaction analyses^a

Genotype	Source ^b	Marker	Mean disease rating ^c								Mean	LSD ^c
			<i>X. campestris</i> pv. <i>phaseoli</i> ^d					<i>X. fuscans</i> subsp. <i>fuscans</i>				
			PR1	Xcp25	Px100	M11	T21	PR8F	M12F	FH61		
Topcrop	A (S)	None	8.2 S	8.7 S	8.0 S	4.5 I	5.5 I	6.9 I	7.3 S	7.5 S	7.1 S	0.50
Sutter Pink	M (S)	None	6.8 I	6.5 I	6.8 I	4.0 I	4.7 I	6.9 I	6.0 I	7.0 S	6.1 I	0.40
G 1320	M	None	5.5 I	5.9 I	5.6 I	2.5 R	3.4 R	5.4 I	5.5 I	6.0 I	5.0 I	0.30
XAN 91	M	None	5.6 I	5.8 I	5.1 I	2.0 R	2.9 R	5.5 I	5.1 I	5.0 I	4.6 I	0.70
ICB 53	<i>Pc</i>	None	4.5 I	6.8 I	6.3 I	<u>2.7 R</u>	<u>4.5 I</u>	5.6 I	5.6 I	6.0 I	5.2 I	0.32
Chase	M	SAP6	3.7 R	7.5 S	6.7 I	4.3 I	4.8 I	6.7 I	6.8 I	6.8 I	5.9 I	0.29
Colima 9	M	SAP6	5.3 I	5.7 I	5.3 I	2.3 R	2.7 R	5.5 I	4.7 I	5.9 I	4.7 I	0.33
Montana 5	M	SAP6	4.4 I	7.2 S	6.3 I	2.4 R	3.5 R	6.6 I	6.0 I	6.7 I	5.4 I	0.30
Montcalm	A	SAP6	5.2 I	8.2 S	7.4 S	4.9 I	4.0 I	6.8 I	6.1 I	6.8 I	6.2 I	0.40
TARS VCI-4B	M	SAP6	3.2 R	6.5 I	5.6 I	2.3 R	3.1 R	5.5 I	6.1 I	6.1 I	4.8 I	0.45
Tamaulipa 9-B	M	SAP6	6.0 I	6.8 I	6.0 I	2.5 R	3.1 R	6.0 I	5.3 I	6.1 I	5.2 I	0.25
ICB 3	<i>Pc</i>	SAP6	3.0 R	5.3 I	4.4 I	2.0 R	2.0 R	5.1 I	4.1 I	5.6 I	3.9 R	0.43
ICB 11	<i>Pc</i>	SAP6	4.9 I	7.6 S	7.0 S	<u>3.0 R</u>	<u>4.3 I</u>	6.1 I	5.4 I	6.3 I	5.6 I	0.35
ICB 67	<i>Pc</i>	SAP6	3.5 R	8.0 S	7.2 S	<u>2.5 R</u>	<u>4.3 I</u>	6.5 I	6.2 I	7.1 S	5.6 I	0.30
VAX 1	<i>Pa</i>	SAP6	2.2 R	4.4 I	4.1 I	2.1 R	2.4 R	4.0 I	3.9 R	4.5 I	3.5 R	0.27
VAX 2	<i>Pa</i>	SAP6	2.3 R	5.1 I	4.5 I	2.0 R	2.0 R	4.9 I	4.4 I	4.9 I	3.8 R	0.30
G 17341	Pyr	SAP6	2.1 R	4.3 I	3.3 I	2.0 R	2.2 R	3.3 R	3.0 R	4.0 I	3.0 R	0.38
G 17344	Pyr	SAP6	2.7 R	4.1 I	4.1 I	2.8 R	2.7 R	3.6 R	3.4 R	4.6 I	3.5 R	0.40
USPT-CBB-1	Pyr	SAP6	2.6 R	5.4 I	5.0 I	2.5 R	3.0 R	4.3 I	4.7 I	4.6 I	4.0 I	0.32
OAC Rex	<i>Pa</i>	SU91	3.3 R	3.8 R	3.5 R	<u>2.1 R</u>	<u>4.2 I</u>	2.9 R	3.1 R	2.7 R	3.2 R	0.28
OAC 88-1	<i>Pa</i>	SAP6+SU91	3.9 R	4.0 I	3.1 R	<u>2.3 R</u>	<u>4.5 I</u>	2.7 R	2.9 R	2.8 R	3.3 R	0.30
USPT-CBB-5	Pyr	SAP6+SU91	3.5 R	4.9 I	4.2 I	3.0 R	3.0 R	4.4 I	3.8 R	4.3 I	3.9 R	0.32
VAX 3	Pyr	SAP6+SU91	2.2 R	2.3 R	2.0 R	2.0 R	2.0 R	2.3 R	2.3 R	2.5 R	2.2 R	0.27
VAX 4	Pyr	SAP6+SU91	2.1 R	2.3 R	2.0 R	2.0 R	2.6 R	2.5 R	2.4 R	2.5 R	2.3 R	0.36
VAX 5	Pyr	SAP6+SU91	2.0 R	3.1 R	2.5 R	2.0 R	2.2 R	2.5 R	2.5 R	2.8 R	2.4 R	0.55
VAX 6	Pyr	SAP6+SU91	2.3 R	2.6 R	2.3 R	2.4 R	2.8 R	2.3 R	2.4 R	2.7 R	2.5 R	0.32
XAN 309	Pyr	SAP6+SU91	2.4 R	2.9 R	2.4 R	2.0 R	2.3 R	3.0 R	3.1 R	2.9 R	2.6 R	0.32
XAN 159	<i>Pa</i>	SU91+BC420	2.9 R	3.6 R	3.0 R	2.5 R	3.2 R	2.8 R	2.5 R	3.0 R	2.9 R	0.28
Wilkinson 2	Pyr	SU91+BC420	3.3 R	3.6 R	3.4 R	2.5 R	3.3 R	3.0 R	2.7 R	3.0 R	3.1 R	0.40
HR 45	Pyr	All three	2.8 R	3.3 R	2.8 R	2.0 R	2.8 R	2.4 R	2.7 R	3.0 R	2.7 R	0.40
Strain Mean	...	...	3.7	5.2	4.7	2.6	3.3	4.5	4.3	4.8	4.1	0.33
LSD (<i>P</i> = 0.05)	...	...	0.43	0.46	0.38	0.42	0.44	0.46	0.38	0.41	0.48	...

^a Experiments were conducted in a greenhouse at the University of California, Davis in 2007. Results represent means of two independent experiments.

^b M = Middle American gene pool, A = Andean gene pool, S = susceptible, Pc = *Phaseolus coccineus*, Pa = *Phaseolus acutifolius*, and Pyr = pyramided resistance.

^c Data based on the 1-to-9 rating scale, where 1 < 4 = resistant (R), 4 < 7 = intermediate (I), and 7–9 = susceptible (S).

^d Numbers shown in bold represent host genotypes for which the highest level of differential virulence was observed (i.e., all three disease responses [R, I, and S] were observed). Underlined numbers show common bean genotypes that differentiated the East African *X. campestris* pv. *phaseoli* genotypes (M11 and T21).

^e Least significant difference (LSD) (*P* = 0.05).

had resistance introgressed from Middle-American sources and had SAP6 or no markers (Table 4).

Genotypes with only the SAP6 marker varied considerably in their response to the pathogen genotypes tested. For example, VAX 1, VAX 2, G 17341, and G 17344 had resistant or intermediate responses to most strains, whereas ICB 11 and ICB 67 had susceptible or intermediate responses (Table 4). In addition, some host genotypes with no markers had resistant or intermediate responses to all strains (e.g., G 1320 [PI 207062], XAN 91, and ICB 53) (Table 4).

A highly significant ($P < 0.01$) host–pathogen interaction was detected in this study, which is fully consistent with the variation in virulence observed for the common blight bacteria. A number of host genotypes revealed evidence of specificity, which is defined as >3 point difference in DSI values for response to specific *X. campestris* pv. *phaseoli* or *X. fuscans* subsp. *fuscans* genotypes, and these included Chase, Montcalm, TARS VCI-4B, and ICB 67. These genotypes all had the SAP6 marker. Other host genotypes were resistant to some pathogen genotypes but had intermediate responses to others. These included genotypes with the SAP6 marker (Colima 9 and G 1320), resistance introgressed from *P. acutifolius* (OAC Rex and OAC 88-1), or pyramided resistance (G 17341, G 17344, and USPT-CBB-5). Consistent with the significant host–pathogen interaction, the relative order of the host genotypes, from most resistant to most susceptible, varied depending on the pathogen genotype. For example, the host order was different for *X. campestris* pv. *phaseoli* strains PR1 and Xcp25 (Table 4, ranking not shown).

DISCUSSION

CBB is an economically important disease of DRK bean in the Midwestern United States, and can cause substantial losses in yield and quality. However, the severity of CBB was low to moderate in the central Wisconsin DRK bean fields surveyed in 2005 and 2006, most likely reflecting unfavorable climatic conditions and the planting of pathogen-free seed. In fact, identification of CBB symptoms in some fields was complicated by the abiotic pseudo-CBB symptoms, which develop due to heat and water stress. Diagnosis of CBB was confirmed by isolation of xanthomonad-like bacteria on the semiselective medium MXP, but this was complicated by the presence of nonpathogenic xanthomonads. These xanthomonads were previously reported associated with common bean in Wisconsin and other bean-growing regions (2,12,15), and must be differentiated from common blight bacteria by genetic or pathogenicity tests. Together, these results highlight the need to perform Koch's postulates to confirm CBB diagnoses in certain field situations.

The majority of common blight bacteria associated with CBB in DRK bean in central Wisconsin were *X. campestris* pv. *phaseoli*. This supports the hypothesis that *X. campestris* pv. *phaseoli* strains are most commonly associated with CBB in large-seeded Andean genotypes (23,34). Interestingly, the *X. campestris* pv. *phaseoli* population was not clonal because two *X. campestris* pv. *phaseoli* phenotypes/genotypes were involved: typical *X. campestris* pv. *phaseoli* strains with yellow mucoid colonies, no brown pigment production, and *X. campestris* pv. *phaseoli*-type rep-PCR fingerprints; and the new Px *X. campestris* pv. *phaseoli* strains, with a less mucoid and flatter colony, brown pigment production, and *X. campestris* pv. *phaseoli*-type rep-PCR fingerprints. Initially, the Px strains were thought to be *X. fuscans* subsp. *fuscans*, based on colony morphology and brown pigment production; however, genetic analyses clearly showed that they were *X. campestris* pv. *phaseoli* strains. Moreover, the Px strains play an important role in CBB in this region, because they were commonly isolated and are highly virulent. Finally, the fact that these two *X. campestris* pv. *phaseoli* phenotypes/genotypes did not seem to coexist in most fields may indicate competition, and it

will be of interest to see whether the population structure changes in the future and whether Px *X. campestris* pv. *phaseoli* strains are identified in other geographic regions.

Brown pigment production is a distinctive characteristic of *X. fuscans* subsp. *fuscans* strains. Thus, the discovery of a brown-pigmented strain of *X. campestris* pv. *phaseoli* was unexpected and revealed phenotypic variability among strains of this bacterium. However, brown pigment production did not enhance virulence, because strain Px100 was slightly less virulent than Xcp25, which has a similar rep-PCR fingerprint but does not produce brown pigment (Table 4). It may enhance bacterial fitness in some other way, such as epiphytic survival. It would be interesting to determine the genetic basis of brown pigment production in the Px *X. campestris* pv. *phaseoli* strains, because they produced substantially less brown pigment than *X. fuscans* subsp. *fuscans* strains. This may reflect horizontal gene transfer between *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* or the activation of an existing biosynthetic pathway, perhaps via a mobile genetic element (13). Finally, these results raise some concerns about using brown pigment production to differentiate *X. campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* strains.

The East African *X. campestris* pv. *phaseoli* genotypes (represented by M11 and T21) were substantially less virulent than typical *X. campestris* pv. *phaseoli* strains, which is consistent with previous reports (23,34). Moreover, the results of the rep-PCR and spacer region sequence analyses performed in the present study indicated that these genetically distinct East African *X. campestris* pv. *phaseoli* strains were more closely related to *X. fuscans* subsp. *fuscans* than *X. campestris* pv. *phaseoli*. This raises the possibility that *X. fuscans* subsp. *fuscans* strains may have originated in Africa, and is consistent with the frequent association of *X. fuscans* subsp. *fuscans* with CBB in East Africa (35). Thus, our results extend the idea that common blight bacteria are a heterogeneous polyphetic group of xanthomonads, which include one or more genotypes or species that have probably evolved independently. Similar findings have been reported for xanthomonads causing bacterial spot disease of tomato (17,18) and black rot of crucifers (14,26,52).

The finding that *X. campestris* pv. *phaseoli* strains were more variable in virulence than *X. fuscans* subsp. *fuscans* strains is in agreement with results of Mutlu et al. (36). However, unlike our findings, Mutlu et al. (36) found that *X. fuscans* subsp. *fuscans* strains were more virulent than *X. campestris* pv. *phaseoli* strains, regardless of geographic origin. This may be explained by the different pathogen strains used in the respective studies. Nonetheless, the substantial variability in virulence among common blight bacteria revealed in the present study and those of others (23,34,36) has practical significance in terms of selecting the appropriate *X. campestris* pv. *phaseoli* or *X. fuscans* subsp. *fuscans* strains for CBB resistance breeding. Use of less virulent strains (e.g., the East African *X. campestris* pv. *phaseoli* strains) or strains with attenuated virulence due to repeated subculturing can lead to selection of “resistant” common bean genotypes that have susceptible or intermediate responses to more virulent strains. For example, a number of common bean genotypes with the SAP6 marker were resistant to the PR1 *X. campestris* pv. *phaseoli* strain but susceptible to the Xcp25 strain; thus, genotypes bred for resistance to PR1 would be susceptible to strains such as Xcp25. This situation may explain why a recently released CBB-resistant DRK breeding line (USDK-CBB-15) was not resistant under high levels of CBB pressure in the field in Wisconsin or when inoculated with the highly virulent Xcp25 (22).

The only known source of CBB resistance from common bean is a QTL derived from Middle-American genotypes that is linked with the SAP6 marker (28). However, some genotypes with only the SAP6 marker were susceptible to some pathogen genotypes,

indicating that SAP6-associated resistance is not sufficient for effective CBB management or that the resistance is not sufficiently closely linked to the marker. The most robust CBB resistance has been introgressed from *P. acutifolius* (via XAN 159) and is associated with the SU91 marker (49). Thus, our finding that all of the most resistant common bean genotypes had this marker is fully consistent with these results (51). However, the highest levels of resistance were associated with genotypes with pyramided resistance (SU91 and one or both of the other markers), indicating that introgression of appropriate combinations of CBB resistance QTL, via marker-assisted selection, can result in genotypes with broad-spectrum resistance to a diversity of pathogen genotypes. This is consistent with other studies, and we are in agreement with Mutlu et al. (36) and Lema et al. (22) that only genotypes with pyramided resistance should be developed and deployed in common bean-production regions where CBB is prevalent (23,25,37). Moreover, having pyramided resistance available in diverse backgrounds (e.g., VAX 3, VAX 4, VAX 5, VAX 6, Wilkinson 2, XAN 159, and XAN 309) should allow for incorporation of CBB resistance into all market classes, including large-seeded Andean cultivars, such as DRK bean. Finally, a strategy based upon multiple QTL should provide more durable resistance than that conferred by single dominant or recessive resistance genes. This is important, because it is clear that multiple pathogen genotypes can exist together in a region or an individual field (Table 1), which could allow for exchange of genetic material among strains and emergence of resistance-breaking strains.

However, it is important to note that genotypes with pyramided resistance varied in levels of CBB resistance and that some genotypes, such as USDK-CBB-15 with the SAP6 and SU91 markers, may be susceptible under high disease pressure (22). This indicates that pyramided resistance does not necessarily confer high levels of resistance in all cases, and highlights the need to use appropriate pathogen strains in CBB resistance breeding. Another potential problem is that the SU91 marker has been associated with a reduction in yield (37). This may complicate its use in CBB resistance-breeding programs unless such undesirable linkages could be broken. Alternatively, additional sources of CBB resistance (and associated markers) could be used. Indeed, our results also indicated the presence of unmarked CBB resistance QTL in some of the common bean genotypes tested based on the variation in response of genotypes with similar marker compositions to certain pathogen genotypes (Table 4). These results are also consistent with the quantitative nature of CBB resistance in common bean, and the identification of CBB resistance QTL on all the common bean linkage groups (22,29).

In conclusion, we confirmed the association of *X. campestris* pv. *phaseoli* with CBB in a major production area of large-seeded Andean bean (DRK) and revealed new phenotypic variability in *X. campestris* pv. *phaseoli* with the identification of a brown-pigmented phenotype and genotype. This extends the concept that common blight bacteria are a heterogeneous group of xanthomonads, and highlights the importance of selecting appropriate pathogen strains for resistance-breeding programs. Our results also support the hypothesis that common bean genotypes with multiple introgressed CBB resistance QTL can be highly resistant to a diversity of common blight bacteria. These genotypes can be used for the development of CBB-resistant common bean cultivars in multiple market classes.

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