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Genetic Diversity and Pathogenic Variation of Common Blight Bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) Suggests Pathogen Coevolution with the Common Bean

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ABSTRACT

Mkandawire, A. B. C., Mabagala, R. B., Guzmán, P., Gepts, P., and Gilbertson, R. L. 2004. Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) suggests pathogen coevolution with the common bean. Phytopathology 94:593-603.

Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*, is one of the most important diseases of common bean (*Phaseolus vulgaris*) in East Africa and other bean-growing regions. Xanthomonad-like bacteria associated with CBB in Malawi and Tanzania, East Africa, and in Wisconsin, U.S., were characterized based on brown pigment production, pathogenicity on common bean, detection with an *X. campestris* pv. *phaseoli*- or *X. campestris* pv. *phaseoli* var. *fuscans*-specific PCR primer pair, and repetitive element polymerase chain reaction (rep-PCR) and restriction fragment length polymorphism (RFLP) analyses. The common bean gene pool (Andean or Middle American) from which each strain was isolated also was determined. In Malawi, *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* were isolated predominantly from Andean or Middle American beans, respectively. In Tanzania, *X. campestris* pv. *phaseoli* var. *fuscans* was most commonly isolated, irrespective of gene pool; whereas, in Wisconsin, only *X. campestris* pv. *phaseoli* was isolated from Andean red kidney beans. Three rep-PCR fingerprints were obtained for *X. campestris* pv. *phaseoli* strains; two were unique to East African strains, whereas the other was associated with strains collected from all other (mostly New World) locations. RFLP analyses with repetitive DNA probes revealed the same genetic diversity among *X. campestris* pv. *phaseoli* strains as did rep-PCR. These probes hybridized with only one or two fragments in the East African strains, but with multiple fragments in the other *X. campestris* pv. *phaseoli* strains. East African *X. campestris* pv. *phaseoli* strains were highly pathogenic on Andean beans, but were significantly less pathogenic on Middle American beans. In contrast, *X. campestris* pv. *phaseoli* strains from New World locations were highly pathogenic on beans of both gene pools. Together, these results indicate the existence of genetically and geographically distinct *X. campestris* pv. *phaseoli* genotypes. The rep-PCR fingerprints of *X. campestris* pv. *phaseoli* var. *fuscans* strains from East African and New World locations were indistinguishable, and were readily distinguished from those of *X. campestris* pv. *phaseoli* strains. Genetic diversity among *X. campestris* pv. *phaseoli* var. *fuscans* strains was revealed by RFLP analyses. East African and New World *X. campestris* pv. *phaseoli* var. *fuscans* strains were highly pathogenic on Andean and Middle American beans. Breeding for CBB resistance in East African beans should utilize *X. campestris* pv. *phaseoli* var. *fuscans* and New World *X. campestris* pv. *phaseoli* strains in order to identify germ plasm with the highest levels of resistance.

Common bean (*Phaseolus vulgaris* L.) is a major food crop that provides an inexpensive source of protein for both rural and urban households in East Africa. However, production of the crop is limited by numerous biotic and abiotic constraints. Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*, is a widespread and destructive disease of common bean in Africa and other bean-growing regions (1,22,24). Common blight bacteria survive between bean crops in association with seed, bean debris, and weeds (10,13,26,32,33). Thus, effective CBB disease management involves the use of certified seed, crop rotation, and sanitation (e.g., deep plowing or removal of debris and weed management). In East Africa, most farmers do not have access to certified seed; and sanitation, weed management, and rotation options are limited (22). Thus, the development of cultivars with durable CBB resistance offers the most promising long-term and economical means of disease management. To identify germ plasm that will be highly resistant to CBB and to facilitate disease resistance breeding for a given region, there is a need to identify the predominant common blight bacteria.

There is evidence of genetic diversity among common blight bacteria, although the significance of this diversity is not well understood. *X. campestris* pv. *phaseoli* var. *fuscans* can be differentiated from *X. campestris* pv. *phaseoli* strains based on phenotypic (i.e., *X. campestris* pv. *phaseoli* var. *fuscans* strains produce a brown melanin-like pigment in culture [14]) and genetic characteristics (5,6,12,16,20). *X. campestris* pv. *phaseoli* var. *fuscans* and *X. campestris* pv. *phaseoli* have been differentiated based upon restriction fragment length polymorphism (RFLP) analyses (6,12,20), a random amplified polymorphic DNA fragment (5), DNA-DNA hybridization (16), and pulse field gel electrophoresis (6). rDNA sequence analysis revealed that strains of *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* were genetically distinct, but were more closely related to each other than to other *X. campestris* pathovars (4). Variability in the pathogenicity of *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains also has been reported (27,37,45);

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however, distinct races of common blight bacteria, based on differential pathogenicity on defined *P. vulgaris* cultivars, have not been identified (10). This is consistent with the fact that CBB resistance is a quantitative trait (10,32,42). *X. campestris* pv. *phaseoli* strains have been differentiated into pathogenic races based on reactions on tepary bean (*P. acutifolius*) lines (27,49), but the significance of this variability in terms of common bean remains to be established.

More recently, DNA fingerprints, generated with the polymerase chain reaction (PCR), have been used to identify genetic diversity among strains of closely related bacteria (21,30). In particular, the repetitive element PCR (rep-PCR) technique has been widely used. This method involves the use of primers based on highly conserved sequences dispersed throughout the bacterial genome and PCR to amplify DNA lying between these elements (47). The rep-PCR method has been used to identify and differentiate many plant-pathogenic bacteria, including pathovars of *Pseudomonas syringae* (19,21,38,48) and *X. campestris* (4,21,31, 34,44,46).

The objectives of this study were to (i) characterize xanthomonad-like bacteria associated with CBB in bean-growing areas of Malawi and Tanzania in East Africa and in Wisconsin, (ii) assess the genetic diversity among these strains, and (iii) determine whether any relationship exists between genetic diversity among *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains and pathogenicity on beans of the Andean and Middle American gene pools. The results of this study are discussed in terms of the development of CBB-resistant common bean cultivars for East Africa.

MATERIALS AND METHODS

Bacterial strains and their identification. Bacterial strains used in this study are listed in Table 1. These strains were recovered from bean leaves or seed collected during surveys conducted in East Africa (Malawi and Tanzania) and Wisconsin. In these surveys, representative bean fields in these regions were randomly selected and surveyed for CBB symptoms by examining

TABLE 1. Xanthomonad-like strains isolated from common bacterial blight samples collected during surveys of East Africa and Wisconsin

Designation	Identity[w]	Geographic origin[x]	Common bean gene pool source[y]	Pathogenicity[z]
M11	*X. campestris* pv. *phaseoli*	Bunda, Malawi	A	+
M12F	*X. campestris* pv. *phaseoli* var. *fuscans*	Bunda, Malawi	A	+
M13F	*X. campestris* pv. *phaseoli* var. *fuscans*	Bunda, Malawi	M	+
M14F	*X. campestris* pv. *phaseoli* var. *fuscans*	Bunda, Malawi	M	+
M15	*Xanthomonas* spp.	Bunda, Malawi	A	–
M16F	*X. campestris* pv. *phaseoli* var. *fuscans*	Bunda, Malawi	M	+
M17	*X. campestris* pv. *phaseoli*	Bunda, Malawi	A	+
M18	*X. campestris* pv. *phaseoli*	Rumphi, Malawi	…	+
M19	*X. campestris* pv. *phaseoli*	Rumphi, Malawi	A	+
M20	*X. campestris* pv. *phaseoli*	Rumphi, Malawi	A	+
M27F	*X. campestris* pv. *phaseoli* var. *fuscans*	Rumphi, Malawi	M	+
M32F	*X. campestris* pv. *phaseoli* var. *fuscans*	Rumphi, Malawi	M	+
M33	*Xanthomonas* spp.	Rumphi, Malawi	A	–
M23F	*X. campestris* pv. *phaseoli* var. *fuscans*	Dedza, Malawi	M	+
M24	*X. campestris* pv. *phaseoli*	Dedza, Malawi	A	+
M25	*X. campestris* pv. *phaseoli*	Mzimba, Malawi	A	+
M29	*X. campestris* pv. *phaseoli*	Mzimba, Malawi	A	+
M31F	*X. campestris* pv. *phaseoli* var. *fuscans*	Mzimba, Malawi	…	+
M26F	*X. campestris* pv. *phaseoli* var. *fuscans*	Kasungu, Malawi	A	+
M30	*X. campestris* pv. *phaseoli*	Kasungu, Malawi	M	+
M28	*Xanthomonas* spp.	Kasungu, Malawi	A	–
T21F	*X. campestris* pv. *phaseoli* var. *fuscans*	Iringa, Tanzania	…	+
T22F	*X. campestris* pv. *phaseoli* var. *fuscans*	Iringa, Tanzania	M	+
T3F	*X. campestris* pv. *phaseoli* var. *fuscans*	Iringa, Tanzania	A	+
T14	*X. campestris* pv. *phaseoli*	Iringa, Tanzania	A	+
T17F	*X. campestris* pv. *phaseoli* var. *fuscans*	Iringa, Tanzania	A	+
T19F	*X. campestris* pv. *phaseoli* var. *fuscans*	Iringa, Tanzania	A	+
T20F	*X. campestris* pv. *phaseoli* var. *fuscans*	Iringa, Tanzania	A	+
T21	*X. campestris* pv. *phaseoli*	Iringa, Tanzania	A	+
T23F	*X. campestris* pv. *phaseoli* var. *fuscans*	Iringa, Tanzania	A	+
T32F	*X. campestris* pv. *phaseoli* var. *fuscans*	Iringa, Tanzania	A	+
T3-3	*Xanthomonas* spp.	Morogoro, Tanzania	A	–
T3-4	*Xanthomonas* spp.	Morogoro, Tanzania	A	–
T5-1	*Xanthomonas* spp.	Lushoto, Tanzania	M	–
T7-8F	*X. campestris* pv. *phaseoli* var. *fuscans*	Lushoto, Tanzania	A	+
T7-9F	*X. campestris* pv. *phaseoli* var. *fuscans*	Lushoto, Tanzania	M	+
T7-10F	*X. campestris* pv. *phaseoli* var. *fuscans*	Lushoto, Tanzania	M	+
T7-11	*Xanthomonas* spp.	Lushoto, Tanzania	M	–
T7-12F	*X. campestris* pv. *phaseoli* var. *fuscans*	Lushoto, Tanzania	M	+
T7-13F	*X. campestris* pv. *phaseoli* var. *fuscans*	Lushoto, Tanzania	M	+
T8-2	*X. campestris* pv. *phaseoli*	Arumeru, Tanzania	A	+
T8-3	*X. campestris* pv. *phaseoli*	Arumeru, Tanzania	A	+
T8-4	*X. campestris* pv. *phaseoli*	Arumeru, Tanzania	A	+
T8-5F	*X. campestris* pv. *phaseoli* var. *fuscans*	Arumeru, Tanzania	M	+
T8-6F	*X. campestris* pv. *phaseoli* var. *fuscans*	Arumeru, Tanzania	M	+
T8-7F	*X. campestris* pv. *phaseoli* var. *fuscans*	Arumeru, Tanzania	M	+

(*continued on next page*)

[w] *Xanthomonas campestris* pv. *phaseoli*, *X. campestris* pv. *phaseoli* var. *fuscans*, and uncharacterized xanthomonad-like bacteria (*Xanthomonas* spp.).
[x] WI = Wisconsin, United States.
[y] A = Andean gene pool and M = Middle American gene pool.
[z] Pathogenicity was determined by inoculating strains onto trifoliolate leaves of 14-day-old plants (cv. Topcrop) with the razor blade method (29).

plants along a zigzag pattern across all or a portion (fields in Wisconsin) of the field. In most cases, a leaf showing typical CBB symptoms (irregular necrotic lesions with yellow borders and water-soaked spots) was collected from each of 5 to 10 plants in each field, and dried between paper towels. For each leaf sample, tissues (≈16 mm^2) were excised from the lesion margin, placed in a drop of sterile distilled water on a microscope slide, and macerated. Loopfuls of macerate were streaked onto 523 medium (18) and the plates were incubated at 28°C. In locations where diseased bean plants were not found (south-central Tanzania), bean seed were collected from local markets and assayed for common blight bacteria with a seed wash dilution plating assay (23).

Four yellow, mucoid, xanthomonad-like colonies were selected from each leaf or seed sample and subcultured on 523 medium. After 2 to 3 days of growth, production of diffusible brown pigment was recorded. To determine if these strains were common blight bacteria, PCR with the *X. campestris* pv. *phaseoli*- or *X. campestris* pv. *phaseoli* var. *fuscans*-specific primer pair X4c (5′-GGC AAC ACC CGA TCC CTA AAC AGG-3′) and X4e (5′-CGC CGG AAG CAC GAT CCT CGA AG-3′) was used. This primer pair directs the amplification of an ≈700-bp fragment from common blight bacteria, but not from other *X. campestris* pathovars, nonpathogenic xanthomonads, or other bacteria associated with common bean (2). Preparation of boiled cell extracts, PCR parameters, and analyses of PCR products have been described previously (2,4).

Additional *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains (or total genomic DNA of these strains) were collected previously from East Africa (Uganda and Kenya), Spain, and various New World locations (Brazil, Colombia, the Dominican Republic, Guatemala, Mexico, Puerto Rico, and the United States) (Table 2).

DNA extraction and rep-PCR. Shake cultures from single colonies were grown in 3 ml of yeast tryptone (YT) broth overnight (12 to 18 h) at 28°C. DNA was extracted from bacterial cells using a modification of Silhavy et al. (40). Cells were washed with NE buffer (50 mM Tris-EDTA and 150 mM NaCl), resuspended in NE buffer with proteinase K at 150 μg/ml and

TABLE 1. (*Continued from preceding page*)

Designation	Identity[w]	Geographic origin[x]	Common bean gene pool source[y]	Pathogenicity[z]
T8-8F	*X. campestris* pv. *phaseoli* var. *fuscans*	Arumeru, Tanzania	M	+
T8-9F	*X. campestris* pv. *phaseoli* var. *fuscans*	Arumeru, Tanzania	M	+
T9-1F	*X. campestris* pv. *phaseoli* var. *fuscans*	Arumeru, Tanzania	M	+
T9-2F	*X. campestris* pv. *phaseoli* var. *fuscans*	Arumeru, Tanzania	M	+
T9-3	*Xanthomonas* spp.	Arumeru, Tanzania	A	–
T9-4	*Xanthomonas* spp.	Arumeru, Tanzania	A	–
T12-3F	*X. campestris* pv. *phaseoli* var. *fuscans*	Njiapanda, Tanzania	A	+
T12-4F	*X. campestris* pv. *phaseoli* var. *fuscans*	Njiapanda, Tanzania	A	+
T12-7F	*X. campestris* pv. *phaseoli* var. *fuscans*	Njiapanda, Tanzania	A	+
T12-8F	*X. campestris* pv. *phaseoli* var. *fuscans*	Njiapanda, Tanzania	A	+
T12-9F	*X. campestris* pv. *phaseoli* var. *fuscans*	Njiapanda, Tanzania	M	+
T12-10F	*X. campestris* pv. *phaseoli* var. *fuscans*	Njiapanda, Tanzania	A	+
T12-11F	*X. campestris* pv. *phaseoli* var. *fuscans*	Njiapanda, Tanzania	A	+
T14-1F	*X. campestris* pv. *phaseoli* var. *fuscans*	Karatu, Tanzania	A	+
T14-2F	*X. campestris* pv. *phaseoli* var. *fuscans*	Karatu, Tanzania	A	+
T14-3F	*X. campestris* pv. *phaseoli* var. *fuscans*	Karatu, Tanzania	M	+
T14-4F	*X. campestris* pv. *phaseoli* var. *fuscans*	Karatu, Tanzania	A	+
T14-5F	*X. campestris* pv. *phaseoli* var. *fuscans*	Karatu, Tanzania	A	+
T/Market 8	*X. campestris* pv. *phaseoli*	Karatu, Tanzania	A	+
T17-1F	*X. campestris* pv. *phaseoli* var. *fuscans*	Babati, Tanzania	A	+
T17-2F	*X. campestris* pv. *phaseoli* var. *fuscans*	Babati, Tanzania	A	+
T17-3F	*X. campestris* pv. *phaseoli* var. *fuscans*	Babati, Tanzania	A	+
T17-4F	*X. campestris* pv. *phaseoli* var. *fuscans*	Babati, Tanzania	A	+
T/Market 9F	*X. campestris* pv. *phaseoli* var. *fuscans*	Babati, Tanzania	M	+
T18-1F	*X. campestris* pv. *phaseoli* var. *fuscans*	Endakasi, Tanzania	A	+
T18-2F	*X. campestris* pv. *phaseoli* var. *fuscans*	Endakasi, Tanzania	A	+
T18-3F	*X. campestris* pv. *phaseoli* var. *fuscans*	Endakasi, Tanzania	A	+
T19-1F	*X. campestris* pv. *phaseoli* var. *fuscans*	Machame, Tanzania	A	+
T19-2F	*X. campestris* pv. *phaseoli* var. *fuscans*	Machame, Tanzania	A	+
T19-3F	*X. campestris* pv. *phaseoli* var. *fuscans*	Machame, Tanzania	A	+
T19-5	*Xanthomonas* spp.	Machame, Tanzania	M	–
W01	*X. campestris* pv. *phaseoli*	Camel, WI	A	+
W02	*X. campestris* pv. *phaseoli*	Camel, WI	A	+
W03	*X. campestris* pv. *phaseoli*	Camel, WI	A	+
W04	*X. campestris* pv. *phaseoli*	Camel, WI	A	+
W05	*X. campestris* pv. *phaseoli*	Camel, WI	A	+
W06	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W07	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W08	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W09	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W10	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W11	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W12	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W13	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W14	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W15	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W16	*X. campestris* pv. *phaseoli*	Luther, WI	A	+
W17	*X. campestris* pv. *phaseoli*	Rock Falls, WI	A	+
W18	*X. campestris* pv. *phaseoli*	Rock Falls, WI	A	+
W19	*X. campestris* pv. *phaseoli*	Test Plot, WI	A	+
W20	*X. campestris* pv. *phaseoli*	Niece, WI	A	+

20% sodium dodecyl sulfate (SDS), and incubated at 50°C for 1 h. The suspension was extracted with phenol/chloroform/isoamyl alcohol (25:24:1), and DNA was precipitated by the addition of 0.1 volume of 3 M sodium acetate and 1 volume of 100% isopropanol. DNA was spooled out of the aqueous phase, washed in 70% ethanol, resuspended in TE buffer (10 mM Tris and 1 mM EDTA), and quantified using a fluorometer (model TK0100-115V; Hoefer Scientific Instruments, San Francisco). The rep-PCR was performed as previously described with the repetitive extragenic palindromic (REP)-PCR primers 1R (5′-III ICG ICG ICA TCI GGC-3′) and 2I (5′-ICG ICT TAT CIG GCC TAC-3′); the enterobacterial repetitive intergenic consensus (ERIC)-PCR primers 1R (5′-ATG TAA GCT CCT GGG GAT TCA C-3′) and 2I (5′-AAG TAA GTG ACT GGG GTG AGC G-3′); and the BOX element 1A (BOX)-PCR primer 1R (5′-CTA CGG CAA GGC GAC GCT GAC G-3′) (21,30,47).

Pathogenicity tests. To prepare inoculum, selected *X. campestris* pv. *phaseoli* or *X. campestris* pv. *phaseoli* var. *fuscans* strains were streaked onto plates of 523 medium and grown for 48 h at 28°C. Cells were suspended in distilled water and adjusted to an optical density at 600 nm = 0.50, which corresponds to $\approx 10^8$ CFU/ml. Two types of pathogenicity tests were performed: (i) basic pathogenicity on common bean, in which strains were inoculated onto the susceptible cv. Topcrop; and (ii) differential pathogenicity, in which strains were inoculated onto genotypes representing the two common bean gene pools. The differential pathogenicity tests involved cvs. Topcrop and White Kidney (Andean), Black Turtle Soup and Sutter Pink (Middle American), and a number of Malawian (22-2 and 12-4 [Andean]; and 1-1, 6-5, and Namajengo [Middle American]) and Tanzanian (Rose Koko [Andean]) genotypes.

Five seed of each genotype were planted in 6-in. pots. The razor blade method (28) was used to inoculate the first fully expanded trifoliolate leaves of bean plants (≈14 days after planting). The floor of the greenhouse was kept wet to generate humidity to favor development of CBB. Symptoms were rated 10 days after inoculation using the following rating scale: 1 = no visual symptoms or slight marginal necrosis; 2 = water-soaking, chlorosis, or necrosis (blight) in <25% of the inoculated area; 3 = 25 to 50% blight; and 4 = >50% blight.

RFLP analysis. Approximately 2 μg of total genomic DNA was digested with *Eco*RI as specified by the manufacturer (Life Technologies, Rockville, MD). Digested DNA was fractionated in 0.7% agarose gels in Tris-Borate-EDTA (TBE) buffer at 35 V for 20 h and stained with ethidium bromide. Southern blotting was performed as previously described (11,35). Recombinant plasmids P2 and P7, which contain repetitive sequences cloned from the *X. campestris* pv. *phaseoli* genome (11,12), were labeled with [α-^{32}P]dCTP by nick translation (Life Technologies) and used as probes. Southern blot hybridization analysis was performed under low-stringency conditions as previously described (11,35). Membranes were air dried and exposed to Fuji Rx NIF X-ray film.

Determination of the common bean gene pool. To determine the gene pool of the bean plants from which common blight bacteria were isolated, total genomic DNA was extracted from ≈10 mg of dried leaf tissue. After grinding dried tissue in liquid nitrogen, DNA was extracted using the Dellaporta method (7), except that an additional chloroform:isoamyl alcohol extraction was performed before DNA was precipitated. Total genomic DNA was used in the PCR with the J_1d_1/J_1d_2 primer pair, which directs the amplification of an ≈1.6-kb DNA fragment from Andean beans and an ≈1.3-kb fragment from Middle American beans

TABLE 2. Additional strains of *Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* used in this study

Strain	Identity	Geographical origin	Source
CNF30[x]	*X. campestris* pv. *phaseoli*	Brazil	11
CNF27[y]	*X. campestris* pv. *phaseoli*	Brazil	11
ES5[y]	*X. campestris* pv. *phaseoli*	Brazil	11
XP15[x]	*X. campestris* pv. *phaseoli*	Brazil	11
147[x]	*X. campestris* pv. *phaseoli*	Brazil	M. A. Pastor-Corrales (CIAT)
151[x]	*X. campestris* pv. *phaseoli*	Brazil	M. A. Pastor-Corrales (CIAT)
153[x]	*X. campestris* pv. *phaseoli*	Brazil	M. A. Pastor-Corrales (CIAT)
X50[x]	*X. campestris* pv. *phaseoli*	Colombia	11
123[x]	*X. campestris* pv. *phaseoli*	Colombia	M. A. Pastor-Corrales (CIAT)
321[x]	*X. campestris* pv. *phaseoli*	Colombia	M. A. Pastor-Corrales (CIAT)
258[x]	*X. campestris* pv. *phaseoli*	Dominican Republic	M. A. Pastor-Corrales (CIAT)
259[x]	*X. campestris* pv. *phaseoli*	Dominican Republic	M. A. Pastor-Corrales (CIAT)
260[x]	*X. campestris* pv. *phaseoli*	Dominican Republic	M. A. Pastor-Corrales (CIAT)
X21[x]	*X. campestris* pv. *phaseoli*	Kenya	R. B. Mabagala
52[x]	*X. campestris* pv. *phaseoli*	Mexico	M. A. Pastor-Corrales (CIAT)
55[x]	*X. campestris* pv. *phaseoli*	Mexico	M. A. Pastor-Corrales (CIAT)
P1[y]	*X. campestris* pv. *phaseoli*	Puerto Rico	R. L. Gilbertson
S659[x]	*X. campestris* pv. *phaseoli*	Spain	R. Lopez Perez
X17[x]	*X. campestris* pv. *phaseoli*	Uganda	R. B. Mabagala
X47[x]	*X. campestris* pv. *phaseoli*	Uganda	11
X45[x]	*X. campestris* pv. *phaseoli*	Florida, USA	11
193[x]	*X. campestris* pv. *phaseoli*	USA	M. A. Pastor-Corrales (CIAT)
332F[x]	*X. campestris* pv. *phaseoli* var. *fuscans*	Brazil	M. A. Pastor-Corrales (CIAT)
91F[x]	*X. campestris* pv. *phaseoli* var. *fuscans*	Colombia	M. A. Pastor-Corrales (CIAT)
110F[x]	*X. campestris* pv. *phaseoli* var. *fuscans*	Colombia	M. A. Pastor-Corrales (CIAT)
XCPFG4[x]	*X. campestris* pv. *phaseoli* var. *fuscans*	Guatemala	11
X25[x]	*X. campestris* pv. *phaseoli* var. *fuscans*	Kenya	R. B. Mabagala
116F[x]	*X. campestris* pv. *phaseoli* var. *fuscans*	Puerto Rico	M. A. Pastor-Corrales (CIAT)
P2F[y]	*X. campestris* pv. *phaseoli* var. *fuscans*	Puerto Rico	R. L. Gilbertson
SITA920[x]	*X. campestris* pv. *phaseoli* var. *fuscans*	Spain	R. Lopez Perez
T123F[z]	*X. campestris* pv. *phaseoli* var. *fuscans*	Tanzania	This study (collected 2002)
MIF1[y]	*X. campestris* pv. *phaseoli* var. *fuscans*	Michigan, USA	11
XCPFN[x]	*X. campestris* pv. *phaseoli* var. *fuscans*	Nebraska, USA	11
WF8[y]	*X. campestris* pv. *phaseoli* var. *fuscans*	Wisconsin, USA	11

[x] These strains were previously identified as *X. campestris* pv. *phaseoli* or *X. campestris* pv. *phaseoli* var. *fuscans*, and total genomic DNA extracted. This DNA was used in repetitive polymerase chain reaction (rep-PCR), Southern blot hybridization, or both.

[y] These strains were used in rep-PCR analyses and in pathogenicity tests.

[z] This strain was isolated and characterized in the present study. Total genomic DNA was extracted and used in Southern blot hybridization analysis.

(17). The PCR profile used was 1 cycle of 94°C for 2 min; 40 cycles of 94°C for 30 s, 58°C for 1 min, and 72°C for 2 min; followed by an extension cycle of 72°C for 5 min. PCR products were fractionated in 1.0% agarose gels in TBE buffer, stained with ethidium bromide, and examined with a gel imaging system (model IS-1000; Alpha Innotech Corporation, San Leandro, CA).

RESULTS

Collection of leaf and seed samples and isolation of common blight bacteria. Three surveys were conducted in East Africa (Malawi and Tanzania) to obtain samples for isolation of common blight bacteria. The incidence of CBB was low in some fields surveyed in south-central Tanzania, and seed samples were collected from local markets and assayed for common blight bacteria. Common blight bacteria were isolated from leaves on 523 medium and classified as xanthomonad-like based on having a yellow, convex, mucoid colony morphology.

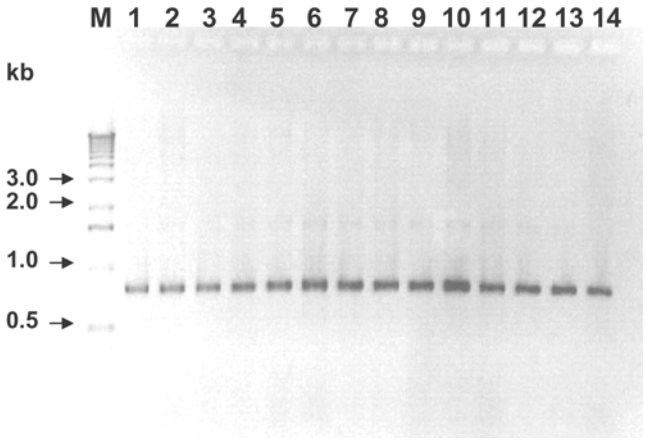

Fig. 1. Agarose gel showing polymerase chain reaction products amplified from total genomic DNA of strains of *Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* with the X4c/X4e primer pair. Lane M: size markers (1-kb ladder, Gibco-BRL). Lane 1: M11 (Malawi *X. campestris* pv. *phaseoli*); lane 2: M12F (Malawi *X. campestris* pv. *phaseoli* var. *fuscans*); lanes 3 to 6: M19, M20, M25, and M30 (Malawi *X. campestris* pv. *phaseoli*); lane 7: T14 (Tanzania *X. campestris* pv. *phaseoli*); lanes 8 to 10: T17F, T19F, and T20F (Tanzania *X. campestris* pv. *phaseoli* var. *fuscans*); lanes 11 and 12: T21 and T8-3 (Tanzania *X. campestris* pv. *phaseoli*); lane 13: P1 (Puerto Rico *X. campestris* pv. *phaseoli*); and lane 14: P2F (Puerto Rico *X. campestris* pv. *phaseoli* var. *fuscans*).

The first survey was conducted in Malawi and Tanzania in 1997. Twenty-one xanthomonad-like strains were isolated from bean leaves from seven fields in five locations in north-central Malawi (strains with 'M' designation in Table 1), and two additional strains were isolated from south-central Tanzania: one from a leaf sample (strain T22F) and another from a seed sample (strain T21F). The second survey (Tanzania I) was conducted in south-central Tanzania in 1998, and eight strains (T3F, T14, T17F, T19F, T20F, T21, T23F, and T32F) (Table 1) were isolated from bean leaves from a number of fields of cv. Rose Koko beans. The third survey (Tanzania II) was conducted in south-central and northern Tanzania in 2000, and 46 strains (remainder of the strains with 'T' designation in Table 1) were isolated from bean leaves from eight fields in different geographical locations. A fourth survey was conducted in north-central Wisconsin in 2002, and 20 strains (strains with 'W' designation in Table 1) were isolated from bean leaves from five fields of dark red kidney beans.

Identification of common blight bacteria. Xanthomonad-like bacteria were subcultured on 523 medium, and strains that produced a brown pigment were tentatively identified as *X. campestris* pv. *phaseoli* var. *fuscans*. Further identification of *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains was based on amplification of the ≈700-bp DNA fragment with the X4c/X4e primer pair (Fig. 1) and pathogenicity on common bean. All brown-pigmented xanthomonad-like bacteria were confirmed as *X. campestris* pv. *phaseoli* var. *fuscans* strains based on being PCR positive (i.e., the ≈700-bp fragment was amplified) and pathogenic on common bean (Table 3). Strains that did not produce a brown pigment were either *X. campestris* pv. *phaseoli* or nonpathogenic xanthomonads. The *X. campestris* pv. *phaseoli* strains were PCR positive and pathogenic on common bean, whereas the nonpathogenic xanthomonads were PCR negative and did not induce symptoms on common bean (Table 3). Together, the results of these tests (i) allowed for the identification of *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains and (ii) provided further evidence that PCR with the X4c/X4e primer pair is an accurate means of detecting common blight bacteria and differentiating them from nonpathogenic xanthomonads that can be associated with common bean (2, 11,13).

Of the 23 strains collected in the first survey (21 from Malawi and 2 from Tanzania), 11 were *X. campestris* pv. *phaseoli*, 9 were *X. campestris* pv. *phaseoli* var. *fuscans* (including T21F and T22F from Tanzania), and 3 were nonpathogenic xanthomonads. Of the 54 strains collected from Tanzania in the Tanzania I and II sur-

TABLE 3. Characterization of xanthomonad-like strains isolated from common bacterial blight samples collected during surveys of East Africa and Wisconsin

Survey, strains[x]	Number	PCR detection/pathogenicity[y]	Common bean gene pool[z]		
			Andean	Mesoamerican	Not determined
Malawi, Tanzania					
X. campestris pv. *phaseoli*	11	11/11	7	1	3
X. campestris pv. *phaseoli* var. *fuscans*	9	9/9	2	7	0
Xanthomonas spp.	3	0/3	3	0	0
Tanzania I					
X. campestris pv. *phaseoli*	2	2/2	2	0	0
X. campestris pv. *phaseoli* var. *fuscans*	6	6/6	6	0	0
Tanzania II					
X. campestris pv. *phaseoli*	4	4/4	4	0	0
X. campestris pv. *phaseoli* var. *fuscans*	35	35/35	21	14	0
Xanthomonas spp.	7	0/7	3	4	0
Wisconsin					
X. campestris pv. *phaseoli*	20	20/20	20	0	0

[x] Strains initially were identified based on a yellow mucoid colony morphology. *Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas* spp. had a yellow mucoid colony morphology, whereas *X. campestris* pv. *phaseoli* var. *fuscans* had a yellow mucoid colony morphology and produced a brown pigment.

[y] Polymerase chain reaction (PCR) detection and pathogenicity. PCR detection was based upon amplification of an ≈700 bp DNA fragment with the X4c/X4e primer pair. Pathogenicity on common bean was determined by inoculating strains onto trifoliolate leaves of 14-day-old plants (cv. Topcrop) with the razor blade method (29). Note that all strains detected by PCR and this primer pair were pathogenic on common bean, whereas strains that were not detected by PCR were not pathogenic.

[z] The gene pool of the common bean plant or seed from which each strain was isolated was determined by PCR with the J_1d_1/J_1d_2 primer pair.

veys, 41 were *X. campestris* pv. *phaseoli* var. *fuscans*, 6 were *X. campestris* pv. *phaseoli,* and 7 were nonpathogenic xanthomonads. All 20 strains collected from red kidney beans in the Wisconsin survey were *X. campestris* pv. *phaseoli* (Table 3).

rep-PCR analysis. Two distinct rep-PCR fingerprints were generated (with all three rep-PCR primers) for *X. campestris* pv. *phaseoli* strains collected in Malawi in 1997, whereas a third fingerprint was generated for a New World *X. campestris* pv. *phaseoli* strain from Puerto Rico (Table 2, strain P1) (Fig. 2 and data not shown). These results indicated that *X. campestris* pv. *phaseoli* is composed of at least three distinct genotypes. Six *X. campestris* pv. *phaseoli* strains were collected from Tanzania in the Tanzania I and II surveys. Three strains (T14, T21, and T/Market 8) had rep-PCR fingerprints identical to one of the two fingerprints of the Malawi *X. campestris* pv. *phaseoli* strains, whereas the other three strains (T8-2, T8-3, and T8-4; collected from a single field in Arumeru in 2000) had fingerprints identical to that of the P1 *X. campestris* pv. *phaseoli* strain (Fig. 3 and data not shown). The 20 *X. campestris* pv. *phaseoli* strains from dark red kidney beans in Wisconsin had rep-PCR fingerprints identical to that of the P1 *X. campestris* pv. *phaseoli* strain.

To determine if the fingerprint of the P1 *X. campestris* pv. *phaseoli* strain was representative of *X. campestris* pv. *phaseoli* strains from other geographical locations, rep-PCR analysis was performed on total genomic DNA of *X. campestris* pv. *phaseoli* strains from various New and Old World locations (Table 2). *X. campestris* pv. *phaseoli* strains from Brazil (seven strains), Colombia (three strains), the Dominican Republic (three strains), Mexico (two strains), Spain (one strain), and the United States (two strains) had rep-PCR fingerprints similar to or indistinguishable from that of the P1 *X. campestris* pv. *phaseoli* strain (Fig. 3 and data not shown). In contrast, strains from Kenya (one strain) and Uganda (two strains) had fingerprints identical to one of the two fingerprints of the Malawi *X. campestris* pv. *phaseoli* strains (Fig. 3 and data not shown). Together, these results indicate that *X. campestris* pv. *phaseoli* is a heterogeneous pathovar composed of three genetically distinct genotypes: two found in East Africa (East African genotypes 1 and 2) and another representing strains from all other locations, including Tanzania.

A different situation was found for *X. campestris* pv. *phaseoli* var. *fuscans* strains. First, the fingerprint for *X. campestris* pv. *phaseoli* var. *fuscans* strains was considerably different from those of the *X. campestris* pv. *phaseoli* strains (all three genotypes) (Fig. 3), which is consistent with *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* being genetically distinct. All *X. campestris* pv. *phaseoli* var. *fuscans* strains from Malawi and Tanzania had rep-PCR fingerprints that were identical or nearly identical to each other (Fig. 4 and data not shown). Furthermore, the fingerprints of these East African *X. campestris* pv. *phaseoli* var. *fuscans* strains also were identical or nearly

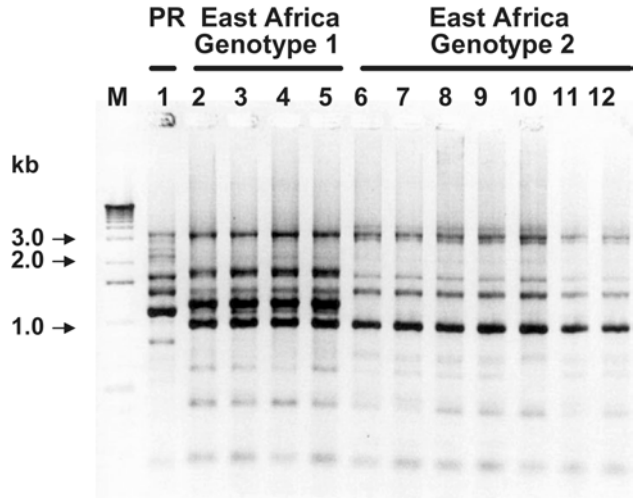

Fig. 2. Agarose gel showing polymerase chain reaction fingerprint patterns generated from total genomic DNA of *Xanthomonas campestris* pv. *phaseoli* strains from East Africa and Puerto Rico with repetitive extragenic palindromic primers. Lane M: size markers (1-kb ladder, Gibco-BRL). Lane 1: P1 (Puerto Rico); lanes 2 to 5: East African genotype 1: M11, M19, M20 (Malawi), and T14 (Tanzania); lanes 6 to 12: East African genotype 2: M17, M18, M24, M25, M29, M30 (Malawi), and T21 (Tanzania).

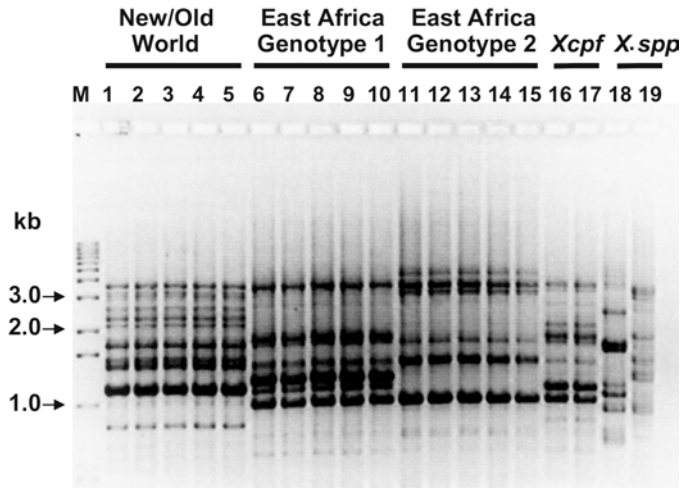

Fig. 3. Agarose gel showing polymerase chain reaction fingerprint patterns generated from total genomic DNA of *Xanthomonas campestris* pv. *phaseoli*, X. *campestris* pv. *phaseoli* var. *fuscans* (*Xcpf*), and nonpathogenic *Xanthomonas* (*X.* spp.) strains with repetitive extragenic palindromic primers. Lane M: size markers (1-kb ladder, Gibco-BRL). Lanes 1 to 5: *X. campestris* pv. *phaseoli* strains: P1 (Puerto Rico), CNF27 (Brazil), 123 (Colombia), 193 (United States), and T8-2 (Tanzania); lanes 6 to 10: East African genotype 1: M11, M19, and M20 (Malawi), T14 (Tanzania), and X17 (Uganda); lanes 11 to 15: East African genotype 2: M17 and M18 (Malawi), X21 (Kenya), and T21 and T/Market 8 (Tanzania); lanes 16 to 17: *X. campestris* pv. *phaseoli* var. *fuscans* strains: M12F and M13F (Malawi); and lanes 18 and 19: *Xanthomonas* spp.: T3-3 and T3-4.

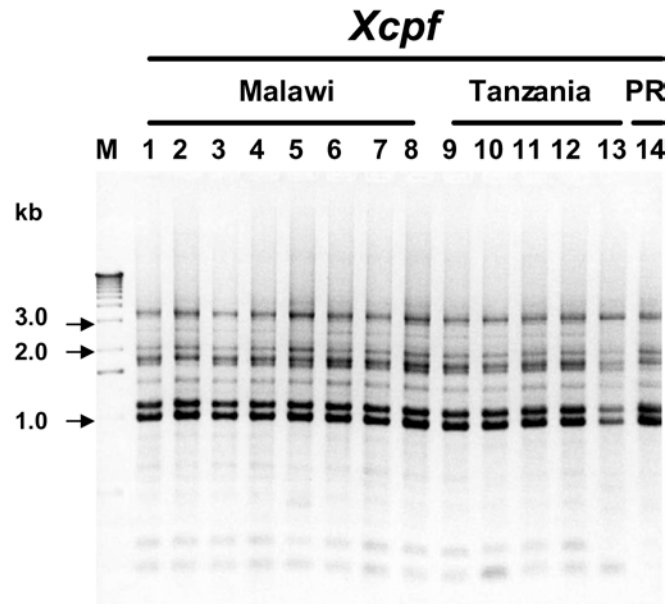

Fig. 4. Agarose gel showing polymerase chain reaction fingerprint patterns generated from total genomic DNA of *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* (*Xcpf*) strains from East Africa and Puerto Rico with repetitive extragenic palindromic primers. Lane M: size markers (1-kb ladder, Gibco-BRL). Lanes 1 to 4 and 7 to 8: M12F, M13F, M14F, M16F, M26F, and M31F from Malawi; lanes 5 to 6 and 9 to 13: T21F, T22F, T17F, T19F, T20F, T23F, and T32F from Tanzania; and lane 14: P2F from Puerto Rico (PR).

identical to fingerprints of *X. campestris* pv. *phaseoli* var. *fuscans* strains from Brazil (one strain), Colombia (two strains), Guatemala (one strain), Kenya (one strain), Puerto Rico (one strain), Spain (one strain), and the United States (three strains) (Fig. 4 and data not shown). Thus, in contrast to *X. campestris* pv. *phaseoli*, *X. campestris* pv. *phaseoli* var. *fuscans* strains from East Africa and the New World composed a genetically homogeneous group.

Analysis of selected nonpathogenic xanthomonads by rep-PCR revealed distinct fingerprints for each strain, and these fingerprints were distinct from those of *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains (Fig. 3 and data not shown). Thus, these results supported those of pathogenicity tests and PCR, indicating that these strains are genetically distinct nonpathogenic xanthomonad-like bacteria.

RFLP analysis. Strains representing the genetic and geographic diversity detected among common blight bacteria by rep-PCR analysis were selected for Southern blot hybridization analysis with the repetitive DNA probes P2 and P7. The P2 probe hybridized to multiple (>10) *Eco*RI fragments of the *X. campestris* pv. *phaseoli* strains from the New World, Spain (strain S659), and Tanzania (T8-3, one of the strains with the New World-type fingerprint), and different hybridization patterns were observed among these strains (Fig. 5). In contrast, this probe hybridized to only a single similar-sized fragment from strains of the two East African *X. campestris* pv. *phaseoli* genotypes (Fig. 5). This was consistent with results of rep-PCR analyses showing genetic diversity between the East African *X. campestris* pv. *phaseoli* genotypes and *X. campestris* pv. *phaseoli* strains from other (mostly New World) locations. The P2 probe did not reveal genetic diversity among strains of the East African genotypes.

The P7 probe also hybridized with multiple DNA fragments of the *X. campestris* pv. *phaseoli* strains from the New World, Spain, and Tanzania (strain T8-3). Some hybridizing fragments were shared among strains, but most were polymorphic, revealing genetic diversity among these *X. campestris* pv. *phaseoli* strains (Fig. 6). The P7 probe hybridized to fewer fragments in the East African genotypes, thereby differentiating these genotypes from other *X. campestris* pv. *phaseoli* strains. This probe also differentiated strains of the two East African genotypes (Fig. 6).

The P2 probe hybridized to multiple DNA fragments (>10) in all *X. campestris* pv. *phaseoli* var. *fuscans* strains, and the hybridization pattern clearly was distinct from those of the *X. campestris* pv. *phaseoli* strains (Fig. 5). Five fragments were shared among all *X. campestris* pv. *phaseoli* var. *fuscans* strains, whereas the others were polymorphic. This indicates genetic diversity among *X. campestris* pv. *phaseoli* var. *fuscans* strains. However, both the rep-PCR and RFLP analyses did not reveal genetically distinct East African *X. campestris* pv. *phaseoli* var. *fuscans* strains. Similar results were obtained for the P7 probe, although it hybridized with considerably fewer *X. campestris* pv. *phaseoli* var. *fuscans* DNA fragments compared with the P2 probe (Fig. 6).

Interaction of *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains with the common bean host. Two approaches were used to analyze the interaction of common blight bacteria and the common bean plant. First, the gene pool of the bean plant from which each strain was isolated was determined using PCR and the J_1d_1/J_1d_2 primer pair, which direct the amplification of different-sized DNA fragments from Andean and Middle American beans. The results are presented in Tables 1 and 3. *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains from Malawi were isolated predominantly from Andean and Middle American beans, respectively (Table 3). In contrast, the majority of common blight bacteria from Tanzania were *X. campestris* pv. *phaseoli* var. *fuscans* (i.e., 43 of 49 strains), and these were isolated from beans of both gene pools. All six strains of *X. campestris* pv. *phaseoli* from Tanzania were isolated from Andean beans, and only *X. campestris* pv. *phaseoli* was isolated from the Andean red kidney bean plants in Wisconsin (Table 3). Finally, the P1 *X. campestris* pv. *phaseoli* and P2F *X. campestris* pv. *phaseoli* var. *fuscans* strains from Puerto Rico were isolated from Andean and Middle American beans, respectively. Taken together, these results indicate that *X. campestris* pv. *phaseoli* was associated predominantly with Andean beans (note that only a single *X. campestris* pv. *phaseoli* strain, M30 from

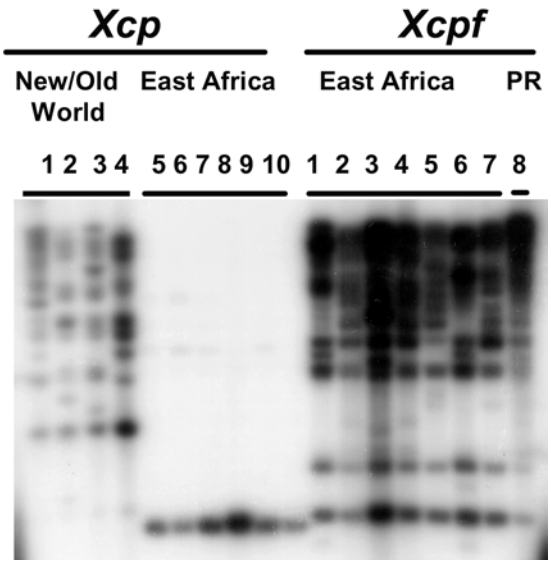

Fig. 5. Southern blot hybridization analysis of *Eco*RI-digested total genomic DNA of strains of *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) and *X. campestris* pv. *phaseoli* var. *fuscans* (*Xcpf*) with a repetitive element probe (P2). *X. campestris* pv. *phaseoli* strains: lanes 1 to 4: P1 (Puerto Rico), S659 (Spain), W01 (United States), and T8-3 (Tanzania); lanes 5 to 7: East African genotype 1: M11 and M19 (Malawi) and T14 (Tanzania); lanes 8 to 10: East African genotype 2: M17 and M30 (Malawi) and T21 (Tanzania); *X. campestris* pv. *phaseoli* var. *fuscans* strains: lanes 1 to 8: M12F and M13F (Malawi), T22F (Tanzania), M31 (Malawi), T17F, T20F, and T123F (Tanzania), and P2F (Puerto Rico, PR).

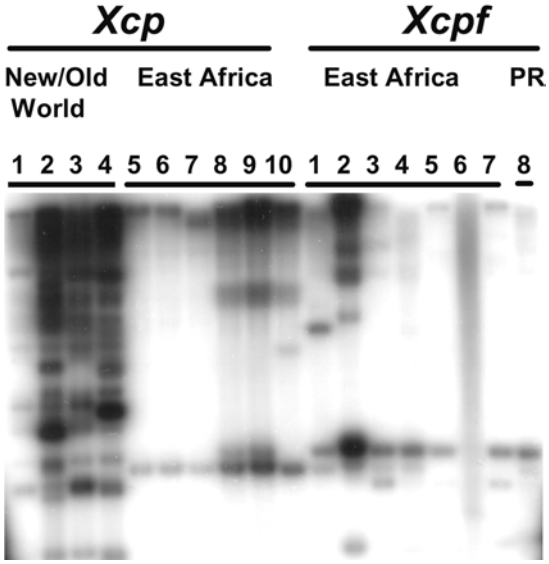

Fig. 6. Southern blot hybridization analysis of *Eco*RI-digested total genomic DNA of strains of *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) and *X. campestris* pv. *phaseoli* var. *fuscans* (*Xcpf*) with a repetitive element probe (P7). *X. campestris* pv. *phaseoli* strains: lanes 1 to 4: P1 (Puerto Rico), S659 (Spain), W01 (United States), and T8-3 (Tanzania); lanes 5 to 7: East African genotype 1: M11 and M19 (Malawi) and T14 (Tanzania); lanes 8 to 10: East African genotype 2: M17 and M30 (Malawi) and T21 (Tanzania); *X. campestris* pv. *phaseoli* var. *fuscans* strains: lanes 1 to 8: M12F and M13F (Malawi), T22F (Tanzania), M31F (Malawi), T17F, T20F, and T123F (Tanzania), and P2F (Puerto Rico, PR).

Malawi, was isolated from Middle American beans). *X. campestris* pv. *phaseoli* var. *fuscans* was the predominant common blight bacterium isolated from Middle American beans, but it also was commonly isolated from Andean beans in Tanzania (Tables 1 and 3).

Pathogenicity tests. The host–pathogen interaction was investigated by inoculating *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains, representing the genetic diversity revealed by rep-PCR and RFLP analyses, onto a series of Andean and Middle American beans. Strain M11 was an East African genotype 1 *X. campestris* pv. *phaseoli* strain, P1 was a New World *X. campestris* pv. *phaseoli* strain, M12F was an East African *X. campestris* pv. *phaseoli* var. *fuscans* strain, and P2F was a New World *X. campestris* pv. *phaseoli* var. *fuscans* strain. Overall, the New World *X. campestris* pv. *phaseoli* strain (P1) was significantly more pathogenic (mean disease rating [MDR] of 3.8 on a 4-point scale) than the East African *X. campestris* pv. *phaseoli* strain (M11) (MDR of 2.9) (Table 4). The New World *X. campestris* pv. *phaseoli* strain was highly pathogenic on Andean and Middle American beans (MDR of 3.8); whereas the East African *X. campestris* pv. *phaseoli* strain was highly pathogenic on Andean beans (MDR of 3.4), but was significantly less pathogenic on Middle American beans (MDR of 2.4). Moreover, the East African *X. campestris* pv. *phaseoli* strain was more pathogenic on Middle American beans from East Africa (MDR of 2.9) than on the two Middle American cultivars from the United States (MDR of 1.6) (Table 4).

In a similar experiment, replicated five times, the pathogenicity of strains M11 and M17, representing the East African *X. campestris* pv. *phaseoli* genotypes 1 and 2, respectively, and two East African *X. campestris* pv. *phaseoli* var. *fuscans* strains (M13F and T22F) were compared with two New World *X. campestris* pv. *phaseoli* strains from Brazil (CNF27 and ES5) and two *X. campestris* pv. *phaseoli* var. *fuscans* strains from the United States (WF8 and MIF1). Both East African *X. campestris* pv. *phaseoli* strains were significantly more pathogenic on Andean beans (MDR of 3.1 for M11 and 3.2 for M17) than on Middle American beans (MDR of 1.7 for M11 and 2.6 for M17). Furthermore, strain M17 (genotype 2) was significantly more pathogenic than M11 (genotype 1) on Middle American beans. In contrast, the two New World *X. campestris* pv. *phaseoli* strains from Brazil were highly pathogenic on Andean and Middle American beans (MDR of 4.0 for CNF27 and 3.9 for ES5).

Strains of *X. campestris* pv. *phaseoli* var. *fuscans* from East Africa (M12F) and the New World (P2F) were highly pathogenic on Andean and Middle American beans, with overall MDRs of 3.8 and 4.0, respectively (Table 4). Similar results were obtained for the two *X. campestris* pv. *phaseoli* var. *fuscans* strains from the Unites States (MDRs of 3.9 for WF8 and MIF1 on Andean and Middle American beans), and for two additional *X. campestris* pv. *phaseoli* var. *fuscans* strains from East Africa (MDRs of 3.9 for M13F and T22F on Andean and Middle American beans).

Together, these results indicate that differences in pathogenicity exist between *X. campestris* pv. *phaseoli* strains representing the East African genotypes and those representing the other (New World) genotype, which is consistent with the genetic diversity detected among these genotypes. No differences in pathogenicity were detected among East African and New World *X. campestris* pv. *phaseoli* var. *fuscans* strains, consistent with the lack of genetic diversity detected among these strains. *X. campestris* pv. *phaseoli* var. *fuscans* strains were significantly more pathogenic than the East African *X. campestris* pv. *phaseoli* strains, but not the New World *X. campestris* pv. *phaseoli* strains (Table 4).

DISCUSSION

Genetic variability of *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains. It long has been

TABLE 4. Mean disease ratings for East African and New World strains of *Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* inoculated onto Andean and Middle American common bean (*Phaseolus vulgaris*) genotypes

	Bacterial strains and pathogenicity tests[x]				
	X. campestris pv. *phaseoli*		*X. campestris* pv. *phaseoli* var. *fuscans*		
Bean gene pool, cultivar, line	M11	P1	M12F	P2F	Overall mean[y]
Andean					
U.S. genotypes					
White Kidney	3.8	3.8	3.3	3.6	3.6 a
Topcrop	3.3	3.9	3.9	3.7	3.7 a
Mean	3.7	3.9	3.6	3.7	3.7
East African genotypes					
Rose Koko	3.5	4.0	3.7	3.7	3.7 a
22-2	3.0	3.8	3.9	3.9	3.6 a
12-4	3.5	3.6	3.3	3.7	3.6 a
Mean	3.3	3.8	3.6	3.4	3.6
Overall Andean mean	3.4	3.8	3.6	3.7	3.6
Middle American					
U.S. genotypes					
Sutter Pink	1.5	3.7	3.6	3.8	3.1 b
Black Turtle Soup	1.7	3.7	3.5	3.7	3.2 b
Mean	1.6	3.7	3.6	3.8	3.2
East African genotypes					
Namajengo	2.9	4.0	4.0	3.5	3.6 a
6-5	2.6	3.9	3.5	3.6	3.4 ab
1-1	3.1	3.5	3.7	3.7	3.5 ab
Mean	2.8	3.8	3.7	3.6	3.5
Overall Middle American mean	2.4	3.8	3.7	3.7	3.4
Overall mean[z]	2.9 b	3.8 a	3.6 a	3.7 a	3.5

[x] Plants were inoculated with an aqueous suspension (1×10^8 CFU/ml) of *X. campestris* pv. *phaseoli* or *X. campestris* pv. *phaseoli* var. *fuscans* strains with the razor blade method. Rating scale: 1 = no visual symptoms or slight marginal necrosis; 2 = water-soaking, chlorosis, or necrosis (blight) in <25% of the inoculated area; 3 = 25 to 50% blight; and 4 = >50% blight. Negative controls consisted of plants inoculated with distilled water and received rating of 1. Numbers represent means of five replicates in each of three independent experiments. Coefficient of variation (%) = 13.1, S_y strains = 0.084, S_y genotypes = 0.133, and S_y strains × genotypes = 0.265.

[y] Means followed by the same letter are not significantly ($P < 0.05$) different between bean cultivars by Duncan's multiple range test.

[z] Means followed by the same letter are not significantly ($P < 0.01$) different between *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains by Duncan's multiple range test.

known that the causal agent of CBB disease exists in two forms, yellow-pigmented *X. campestris* pv. *phaseoli* and the melanin-like (brown)-pigmented *X. campestris* pv. *phaseoli* var. *fuscans*. Furthermore, it has been established that these two forms are genetically distinct (5,6,11,12,20), although the biological and taxonomic significance of this variability is not well understood. The development of molecular tools for genetic fingerprinting of bacteria (e.g., rep-PCR and RFLP analyses) has allowed for the examination of genetic diversity among *X. campestris* pathovars and strains within pathovars. These studies have revealed that pathovars of *X. campestris* are genetically distinct (20,21), but that some pathovars are heterogeneous (i.e., composed of distinct genotypes). For example, *X. campestris* pv. *vesicatoria* is composed of two distinct types (43), whereas *X. campestris* pv. *hederae* is composed of a number of distinct genotypes (25,44).

In this study, genetic fingerprinting tools were used to examine variability among common blight bacteria, and an attempt was made to correlate variability with biological properties (e.g., pathogenicity). Results of surveys conducted in Malawi and Tanzania revealed that both *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* were associated with CBB, but that *X. campestris* pv. *phaseoli* var. *fuscans* was more common in Tanzania. More extensive and systematic sampling could establish whether both *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* predominate in Malawi, whereas *X. campestris* pv. *phaseoli* var. *fuscans* predominates in Tanzania. Nonetheless, the results of these surveys are consistent with those of Opio et al. (27), who found *X. campestris* pv. *phaseoli* var. *fuscans* (60%) and *X. campestris* pv. *phaseoli* (40%) associated with CBB in East Africa (primarily Uganda). *X. campestris* pv. *phaseoli* var. *fuscans* also commonly is associated with common blight in South Africa (D. Fourie, *personal communication*). Thus, it is clear that *X. campestris* pv. *phaseoli* var. *fuscans* is a major causal agent of CBB in East Africa.

Genetic fingerprinting of *X. campestris* pv. *phaseoli* strains revealed three distinct genotypes, two of which were specific for strains from East Africa (e.g., Kenya, Malawi, Tanzania, and Uganda). The third genotype included strains from the New World (e.g., Brazil, Colombia, the Dominican Republic, Mexico, Puerto Rico, and the United States), a strain from Spain, and three strains from a single field in Tanzania (T8-2, T8-3, and T8-4) (Table 1). Irrespective of genotype or geographical origin, *X. campestris* pv. *phaseoli* strains predominantly were isolated from Andean beans. The association between *X. campestris* pv. *phaseoli* and Andean beans was demonstrated further by the finding that the two East African *X. campestris* pv. *phaseoli* genotypes (represented by strains M11 and M17) were significantly more pathogenic on Andean than Middle American beans. The fact that this differential pathogenicity was not observed for the three New World *X. campestris* pv. *phaseoli* strains examined (P1, CNF27, and ES5) revealed a biological difference between the genetically distinct East African and New World *X. campestris* pv. *phaseoli* genotypes. It will be important to confirm these findings with a larger number of *X. campestris* pv. *phaseoli* strains from East Africa and other locations.

The finding of differential pathogenicity for the East African *X. campestris* pv. *phaseoli* genotypes on the two common bean gene pools, although obtained with a relatively small number of strains, supports the hypothesis that these strains have adapted to be pathogenic on Andean beans. This hypothesis also is supported by the predominance of Andean beans in East Africa, ever since the introduction of the common bean ≈400 years ago. The high level of pathogenicity of the New World *X. campestris* pv. *phaseoli* strains on Andean and Middle American beans likely reflects the cultivation of beans of both gene pools in many bean-growing regions of the New World and, thus, selection pressure to evolve pathogenicity on both types of beans.

The genetic diversity between East African *X. campestris* pv. *phaseoli* strains and those from other locations indicates geographical isolation of the East African *X. campestris* pv. *phaseoli* genotypes. These *X. campestris* pv. *phaseoli* genotypes may have evolved from a common ancestor, with the East African genotypes evolving independently on Andean beans after an initial introduction, possibly in association with seed. Alternatively, the East African *X. campestris* pv. *phaseoli* genotypes may represent indigenous xanthomonads that independently evolved to be pathogenic on Andean beans. The latter scenario may be favored by the finding of two distinct East African genotypes. The detection of *X. campestris* pv. *phaseoli* strains with fingerprints similar to those from New World locations from a single field in Tanzania probably reflects introduction of these strains with seed. It will be of interest to determine if these *X. campestris* pv. *phaseoli* strains become established in Tanzania and how well they compete with *X. campestris* pv. *phaseoli* var. *fuscans*.

Results of the surveys in Tanzania revealed that *X. campestris* pv. *phaseoli* var. *fuscans* strains were associated with Andean and Middle American beans. Consistent with this finding, *X. campestris* pv. *phaseoli* var. *fuscans* strains from East Africa, as well as those from the New World, were highly pathogenic on Andean and Middle American beans. Taken together with the finding of relatively little genetic diversity among the *X. campestris* pv. *phaseoli* var. *fuscans* strains examined in this study (i.e., no distinct East African *X. campestris* pv. *phaseoli* var. *fuscans* genotypes were identified), these results indicate that *X. campestris* pv. *phaseoli* var. *fuscans* is more homogeneous than *X. campestris* pv. *phaseoli*. It is possible that *X. campestris* pv. *phaseoli* var. *fuscans* was more recently introduced into East Africa, perhaps via seed of Middle American beans, and that the prevalence of *X. campestris* pv. *phaseoli* var. *fuscans* in Tanzania indicates displacement of East African *X. campestris* pv. *phaseoli* genotypes by the more pathogenic *X. campestris* pv. *phaseoli* var. *fuscans*.

Genetic diversity among *X. campestris* pathovars (20) and strains within pathovars (4,6,12) can be revealed by RFLP analyses. The results of RFLP analysis of common blight bacteria with repetitive DNA probes generally supported results of rep-PCR analyses. First, *X. campestris* pv. *phaseoli* var. *fuscans* and *X. campestris* pv. *phaseoli* strains were readily distinguished by both the P2 and P7 probes, consistent with previous results (11,12,20). Second, both the P2 and P7 probes readily differentiated the East African *X. campestris* pv. *phaseoli* genotypes from other (mostly New World) *X. campestris* pv. *phaseoli* strains, with considerably more hybridization (i.e., copies of the repetitive elements) detected in the New World genotype (>10 hybridizing fragments) compared with East African genotypes (1 to 2 hybridizing fragments). Finally, the P7 probe also differentiated strains of the two East African *X. campestris* pv. *phaseoli* genotypes. The low copy number of these repetitive elements in the East African *X. campestris* pv. *phaseoli* genotypes provides additional evidence that these strains are genetically distinct from the New World *X. campestris* pv. *phaseoli* strains, and supports the concept of geographical isolation of the East African *X. campestris* pv. *phaseoli* genotypes. The difference in the copy number of these repetitive elements in these *X. campestris* pv. *phaseoli* genotypes may reflect different selection pressures on these bacteria. However, the significance of these elements, in terms of the biological properties of common blight bacteria, is not known. In contrast to rep-PCR results, the RFLP analysis revealed genetic diversity among *X. campestris* pv. *phaseoli* var. *fuscans* strains, indicating that *X. campestris* pv. *phaseoli* var. *fuscans* strains are not clonal.

Bean gene pool (host) and pathogen relationship. Genetic diversity in the common bean exists on a number of levels. The most significant level reflects the two major domestication sites of this crop: Mesoamerica or Middle America (e.g., Colombia, Mexico, and countries of Central America) and Andean South America (e.g., Peru, Bolivia, and northern Argentina). This led to

the emergence of the two major common bean gene pools: Andean and Middle American (9). Andean and Middle American beans can be differentiated based upon morphological and molecular characteristics, as well as partial reproductive isolation (8,9,41).

It now has been established that genetic diversity within populations of certain fungal pathogens of common bean (e.g., *Phaeoisariopsis griseola*, causal agent of angular leafspot [15, 29]; *Colletotrichum lindemuthianum*, causal agent of anthracnose [3,39]; and *Uromyces appendiculatus*, causal agent of bean rust [36]) may parallel genetic diversity in the common bean host, particularly that between the two major common bean gene pools. In these cases, genetically distinct pathogen genotypes are associated with the two common bean gene pools and, in general, pathogen genotypes associated with a particular gene pool tend to be more pathogenic on beans of that gene pool than on beans of the other gene pool (i.e., isolates from Andean beans are more pathogenic on Andean than Middle American beans and vice versa). Conversely, resistance to a pathogen genotype may be found in germ plasm of the common bean gene pool that this genotype is not associated with (i.e., resistance to isolates from Andean beans may be found in Middle American germ plasm and vice versa). This has led to the concept that these pathogens have coevolved with the common bean host toward an increased level of pathogenicity. Thus, our results with the East African *X. campestris* pv. *phaseoli* genotypes provide an example of a prokaryote pathogen that has coevolved (coadapted) with the common bean host in a similar manner (Table 4).

Although our results indicate that Middle American beans have some resistance to CBB induced by East African *X. campestris* pv. *phaseoli* genotypes, they also support previous reports that high levels of CBB resistance are not found in common bean (42). Our results also demonstrate the importance of identifying the type or types of common blight bacteria prevalent in a region in order to select appropriate strains for breeding for resistance. In the case of East Africa, breeding programs should include *X. campestris* pv. *phaseoli* var. *fuscans* as well as New World *X. campestris* pv. *phaseoli* strains in order to assure identification and incorporation of resistance to the most pathogenic common blight bacteria found in that region. To this end, the VAX lines, which combine various sources of CBB resistance (42), showed high levels of resistance to New World *X. campestris* pv. *phaseoli* and East African *X. campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans* strains (*unpublished data*). Thus, these lines offer promising sources of resistance for CBB breeding programs in East Africa and other regions.

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