Detection of the Cassava Bacterial Blight Pathogen, *Xanthomonas axonopodis* pv. *manihotis*, by Polymerase Chain Reaction

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ABSTRACT

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Cassava bacterial blight, caused by *Xanthomonas axonopodis* pv. *manihotis*, is of significant concern wherever cassava is grown. The movement of infected, asymptomatic stems is a major means of pathogen dispersal. A reliable and sensitive diagnostic procedure is necessary for the safe movement of cassava planting material. We used a cloned and sequenced pathogenicity gene of *X. axonopodis* pv. *manihotis* to develop a polymerase chain reaction (PCR) test for this pathogen. A set of primers directed the amplification of an 898-bp fragment in all 107 pathogenic strains of *X. axonopodis* pv. *manihotis* tested. PCR products were not observed when genomic DNA was tested for 27 strains of other xanthomonads, for saprophytic bacteria, or for five nonpathogenic strains of *X. axonopodis* pv. *manihotis*. The primers worked well for pathogen detection in direct PCR assays of *X. axonopodis* pv. *manihotis* colonies grown on liquid medium and in PCR assays of extracts from leaf and stem lesions. The minimum number of cells that could be detected from cassava stem and leaf lesions was 3×10^2 to 10^4 CFU/ml. The PCR assays proved to be relatively sensitive and could become very useful in detecting the pathogen in cassava planting material.

Cassava (*Manihot esculenta* Crantz) is a starchy root crop that provides food and income for about 500 million people living in the tropics. *Xanthomonas axonopodis* pv. *manihotis* Dye (42) is the causal agent of cassava bacterial blight, an economically important disease with a worldwide distribution (20,24). The disease was first reported in South America and has subsequently been reported in Africa and Asia (5,20); it is now a target for international phytosanitary quarantine efforts (20). The disease has a wide spectrum of symptoms, including angular leaf spots, blighting, wilting, vascular necrosis of the stem, dieback, and production of exudates (24). Crop losses of 12 to 100% have been reported (20). This vascular disease also affects the quality of the planting material, leading to reductions in seed yield (5,20). Cassava is propagated by planting pieces of stem, and the pathogen is thus disseminated primarily through infected planting material (5,20). Previous studies have shown that *X. axonopodis* pv. *manihotis* is also seedborne and is carried either on the seed coat or in the embryo (29). This contamination, whether internal or external, is an important means of survival for this bacterium (8,9,11). The pathogen also affects the germination of infected seeds (11).

The disease can be controlled through cultural practices, including the use of uninfected planting material and resistant cultivars (5,20). Although the production of healthy planting material in inspected nurseries can reduce the incidence of the disease, this method has its limitations because *X. axonopodis* pv. *manihotis* can survive in tissue without causing symptoms (5). Thus, sensitive detection techniques are needed to prevent the introduction of the pathogen into regions free of bacterial blight. As a consequence of quarantine procedures, international exchange of cassava germ plasm is limited and occurs through true seeds or material propagated in vitro (20). The success of a cassava seed certification program will depend on the availability of reliable tests for detecting the pathogen in true seeds and vegetative material.

Current methods for identifying and detecting *X. axonopodis* pv. *manihotis* rely on isolating the bacterium and conducting immunoassays of tissue extracts (8,9,11). Enzyme-linked immunosorbent assays for *X. axonopodis* pv. *manihotis* may improve detection and are rapid, but immunological reactions are not entirely specific because of cross-reactions with some strains of other pathovars of *X. axonopodis* and one strain of *X. campestris* pv. *euphorbiae* (6). Newer molecular tools, including Southern hybridization probes, are very efficient for identifying plant pathogens (12,23). Recently, DNA probes derived from genomic and plasmid DNA have been developed for *X. axonopodis* pv. *manihotis* and have been useful in restriction fragment length polymorphism (RFLP) studies (41). The genetic variability among strains of *X. axonopodis* pv. *manihotis* has been characterized in detail (39,41), revealing that the African strains are homogenous, whereas in South America, the pathogen is highly diverse.

The polymerase chain reaction (PCR) method has been widely used to identify and detect phytopathogenic bacteria (2,3, 10,13,15,30). Various PCR methods have been developed for different phytopathogenic bacteria based on the amplification of *hrp* sequences (16,18), repetitive bacterial sequences (rep-PCR) (27,31), tRNA intergenic and rDNA spacer sequences (15,19,21,30), specific plasmid sequences (3,13), and specific random amplified polymorphic DNA products (23,31).

The pathogenicity of *X. axonopodis* pv. *manihotis* is associated with a plasmid (p44) that is widely distributed within *X. axonopodis* pv. *manihotis* strains (38). In a nonpathogenic *X. axonopodis* pv. *manihotis* strain, a 8-kb region is missing from the plasmid, suggesting that this sequence is involved in pathogenicity. This sequence is included in a 12-kb *Hin*dIII plasmid fragment (psF2) (40). We established a restriction map for the plasmid fragment psF2, subcloned selected fragments, and determined the nucleotide sequences of the fragments (40). For this study, we developed primers for PCR based on the sequence of one of these, a 1.2-kb *X. axonopodis* pv. *manihotis* plasmid fragment. We thus developed a sensitive and rapid PCR-based assay for detecting the blight-causing bacterium in cassava stem and leaf tissues.

MATERIALS AND METHODS

Bacterial strains and DNA extraction. All of the 158 bacterial strains we used are listed in Table 1. Bacteria living as epi-

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phytes on cassava foliage were recovered from collected leaves and used for testing primer specificity. Colombian isolates of *X. axonopodis* pv. *manihotis* (CIO strains), collected during 1995 in different edaphoclimatic zones, were included. Sixteen *Xanthomonas* strains were used, as well as 19 strains representing 14 pathovars of *X. axonopodis*. All cultures were stored at −70°C in nutrient broth containing 60% glycerol. Strains were cultured routinely at 30°C on LPG medium (5 g of glucose, 5 g of yeast extract, 5 g of peptone, and 15 g of agar per liter). Freshly grown, single colonies on LPG media were used to start broth cultures. Bacterial cultures were grown at 30°C for 12 h, and cells from 3 ml of broth were used for DNA extraction. Total genomic DNA was isolated as described by Berthier et al. (4).

PCR reaction conditions. Primers for the PCR were designed with the software OLIGO, version 4.0 (National Biosciences, Plymouth, NC), based on the sequence of *X. axonopodis* pv. *manihotis*'s 1,276-nucleotide *Bam*HI-*Eco*RI insert in pBSF2. The primers were obtained commercially from Eurogentec (Louvain, Belgium). Sequence analysis was performed with the Bisance service of CITI2 (Faculté de Médecine, Paris, France). The sequence from which the primers were selected showed no homology to other available DNA se-

Table 1. Bacterial strains used in this study

Strain	Isolates[a]	Location
Xanthomonas axonopodis pv. *manihotis*	I67, I71, I70	Ghana
	I89, K1, K7	Nigeria
	I1, I10, I13, I25, I36, I41, I42, I63	Benin
	Orst17, Orst151, Orst161, Orst178, Orst200	Congo
	OrstX27, OrstX102, OrstX75, OrstX84, OrstX80	Togo
	LMG769, Orst44, Orst50, Orst186, UPB009	Zaire
	Orst267, Orst212, Orst213, Orst214, Orst215, Orst216	Cameroon
	Orst324, Orst241, LMG780, Orst242, Orst243	Uganda
	Orst56, LMG5249	Ivory Coast
	Orst39, Orst40, Orst41	Central Africa
	LMG5288	Niger
	LMG783	Kenya
	CFBP2624, Orst227, Orst228	Réunion
	CIO46 + 25 other CIO strains, CIO188,* CIAT1059*	Colombia
	CIAT1063,* CIAT1066, Orst4,* CFBP1851	Colombia
	NCPPB348, CIO286, CIO287, CIO288, CIO289, CIO290	Brazil
	NCPPB1159, LMG 777, UPB079,* UPB070	Brazil
	CIAT1241, INTA1, INTA2, INTA3, INTA4, INTA5	Argentina
	CIAT1129, CIO191, CIO237, CIO231, CIO246	Venezuela
	CIAT1162	Mexico
	CIAT1135	China
	CIAT1161	Thailand
	CIAT1163	Sumatra (Indonesia)
	CIAT1160, LMG765	Malaysia
	CIAT1181	Philippines
	CIAT1203	Dominican Republic
	UPB071	Mauritius
X. axonopodis pv. *citri*	CFBP1814	Réunion
X. axonopodis pv. *campestris*	CFBP10412	United Kingdom
X. axonopodis pv. *aracearum*	NCPPB2832	Guadeloupe
X. axonopodis pv. *begoniae*	NCPPB3003	Netherlands
X. axonopodis pv. *hordei*	UPB684	Iran
X. axonopodis pv. *diffenbachiae*	NCPPB1833	Brazil
X. axonopodis pv. *glycines*	NCPPB554, Orst1144	Sudan, Congo
X. axonopodis pv. *vesicatoria*	CFBP10601	Unknown
X. axonopodis pv. *malvacearum*	NCPPB633, Orst57	Ivory Coast
X. axonopodis pv. *vasculorum*	CFBP1289	Réunion
X. axonopodis pv. *phaseoli*	CIAT306, CIAT072	Argentina, United States
X. axonopodis pv. *vignicola*	I2	Niger
X. axonopodis pv. *poinsetticola*	UPB073, LMG5403	India, New Zealand
X. axonopodis pv. *ricini*	UPB075, UPB076	Ethiopia, Hong Kong
X. campestris pv. *incanae*	CFBP1438	United States
X. campestris pv. *mangiferaeindicae*	NCPPB490, 2623, 1117, 2926	Réunion
X. campestris pv. *euphorbiae*	LMG863, LMG7402	Sudan
X. horturum pv. *pelargonii*	NCPPB2985, CFBP10342	New Zealand, France
X. translucens pv. *translucens*	UPB545	Mexico
X. translucens pv. *undulosa*	UPB882	Yemen
X. oryzae pv. *oryzae*	CFBP1948	Cameroon
X. albilineans	G7, R8	Guadeloupe
X. cassavae	NCPPB101, UPB037	Malawi, Rwanda
Other bacterial species		
Ralstonia solanacearum	Orst1153, Orst1155, GMI1000	Congo, Guyana
Epiphytic strains	Eight field isolates	Congo

[a] CFBP = Collection Française de Bactéries Phytopathogènes, Angers, France; CIAT = *Xanthomonas* collection, Centro Internacional de Agricultura Tropical, Cali, Colombia; CIO = ORSTOM collection, CIAT, Cali, Colombia; G7 and R8 = Collection CIRAD-CA de Bactéries Phytopathogènes, Montpellier, France; INTA = Collection Instituto Nacional de Tecnologia Agropecuaria, Bella Vista, Argentina; I and K = IITA, International Institute of Tropical Agriculture, Collection Laboratoire de Phytopathologie, Cotonou, Benin; LMG = Laboratorium voor Microbiologie Gent culture collection, Universiteit Gent, Belgium; NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, England; Orst = ORSTOM, Collection de Bactéries Phytopathogènes, Laboratoire de Phytopathologie, Montpellier, France; and UPB = Collection Unité de Phytopathologie, Louvain-La-Neuve, Belgium. Strains 2623, 1117, and 2926 of *X. campestris* pv. *mangiferaeindicae* were kindly provided by O. Pruvost (CIRAD-FLHOR, Saint Pierre, La Réunion, France). * = Nonpathogenic strain, according to this assay and as previously described (39).

quences in the nucleotide sequence databases (EMBL, PDB, or GenBank library searches, subdivision bacterial, programs Blast and Fasta) (1). PCR assays were performed with a DNA thermal cycler (PTC-100, MJ Research, Watertown, MA). PCR reactions contained 1.5 mM $MgCl_2$, primer (12.5 pmol), 200 μM nucleotides, and 1.25 units of *Taq* DNA polymerase per 50-μl reaction. The PCR temperature profile comprised an initial denaturation step at 95°C for 5 min, 30 cycles of 95°C for 30 s, 61°C for 30 s, and 72°C for 1.5 min; and a final extension at 72°C for 5 min. Nucleotide sequences of primers used in the PCR reaction are 5′-TTC-GGC-AAC-GGC-AGT-GAC-CAC-C-3′ (primer XV) and 5′-TCA-ATC-GGA-GAT-TAC-CTG-AGC-G-3′ (primer XK). PCR amplification assays were routinely performed with 20 ng of bacterial genomic DNA. Aliquots containing 20 μl of the PCR amplification product were removed and subjected to electrophoresis in 1% agarose gels at 50 V for 1 h and visualized by ultraviolet light after staining with ethidium bromide.

Detection of *X. axonopodis* pv. *manihotis* in plant samples. Cassava stems and leaves were collected from infected fields. Leaves were sampled by cutting out the angular lesions, macerating the tissue, and suspending it in 0.5 ml of sterile distilled water. Stems were crushed and treated as described above. The extract from each sample was diluted serially in 10-fold increments in distilled water, and 10 μl was used in the PCR assay. Leaves of greenhouse-grown cassava were inoculated by placing 10 μl of *X. axonopodis* pv. *manihotis* strains (about 10^8 CFU/ml; optical density at 600 nm = 0.1) on the leaf surface in a hole (2-mm diameter) made with a cork borer (39). In total, eight pathogenic and nonpathogenic *X. axonopodis* pv. *manihotis* strains were tested with this assay. After 7 days, individual lesions and control tissues were removed around the point of inoculation and macerated in 300 μl of distilled sterile water. The presence of typical water-soaked angular lesions indicated pathogenicity of an isolate, whereas no reaction was visible with nonpathogenic isolates (39). Of the resulting extracts and dilution series in sterile water, 10 μl were used as a source of template DNA and plated on LPG agar, and the colonies that developed were counted.

Assessment of sensitivity thresholds. Aliquots from bacterial suspensions made in sterile water from freshly streaked LPG plates were used as templates in PCR assays. Bacteria were adjusted to A_{600nm} = 0.1 (about 1.2×10^8 CFU/ml), serially diluted in sterile water, and plated on LPG medium to determine the bacterial concentration. From each dilution, 10 μl was used for direct amplification in the PCR assay. DNA concentrations were estimated by spectrophotometry at 260 and 280 nm. A dilution series of DNA from *X. axonopodis* pv. *manihotis* strain CIO46, in the range of 20 ng to 1 fg, was prepared and assayed to estimate the limit for detection for purified DNA.

Nucleotide sequence accession number. The nucleotide sequence has been submitted to GenBank and assigned accession number AF012325. The 898-bp target sequence corresponds to nucleotides 4996 to 5894.

RESULTS

Primer specificity for detecting *X. axonopodis* pv. *manihotis*. Primer pair XV-XK directed the amplification of the 898-bp target sequence in DNA from 107 *X. axonopodis* pv. *manihotis* strains originally isolated in 25 countries (Table 1 and Fig. 1). Five nonpathogenic *X. axonopodis* pv. *manihotis* strains were tested with the primer pair; the assay produced no amplification product (*data not shown*). No amplicons were produced from DNA extracted from 46 other strains of bacteria, including 16 strains of six other *Xanthomonas* species, 19 strains of 14 other pathovars of *X. axonopodis*, three strains of *Ralstonia solanacearum*, or eight epiphytic strains isolated from cassava leaf tissue (Table 1 and Fig. 1; *some data not shown*).

Sensitivity of PCR amplification. A dilution series of freshly cultured cells of *X. axonopodis* pv. *manihotis* strain CIO46 yielded a limit of detection of about 1.2×10^2 viable cells per reaction, as determined by dilution plating (Fig. 2A). A dilution series of total DNA of *X. axonopodis* pv. *manihotis* strain CIO46 was prepared and used as template for PCR amplification. Aliquots that contained 10 fg of genomic DNA were successfully detected after amplification with the primer pair XV-XK (Fig. 2B).

Detection of *X. axonopodis* pv. *manihotis* in plant tissues. PCR was used to detect bacteria in leaf and stem tissues obtained from inoculated plants grown in the greenhouse or from plant samples collected in the field. PCR products were generated from single leaf lesions that were collected in the field, diluted 1,000 times, and then amplified (Fig. 3). The minimum number of viable cells detected by PCR was six per reaction (Fig. 3). The appropriate PCR product was also generated from 8-month-old dry leaf lesions collected in field experiments. The minimum number of cells detected was 3 CFU per reaction in these experiments (*data not shown*). Amplification of the expected fragment also occurred from extracts of lesions induced by inoculation with different *X. axonopodis* pv. *manihotis* strains 7 days earlier (*data not shown*). No prod-

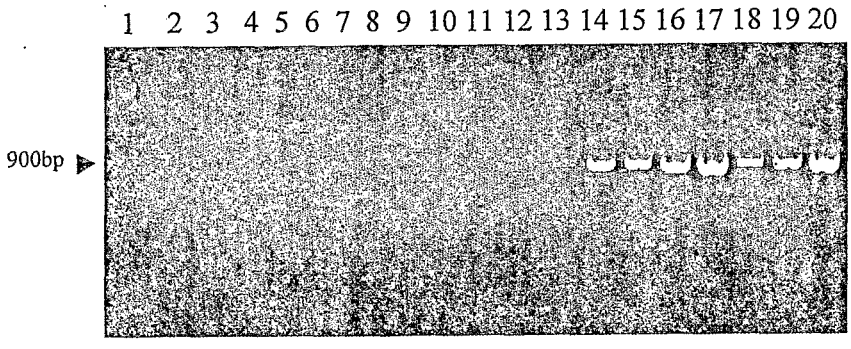

Fig. 1. Agarose gel, stained with ethidium bromide, of DNA products extracted from *Xanthomonas* strains and amplified by polymerase chain reaction. Lane 1, 100-bp ladder; lane 2, *X. albilineans* G7; lane 3, *X. oryzae* pv. *oryzae* CFBP1948; lane 4, *X. axonopodis* pv. *campestris* CFBP10412; lane 5, *X. axonopodis* pv. *aracearum* NCPPB2832; lane 6, *X. axonopodis* pv. *glycines* NCPPB554; lane 7, *X. axonopodis* pv. *citri* CFBP1814; lane 8, *X. axonopodis* pv. *vesicatoria* CFBP10601; lane 9, *X. axonopodis* pv. *malvacearum* ORST57; lane 10, *X. axonopodis* pv. *ricini* UPB075; lane 11, *X. cassavae* UPB037; lanes 12–20, *X. axonopodis* pv. *manihotis* strains I89, Orst200, Orst151, OrstX84, Orst214, Orst215, LMG780, Orst50, and Orst186, respectively.

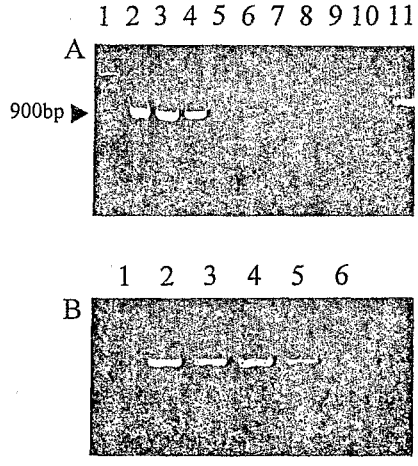

Fig. 2. Agarose gel, stained with ethidium bromide, of products from polymerase chain reaction amplification for a sensitivity assay with *Xanthomonas axonopodis* pv. *manihotis* strain CIO46. **(A)** Lane 1, 100-bp ladder; lane 2, 1.2×10^8 CFU/10 μl; lane 3, 1.2×10^7 CFU/10 μl; lane 4, 1.2×10^6 CFU/10 μl; lane 5, 1.2×10^5 CFU/10 μl; lane 6, 1.2×10^4 CFU/10 μl; lane 7, 1.2×10^3 CFU/10 μl; lane 8, 1.2×10^2 CFU/10 μl; lanes 9 and 10, negative controls; and lane 11, DNA strain CIO46 as positive control. **(B)** Different DNA concentrations of CIO46 strain: lane 1, 100-bp ladder; lane 2, 20 ng/μl; lane 3, 10 ng/μl; lane 4, 10 pg/μl; lane 5, 10 fg/μl; and lane 6, negative control (distilled water).

ucts were observed from leaf tissue inoculated with the nonpathogenic *X. axonopodis* pv. *manihotis* strain Orst4, nor were viable bacteria recovered from this tissue (*data not shown*). No products were observed from healthy noninoculated leaf disks assayed as controls (Fig. 3). Extracts prepared from stem lesions of naturally infected plants collected in the field were positive by PCR; a bacterial concentration of about 1.2×10^3 CFU per reaction was detected (*data not shown*).

DISCUSSION

We have developed a PCR-based assay for *X. axonopodis* pv. *manihotis*, an important and worldwide pathogen of cassava that is subject to international phytosanitary quarantine (20). We used the sequence data of a *X. axonopodis* pv. *manihotis* pathogenicity gene to design primers. No similarities were found within the 1.2-kb *Bam*HI-*Eco*RI fragment to known sequences in data banks. This DNA sequence has provided primers specific for *X. axonopodis* pv. *manihotis*. The expected fragment was amplified only from *X. axonopodis* pv. *manihotis* strains. No DNA fragments were amplified from the 46 other bacterial strains tested, including six unidentified epiphytes.

The 112 *X. axonopodis* pv. *manihotis* strains tested in this study are from diverse geographic origins (25 countries in South America, Africa, and Asia). The expected fragment was amplified from several Colombian *X. axonopodis* pv. *manihotis* strains collected in different edaphoclimatic zones. PCR reactions from the 107 pathogenic strains of *X. axonopodis* pv. *manihotis* tested contained an amplification product of the expected size after gel electrophoresis. In this study, PCR products were not observed for the five nonpathogenic *X. axonopodis* pv. *manihotis* strains. Little is known about the epidemiological importance of such strains in cassava bacterial blight (25,39,41). The nonpathogenic *X. axonopodis* pv. *manihotis* strain Orst4 carries a deletion in the 1.2-kb *Bam*HI-*Eco*RI fragment (40); thus, the DNA target sequence is absent and not integrated with another part of the genome (40). Whether other nonpathogenic *X. axonopodis* pv. *manihotis* strains also lack the target plasmid sequence is of research interest.

In practical diagnosis, the ability to differentiate rapidly the nonpathogenic from pathogenic strains (37) is necessary, but very often techniques for such differentiation are not readily available. While the amplification of *hrp* sequences by PCR is useful for detecting and identifying plant-pathogenic xanthomonads (16,17,35), it is the lack of *hrp* DNA sequences among nonpathogenic xanthomonads that makes this tool efficient (18).

Several authors have developed plasmid-based PCR assays for detecting phytopathogenic bacteria (2,14,36). The risks of using plasmid DNA as a target sequence, either in PCR assays or in epidemiological studies, have been well documented (7,14). However, the sensitivity of detection assays may be enhanced because the plasmid-borne sequence can be present in multiple copies per bacterium (14,22). As suggested by Hartung et al. (14), a PCR-based assay that relies on a plasmid DNA target could be misleading if the plasmid is transmissible. Their results tend to demonstrate that plasmids are stably maintained in *X. axonopodis* pv. *citri* (14,32). For example, cluster analysis with RFLP data of strains of *X. axonopodis* pv. *citri* reveals similar phylogenies for plasmid and genomic DNA, which is consistent with the plasmids being stably maintained in *X. axonopodis* pv. *citri* (32). Similar results have been observed with strains of *X. axonopodis* pv. *manihotis* (33).

The PCR procedure is simple to perform, and a 30-cycle, three-step PCR program takes 2 h to complete. This rapid method is easily adapted for confirming *X. axonopodis* pv. *manihotis* bacterial colonies and for testing infected plant tissue. PCR detection of *X. axonopodis* pv. *manihotis* from leaf spots and stem lesions on cassava was successfully conducted without prior DNA extraction. In practice, preparing tissue extracts suitable for PCR requires less than 1 min per sample (crushing), but the time could perhaps be reduced by simply boiling the samples in distilled water (3). The PCR method we used allowed us to detect three viable cells per reaction or the equivalent of 300 CFU/ml in plant tissue. This is comparable to the levels obtained from PCR techniques available for detecting other phytopathogenic bacteria (13,14, 26,28,34).

With its specificity and sensitivity, the short PCR assay described here has considerable potential as a reliable procedure for detecting and identifying the cassava bacterial blight pathogen in infected plant tissue. This method will also be applied to detect *X. axonopodis* pv. *manihotis* in cassava true seeds.

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LITERATURE CITED

1. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.
2. Audy, P., Braat, C. E., Saindon, G., Huang, H. C., and Laroche, A. 1996. A rapid and sensitive PCR-based assay for concurrent detection of bacteria causing common and halo blights in bean seed. Phytopathology 86:361-366.
3. Audy, P., Laroche, A., Saindon, G., Huang, H. C., and Gilbertson, R. L. 1994. Detection of the bean common blight bacteria, *Xanthomonas campestris* pv. *phaseoli* and *X. c. phaseoli* var. *fuscans* using the polymerase chain reaction. Phytopathology 84:1185-1192.
4. Berthier, Y., Verdier, V., Guesdon, J. L., Chevrier, D., Denis, J. B., Decoux, G., and Lemattre, M. 1993. Characterization of *Xanthomonas campestris* pathovars by rRNA gene restriction patterns. Appl. Environ. Microbiol. 59:851-859.
5. Boher, B., and Verdier, V. 1994. Cassava bacterial blight in Africa. The state of knowledge and implications for designing control strategies. Afr. Crop Sci. J. 2:1-5.
6. Boher, B., Nicole, M., Potin, M., and Geiger, J. P. 1997. Extracellular polysaccharides from *Xanthomonas axonopodis* pv. *manihotis* interact with cassava cell walls during pathogenesis. Mol. Plant-Microbe Interact. 10:803-811.

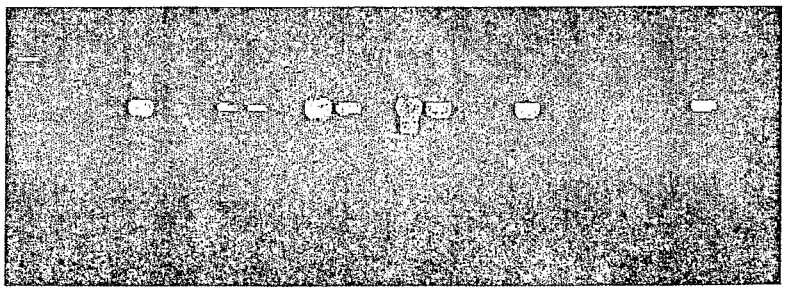

Fig. 3. Agarose gel, stained with ethidium bromide, of polymerase chain reaction amplification products for detecting *Xanthomonas axonopodis* pv. *manihotis* in infected cassava leaf tissue. Lane 1, 100-bp DNA ladder; lane 2, negative control (distilled water); lanes 3 and 4, noninoculated leaf tissues; lanes 5–7, 10-fold dilution series from lesion 1, containing 6.4×10^4 to 6.4×10^2 CFU per reaction; lanes 8–10, 10-fold dilution series from lesion 2, containing 4.5×10^4 to 4.5×10^2 CFU per reaction; lanes 11–13, 10-fold dilution series from lesion 3, containing 9.2×10^3 to 92 CFU per reaction; lanes 14–16, 10-fold dilution series from lesion 4, containing 9.4×10^4 to 9.4×10^2 CFU per reaction; lanes 17–19, 10-fold dilution series from lesion 5, containing 6×10^2 to 6 CFU per reaction; lanes 20–22, 10-fold dilution series from lesion 6, containing 1.7×10^4 to 1.7×10^2 CFU per reaction; lanes 23–25, 10-fold dilution series from lesion 7, containing 1×10^4 to 1×10^2 CFU per reaction; and lane 26, positive control of DNA from *X. axonopodis* pv. *manihotis* strain CIO46.

7. Canteros, B. I., Minsavage, G. V., Jones, J. B., and Stall, R. E. 1995. Diversity of plasmids in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 85:1482-1486.
8. Daniel, J. F., and Boher, B. 1981. Contamination of cassava flowers, fruits and seeds by *Xanthomonas campestris* pv. *manihotis*. Pages 614-617 in: Proc. Int. Conf. Plant Pathog. Bact., 5th. J. C. Lozano, ed. Centro Internacional de Agricultura Tropical, Cali, Colombia.
9. Daniel, J. F., and Boher, B. 1981. Fluorescent antibody technique for detection of *Xanthomonas campestris* pv. *manihotis*. Pages 176-180 in: Proc. Int. Conf. Plant Pathog. Bact., 5th. J. C. Lozano, ed. Centro Internacional de Agricultura Tropical, Cali, Colombia.
10. De Boer, S. H., and Ward, L. J. 1995. PCR detection of *Erwinia carotovora* subsp. *atroseptica* associated with potato tissue. Phytopathology 85:854-858.
11. Elango, F., and Lozano, J. C. 1980. Transmission of *Xanthomonas manihotis* in seed of cassava (*Manihot esculenta*). Plant Dis. 64: 784-786.
12. Hartung, J. S. 1992. Plasmid-based hybridization probes for detection and identification of *Xanthomonas campestris* pv. *citri*. Plant Dis. 76:889-893.
13. Hartung, J. S., Daniel, J. F., and Pruvost, O. P. 1993. Detection of *Xanthomonas campestris* pv. *citri* by the polymerase chain reaction method. Appl. Environ. Microbiol. 59:1143-1148.
14. Hartung, J. S., Pruvost, O. P., Villemot, I., and Alvarez, A. 1996. Rapid and sensitive colorimetric detection of *Xanthomonas axonopodis* pv. *citri* by immunocapture and a nested-polymerase chain reaction assay. Phytopathology 86:95-101.
15. Honeycutt, R., and McClelland, M. 1996. Application of the polymerase chain reaction to the detection of plant pathogens. Pages 187-201 in: The Impact of Plant Molecular Genetics. B. W. S. Sobral, ed. Birchhaüser, Boston.
16. Leite, R. P., Jr., Egel, D. S., and Stall, R. E. 1994. Genetic analysis of *hrp*-related DNA sequences of *Xanthomonas campestris* strains causing diseases of citrus. Appl. Environ. Microbiol. 60:1078-1086.
17. Leite, R. P., Jr., Jones, J. B., Somodi, G. C., Minsavage, G. V., and Stall, R. E. 1995. Detection of *Xanthomonas campestris* pv. *vesicatoria* associated with pepper and tomato seed by DNA amplification. Plant Dis. 79: 917-922.
18. Leite, R. P., Jr., Minsavage, G.V., Bonas, U., and Stall, R. E. 1994. Detection and identification of phytopathogenic *Xanthomonas* strains by amplification of DNA sequences related to the *hrp* genes of *Xanthomonas campestris* pv. *vesicatoria*. Appl. Environ. Microbiol. 60:1068-1077.
19. Li, X., and De Boer, S. H. 1995. Selection of polymerase chain reaction primers from an RNA intergenic spacer region for specific detection of *Clavibacter michiganensis* subsp. *sepedonicus*. Phytopathology 85:837-842.
20. Lozano, J. C. 1986. Cassava bacterial blight: A manageable disease. Plant Dis. 70:1089-1093.
21. Maes, M., Garbeva, P., and Kamoen, O. 1996. Recognition and detection in seed of the *Xanthomonas* pathogens that cause cereal leaf streak using rDNA spacer sequences and polymerase chain reaction. Phytopathology 86:63-69.
22. Mahoney, J. B., Luinstra, K. E., Sellors, J. W., and Cheresky, M. A. 1993. Comparison of plasmid-based and chromosome-based polymerase chain reaction assays for detecting *Chlamydia trachomatis* nucleic acids. J. Clin. Microbiol. 31:1753-1758.
23. Manulis, S., Valinsky, L., Lichter, A., and Gabriel, D. W. 1994. Sensitive and specific detection of *Xanthomonas campestris* pv. *pelargonii* with DNA primers and probes identified by random amplified polymorphic DNA analysis. Appl. Environ. Microbiol. 60:-4094-4099.
24. Maraite, H. 1993. *Xanthomonas campestris* pathovars on cassava: Cause of bacterial blight and bacterial necrosis. Pages 18-24 in: *Xanthomonas*. J. G. Swings and E. L. Civerolo, eds. Chapman and Hall, London.
25. Maraite, H., Weyns, J., Yimkwan, O., Lipembra, P., and Perreaux, D. 1981. Physiological and pathogenic variations in *Xanthomonas campestris* pv. *manihotis*. Pages 358-368 in: Proc. Int. Conf. Plant Pathog. Bact., 5th. J. C. Lozano, ed. Centro Internacional de Agricultura Tropical, Cali, Colombia.
26. McManus, P. S., and Jones, A. L. 1995. Detection of *Erwinia amylovora* by nested PCR and PCR-dot-blot and reverse-blot hybridizations. Phytopathology 85:618-623.
27. Opgenorth, D. C., Smart, C. D., Lows, F. J., de Bruijn, F. J., and Kirkpatrick, B. C. 1996. Identification of *Xanthomonas fragariae* field isolates by rep-PCR genomic fingerprinting. Plant Dis. 80:868-873.
28. Pan, Y.-B., Grisham, M. P., and Burner, D. M. 1997. A polymerase chain reaction protocol for the detection of *Xanthomonas albilineans*, the causal agent of sugarcane leaf scald disease. Plant Dis. 81:189-194.
29. Persley, G. J. 1979. Studies on the survival and transmission of *Xanthomonas manihotis* on cassava seed. Ann. Appl. Biol. 93:159-166.
30. Pooler, M. R., and Hartung, J. S. 1995. Specific PCR detection and identification of *Xylella fastidiosa* strains causing citrus variegated chlorosis. Curr. Microbiol. 31:377-381.
31. Pooler, M. R., Ritchie, D. F., and Hartung, J. S. 1996. Genetic relationships among strains of *Xanthomonas fragariae* based on random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus PCR data and generation of multiplexed PCR primers useful for the identification of this phytopathogen. Appl. Environ. Microbiol. 62:3121-3127.
32. Pruvost, O., Hartung, J. S., Civerolo, E. L., Dubois, C., and Perrier, X. 1992. Plasmid DNA fingerprints distinguish pathotypes of *Xanthomonas campestris* pv. *citri*, the causal agent of citrus bacterial canker disease. Phytopathology 82:485-490.
33. Restrepo, S., and Verdier V. 1997. Geographical differentiation of the population of *Xanthomonas axonopodis* pv. *manihotis* in Colombia. Appl. Env. Microbiol. 63:4427-4434.
34. Roberts, P. D., Jones, J. B., Chandler, C. K., Stall, R. E., and Berger, R. D. 1996. Survival of *Xanthomonas fragariae* on strawberry in summer nurseries in Florida detected by specific primers and nested polymerase chain reaction. Plant Dis. 80:1283-1288.
35. Stall, R. E., and Minsavage, G. V. 1990. The use of *hrp* genes to identify opportunistic xanthomonads. Pages 369-374 in: Proc. Int. Conf. Plant Pathog. Bact., 7th. Z. Klement, ed. Akadémiai Kiadó, Budapest.
36. Takahashi, Y., Omura, T., Hibino, H., and Sato, M. 1996. Detection and identification of *Pseudomonas syringae* pv. *atropurpurea* by PCR amplification of specific fragments from an indigenous plasmid. Plant Dis. 80:783-788.
37. Vauterin, L., Yang, P., Alvarez, A., Takikawa, Y., Roth, D. A., Vidaver, A. K., Stall, R. E., Kersters, K., and Swings, J. G. 1996. Identification of non-pathogenic *Xanthomonas* strains associated with plants. Syst. Appl. Microbiol. 19:96-105.
38. Verdier, V. 1988. Evaluation de la variabilité de *Xanthomonas campestris* pv. *manihotis*, agent causal de la bactériose vasculaire du manioc (*Manihot esculenta* Crantz). Ph.D. thesis. Université de Paris Sud-Orsay, Paris.
39. Verdier, V., Boher, B., Maraite, H., and Geiger, J. P. 1994. Pathological and molecular characterization of *Xanthomonas campestris* strains causing diseases of cassava (*Manihot esculenta*). Appl. Environ. Microbiol. 60: 4478-4486.
40. Verdier, V., Cuny, G., Assigbétsé, K., Geiger, J. P., and Boucher, C. 1996. Characterization of pathogenicity gene in *Xanthomonas campestris* pv. *manihotis*. Abstract G-71 in: Proc. Int. Congr. Mol. Plant Microbe Interact., 8th. G. Stacey, B. Mullin, and P. Gresshoff, eds. Knoxville, TN.
41. Verdier, V., Dongo, P., and Boher, B. 1993. Assessment of genetic diversity among strains of *Xanthomonas campestris* pv. *manihotis*. J. Gen. Microbiol. 139:2591-2601.
42. Young, J. M., Saddler, G. S., De Boer, S. H., Vauterin, L., Gardan, L., Gvozdyak, R. I., and Stead, D. E. 1996. Names of plant pathogenic bacteria 1864-1995. Rev. Plant Pathol. 75: 721-763.

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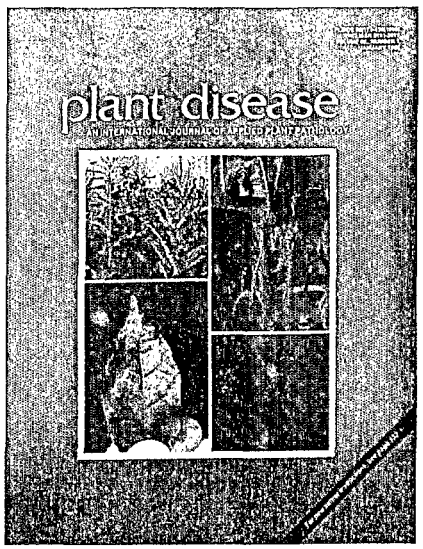

COVER

(*Clockwise from upper right*): Banana streak badnavirus in plantain (courtesy G. Dahal, see page 16); powdery mildew on poinsettia caused by an *Oidium* sp. (courtesy S. T. Koike, see page 128); leaf scorch of coffee caused by a strain of *Xylella fastidiosa* (courtesy J. E. O. de Lima et al., see page 94); sweet corn resistant and susceptible to northern leaf blight caused by *Exserohilum turcicum* (courtesy J. K. Pataky et al., see page 57).

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