A Polymerase Chain Reaction Assay for the Detection of *Xanthomonas campestris* pv. *musacearum* in Banana

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ABSTRACT

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Polymerase chain reaction (PCR) primers (BXW-1 and BXW-3) for conventional PCR were developed from conserved sequences in the *hrpB* operon of the *hrp* gene cluster from *Xanthomonas campestris* pv. *musacearum*, the causative agent of banana Xanthomonas wilt (BXW). All 50 strains of *X. campestris* pv. *musacearum*, isolated from Uganda, Rwanda, and Tanzania, produced a 214-bp amplicon when whole cells, bacterial ooze from infected tissue, and genomic DNA purified from bacterial ooze or infected tissue were used as template. The BXW primers also detected strains of *X. axonopodis* pv. *vasculorum* isolated from sugarcane and maize and strains of *X. vasicola* pv. *holcicola* isolated from sorghum. All of the strains of *X. campestris* pv. *musacearum* were clonal when compared using enterobacterial repetitive intergenic consensus PCR.

Banana Xanthomonas wilt (BXW), also known as banana bacterial wilt (BBW) (57), is a devastating disease of banana (*Musa* spp.) and enset (*Ensete ventricosum*) in many African countries, including Ethiopia (57,58), Democratic Republic of Congo (33,34), Uganda (54,55), Rwanda (42), Tanzania (32), Kenya (31), and Burundi (49). The disease is caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (15,60), formerly known as *X. musacearum* (57) and proposed as *X. vasicola* pv. *musacearum* (3). The pathogen's rapid spread through East Africa has threatened the livelihood of millions of Africans who rely on banana fruit for food and export trade. Annual banana production in Africa is currently at about 603,000 metric tons, with approximately 4% of that being cultivated for export trade (19). BXW attacks all cultivars of banana (7), causing annual losses of over 500 million dollars across East and Central Africa (4). Once the disease is established in an area, total yield loss may occur (7). One of the first characteristic symptoms of the disease is the shriveling of the male flower bud, followed by yellowing and wilting of leaves and premature fruit ripening and discoloration. A sign of the disease is bacterial ooze from vascular tissue and fruit. There are currently no commercial chemicals, biocontrol agents, or resistant cultivars available to control the pathogen (7).

Many symptoms of BXW are similar to those of other bacterial and fungal wilts of banana, including Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*) and Moko/bugtok disease (*Ralstonia solanacearum*) (53). Currently, BXW is identified by isolation of bacteria from infected tissue followed by fatty acid and metabolic analyses (55). Although these methods are acceptable, they are generally only used once symptoms are visible. However, by the time the plant is showing visible symptoms, the BXW pathogen has established itself within the plant, and control and eradication become very difficult. In addition, latent infections in host tissue can result in widespread distribution of the pathogen through movement of infected planting material.

Polymerase chain reaction (PCR) with pathogen-specific primers has been used for the rapid identification of many phytopathogenic bacteria (5,9,10,14,20,24,25, 30,35–37,39,50). Variable spacer regions within the 16S and 23S ribosomal DNA (rDNA) genes have been used extensively as targets for the development of hybridization probes and PCR primers (8,13,27, 44,48). However, in some phytobacteria, such as *Ralstonia* spp. (43,48,52) and *Xanthomonas* spp. (21), variability in the rDNA sequences is limited and alternative targets are needed. Primers targeting functional genes, such as *gyrB* (44), endogluconase (40), the coronatine (COR) biosynthetic gene cluster, the phaseolotoxin (tox) gene cluster (5,10,11,41), *fliC* (47), and the *hrp* gene cluster (6,22,24,25,35,38,61,62), have a higher resolution than rDNA-based genes and are good candidates for species-specific primer development.

In *Xanthomonas* spp. and other phytopathogenic bacteria, *hrp* genes determine pathogenicity in hosts and hypersensitivity in resistant or nonhosts (1,56) but are not found in nonpathogenic species of *Xanthomonas* (25,28,51). Previous observations from this laboratory (*unpublished*) and from studies in Ontario, Canada (10) showed that *X. campestris* pv. *vesicatoria*-specific primers (RST65/RST69) developed from the *hrpB* gene (35) cross-reacted with other pathovars of *X. campestris*, including *X. campestris* pv. *musacearum* strains from this study. Unexpected, however, was the amplification of a unique additional lower molecular weight band with the *X. campestris* pv. *musacearum* strains. This additional band was not previously observed in our laboratory and was not reported in the study by Cuppels et al. (10). Based on these observations, we hypothesized that an *X. campestris* pv. *musacearum*-specific PCR assay could be developed to detect *X. campestris* pv. *musacearum* in host banana plants. Hence, the objective of this study was to design and evaluate a robust, sensitive PCR assay for the early detection of BXW.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in this study are listed in Table 1. All strains were maintained on yeast dextrose calcium carbonate (YDC) medium or yeast extract peptone glucose (YPG) medium (*Clavibacter* and *Xanthomonas* spp.) (26,46), Pseudomonas F (PF) medium (*Pseudomonas* spp.) or nutrient broth yeast extract (NBY) agar medium (*Ralstonia* spp.) and stored long term in NBY broth containing 15% glycerol at –80°C.

Isolation of *X. campestris* pv. *musacearum* from infected plant tissue. Bacteria were isolated from pseudostems of symptomatic banana plants (cv. Pisang Awak) from farmer-managed fields in Uganda, Tanzania, and Rwanda. From each plant, a 30-cm portion of infected pseudostem was removed and transported to the laboratory in a polythene bag. The two outermost sheaths on the pseudostem were peeled off and discarded. The re-

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maining exposed surface was sterilized with 75% ethyl alcohol and dried on sterile absorbent tissue paper under a laminar flow hood. Sterilized stems were cut aseptically into two cross-section pieces and kept at a slight slant until bacterial ooze was observed (10 to 15 min). Bacterial ooze was picked and directly streaked onto YPG medium and incubated at 25°C for 3 days. Colonies resembling *Xanthomonas* spp. were purified on YPG medium and maintained as described above.

Pathogenicity testing. Pathogenicity tests were performed in the Lake Victoria basin in Uganda, Africa on all isolates recovered from banana tissue. An approximately 10^8 CFU/ml bacterial suspension (1 ml) was injected into the stem (5 cm above the soil line) of 10 replicate 3-month-old tissue cultured banana plantlets (cv. Pisang Awak*)* using a sterile syringe with a 21-gauge needle per isolate. Inoculated plants were incubated in a screenhouse at ambient temperature (approximately 28°C) and observed for symptom development over a 28-day period. Control plants were inoculated with sterile distilled water (1 ml) and maintained under similar conditions. The number of wilted plants at the end of the 28-day period was recorded. To fulfill Koch's postulates, the pathogen was reisolated from all plants showing symptoms. A 5 cm-long piece of stem was cut 5 cm above the point of injection and surface sterilized in 75% ethyl alcohol for 30 s, then passed briefly through a flame. Each piece of stem was macerated in 10 ml of 10 mM $MgSO_4$ buffer. A 100-µl volume of the resulting suspension was spread plated onto YPG and incubated at 25°C for 3 days. Recovery of bacterial colonies similar to those of the inoculated isolate was taken as confirmation of Koch's postulates.

DNA extraction. Genomic DNA was extracted from banana tissue, bacterial ooze, and pure cultures grown on either YDC or YPG media using a cetyltrimethylammonium bromide solution as described previously (45). Purified DNA was diluted 1:10 (Africa tests) or to 50 ng/µl (United States tests) for use in PCR.

DNA fingerprinting. *X. campestris* pv. *musacearum* strains were fingerprinted using the enterobacterial repetitive intergenic consensus (ERIC) primers ERIC1R and ERIC2 (12). ERIC-PCR was performed using the conditions described by Louws et al. (29) in a PTC-100 thermocycler (MJ Research Inc., Waltham, MA) with whole-cell template. Whole-cell template was prepared by suspending one loopful of bacteria from a 48-h YDC agar plate into 200 µl of sterile distilled water. Suspensions were incubated at –20°C until completely frozen (at least 2 h) and thawed prior to use. Amplified PCR products (10 µl) were separated by horizontal gel electrophoresis in 1.5% agarose gels at 12°C in 1× Tris-acetate-EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.3) for 7.5 h at 50 V. Gels were stained in di-

Table 1. Strains and their host and source that were used in this study

Pathogen, strain[a]	Host	Source
Xanthomonas campestris pv. *musacearum*		
R11-06, R12-06, R13-06, R14-06, R15-06, R17-06, R18-06	Banana	This study, Rwanda
T1-06, T2-06, T3-06, T4-06, T5-06, T6-06, T7-06, T8-06, T9-06, T10-06	Banana	This study, Tanzania
UG1-06, UG2-06, UG3-06, UG4-06, UG6-06, UG7-06, UG8-06, UG9-06, UG10-06, UG11-06, UG12-06, UG13-06, UG14-06, UG15-06, UG17-06, UG18-06, UG3-019, UG21-06, UG22-06, UG23-06, UG24-06, UG25-06, UG27-06, UG28-06, UG29-06, UG30-06, UG31-06, UG32-06, UG33-06, UG34-06, UG35-06, UG36-06	Banana	This study, Uganda
X. campestris pv. *vesicatoria*		
110c, 17b	Pepper	S. Miller
761, 767, 794a, 788, 791, 1220	Tomato	S. Miller
X. campestris pv. *vitians*		
700a, 701a	Lettuce	S. Miller
X. campestris pv. *campestris*		
A4956, A4964, A4953, A4947, A4962	Cabbage	A. Alvarez
Cb8	Cabbage	G. Tusiime
Br5	Broccoli	G. Tusiime
NCPPB 528	Brussels sprouts	G. Tusiime
X. campestris pv. *amoracea*		
704b	Radish	S. Miller
756c, HW9, 704e	Radish	S. Kamoun
X. campestris pv. *raphani*		
DC91-1	Tomato	D. Cuppels
737, 738, 741	Tomato	S. Miller
X. axonopodis pv. *vasculorum*		
NCPPB 702, NCPPB 796, NCPPB 1326, MA2771, XV24, W4, UVZ011	Sugarcane	G. Tusiime, C. Webb
NCPPB 206	Maize	G. Tusiime, C. Webb
NCPPB 892, PDDCC327	Tiger grass	G. Tusiime, C. Webb
X. vasicola pv. *holcicola*		
NCPPB 2417, Z1, Z5, SAS211, TX1, Mexico1A, NCPPB 1241	Sorghum	G. Tusiime, C. Webb
ATCC 13461	Holcus	C. Webb
X. gardneri		
XCGA2, XV444, XV451, DC00T20A, DC03T4	Tomato	D. Cuppels
X. oryzae pv. *oryzae*		
J17, J12, J22, J6, R6	Rice	G. Wang
Clavibacter michiganensis subsp. *michiganensis*		
C290, A226	Tomato	D. Francis
Pseudomonas syringae pv. *tomato*		
DC3000	Tomato	D. Cuppels
P. syringae pv. *maculicola*		
88-10	Tomato	D. Cuppels
P. syringae pv. *syringae*		
SM22-01	Tomato	S. Miller
Ralstonia solanacearum race 1, biovar 3		
NO65-01	Eggplant	S. Miller

[a] *Xanthomonas* nomenclature is according to the International Society of Plant Pathology (59) or the International Journal of Systematic and Evolutionary Microbiology.

lute ethidium bromide (2 µg/ml), and DNA was visualized under UV light and photographed using the Kodak Electrophoresis Documentation and Analysis System 290 (Eastman Kodak Company, New Haven, CT).

***X. campestris*-specific PCR.** The PCR primers RST65 (5′GTCGTCGTTACG GCAAGGTGGTCG3′) and RST69 (5′TCG CCCAGCGTCATCAGGCCATC3′) generated from conserved sequences in the *hrpB* operon of the *hrp* gene cluster from *X. campestris* pv. *vesicatoria* group A, B, C, and D strains (35) were used to amplify all the strains listed in Table 1. Each reaction contained 1 µl of whole-cell template (whole-cell preparation described above) or purified genomic DNA and 24 µl of master mix (1.25 µl of each 10 µM primer, 12.5 µl of GoTaq Green Master Mix [GoTaq DNA polymerase at 100 units/ml, 400 µM dNTP, and 3 mM $MgCl_2$; Promega Corporation, Madison, WI] and 10 µl of sterile milliQ water). PCR was performed in a PTC-100 thermocycler (MJ Research Inc.) using the following program: 5 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 63°C, and 40 s at 72°C; and then a 5-min final extension at 72°C. Water was used as a negative control for all amplifications. PCR products (10 µl) were separated by horizontal gel electrophoresis in 1.5% agarose in 0.5× Tris-borate-EDTA (TBE) buffer at 50 V for 60 min. Gels were stained and DNA visualized as described above.

Nucleotide sequencing. The 422-bp amplicon from *X. campestris* strains 110C, 791, and 1220 and *X. campestris* pv. *musacearum* strains T3-06, T6-06, R17-06, and R18-06, produced by PCR with the RST65 and RST69 primers, were gel purified using the Qiagen QIAquick spin kit (Qiagen Inc, Chatsworth, CA) according to the manufacturer's instructions. Direct sequencing in both the forward and reverse directions of gel-purified amplification products was performed at the Plant-Microbe Genomics Facility, The Ohio State University, Columbus, using an automated 3730 DNA Analyzer (Applied Biosystems, Inc.). Base calling and sequence quality were determined for both strands of the sequences generated using Mac Phred-Mac Phrap software (17,18). The software Sequencher (Sequencher 3.0; Gene Codes Corporation, Ann Arbor, Michigan) was used to assemble, edit, trim, and generate high-quality consensus sequences. Sequence alignments of the consensus sequences were performed and similarity values were calculated with the ClustalW algorithm in Mac Vector (Mac vector 6.1; Oxford Molecular Ltd., Beaverton, OR) using default settings.

BXW primer design and PCR. *X. campestris* pv. *musacearum*-specific oligonucleotide primers were designed from regions of the RST65/69 consensus sequences that had minimal variability within each of the two groups (*X. campestris* and *X. campestris* pv. *musacearum* strains) but a high degree of variability between the two groups. A similarity matrix was calculated using MEGA 3.1 with default settings to determine the number of nucleotide differences within and between the two groups (23). Primers were initially tested using Amplify 3.1X software (16). The *X. campestris* pv. *musacearum*-specific PCR primers (BXW-1: 5′GTC GTTGGCACCATGCTCA3′ and BXW-3: 5′TCCGACCGATACGGCT3′) were used in PCR with whole-cell preparations of all the strains listed in Table 1, bacterial ooze from infected tissue, and genomic DNA (1:10) purified from bacterial ooze or infected tissue. Each reaction contained 1 µl of template and 24 µl of master mix (1.25 µl of each 10 µM primer, 12.5 µl of GoTaq Green Master Mix [GoTaq DNA polymerase at 100 units/ml, 400 µM dNTP, and 3 mM $MgCl_2$; Promega Corporation], and 10 µl of sterile milliQ water). PCR was performed in a PTC-100 thermocycler (MJ Research Inc.) using the following program: 5 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C; and then a 5-min final extension at 72°C. Water was used as a negative control for all amplifications. PCR products (10 µl) were separated by horizontal gel electrophoresis in 1.5% agarose in 0.5× TBE buffer at 50 V for 60 min. Gels were stained and DNA visualized as described above.

The sensitivity of the BXW primers using the PCR assay described above was determined using a pure culture of *X. campestris* pv. *musacearum* (strain R11-06). A 1-ml bacterial suspension (optical density at 600 nm = 0.23) was prepared in sterile distilled water and diluted to 10^{-9} using 10 fold serial dilutions. Each dilution (1 µl) was used as template in PCR and 100 µl was plated onto YDC in triplicate. The plates were incubated for 72 h at 28°C and counted, and the CFU/ml was calculated.

RESULTS

Isolation and pathogenicity of *X. campestris* pv. *musacearum* strains. Fifty isolates were collected and purified from naturally infected banana plants from Uganda, Tanzania, and Rwanda. All of the strains caused wilt within 28 days when inoculated into cv. Pisang Awak plantlets

Table 2. Polymerase chain reaction (PCR) amplification results using *Xanthomonas campestris*-specific and *X. campestris* pv. *musacearum*-specific primers

Pathogen	No. of strains evaluated	*X. campestris*-specific primers[a]	BXW-specific primers[b]
X. campestris pv. *musacearum*	50	+ (2)	+
X. campestris pv. *vesicatoria*	8	+	–
X. campestris pv. *vitians*	2	+	–
X. campestris pv. *campestris*	8	+	–
X. campestris pv. *amoracea*	4	+	–
X. campestris pv. *raphani*	4	+	–
X. axonopodis pv. *vasculorum*	10		
NCPPB 796, NCPPB 892, MA2771	…	+	–
PDDCC327, XV24	…	+	–
W4, NCPPB 702	…	+	+
UVZ011, NCPPB 206, NCPPB 1326	…	+ (2)	+
X. vasicola pv. *holcicola*	8		
ATCC 13461, TX1	…	–	–
SAS211, NCPPB 1241	…	+	–
Z5	…	+	+
Z1, NCPPB 2417, Mexico1A	…	+ (2)	+
X. oryzae pv. *oryzae*	5	+	–
X. gardneri	5	+	–
Clavibacter michiganensis subsp. *michiganensis*	2	–	–
Pseudomonas syringae pv. *tomato*	1	–	–
P. syringae pv. *maculicola*	1	–	–
P. syringae pv. *syringae*	1	–	–
Ralstonia solanacearum race 1, biovar 3	1	–	–

[a] RST65 and RST69 (35); + indicates that a single amplicon of 287 bp (*X. campestris*-specific PCR) or 214 bp (banana Xanthomonas wilt [BXW]-specific PCR) was produced; +(2) indicates that two amplicons (422 and 287 bp) were produced; and – indicates that no amplicon was produced.

[b] BXW-1 and BXW-3 (this study).

and were successfully recovered from the plantlets, thereby fulfilling Koch's postulates. None of the noninoculated control plantlets developed symptoms.

***X. campestris*-specific-PCR.** All but two (ATCC 13461 and TX1, identified as *X. vasicola* pv. *holcicola*) of the *Xanthomonas* strains tested produced the expected 422-bp product when amplified with the RST65 and RST69 PCR primers (RST primers). In addition to the 422-bp product, all of the *X. campestris* pv. *musacearum* strains, 3 of the 10 *X. campestris* pv. *vasculorum* strains, and 3 of the 8 *X. vasicola* pv. *holcicola* strains produced a smaller, 287-bp product (Table 2). No amplicons were produced for the *Pseudomonas*, *Clavibacter*, or *Ralstonia* strains or the water controls (Table 2). PCR amplification of representative strains of *X. campestris* pv. *musacearum* from Uganda, Tanzania, and Rwanda with RST primers is shown in Figure 1.

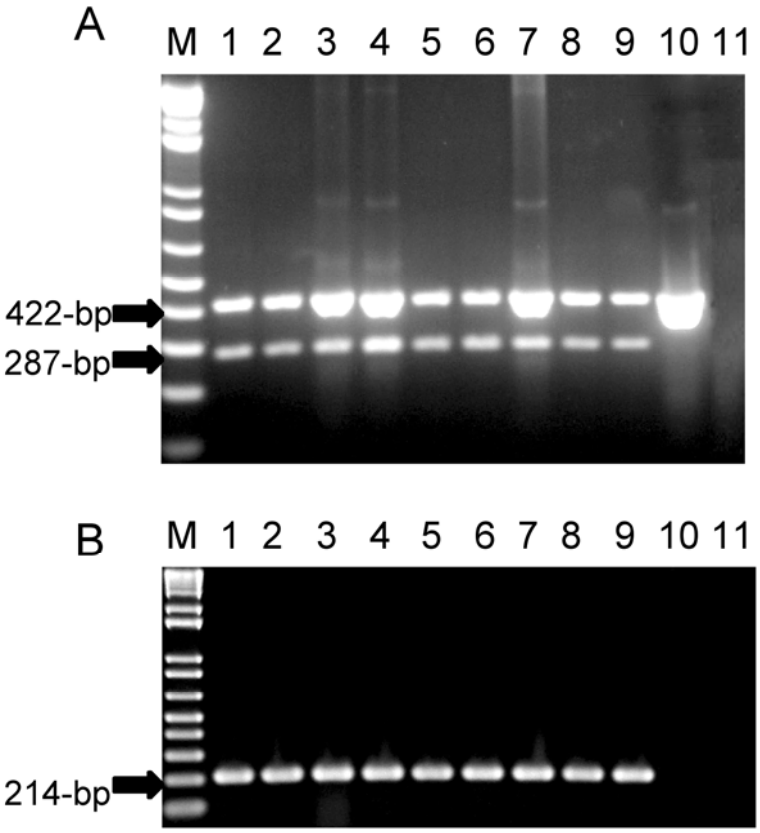

Fig. 1. Polymerase chain reaction (PCR) amplification of representative strains of *Xanthomonas vasicola* pv. *musacearum* from Uganda (UG), Tanzania (T), and Rwanda (R) with **A,** RST primers and **B,** banana Xanthomonas wilt primers. PCR was performed with whole-cell template as described in the Materials and Methods. Lane M, 1-kb plus ladder (Invitrogen Corp., Carlsbad, CA); lane 1, UG2-06; lane 2, UG15-06; lane 3, UG34-06; lane 4, T3-06; lane 5, T5-06; lane 6, T8-06; lane 7, R11-06; lane 8, R15-06; lane 9, R18-06, lane 10, *X. campestris* pv. *vesicatoria* 791; lane 11, *Pseudomonas syringae* pv. *tomato* DC3000.

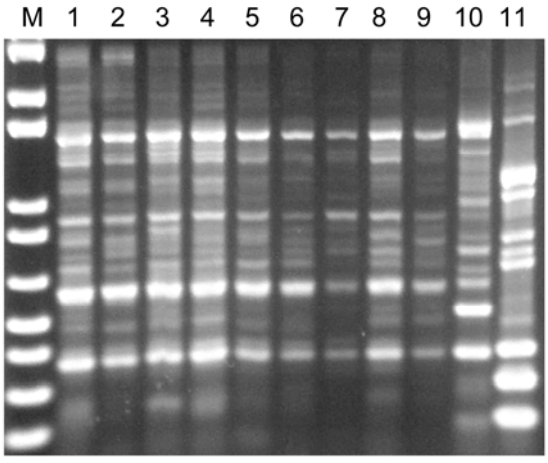

Fig. 2. Repetitive sequence-based polymerase chain reaction fingerprinting patterns using the enterobacterial repetitive intergenic consensus (ERIC) primers ERIC1R and ERIC2 generated for representative strains of *Xanthomonas campestris* pv. *musacearum* from Uganda (UG), Tanzania (T), and Rwanda (R) using ERIC primers. Lane M, 1-kb plus ladder (Invitrogen Corp., Carlsbad, CA); lane 1, UG2-06; lane 2, UG15-06; lane 3, UG34-06; lane 4, T3-06; lane 5, T5-06; lane 6, T8-06; lane 7, R11-06; lane 8, R15-06; lane 9, R18-06, lane 10, *X. campestris* pv. *vesicatoria* 791; lane 11, *Pseudomonas syringae* pv. *tomato* DC3000.

BXW primer design and PCR. To develop specific primers for the *X. campestris* pv. *musacearum* strains, the 422-bp amplicon generated from the RST primers was sequenced for three selected *X. campestris* strains (110C, 791, and 1220) and four selected strains of *X. campestris* pv. *musacearum* (T3-06, T6-06, R17-06, and R18-06). After editing and trimming of the sequenced products, a 399-nucleotide (nt), high-quality consensus sequence was generated. Aligned sequences were examined to determine regions of variability between the groups of strains generating one (*X. campestris* group) or two (*X. campestris* pv. *musacearum* group) amplicons when amplified by PCR with the RST primers (Table 2). The two groups differed by an average of 48.7 nt. Within the *X. campestris* and *X. campestris* pv. *musacearum* groups, there were, on average, 38.3 and 0 nt differences, respectively. In the 200- to 295-nt region of the 399-nt sequence, there were, on average, 19 nt differences that accounted for 39% (19/48.7) of the total variability between the two groups. The forward primer (BXW-1) for the *X. campestris* pv. *musacearum* PCR was developed 6 bp upstream from the 5′ end of the RST65 primer sequence and the reverse primer (BXW-3) was developed within the variable 200- to 295-nt region of the sequences; specifically from nucleotides 210 to 220. All of the *X. campestris* pv. *musacearum* strains from this study produced a single, 214-bp product by PCR with primers BXW-1 and BXW-3 (Table 2; Fig. 1). In addition to these strains, 50% of all of the *X. campestris vasculorum* and all of the *X. campestris holcicola* strains tested produced a 214-bp product with the BXW primers (Table 2). No amplicon was produced for any of the other *Xanthomonas* strains or the *Pseudomonas*, *Clavibacter*, or *Ralstonia* strains tested (Table 2), or the water controls (*data not shown*). A 214-bp product was generated regardless of the type of template (genomic DNA from symptomatic tissue, genomic DNA from bacterial ooze, and bacterial ooze from BXW-diseased plants) used for PCR. No PCR products were amplified from genomic DNA from healthy tissue (*data not shown*). The detection limit for whole *X. campestris* pv. *musacearum* (R11-06) cells from pure culture used directly as template in PCR with the BXW primers was 10^4 to 10^5 CFU/ml (*data not shown*).

ERIC-PCR genomic fingerprinting analysis of *X. campestris* pv. *musacearum* strains. ERIC-PCR generated complex fingerprinting patterns consisting of 11 or more distinct bands ranging in size from 0.35 kb to approximately 3 kb. Fingerprint patterns, under our reaction conditions, from *X. campestris* pv. *musacearum* strains isolated from Uganda, Rwanda, and Tanzania were identical (Fig. 2), indicating that the strains causing disease in these countries are clonal. None of the other *Xanthomonas* strains, including the *vasculorum* and *holcicola* pathovar strains that produced a 214-bp product with the BXW primers, were identical to the *musacearum* strains (*data not shown*). The *vasculorum* and *holcicola* strains (Table 1) had two to three bands and one to two bands in common with the *musacearum* strains, respectively (*data not shown*).

DISCUSSION

We developed a rapid whole-cell PCR assay using primers specific to a region of the *hrpB* gene cluster to detect *X. campestris* pv. *musacearum*. The use of these primers with PCR will allow for the early detection of this pathogen, which is critical due to its rapid and uncontrolled spread on symptomatic and asymptomatic crop material and through insect vectors throughout Africa. The presence of an additional, apparently unique amplicon in PCR assays using the primers (RST65 and RST69) designed to be specific to strains of *X. campestris* pv. *vesicatoria* isolated from pepper and tomato (35) suggested that a specific primer for *X. campestris* pv. *musacearum* could be developed from the same region of the *hrpB* gene from which the RST primers were developed. Sequence analysis of the 422-bp fragment revealed that the RST reverse primer (RST69) had two binding sites in strains of *X. campestris* pv. *musacearum*. Using this sequence information, a new reverse primer, internal to the smaller 287-bp product, was designed. In addition, the forward RST primer (RST65) was slightly modified to increase its specificity. The new primers (BXW-1 and BXW-3) were then evaluated for cross-reactivity to other phytopathogenic *Xanthomonas* spp. and pathovars. The BXW1 and BXW3 primers cross-reacted with some but not all of the strains of *X. vasicola* pv. *holcicola* and *X. axonopodis* pv. *vasculorum* tested in this study. Interestingly, three of the *X. vasicola* pv. *holcicola* strains (Z1, NCPPB 2417, and Mexico1A) and three of the *X. axonopodis* pv. *vasculorum* strains (UVZ011, NCPPB 206, and NCPPB 1326) that cross-reacted with the BXW primers produced two amplicons with the RST primers. Based on these findings, it was hypothesized that these strains may be very similar to *X. campestris* pv. *musacearum*. Although ERIC fingerprint patterns for these strains had little similarity (approximately 9 to 27% similarity) to *X. campestris* pv. *musacearum* strains, recent taxonomic studies using fatty acid profiles, repetitive sequence-based PCR, and sequencing of *gyrB* showed that *X. campestris* pv. *musacearum* is very similar to *X. vasicola* pv. *holcicola* isolated from sugarcane and maize from Africa (2) and sorghum from New Zealand (28). Aritua et al. (2) also showed that *X. axonopodis* pv. *vasculorum* strains isolated from sugarcane and maize were very similar to strains of *X. campestris* pv. *musacearum*. However, a more recent study by Parkinson et al. (38) showed that the *gyrB* sequence of *X. axonopodis* pv. *vasculorum* was dissimilar to that of a strain of *X. campestris* pv. *musacearum* from Ethiopia. Aritua et al. (3) proposed reclassification of *X. campestris* pv. *musacearum* to *X. vasicola* but this reclassification has not yet been accepted. Based on these two studies, it is not unexpected that our BXW primers cross-reacted with *X. vasicola* pv. *holcicola*. Although the BXW primers can cross-react with both *X. vasicola* pv. *holcicola* and *X. axonopodis* pv. *vasculorum*, neither of these pathogens has been shown to cause disease in banana using artificial inoculations (2). In addition, to the best of our knowledge, there have been no reports of these two pathogens being isolated from diseased banana trees or enset found in the field; therefore, the practical utility of this assay is not likely to be compromised on the target crops. Furthermore, the *X. campestris* pv. *musacearum* strains from Uganda, Tanzania, Rwanda, and Burundi (M. L. Lewis Ivey and S. A. Miller, *unpublished*) were clonal, indicating that false negatives are unlikely due to the lack of strain variation. Finally, any nonpathogenic *Xanthomonas* spp. that may be present on asymptomatic or symptomatic tissue will not be detected because *hrp* gene sequences are not present in their genomes. The BXW primers target the *hrpB* gene sequence of the *hrp* gene cluster. Among pathovars of *X. campestris*, *hrp* sequences are both functionally and physically similar, and finding a uniquely conserved portion can be difficult. Once the classification of *X. campestris* pv. *musacearum* is resolved, it may be worthwhile to investigate a more specific region in the *hrp* gene cluster sequence for primer development. Currently, this assay is being used in Africa to diagnose BXW and has been especially useful because the disease is moving into new areas, where symptoms are unfamiliar. The widespread availability of this assay in Africa will serve as a critical tool to help monitor the spread of this damaging plant disease, allowing appropriate management techniques to be initiated.

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