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Leaf blight and defoliation caused by two new pathovars of *Xanthomonas axonopodis* on *Schinus terebinthifolius* and *Mabea fistulifera*

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Schinus terebinthifolius and *Mabea fistulifera* have been used for forest repositioning and urban forestry in Brazil. In October 2012, in a routine inspection at the research nursery of the Forestry Department of the Universidade Federal de Viçosa, in Minas Gerais, Brazil, a mortality of approximately 40% of the seedlings was observed as a result of diseases characterized by leaf blight and intense defoliation, which culminated in the death of the plants. Microscopy observations revealed oozing from the infected tissue and isolations revealed a bacterial aetiology for both diseases. Bacterial cells that formed bright yellow mucoid colonies with round edges were routinely isolated from lesion margins. Inoculation of isolated strains into healthy seedlings reproduced the symptoms observed under natural conditions. Bacterial cells showing the same morphological, biochemical and molecular characteristics as those originally isolated from naturally infected plants were reisolated from inoculated plants. Morphological, physiological and biochemical tests as well as 16S rDNA sequencing and multilocus sequence analysis using four housekeeping genes, *dnaK*, *fyuA*, *gyrB* and *rpoD*, confirmed the newly isolated strains belong to *Xanthomonas axonopodis*. Plant cross-inoculations showed the strains did not belong to any known phylogenetically related pathovar. Pathovars *X. axonopodis* pv. *schini* pv. nov. and *X. axonopodis* pv. *mabeae* pv. nov. are proposed as the causal agents of bacterial leaf blight on *S. terebinthifolius* and *M. fistulifera*, respectively.

Keywords: bacterial disease, fatty acid profiling, housekeeping genes, metabolic fingerprinting, multilocus sequence analysis

Introduction

Schinus terebinthifolius and *Mabea fistulifera* are trees native to Brazil. *Schinus terebinthifolius* is also native to Argentina, Paraguay and Uruguay. However, both trees have been introduced in tropical and subtropical areas around the world (Ewe & Sternberg, 2002). In Florida and Hawaii, *S. terebinthifolius* was introduced as an ornamental plant and has become one of the most aggressive invasive species (Ewe & Sternberg, 2002; Williams *et al.*, 2005). In Brazil, *S. terebinthifolius* is known as *aroeirinha*, *aroeira da praia*, *aroeira negra*, *aroeira vermelha*, *aroeira de Minas* and *corneiba*. In the United States, it is known as Brazilian peppertree and Florida holly (Morton, 1978). It is used mainly in urban forestry as an ornamental plant and in forest repositioning. Its pink/red seeds are used as a condiment due to their mild and slightly spicy flavour, and the seeds have also been used as anti-inflammatory, antipyretic, analgesic and cleansing agents (Carvalho *et al.*, 2013). *Mabea fistulifera* occurs widely in the Brazilian Cerrado and in transition areas of semideciduous forests in the states of Rio de Janeiro, Minas Gerais and São Paulo. It is an undemanding species that grows well in low-fertility soils and could be used in the recovery of degraded areas where recolonization is facilitated by several animal species that serve as potential dispersers of plant species (Leal Filho & Borges, 1992).

In October 2012, in a routine inspection at the Research Nursery of the Forestry Department of the Universidade Federal de Viçosa, in Minas Gerais, Brazil, approximately 40% of the seedlings of *S. terebinthifolius* and *M. fistulifera* were dying as a result of diseases characterized by leaf blight and intense defoliation. Previous reports indicated that *S. terebinthifolius* can be an alternative host for *Xanthomonas campestris* pv. *viticola*, which is the causal agent of grapevine canker (Peixoto *et al.*, 2007), and *Xanthomonas axonopodis* pv. *mangiferaindicae*, which is the causal agent of mango bacterial black spot (Pruvost *et al.*, 1992). One study has also reported that *Enterobacter cowanii* causes leaf spot on *M. fistulifera* (Furtado *et al.*, 2012).

The identification of *Xanthomonas* species has been based primarily on morphological, physiological and biochemical characteristics (Van den Mooter & Swings, 1990; Schaad *et al.*, 2001). However, these conventional

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methods are not adequate for distinguishing between pathovars of xanthomonads (Dye, 1962). Significant progress in the taxonomy of xanthomonads has been achieved with the use of DNA–DNA reassociation techniques (Vauterin *et al.*, 1995). The study by Vauterin *et al.* (1995) resulted in the recognition of 20 *Xanthomonas* species. Additionally, eight species of xanthomonads have been reclassified, including *X. cynarae* (Trébaol *et al.*, 2000), *X. euvesicatoria*, *X. gardneri*, *X. perforans* (Jones *et al.*, 2004) and *X. dyei* (Young *et al.*, 2010). Additional newly reclassified xanthomonads include *X. fuscans*, *X. fuscans* subsp. *fuscans*, *X. fuscans* subsp. *aurantifolii*, *X. alfalfae*, *X. alfalfae* subsp. *alfalfae*, *X. alfalfae* subsp. *citrumelonis* and *Xanthomonas citri* subsp. *malvacearum* (Schaad *et al.*, 2006). Currently, various other molecular techniques, such as 16S rDNA sequencing (Hauben *et al.*, 1997), repetitive sequence-based PCR (rep-PCR), enterobacterial repetitive intergenic consensus sequence (ERIC) PCR, box sequences (BOX) PCR (Rademaker *et al.*, 2005) and multilocus sequence analysis (MLSA; Almeida *et al.*, 2010; Young *et al.*, 2008) are used in phylogenetic studies of xanthomonads. Among these techniques, MLSA, which is based on sequencing several housekeeping genes, has been widely used for delimitation of plant pathogenic bacterial species (Young *et al.*, 2008, 2010; Ah-You *et al.*, 2009; Samanta *et al.*, 2013). MLSA is a simple method for rapidly and accurately allocating strains in their corresponding species within the genus, which reflects their allocation based on DNA–DNA reassociation (Young *et al.*, 2008). The objective of this study was to identify the causal agents responsible for bacterial leaf blight of *S. terebinthifolius* and *M. fistulifera*. The results indicated that bacteria belonging to two new pathovars of *X. axonopodis* were responsible for these diseases.

Materials and methods

Disease symptoms and bacterial isolation, cultivation and storage

Naturally infected tissue of plants growing in a nursery was collected from both host species between October and November of 2012. Microscopic oozing from leaf fragments (0.5 × 0.5 cm) collected from the lesion margins and placed on a drop of water was observed under a light microscope (×200). Samples showing bacterial oozing were disinfected in 50% ethanol and then in sodium hypochlorite containing 2% chlorine. After 3 min, the fragments were rinsed in sterile distilled water and then macerated in saline solution (0.85% NaCl). Loops of the macerate were streaked onto solid 523 medium (Kado & Heskett, 1970), and the plates were incubated at 28 °C in the dark. After 24–48 h of incubation, individual yellow colonies were transferred to fresh solid 523 medium. Purified cultures were maintained on medium 523 at 4 °C and stored in 30% glycerol at −80 °C for later use.

Pathogenicity tests

Sixty-day-old seedlings of *S. terebinthifolius* and *M. fistulifera* were transplanted to 2 L plastic bags containing Carolina Soil substrate (70% sphagnum, 20% roasted rice straw, 10% perlite; Carolina Soil do Brasil), enriched with superphosphate (6 kg m^{-3}) and Osmocote (19:6:10 at 1.5 kg m^{-3}; Scotts Australia Pty Ltd). Plants were maintained in a greenhouse and fertilized every 2 weeks with 100 mL of a 7.5 g L^{-1} Ouro Verde solution (15% N, 15% P_2O_5, 20% K_2O) at 6 g L^{-1} per plant.

The seedlings were inoculated 30 days after transplanting. Cultures were grown on solid 523 medium at 28 °C for 48 h in the dark. Five seedlings were separately spray inoculated with bacterial suspensions adjusted to 10^8 CFU mL^{-1} for each strain (Table 1). The inoculum was evenly sprayed on both sides of the leaves before reaching the surface run-off point. Following inoculation, the plants were incubated in a moist chamber at 25 °C for 24 h and subsequently maintained in a greenhouse (temperature 20 ± 5 °C and relative humidity 75 ± 5%). The pots were irrigated daily using tap water. Seedlings sprayed with sterile distilled water served as controls.

16S rDNA amplification and sequencing

DNA was extracted from bacterial cultures grown in liquid 523 medium at 28 °C for 48 h in the dark using the Wizard Genomic DNA purification kit (Promega) according to the manufacturer's instructions. The 16S rDNA gene region was amplified by PCR using primers fd2 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rP1 (5′-ACGGTTACCTTGTTACGACTT-3′) (Weisburg *et al.*, 1991). PCRs were performed in a 25 μL reaction containing 12.5 μL of Dream Taq PCR Master Mix 2× (MBI Fermentas), 1.5 μL of each primer (10 μM) and 2 μL of genomic DNA (10 ng μL^{-1}). Amplification was performed with an initial denaturation at 94 °C for 2 min; then 35 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 2 min; and a final extension at 72 °C for 5 min. The expected amplicon size was confirmed by agarose gel electrophoresis using 5 μL of PCR product. Fragments of the expected size were purified with the illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare). Sequencing was performed in a 3500XL Series Genetic Analyzer (Applied Biosystems) using the same primers used for PCR amplification. Subsequently, the sequences were compared with sequences available in GenBank (http://www.ncbi.nlm.nih.gov) using BLAST.

Biochemical and physiological tests

The strains were subjected to the following tests in the Laboratory Guide for Identification of Plant Pathogenic Bacteria (Schaad *et al.*, 2001): (i) Gram typing using Ryu's non-staining KOH test; (ii) formation of yellow colonies on yeast extract-dextrose-calcium carbonate agar medium (YDC); (iii) oxidase activity; (iv) starch hydrolysis; (v) catalase activity; (vi) gelatin liquefaction; (vii) H_2S production from cysteine; (viii) esculin hydrolysis; (ix) urease activity; (x) nitrate reduction; (xi) utilization of asparagine as a sole source of carbon and nitrogen; (xii) anaerobic growth; (xiii) production of fluorescent pigment on solid King's B medium; and (xiv) xanthomonadin production.

Metabolic fingerprinting

Strains were tested for their ability to utilize carbon sources using the Biolog GN microtitre plate system (Microlog 2, v. 3.5; Biolog Inc.). A single colony from cultures grown on nutrient agar (NA) medium (Difco) was transferred to trypticase soy agar (TSA) medium and incubated at 28 °C for 24 h. Bacterial

Table 1 *Xanthomonas* strains used in multilocus sequence analysis

Bacterial strain	Species	Synonym	GenBank accession number			
			dnaK	*fyuA*	*gyrB*	*rpoD*
ICMP 196[a]	*Xanthomonas albilineans*		—	—	EU498963	EU499082
ICMP 35[a]	*X. arboricola* pv. *juglandis*		EU498750	EU498852	EU498951	EU499070
ICMP 50[a]	*X. axonopodis* pv. *axonopodis*		EU498751	EU498853	EU498952	EU499071
ICMP 5718[b]	*X. axonopodis* pv. *alfalfae*	*X. alfalfae* subsp. *alfalfae*	EU498792	EU498894	EU499001	EU499120
ICMP 4765	*X. axonopodis* pv. *alfalfae*	*X. alfalfae* subsp. *alfalfae*	EU498788	EU498890	EU498997	EU499116
ICMP 8432	*X. axonopodis* pv. *aurantifolii*	*X. fuscans* subsp. *aurantifolii*	EU498811	EU498913	EU499027	EU499146
ICMP 194[b]	*X. axonopodis* pv. *begoniae*		EU498757	EU498859	EU498962	EU499081
ICMP 444[b]	*X. axonopodis* pv. *cajani*		EU498767	EU498869	EU498973	EU499092
ICMP 24[a]	*X. axonopodis* pv. *citri*	*X. citri* subsp. *citri*	EU498749	EU498851	EU498950	EU499069
ICMP 10009	*X. axonopodis* pv. *citrumelo*	*X. alfalfae* subsp. *citrumelonis*	EU498826	EU498926	EU499042	EU499162
ICMP 10010	*X. axonopodis* pv. *citrumelo*	*X. alfalfae* subsp. *citrumelonis*	EU498827	EU498927	EU499043	EU499163
ICMP10014	*X. axonopodis* pv. *citrumelo*	*X. alfalfae* subsp. *citrumelonis*	EU498828	EU498928	EU499044	EU499164
ICMP 5732[b]	*X. axonopodis* pv. *glycines*		EU498794	EU498896	EU499003	EU499122
ICMP 217	*X. axonopodis* pv. *malvacearum*	*X.citri* subsp. *malvacearum*	EU498760	EU498862	EU498966	EU499085
ICMP 5741[b]	*X. axonopodis* pv. *manihotis*		EU498796	EU498898	EU499006	EU499125
ICMP 5834[b]	*X. axonopodis* pv. *phaseoli*	*X. phaseoli*	EU498802	EU498904	EU499015	EU499134
ICMP 239[a]	*X. axonopodis* pv. *phaseoli* biovar *fuscans*	*X. fuscans* subsp. *fuscans*	EU498761	EU498863	EU498967	EU499086
ICMP 3031	*X. axonopodis* pv. *ricini*		EU498782	EU498884	EU498991	EU499110
ICMP 5757[b]	*X. axonopodis* pv. *vasculorum*		EU498798	EU498900	EU499011	EU499130
ICMP 333[b]	*X. axonopodis* pv. *vignicola*		EU498764	EU498866	EU498970	EU499089
ICMP 109[a]	*X. axonopodis* pv. *vesicatoria*	*X. euvesicatoria*	EU498754	EU498856	EU498955	EU499074
ICMP 1663	*X. axonopodis* pv. *vesicatoria*	*X. euvesicatoria*	EU498777	EU498879	EU498986	EU499105
ICMP 4739	*X. axonopodis* pv. *vesicatoria*	*X. euvesicatoria*	EU498790	EU498892	EU498999	EU499118
ICMP 8037	*X. axonopodis* pv. *vesicatoria*	*X. euvesicatoria*	EU498810	EU498912	EU499026	EU499145
ICMP 12545[a]	*X. bromi*		EU498837	EU498937	EU499052	EU499172
ICMP 13[a]	*X. campestris* pv. *campestris*		EU498747	EU498849	EU498948	EU499067
ICMP 204[a]	*X. cassavae*		EU498759	EU498861	EU498965	EU499084
ICMP 9513[a]	*X. codiaei*		EU498822	EU498922	EU499038	EU499158
ICMP 2299[a]	*X. cucurbitae*		EU498780	EU498882	EU498989	EU499108
ICMP 16775[a]	*X. cynarae*		EU498846	EU498946	EU499061	EU499181
ICMP 2415[a]	*X. dyei* pv. *dysoxyli*		EU498781	EU498883	EU498990	EU499109
ICMP 5715[a]	*X. frageriae*		EU498791	EU498893	EU499000	EU499119
ICMP 16689[a]	*X. gardneri*		EU498843	EU498943	EU499058	EU499178
ICMP 453[a]	*X. hortorum*		EU498769	EU498871	EU498975	EU499094
ICMP 189[a]	*X. hyacinthi*		—	—	EU498960	EU499079
ICMP 8683	*X. melonis*		EU498816	EU498916	EU499032	EU499152
ICMP 3125[a]	*X. oryzae* pv. *oryzae*		EU498784	EU498886	EU498993	EU499112
ICMP 16690[a]	*X. perforans*		EU498844	EU498944	EU499059	EU499179
ICMP 570[a]	*X. pisi*		EU498770	EU498872	EU498976	EU499095
ICMP 5816[a]	*X. populi*		EU498801	EU498903	EU499014	EU499133
ICMP 16916[a]	*X. sacchari*		—	—	EU499063	EU499183
ICMP 6774[a]	*X. theicola*		—	—	EU499020	EU499139
ICMP 5752[a]	*X. translucens* pv. *translucens*		—	—	EU499009	EU499128
ICMP 3103[a]	*X. vasicola*		EU498783	EU498885	EU498992	EU499111
ICMP 63[a]	*X. vesicatoria*		EU498753	EU498855	EU498954	EU499073
LPF 603	*X. axonopodis* (*Schinus terebinthifolius*)		KY205644	KY205653	KY273415	KY273424
LPF 604	*X. axonopodis* (*S. terebinthifolius*)		KY205645	KY205654	KY273416	KY273425
LPF 605	*X. axonopodis* (*S. terebinthifolius*)		KY205646	KY205655	KY273417	KY273426
LPF 606	*X. axonopodis* (*S. terebinthifolius*)		KY205647	KY205656	KY273418	KY273427
LPF 607	*X. axonopodis* (*Mabea fistulifera*)		KY205648	KY205657	KY273419	KY273428
LPF 608	*X. axonopodis* (*M. fistulifera*)		KY205649	KY205658	KY273420	KY273429
LPF 609	*X. axonopodis* (*M. fistulifera*)		KY205650	KY205659	KY273421	KY273430
LPF 610	*X. axonopodis* (*M. fistulifera*)		KY205651	KY205660	KY273422	KY273431
LPF 611	*X. axonopodis* (*M. fistulifera*)		KY205652	KY205661	KY273423	KY273432
ICMP 17033[a]	*Stenotrophomonas maltophilia*		EU498848	—	EU499066	EU499186

[a]Type strain of species.
[b]Pathotype strain.

colonies were harvested with a moistened sterile cotton swab suspended in sterile saline solution, and the optical density at 590 nm was adjusted to match the Biolog GN MicroPlate system's turbidity standards. Then, 150 μL of bacterial suspensions were pipetted into the wells of the microplates. Reactions were rated as positive or negative 72 h after incubation of the Biolog GN plates at 28 °C.

Fatty acid profiling

Whole cell fatty acid methyl ester (FAME) profiles were determined using the Microbial Identification System (MIS; MIDI Inc.). Bacterial strains were grown on TSA medium at 28 °C for 1 day. Fatty acids were extracted following the procedures described in the MIS Handbook and analysed with a 7890 gas chromatograph (Hewlett Packard). Fatty acid identification and quantification were automatically provided by the MIS software by comparison with internal FAME standards. Fatty acid profiles were compared to reference strains deposited in the MIS database, and an index that expressed the similarity of profiles of the test strains with profiles of known *Xanthomonas* species and pathovars deposited in the database were generated.

Multilocus sequence analysis

Phylogenetic analyses were performed using the four housekeeping genes *dnaK*, *fyuA*, *gyrB* and *rpoD* (Young *et al.*, 2008). The reaction mixture used for PCR was the same as for amplification of the 16S rDNA region, except for the sequence of the primers (Young *et al.*, 2008). PCR amplifications were performed with initial denaturation at 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min; and final extension at 72 °C for 10 min. Confirmation of amplicon sizes, amplicon purification and sequencing was conducted as described above.

Two phylogenetic analyses were performed. In the first analysis, nucleotide sequences of genes *dnaK*, *fyuA*, *gyrB* and *rpoD* of strains used in this study as well as sequences of the same four genes obtained from validly published *Xanthomonas* species (Bull *et al.*, 2010, 2012) were used. In the second analysis, only nucleotide sequences of *X. axonopodis* pathovars were used. Nucleotide sequences not determined in this study and used in both phylogenetic analyses were obtained from GenBank (Table 1). In this study, the *Xanthomonas* classification and nomenclature proposed by Vauterin *et al.* (1995) was adopted.

Maximum parsimony (MP) and Bayesian inference (BI) methods were used to construct phylogenetic trees. Analyses were conducted using the concatenated nucleotide sequences of the *dnaK*, *fyuA*, *gyrB* and *rpoD* genes. Maximum parsimony analysis was performed using PAUP* v. 4.0b10 using a heuristic search with the TBR (tree bisection reconnection) algorithm to switch the branches. Nucleotide sequences were added randomly by a stepwise method with 100 repetitions. Branch stability was assessed by bootstrap with 1000 replications. Bayesian inference was performed with MRBAYES v. 3.1.2 (Ronquist & Huelsenbeck, 2003). The substitution model was selected based on the Akaike information criterion (AIC) using MRMODELTEST v. 3.2. The probability of *a posteriori* tree distribution was calculated using MCMCMC (Metropolis-coupled Markov chain Monte Carlo) of two chains from a random tree with 10 million generations and discarding 25% of the first trees. The MCMCMC convergence and effective sample size were examined using the TRACER v. 1.4 program. Phylogenetic trees were viewed and edited with FIGTREE v. 1.3.1. (http://tree.bio.ed.ac.uk/software/figtree).

Plant cross-inoculation with *X. axonopodis* pathovars

Cross-inoculation of host plants was performed with pathovars of *X. axonopodis* that were phylogenetically more closely related to the strains isolated from *S. terebinthifolius* and *M. fistulifera* in this study. Cross-inoculations were conducted using representative and designated type strains *X. axonopodis* pv. *schini* LPF 603 and *X. axonopodis* pv. *mabeae* LPF 607. Based on the results of MLSA, strain LPF 603 was inoculated onto plants of cassava (*Manihot esculenta*), common bean (*Phaseolus vulgaris*) and *M. fistulifera*. Strain LPF 607 was inoculated onto sweet pepper (*Capsicum annuum*), castor bean (*Ricinus communis*) and *S. terebinthifolius*. Representative strains of *X. axonopodis* pv. *manihotis* (IBSBF 278 = ICMP 5741 = LMG 784) and *X. axonopodis* pv. *phaseoli* (*X. phaseoli*) (IBSBF 1346 = ICMP 5834) were inoculated onto *S. terebinthifolius* as well as onto susceptible plants of their corresponding host species. In addition, representative strains of *X. axonopodis* pv. *vesicatoria* (*X. euvesicatoria*) (IBSBF 345 = ICMP 8037) and *X. axonopodis* pv. *ricini* (IBSBF 318 = ICMP 7463) were inoculated onto *M. fistulifera* as well as onto susceptible plants of their corresponding host species. Plant maintenance, inoculum preparation and inoculation were conducted as described for pathogenicity tests. Bacterial strains inoculated onto their respective host species served as positive controls for disease.

Results

Disease symptoms and bacterial isolation

On both plant species, disease symptoms were characterized by water-soaked, angular and interveinal lesions on the leaves. As the disease progressed, the lesions coalesced and ranged from brown to black, and often occupied a large portion of the leaf area (Fig. 1a,b). Leaf defoliation was observed at advanced stages of infection, and in some cases the seedlings died. Bacteria were easily isolated on solid 523 medium from disease lesions of both host species and formed bright yellow colonies after 24–48 h of incubation at 28 °C. Four strains recovered from *S. terebinthifolius* (LPF 603, LPF 604, LPF 605 and LPF 606) and five recovered from *M. fistulifera* (LPF 607, LPF 608, LPF 609, LPF 610 and LPF 611) were selected for further characterization in this study.

Inoculation of healthy host plants reproduced the disease symptoms observed under natural infection. Leaf blight symptoms were observed 10 days after inoculation (dai) of *S. terebinthifolius* with LPF 603 (Fig. 1c) and 7 dai of *M. fistulifera* with LPF 607 (Fig. 1d). At approximately 20 dai, defoliation was observed on both host species. Plants sprayed with water showed no disease symptoms. Bacterial colonies with the same morphological characteristics as those of strains initially recovered from natural infections were reisolated from inoculated plants and their identities were confirmed to complete Koch's postulates.

16S rDNA sequence analysis

To determine the taxonomic position of the strains recovered from *S. terebinthifolius* and *M. fistulifera*, their

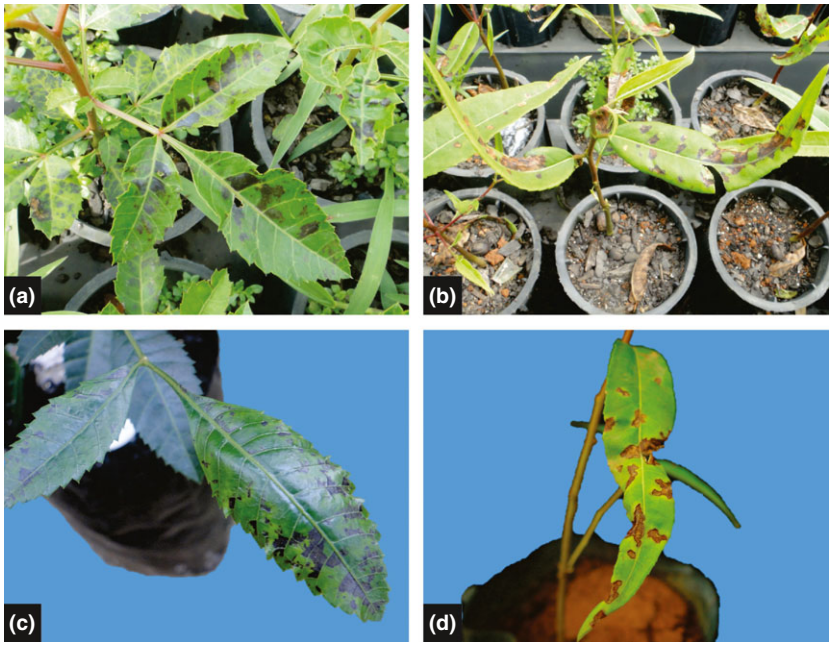

Figure 1 Symptoms of leaf blight on *Schinus terebinthifolius* and *Mabea fistulifera*. (a) Leaf blight on *S. terebinthifolius* seedlings caused by natural infection in nurseries, (b) leaf blight on *M. fistulifera* seedlings caused by natural infection in nurseries, (c) symptoms caused by strain LPF 603 after artificial inoculation on *S. terebinthifolius*, and (d) symptoms caused by strain LPF 607 after artificial inoculation on *M. fistulifera*. [Colour figure can be viewed at wileyonlinelibrary.com]

partial 16S rDNA sequences were first compared to sequences present in the NCBI nonredundant databases using BLAST. The 16S rDNA sequence of LPF 603 showed higher than 99% identity with sequences of *X. citri* subsp. *citri* (GeneBank accession no. CP008989), *X. axonopodis* pv. *glycines* (KM593178) and *X. campestris* pv. *viticola* (JQ513818). The 16S rDNA sequence of LPF 607 showed higher than 99% identity with the sequences of *X. fuscans* subsp. *fuscans* (FO681494), *X. axonopodis* pv. *diffenbachiae* (KM576803) and *X. campestris* pv. *coriandri* (AY601494). Therefore, LPF 603 and LPF 607 were identified as belonging to the genus *Xanthomonas*.

Biochemical and physiological characterization

Bacterial strains recovered from both hosts were typical of *Xanthomonas* spp. They were Gram-negative and formed yellow and mucilaginous colonies on YDC agar medium. They were positive for starch utilization, catalase, gelatine liquefaction, H_2S production and esculin hydrolysis. All strains produced xanthomonadin and were negative for oxidase, urease, nitrate reductase and asparaginase. They did not grow under anaerobic conditions and did not produce fluorescent pigments on King's B medium.

Metabolic fingerprinting

The metabolic profile of strains recovered from both hosts determined using the Biolog system was the same as previously described for *X. axonopodis* (Vauterin *et al.*, 1995). The only exception with the newly isolated strains was their inability to metabolize succinamic acid. Strains from *S. terebinthifolius* differed in the profile of carbon source utilization from strains recovered from *M. fistulifera* (Table 2). Strains from *S. terebinthifolius* showed positive reactions for 38 carbon sources, whereas strains isolated from *M. fistulifera* were able to utilize 34 out of 95 carbon sources tested in the Biolog system (Table 2). Strains isolated from *M. fistulifera* (LPF 607–611) had a positive reaction for all carbon sources used by *S. terebinthifolius* strains (LPF 603–606), except for acetic acid, α-ketobutyric acid, propionic acid and L-aspartic acid.

Fatty acid analysis

Fatty acid profiles of the strains isolated from both hosts were the same as the profiles for *X. axonopodis* (Table 3) reported by Vauterin *et al.* (1996). The profile of strains recovered from *S. terebinthifolius* more closely resembled the profile of *X. axonopodis* pv. *glycines* and *X. axonopodis* pv. *manihotis* with similarity indices (SI) of 0.76 and 0.71, respectively. The profiles of strains obtained from *M. fistulifera* were more similar to *X. axonopodis* pv. *glycines* (SI = 0.75) and *X. axonopodis* pv. *manihotis* (SI = 0.73). In contrast, *X. axonopodis* pv. *phaseoli* var. *fuscans* (*X. fuscans* subsp. *fuscans*) had a SI of only 0.50.

Multilocus sequence analysis

Two phylogenetic analyses were performed. The first was to position the bacterial strains recovered from *S. terebinthifolius* and *M. fistulifera* in the genus *Xanthomonas*, and the second was to position them within the species *X. axonopodis*. In both phylogenetic analyses, partial sequences of 928, 698, 829 and 856 bp were used for *dnaK*, *fyuA*, *gyrB* and *rpoD*, respectively. The total length of the alignment obtained with concatenated

Table 2 Characterization of *Xanthomonas axonopodis* strains based on the Biolog test

Carbon source	*Schinus terebinthifolius* (LPF 603–606)	*Mabea fistulifera* (LPF 607–611)	Vauterin *et al.* (1995)[a]
α-cyclodextrin	–	–	–
Dextrin	+	+	+
Glycogen	+	+	V
Tween 40	+	+	V
Tween 80	–	–	V
N-acetyl-D-galactosamine	–	–	V
N-acetyl-D-glucosamine	+	+	V
Adonitol	–	–	–
L-arabinose	–	–	V
D-arabitol	–	–	–
D-cellobiose	+	+	+
meso-erythritol	–	–	–
D-fructose	+	+	+
L-fucose	+	+	V
D-galactose	+	+	V
Gentiobiose	+	+	+
α-D-glucose	+	+	+
m-inositol	–	–	–
α-D-lactose	–	–	V
Lactulose	+	+	V
Maltose	+	+	+
D-mannitol	–	–	V
D-mannose	+	+	+
D-melibiose	–	–	V
β-methyl-D-glucoside	–	–	–
D-psicose	+	+	+
D-raffinose	–	–	V
L-rhamnose	–	–	–
D-sorbitol	–	–	V
Sucrose	+	+	V
D-trehalose	+	+	+
Turanose	–	–	V
Xylitol	–	–	–
Methyl pyruvate	+	+	+
Monomethyl succinate	+	+	+
Acetic acid	+	–	V
Cis-aconitic acid	+	+	V
Citric acid	+	+	V
Formic acid	–	–	–
D-galactonic acid lactone	–	–	–
D-galacturonic acid	–	–	–
D-gluconic acid	–	–	V
D-glucosaminic acid	–	–	–
D-glucuronic acid	–	–	–
α-hydroxybutyric acid	–	–	V
β-hydroxybutyric acid	–	–	V
γ-hydroxybutyric acid	–	–	–
p-hydroxyphenylacetic acid	–	–	–
Itaconic acid	–	–	–
α-ketobutyric acid	+	–	V
α-ketoglutaric acid	+	+	+
α-ketovaleric acid	–	–	–
DL-lactic acid	–	–	V
Malonic acid	+	+	V
Propionic acid	+	–	V
Quinic acid	–	–	–
D-saccharic acid	–	–	V
Sebacic acid	–	–	–
Succinic acid	+	+	+
Bromosuccinic acid	+	+	+
Succinamic acid	–	–	+
Glucuronamide	–	–	–
L-alaninamide	+	+	V
D-alanine	+	+	+
L-alanine	+	+	+
L-alanylglycine	+	+	+
L-asparagine	–	–	V
L-aspartic acid	+	–	V
L-glutamic acid	+	+	+
Glycyl-L-aspartic acid	–	–	V
Glycyl-L-glutamic acid	+	+	+
L-histidine	–	–	V
Hydroxy-L-proline	+	+	V
L-leucine	–	–	V
L-ornithine	–	–	V
L-phenylalanine	–	–	–
L-proline	+	+	V
L-pyroglutamic acid	–	–	–
D-serine	–	–	–
L-serine	+	+	V
L-threonine	–	–	V
DL-carnitine	–	–	–
γ-aminobutyric acid	–	–	–
Urocanic acid	–	–	V
Inosine	–	–	V
Uridine	–	–	V
Thymidine	–	–	–
Phenyethylamine	–	–	–
Putrescine	–	–	–
2-aminoethanol	–	–	–
2,3-butanediol	–	–	–
Glycerol	+	+	V
DL-α-glycerol phosphate	–	–	V
Glucose-1-phosphate	–	–	V
Glucose-6-phosphate	–	–	V

[a]Strains of *X. axonopodis* used by Vauterin *et al.* (1995): +, ≥90% of strains are positive; –, ≥90% of strains are negative; V, 11–89% of strains were able to metabolize the carbon source.

sequences of the four genes was 3311 bp. In the first phylogenetic analysis, including multiple *Xanthomonas* species, the number of conserved sites was 705, 471, 416 and 504 and the number of variable sites was 223, 227, 413 and 351 for *dnaK*, *fyuA*, *gyrB* and *rpoD*, respectively. The number of parsimony informative sites was 92, 152, 248 and 230 for *dnaK*, *fyuA*, *gyrB* and *rpoD*, respectively. The best evolutionary model selected by MrModeltest v. 2.3 for Bayesian analysis by AIC was the general time reversible plus invariant site plus gamma

Table 3 Summary of the fatty acid composition of strains from *Schinus terebinthifolius* (LPF 603–606) and *Mabea fistulifera* (LPF 607–611)

Compound	*S. terebinthifolius*	*M. fistulifera*
Saturated fatty acids		
10:0	1.09 ± 0.09	1.10 ± 0.17
14:0	1.90 ± 0.22	2.04 ± 0.24
16:0	6.70 ± 0.52	6.96 ± 0.39
17:0	0.18 ± 0.01	0.10 ± 0.00[c]
Unsaturated fatty acids		
15:1 w6c	0.58 ± 0.05	0.32 ± 0.02
16:1 w7c/16:1 w6c	23.56 ± 1.45	25.07 ± 1.51
16:1 w5c	0.16 ± 0.03[a]	0.12 ± 0.04[b]
16:0 10-methyl/17:1 iso w9c	4.24 ± 0.34	4.27 ± 0.51
17:1 w8c	1.39 ± 0.09	0.90 ± 0.06
17:1 w6c	0.26 ± 0.01	0.19 ± 0.01
18:1 w9c	0.57 ± 0.03	0.61 ± 0.03
18:1 w7c/18:1 w6c	0.76 ± 0.05	0.68 ± 0.03
Hydroxy fatty acids		
10:0 3OH	0.41 ± 0.03	0.42 ± 0.04
10:0 2OH	0.21 ± 0.03	0.21 ± 0.03
11:0 3OH	0.35 ± 0.01	0.24 ± 0.01
12:0 3OH	4.08 ± 0.31	4.03 ± 0.60
16:0 3OH	0.17 ± 0.04	0.20 ± 0.06
Branched-chain fatty acids		
11:0 iso	4.32 ± 0.26	4.00 ± 0.16
13:0 iso	0.28 ± 0.01	0.26 ± 0.01
14:0 iso	0.27 ± 0.01	0.27 ± 0.01
15:0 iso	27.83 ± 0.69	27.25 ± 0.87
15:0 anteiso	5.53 ± 0.41	6.53 ± 0.41
16:0 iso	1.04 ± 0.07	1.06 ± 0.10
17:0 iso	6.77 ± 0.48	6.15 ± 0.63
17:0 anteiso	0.30 ± 0.03	0.35 ± 0.04
Branched-chain unsaturated fatty acids		
15:1 iso F	0.23 ± 0.02	0.27 ± 0.05
Branched-chain hydroxy fatty acids		
12:0 iso 3OH	0.18 ± 0.01[a]	0.18 ± 0.01[c]
17:0 iso 3OH	0.29 ± 0.02	0.28 ± 0.03

[a]Fatty acid is absent in one strain.
[b]Fatty acid absent in two strains.
[c]Fatty acid absent in three strains.

(GTR + I + G) for all four genes included in this species-level analysis. The results indicate that strains recovered from both plant species cluster together with *X. axonopodis* (Fig. 2).

In the second phylogenetic analysis, which included several species and pathovars of *X. axonopodis*, the numbers of conserved and variable sites were 843 and 87 for *dnaK*, 572 and 126 for *fyuA*, 672 and 158 for *gyrB*, and 750 and 107 for *rpoD*, respectively. The number of parsimony informative sites was 58 for *dnaK*, 59 for *fyuA*, 69 for *gyrB*, and 54 for *rpoD*. In this case, MrModeltest v. 2.3 selected the GTR + G model for all genes, except *dnaK*, for which the best fit was the GTR + I + G model. The results showed the strains recovered from *S. terebinthifolius* (LPF 603–606) grouped together in RG 9.4 (Rademaker *et al.*, 2005) and were phylogenetically more closely related to *X. axonopodis* pv. *manihotis* and *X. axonopodis* pv. *phaseoli* (*X. phaseoli*, Gabriel *et al.*, 1989). Strains recovered from *M. fistulifera* (LFP 607–611) grouped together in RG 9.2 (Rademaker *et al.*, 2005) and were more closely related to *X. axonopodis* pv. *vesicatoria* (*X. euvesicatoria*, Jones *et al.*, 2004), *X. axonopodis* pv. *ricini*, *X. axonopodis* pv. *alfalfae* (*X. alfalfae*, Schaad *et al.*, 2006), *X. axonopodis* pv. *citrumelo* (*X. alfalfae* subsp. *citrumelonis*, Schaad *et al.*, 2006) and *X. perforans* (Jones *et al.*, 2004) (Fig. 3).

Plant cross-inoculations

Taken together, the results of the MLSA and fatty acid profiling indicated that the newly isolated strains were more closely related to *X. axonopodis* pv. *manihotis*, *X. axonopodis* pv. *phaseoli* (*X. phaseoli*), *X. axonopodis* pv. *vesicatoria* (*X. euvesicatoria*) and *X. axonopodis* pv. *ricini*, but no study has reported that any of these pathovars were able to cause disease on *S. terebinthifolius* and/or *M. fistulifera*. Therefore, it was decided to inoculate susceptible host plants with their cognate pathovars as well as with LPF 603 and LPF 607. *Mabea fistulifera* and *S. terebinthifolius* were also inoculated with each bacterial strain that was tested. All strains belonging to previously reported pathovars caused disease on their respective host species, but did not cause disease on *M. fistulifera* or on *S. terebinthifolius*. LPF 603 caused disease only on *M. fistulifera* whereas LPF 607 was able to infect only *S. terebinthifolius* (Table 4). These results indicate that the strains newly isolated from *M. fistulifera* and *S. terebinthifolius* belong to two pathovars that have not been previously reported.

Discussion

In an attempt to identify the causal agents responsible for leaf blight observed on *M. fistulifera* and *S. terebinthifolius* during an inspection of a nursery in the Brazilian state of Minas Gerais, yellow-pigmented bacteria were isolated, from which the disease symptoms could be reproduced by inoculation into healthy plants and completing Koch's postulates. Based on physiological and biochemical tests as well as the sequence of their 16S rDNA region, the newly isolated strains were tentatively identified to be xanthomonads. The strains could not be assigned to a defined species within *Xanthomonas* either by comparison of the 16S rDNA sequences with sequences deposited in the databases or from the results of the biochemical and physiological tests (Hauben *et al.*, 1997).

Metabolic fingerprinting showed that all strains isolated in this study were able to utilize the same carbon sources as previously described for *X. axonopodis* (Vauterin *et al.*, 1995). Additionally, fatty acid profiles were most similar to profiles of other pathovars of the species *X. axonopodis*. Nevertheless, it was not possible to conclusively assign the newly isolated strains to any pathovar.

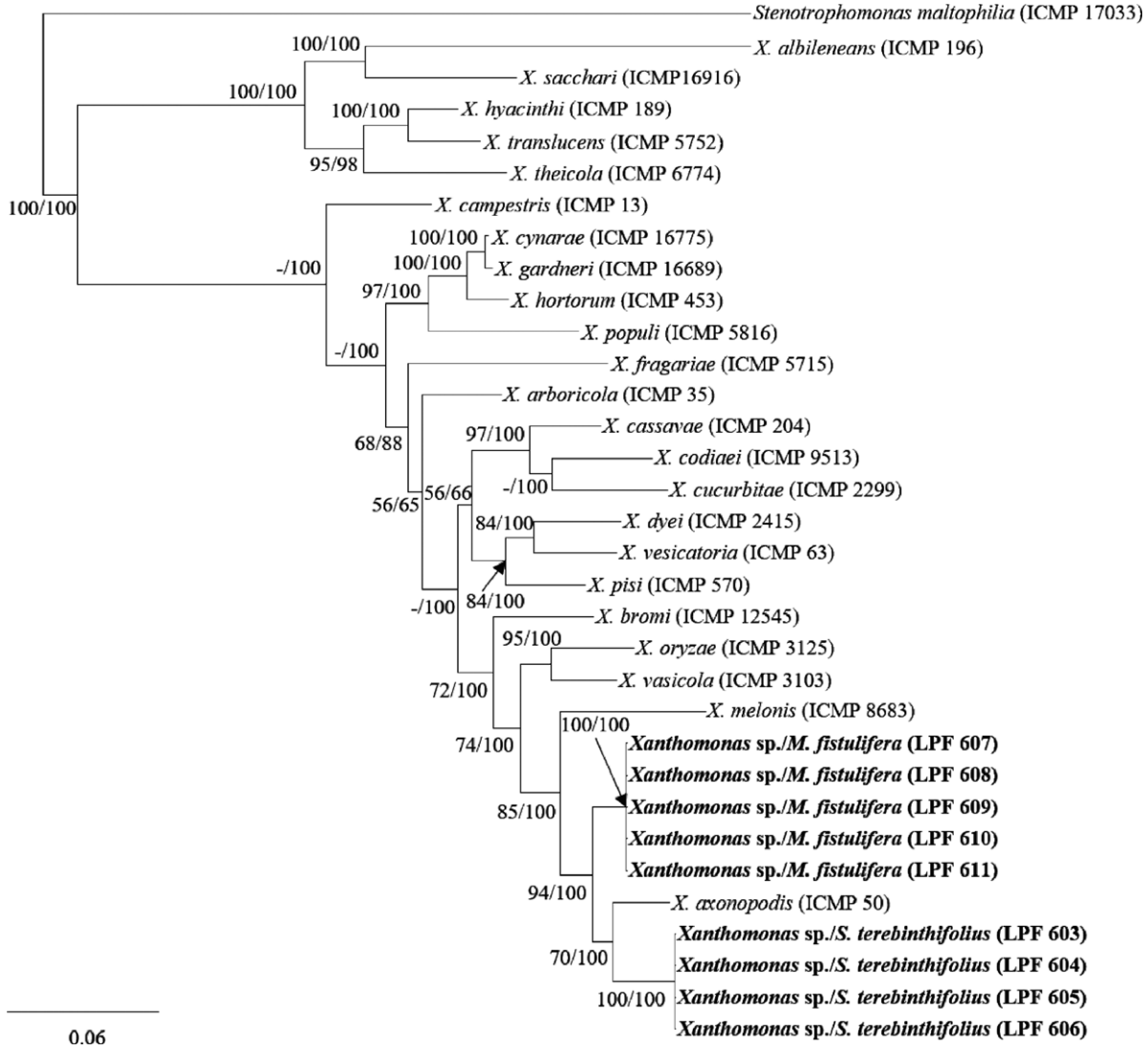

Figure 2 Phylogenetic tree of concatenated *dnaK*, *fyuA*, *gyrB* and *rpoD* partial nucleotide sequences constructed by Bayesian inference. Bootstrap values are indicated above the branches while posterior probabilities are indicated after the slashes. Bar indicates the fraction of substitutions per site. The *Xanthomonas* species included in this analysis are the same as those included in Young *et al.* (2008). *Stenotrophomonas maltophilia* (ICMP 17033) is used as an out-group.

MLSA was then used to delimit the strains isolated from *S. terebinthifolius* and *M. fistulifera* within *X. axonopodis*. Strains isolated from *S. terebinthifolius* clustered in group RG 9.4 (Rademaker *et al.*, 2005) along with the *X. axonopodis* pv. *manihotis* and *X. axonopodis* pv. *phaseoli* (*X. phaseoli*) and strains from *M. fistulifera* grouped in RG 9.2 along with pathovars *X. axonopodis* pv. *ricini* and *X. axonopodis* pv. *vesicatoria* (*X. euvesicatoria*). Rademaker *et al.* (2005) previously showed that the species *X. axonopodis* could be divided into groups (RG) 9.1–9.6 based on rep-PCR analysis. Group RG 9.2 was composed of a large number of pathovars including *alfalfae* (*X. alfalfae* subsp. *alfalfae*), *cassavae*, *cassiae*, *citrumelo* (*X. alfalfae* pv. *citrumelonis*), *coracanae*, *cyamopsidis*, *desmodii*, *desmodiigangetici*, *desmodiirotundifolii*, *erythrinae*, *lespedezae*, *patelii*, *phyllanthi*, *poinsettiicola*, *ricini*, *tamarindi* and *vesicatoria* (*X. euvesicatoria*); while RG 9.4 included pathovars *manihotis*, *phaseoli* and *dieffenbachiae* (Rademaker *et al.*, 2005). Other studies have proposed the reclassification of several pathovars of group RG 9.2 to the species level based on results of more recent DNA–DNA reassociation. Jones *et al.* (2004) proposed the reclassification of phenotypic groups A and C of *X. axonopodis* pv. *vesicatoria* to the species *X. euvesicatoria* and *X. gardneri*, respectively. Similarly, *X. axonopodis* pv. *alfalfae* was reclassified to *X. alfalfae* subsp. *alfalfae* (Schaad *et al.*, 2005, 2006). It was also proposed to reclassify the *fuscans* variant of *X. axonopodis* to *X. fuscans* subsp. *fuscans* (Schaad *et al.*,

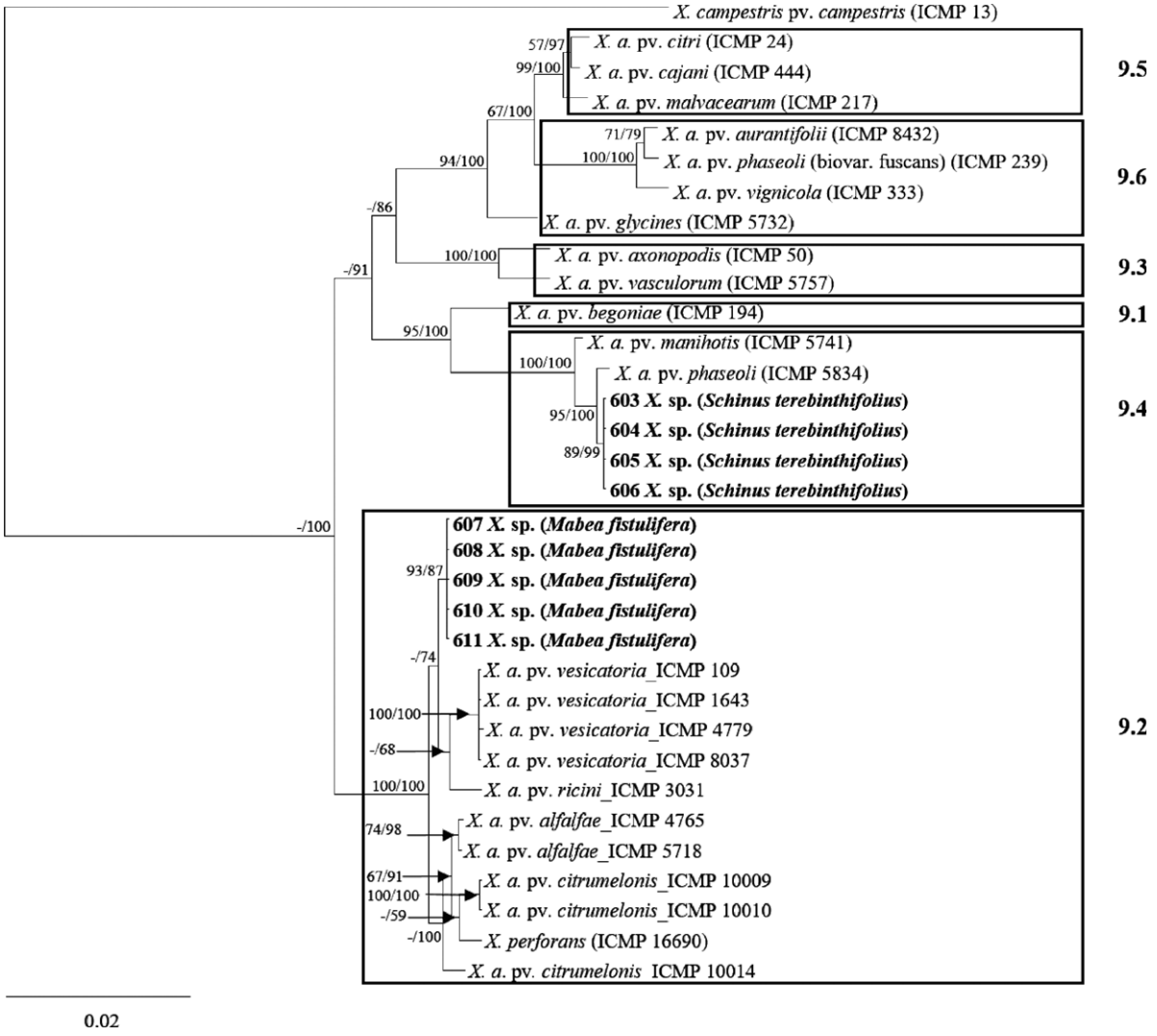

Figure 3 Phylogenetic tree of concatenated *dnaK*, *fyuA*, *gyrB* and *rpoD* partial nucleotide sequences constructed by Bayesian inference. Bootstrap values are indicated above the branches while posterior probabilities are indicated after the slashes. *Xanthomonas axonopodis* (*X. a.*) pathovars are grouped according to Rademaker *et al.* (2005). The bar indicates the number of substitutions per site. *Xanthomonas campestris* pv. *campestris* (ICMP 13) is used as an out-group.

2005, 2006). *Xanthomonas citri* subsp. *citri* and *X. citri* subsp. *malvacearum* have been validly published (Gabriel *et al.*, 1989). According to Bull *et al.* (2010), there are currently at least 41 validly published names for pathovars of *X. axonopodis*. In this study, the taxonomic classification and nomenclature widely accepted and used by Vauterin *et al.* (1995) and Rademaker *et al.* (2005) was adopted to assign a taxonomic position to the pathogenic strains isolated from *S. terebinthifolius* and *M. fistulifera* within the genus *Xanthomonas*. Overall, the results suggested that strains isolated from these plant species belonged to two newly defined *X. axonopodis* pathovars.

To position strains from *M. fistulifera* and *S. terebinthifolius* into specific pathovars within *X. axonopodis*, the sequences of four housekeeping genes were compared with sequences of well-characterized *X. axonopodis* pathovars. Strains isolated from *M. fistulifera* were found to be more closely related to the pathovars *vesicatoria* (*X. euvesicatoria*) and *ricini*, and those isolated from *S. terebinthifolius* were more closely related to pathovars *manihotis* and *phaseoli* (*X. phaseoli*, Gabriel *et al.*, 1989). However, no *X. axonopodis* pathovar has been reported to cause disease on *M. fistulifera*. On the other hand, there was one report indicating that *S. terebinthifolius* could be an alternate host for *X. campestris* pv. *viticola*, which is the causal agent of bacterial canker in grapevine (Peixoto *et al.*, 2007). Additionally, pathovars *mangiferaeindicae*, *anacardii* and *spondiae* are pathogenic to some members of the family Anacardiaceae. These pathovars can also infect *S. terebinthifolius*, which belongs to the same botanical family (Ah-You *et al.*, 2007), but only *X. axonopodis*

Table 4 Plant cross-inoculations

Pathogen	Strain	Source plant	Inoculated plant					
			Schinus terebinthifolius	*Mabea fistulifera*	*Capsicum annuum*	*Manihot esculenta*	*Phaseolus vulgaris*	*Ricinus communis*
Xanthomonas axonopodis	LPF 603	*S. terebinthifolius*	+	–	ND	–	–	ND
X. axonopodis	LPF 607	*M. fistulifera*	–	+	–	ND	ND	–
X. axonopodis pv. *vesicatoria*	IBSBF 345/ICMP 8037	*C. annuum*	ND	–	+	ND	ND	ND
X. axonopodis pv. *manihotis*	IBSBF 278/ICMP 5741/LMG 784	*M. esculenta*	–	ND	ND	+	ND	ND
X. axonopodis pv. *phaseoli*	IBSBF 2930/ATCC 9563/NCPPB 3035/LMG 7455/CFBP 2534	*P. vulgaris*	–	ND	ND	ND	+	ND
X.axonopodis pv. *ricini*	IBSBF 2939/ICMP 5747	*R. communis*	ND	–	ND	ND	ND	+

+, Disease symptoms; –, no symptoms; ND, not determined.

pv. *mangiferaeindicae* was found to naturally cause disease on *S. terebinthifolius* (Pruvost *et al.*, 1992). Pathovars *mangiferaeindicae* and *anacardii* belong to RG 9.5 and RG 9.6, respectively (Ah-You *et al.*, 2007), which ruled out the possibility that the strains isolated from *S. terebinthifolius* in this study, which clustered in RG 9.4, belong to either of these two pathovars. Furthermore, *X. axonopodis* pv. *spondiae*, which causes leaf spots on mombins (*Spondias dulcis*), belongs to the same RG as the strains isolated from *S. terebinthifolius*. However, colonies of *X. axonopodis* pv. *spondiae* are white (atypical of a xanthomonad), whereas the strains recovered from *S. terebinthifolius* are yellow. Moreover, *X. axonopodis* pv. *spondiae* has only been reported in the French Antilles (Ah-You *et al.*, 2007). Those strains isolated from *M. fistulifera* or *S. terebinthifolius* failed to cause disease on *C. annuum*, *M. esculenta*, *P. vulgaris* or *R. communis*, which therefore rules out the possibility that they belong to the previously reported pathovars *vesicatoria* (*X. euvesicatoria*), *manihotis*, *phaseoli* (*X. phaseoli*) or *ricini*. Furthermore, strains representative of these pathovars did not cause disease on either *M. fistulifera* or *S. terebinthifolius*. The reproduction of disease symptoms only in their respective source plant species indicated that these bacteria showed host specificity and belonged to different pathovars.

The polyphasic approach used in this study was a robust method for bacterial taxonomy (Young *et al.*, 2008; Samanta *et al.*, 2013). This approach encompassed morphological, physiological, biochemical, molecular and pathogenic characterization of the bacterial strains recovered from S. *terebinthifolius* and *M. fistulifera*. Comparisons of the results of these analyses with other xanthomonads revealed that they were not identical to any other pathovar that has been previously reported, and most importantly, they were distinctively pathogenic in the plant species from which they were isolated. Consequently, two new pathovars, *X. axonopodis* pv. *schini* pv. nov. infecting *S. terebinthifolius* and *X. axonopodis* pv. *mabeae* pv. nov. infecting *M. fistulifera* are proposed and described in this study.

Description of *Xanthomonas axonopodis* pv. *schini* pv. nov

Xanthomonas axonopodis pv. *schini* (shi'ni. N.L. gen. n. *schini* of *Schinus*, referring to the isolation source of the type strain).

The phenotypic description of the pathovar is based on representative strain LPF 603. The cells are Gram-negative and form yellow and mucilaginous colonies with abundant slime formation on YDC medium. The strains were positive for starch utilization, catalase, gelatine liquefaction, H_2S production and esculin hydrolysis. They produced xanthomonadin on NA medium; were negative for oxidase, urease, nitrate reductase; did not grow under anaerobic conditions; and did not produce fluorescent pigments on King's B medium. They were not able to utilize asparagine as a sole source of carbon and nitrogen.

The strains had positive auxanographic reactions for dextrin, glycogen, Tween 40, *N*-acetyl-D-glucosamine, D-cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, lactulose, maltose, D-mannose, D-psicose, sucrose, D-trehalose, methyl pyruvate, monomethyl-succinate, acetic acid, *cis*-aconitic acid, citric acid, α-ketobutyric acid, α-ketoglutaric acid, malonic acid, propionic acid, succinic acid, bromosuccinic acid, L-alaninamide, D-alanine, L-alanine, L-alanylglycine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, hydroxy-L-proline, L-proline, L-serine and glycerol. Negative reactions were observed for α-cyclodextrin, Tween 80, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *meso*-erythritol, *m*-inositol, α-D-lactose, D-mannitol, β-methyl-D-glucoside, D-raffinose, L-rhamnose, D-sorbitol, turanose, xylitol, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-

gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketovaleric acid, DL-lactic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, L-asparagine, glycyl-L-aspartic acid, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenyethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, DL-α-glycerol phosphate, glucose-1-phosphate and glucose-6-phosphate.

The type strain is LPF 603, which caused bacterial leaf blight on *Schinus terenbithifolius* and was isolated from infected tissue in the municipality of Viçosa in the state of Minas Gerais, Brazil. GenBank accession numbers of the 16S rDNA, *dnaK*, *fyuA*, *gyrB* and *rpoD* sequences of the type strain are KY271338, KY205644, KY205653, KY273415 and KY273424, respectively.

Description of *X. axonopodis* pv. *mabeae* pv. nov

Xanthomonas axonopodis pv. *mabeae* (ma.be'ae. N.L. gen. n. *mabeae* of *Mabea*, referring to the isolation source of the type strain).

The phenotypic description of the pathovar is based on representative strain LPF 607. Cells exhibited the same morphological, physiological and biochemical characteristics as the cells described above for pathovar *schini*, except they showed negative auxotrophic reactions for acetic acid, α-ketobutyric acid, propionic acid and L-aspartic acid. This pathovar also differs from pathovar *schini* because of its distinctive pathogenicity on *M. fistulifera* and not on *S. terebinthifolius*.

The type strain is LPF 607, which caused bacterial leaf blight on *M. fistulifera* and was isolated from infected tissue in the municipality of Viçosa in the state of Minas Gerais, Brazil. GenBank accession numbers of the 16S rDNA, *dnaK*, *fyuA*, *gyrB* and *rpoD* sequences of the type strain are KY287944, KY205648, KY205657, KY273419 and KY273428, respectively.

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