Structure and Origin of *Xanthomonas arboricola* pv. *pruni* Populations Causing Bacterial Spot of Stone Fruit Trees in Western Europe

Sylvain Boudon, Charles Manceau, and Jean-Loup Nottéghem

First and third authors: UMR BGPI, Campus International de Baillarguet, TA 41/K, 34398 Montpellier Cedex 05, France; and second author: UMR PaVé: INRA, 42 rue Georges Morel—BP 60057 49071, Beaucouzé Cedex, France.
Accepted for publication 6 May 2005.

ABSTRACT

Boudon, S., Manceau, C., and Nottéghem, J.-L. 2005. Structure and origin of *Xanthomonas arboricola* pv. *pruni* populations causing bacterial spot of stone fruit trees in Western Europe. *Phytopathology* 95:1081-1088.

Xanthomonas arboricola pv. *pruni*, the causal agent of bacterial spot on stone fruit, was found in 1995 in several orchards in southeastern France. We studied population genetics of this emerging pathogen in comparison with populations from the United States, where the disease was first described, and from Italy, where the disease has occurred since 1920. Four housekeeping genes (*atpD*, *dnaK*, *efp*, and *glnA*) and the intergenic transcribed spacer region were sequenced from a total of 3.9 kb of sequences, and fluorescent amplified fragment length polymorphism

(FAFLP) analysis was performed. A collection of 64 *X. arboricola* pv. *pruni* strains, including 23 strains from France, was analyzed. The *X. arboricola* pv. *pruni* population had a low diversity because no sequence polymorphisms were observed. Population diversity revealed by FAFLP was lower for the West European population than for the American population. The same bacterial genotype was detected from five countries on three continents, a geographic distribution that can be explained by human-aided migration of bacteria. Our data support the hypothesis that the pathogen originated in the United States and subsequently has been disseminated to other stone-fruit-growing regions of the world. In France, emergence of this disease was due to a recent introduction of the most prevalent genotype of the bacterium found worldwide.

Xanthomonas arboricola pv. *pruni* (46), previously named *Xanthomonas campestris* pv. *pruni* (40), is the causal agent of bacterial spot of stone fruit (BSSF), one of the most important diseases of peach, nectarine, Japanese plum, apricot, and almond (34). The disease was first described in North America in 1903 (40). The disease, now widespread on cultivated stone fruit, may have developed due to the spread of endemic populations from wild stone fruit (47). Since its initial description in North America, the disease has been observed on four continents, in countries including Australia, New-Zealand, South Africa, Brazil, and several European countries. In Europe, the disease first was detected in Italy in 1920 and considered endemic there since the 1970s (6). It also was reported in Romania in 1941 (37). The first documented observation of the disease in France was in 1995 in the departments (i.e., regions) of Drôme and Gard. The disease was very severe in 2000 and has expanded to new stone fruit orchards every year. *X. arboricola* pv. *pruni* is differentially virulent on stone fruit species, being common and severe on peach and plum but causing less damage on apricot (8). Control methods are limited to copper sprays supplemented with cultural practice modifications such as optimization of irrigation to reduce extended wetting of the foliage. Although less-susceptible cultivars are available, none have complete resistance. Furthermore, market preferences are often for fruit of highly susceptible cultivars. A strategy to limit the spread of *X. arboricola* pv. *pruni* is in place by its inclusion on the European list of quarantine pathogen agents (annex A2 of the European Union plant health directive 2000/29/EC).

Because the pathogen overwinters in buds (6) or leaf scars (51), it has been hypothesized that dissemination occurs as the result of latent infections or pathogen colonization of vegetative material

used for propagation. The geographic origin of the pathogen might be North America, where it was first described (40). Nevertheless, local adaptation to a new host of a local bacterial species from a wild host also may have occurred in multiple locations throughout the world.

When the same disease occurs in different geographical areas, analysis of the genetic structure of the pathogen populations from these areas should provide information about the genetic diversity of populations occurring in different locations. It will support the transfer of control strategies from countries where the disease has occurred for a long time to countries where it has been recently discovered. Literature about genetic diversity of *X. arboricola* pv. *pruni* is restricted to an analysis of the population from Italy (50). Our objective was to genetically analyze the population structure of *X. arboricola* pv. *pruni* strains from cultivated stone fruit in France and compare this population with that of *X. arboricola* pv. *pruni* from Italy and North America.

X. arboricola is a species consisting of a number of pathovars, including *corylina*, *fragariae*, *juglandis*, *poinsetticola* type C strains, *populi*, and *pruni* (18,46). Analysis of 16S ribosomal DNA sequences facilitates evaluation of the genetic diversity between different species; however, this technique is not appropriate to classify members of the genus *Xanthomonas* at the subspecies level (14). DNA-DNA hybridization (49) often is used in taxonomic studies, but is not considered sensitive enough to detect close relationships between populations within a bacterial species (48). Housekeeping genes share conserved sequences in different species and commonly are sequenced for molecular epidemiology of human pathogenic bacteria such as *Neisseria meningitidis* (10), *Salmonella* spp. (20), and *Helicobacter pylori* (9,42). Sequence data of seven to eight housekeeping genes have been used to develop multilocus sequence typing (MLST) for the characterization of bacterial populations (24,41).

Others tools, including analysis of insertion sequence (IS) polymorphisms, spoligotyping, polymorphisms at variable numbers of tandem repeats (VNTR), and mycobacterial interspersed repeti-

Corresponding author: J.-L. Nottéghem; E-mail address: nottege@ensam.inra.fr

DOI: 10.1094/PHYTO-95-1081

© 2005 The American Phytopathological Society

tive units (MIRU), have been used to evaluate genetic diversity of bacterial species, but have been shown to be less accurate than amplified fragment length polymorphism (AFLP) for phylogenetic analysis (3). AFLP and fluorescent AFLP (FAFLP) were used successfully to analyze population structures of several *Xanthomonas* spp., such as *X. axonopodis* pv. *manihotis* (33), and *X. arboricola* pv. *juglandis* (22), or to identify a *Xanthomonas* sp. isolated from onion as a pathovar of *X. axonopodis* (35). AFLP previously was used to study the population structure of the Italian strains of *X. arboricola* pv. *pruni* (50). AFLP appeared to be a rapid and highly discriminatory technique to determine the genomic diversity of bacterial populations (19,48); thus, the technique seemed appropriate for analysis of our *X. arboricola* pv. *pruni* populations.

In this study, we used three complementary genomic tools to characterize populations of *X. arboricola* pv. *pruni* from different continents for comparison with strains isolated from stone fruit in France. We sequenced the intergenic transcribed spacer (ITS) region of the *rrn* operon and four housekeeping genes for the evaluation of the diversity level of this pathovar. On the ITS, few mutations were expected because this region is highly conserved. Similarity between species of the *Xanthomonas* genus ranged from 63 to 99% (12). We partially sequenced the four housekeeping genes *atpD*, *dnaK*, *efp*, and *glnA* described below. These genes are some of the most informative ones in human bacterial genetics studies, and references for plant pathogenic bacteria are not yet available. The gene *atpD* encodes F₁-F₀-ATPase β subunit (25). This gene was used for a phylogenetic analysis on *H. pylori* (42). The gene *dnaK* encodes a 70-kDa heat shock protein (Hsp70). This gene has been used frequently in evolutionary studies because it codes for an essential protein and its sequences are conserved among various species (13). The gene *efp* encodes the elongation factor P protein that stimulates the peptidyltransferase activity of fully assembled 70 S prokaryotic ribosomes and enhances the synthesis of certain dipeptides initiated by N-formylmethionine (5). This gene was used by Achtman et al. (1) to study population genetic structure and diversity of *H. pylori*. The gene *glnA* encodes for the glutamine synthetase I (GSI) and has been used for studying bacterial evolution (21,29,30).

Additionally, we performed FAFLP analysis to assess the phylogenetic structure of *X. arboricola* pv. *pruni* and to investigate the origin of French bacterial populations.

MATERIALS AND METHODS

Strains of *X. arboricola*. The origin of strains is given in Table 1. They were obtained from other collections or were isolated from diseased *Prunus* spp. in the south of France. Strains were collected from various hosts and locations and at different times. *X. arboricola* pv. *corylina* strain CFBP 1846 was used as an out strain. The strains all were deposited in the Collection Française de Bactéries Phytopathogènes (CFBP, Institut National de la Recherche Agronomique, Angers, France), where they were characterized as *X. arboricola* pv. *pruni*. Bacteria were grown at 27 to 28°C on yeast-peptone-glucose agar (YPGA) medium consisting of yeast extract (7 g/liter), peptone (7 g/liter), glucose (7 g/liter), and agar (15 g/liter) at pH 7. For long-term conservation, bacteria were freeze dried. For short-term storage, bacteria were kept at -80°C in YPG broth supplemented with glycerol (30% wt/vol).

Pathogenicity tests. Pathogenicity of each strain was tested using a detached leaf assay. The peach cv. O'Henry, susceptible to BSSF, was used in all experiments. Peach trees were grown in 30-liter pots in soil (pH 6) composed of 50% Uminsubstrat garden soil (Proveg, La Rochelle, France), 25% peat moss (Planta flor humus, Vechta, Germany), and 25% sand. Leaves were collected between the third and seventh nodal positions from the top of branches. Overnight cultures of bacteria, grown on YPGA plates, were suspended in sterile water to approximately 5 ×

10⁸ CFU/ml (optical density at 450 nm = 0.5) as adjusted using a spectrophotometer (spectronic 20D; Milton Roy Company, Ivyland, PA). Suspensions were diluted 10-fold in water to obtain an inoculum of 5 × 10⁸ CFU/ml. Peach leaf surfaces were disinfected by immersing in a 70% alcohol solution for 30 s and rinsing them three times in sterile water. These leaves then were immersed in a bacterial suspension and a 0.3-bar vacuum was applied for 5 min. Inoculated leaves were placed in a sterile tube with the leaf upright, and the petiole immersed in 6% agar. Inoculated leaves were kept in a growth chamber at 24°C with a 12-h photoperiod. Symptom development was recorded daily for 3 weeks after inoculation. The experiment was repeated once.

DNA extraction. Genomic DNA was extracted and purified using the DNeasy tissue kit (Quiagen, Courtaboeuf, France). Quality and quantity of DNA was evaluated on a 0.7% agarose electrophoresis gel. DNA was stored at 20 ng/μl in sterile distilled water at 4°C until use.

Amplification and sequencing of conserved DNA regions. For each studied gene, *atpD*, *dnaK*, *efp*, and *glnA*, published sequences of *X. axonopodis* pv. *citri* strain 306 (GenBank reference sequence AE008923) and *X. campestris* pv. *campestris* strain ATCC 33913 (GenBank reference sequence AE008922) were compared. In order to design useful forward and reverse primers (Table 2), conserved sequences between these two *Xanthomonas* spp. were identified.

The 16S-23S rDNA ITS region, including tRNA^{Ala} and tRNA^{Ile} genes interspersed within the sequence and partial sequence of 16S RNA and 23S RNA flanking the ITS, was sequenced for six strains (CFBP: 3894, 3900, 3901, 3902, 5530, and 6653) and the four housekeeping genes were sequenced for four strains (CFBP: 3894, 3900, 3901, and 6653).

DNA amplifications from ITS regions were performed in a total volume of 25 μl using 30 to 50 ng of template DNA, 100 μM dNTPs, 0.5 μM each primer, and 0.75 U of *Taq* polymerase (Promega Corp., Madison WI) in 1× buffer with 1.5 mM MgCl₂. Polymerase chain reaction (PCR) conditions consisted of one cycle at 94°C for 5 min and 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min. Amplification of the ITS region was carried out using primers P-X-16S-F (16S uni) and P-X-23S-R (23S uni 322 anti) as described by Honneycutt et al. (15) (Table 2).

Amplifications from housekeeping genes were performed in a total volume of 25 μl using 5 ng of DNA, 200 μM dNTPs, 0.2 μM each primer, and 0.75 U of *Taq* polymerase (Promega Corp.) in 1× buffer with 1.5 mM MgCl₂. Cycling conditions consisted of one cycle of 94°C for 5 min and 40 cycles of 94°C for 30 s, 60°C for the *dnaK* region or 56°C for others for 30 s, and 72°C for 1 min. Amplification reactions were performed in a PE-9700 thermocycler (Applied Biosystems, Foster City, CA).

DNA amplicons were separated in 1% agarose gels by electrophoresis. Appropriately sized DNA fragments were purified using QIAquick gel extraction kit (Qiagen). Diluted fragments then were sent to Genome Express Company (Meylan, France) for double-strand sequencing. Each electrophoregram was verified before alignment. Sequences were aligned using the CLUSTAL W program (45). All the sequences determined in this work were deposited in the GenBank database under the accession numbers shown in Table 3.

FAFLP analysis. The analyses were performed as described by Roumagnac et al. (35) with the addition of a DNA predigestion step. For predigestions, the reaction mixture was prepared in a total volume of 10 μl containing 1 μl of genomic DNA (20 ng), 12 U of restriction enzyme *EcoRI* (Eurogentec, Liege, Belgium), 1 μl of 10× digestion buffer, and 0.1 μl of 100× bovine serum albumen (BSA) solution (Biolabs, Beverly, MA). The reaction mixture was incubated for 1 h at 37°C. After incubation, 5 μl of the reaction mixture was used for DNA digestion and ligation. Two reaction mixtures were prepared for each strain for digestion and ligation. For the first mixture, 5 μl of genomic DNA (2 ng μl⁻¹)

TABLE 1. Bacterial strains of *Xanthomonas arboricola* pv. *pruni* used for analysis^a

Strain	Other collection number	Host	Geographical origin	Year of isolation	FAFLP group ^b
CFBP 411	ATCC 10016	Unknown	United States	1963	A
CFBP 1706	LNPV 17.06	<i>Prunus persica</i>	Vallée du Rhône, France	1996	F
CFBP 2209	LNPV 22.09	<i>P. persica</i>	France	1999	A
CFBP 3893	VR 85/	<i>P. persica</i>	Veneto, Italia	1989	L
CFBP 3894	NCPBP 416, ATCC 19316, ICMP 51	<i>P. salicina</i>	New-Zealand	1953	J
CFBP 3895	OMP-BO 576	<i>P. persica</i>	Emilia Romagna, Italy	1991	A
CFBP 3897	OMP-BO 691.1	<i>P. armeniaca</i>	Emilia Romagna, Italy	1991	F
CFBP 3898	X120 ^c	<i>P. dosmetica</i>	United States	1989	A
CFBP 3899	X121 ^c	<i>P. persica</i>	United States	1989	C
CFBP 3900	X122 ^c	<i>P. persica</i>	United States	1987	B
CFBP 3901	X123 ^c	<i>P. armeniaca</i>	United States	1987	I
CFBP 3902	X124 ^c	<i>P. persica</i>	United States	1986	C
CFBP 3903	IPV-BO 2294	<i>P. domestica</i>	Piemonte, Italy	1994	D
CFBP 3904	IPV-BO 2293	<i>P. persica</i>	Zevio, Italy	1993	A
CFBP 3905	IPV-BO 2616	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3906	IPV-BO 2617	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3907	IPV-BO 2618	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3908	IPV-BO 2620	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3909	IPV-BO 2625	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3910	IPV-BO 2626	<i>P. persica</i>	Veneto, Italy	1992	K
CFBP 3911	IPV-BO 2635	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3912	IPV-BO 2636	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3913	IPV-BO 2638	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3914	IPV-BO 2639	<i>P. persica</i>	Veneto, Italy	1992	A
CFBP 3915	IPV-BO 3015	<i>P. persica</i>	Emilia Romagna, Italy	1996	A
CFBP 3916	IPV-BO 3020	<i>P. persica</i>	Emilia Romagna, Italy	1996	A
CFBP 3917	...	<i>P. persica</i>	Veneto, Italy	1996	A
CFBP 3918	...	<i>P. persica</i>	Veneto, Italy	1996	A
CFBP 3919	...	<i>P. persica</i>	Veneto, Italy	1996	A
CFBP 3920	...	<i>P. persica</i>	Veneto, Italy	1996	D
CFBP 3921	...	<i>P. persica</i>	Veneto, Italy	1996	A
CFBP 5529	NCPBP 1607	<i>P. persica</i>	Australia	1964	A
CFBP 5530	VR 69	<i>P. persica</i>	Veneto, Italy	1989	A
CFBP 5562	LNPV 13.59	<i>P. persica</i>	Gard, France	1995	A
CFBP 5563	LNPV 20.72	<i>P. persica</i>	Gard, France	1998	A
CFBP 5564	LNPV 20.78	<i>P. persica</i>	Gard, France	1998	A
CFBP 5565	LNPV 20.81	<i>P. persica</i>	Gard, France	1998	A
CFBP 5566	LNPV 21.76	<i>P. persica</i>	Gard, France	1999	H
CFBP 5567	LNPV 20.73	<i>P. persica</i>	Gard, France	1998	A
CFBP 5569	LNPV 20.74	<i>P. persica</i>	Gard, France	1998	A
CFBP 5570	LNPV 22.06	<i>P. persica</i>	Gard, France	1999	A
CFBP 5571	LNPV 21.05	<i>P. persica</i>	Gard, France	1998	A
CFBP 5572	LNPV 23.57	<i>P. persica</i>	Gard, France	2000	A
CFBP 5573	LNPV 23.37	<i>P. armeniaca</i>	Gard, France	2000	A
CFBP 5574	LNPV 23.42	<i>P. armeniaca</i>	Gard, France	2000	A
CFBP 5575	LNPV 23.62	<i>P. armeniaca</i>	Gard, France	2000	G
CFBP 5577	LNPV 16.12	<i>P. persica</i>	Drôme, France	1996	A
CFBP 5578	LNPV 22.30	<i>P. persica</i>	Drôme, France	1999	A
CFBP 5579	LNPV 23.41	<i>P. persica</i>	Drôme, France	2000	A
CFBP 5580	LNPV 23.66	<i>P. salicina</i>	Lot et Garonne, France	2000	A
CFBP 5581	LNPV 16.13	<i>P. persica</i>	Vallée du Rhône, France	1996	A
CFBP 5582	LNPV 22.04	<i>P. persica</i>	France	1999	A
CFBP 5583	LNPV 23.53	<i>P. persica</i>	Gard, France	2000	A
CFBP 5716 ^d	...	<i>P. persica</i>	North Carolina, United States	1980	E
CFBP 5717 ^d	...	<i>P. persica</i>	Georgia, United States	1982	A
CFBP 5718 ^d	...	<i>P. persica</i>	North Carolina, United States	1975	E
CFBP 5719 ^d	...	<i>P. persica</i>	Virginia, United States	1983	B
CFBP 5720 ^d	...	<i>P. persica</i>	South Carolina, United States	1983	A
CFBP 5721 ^d	...	<i>P. armeniaca</i>	North Carolina, United States	1989	A
CFBP 5722 ^d	...	<i>P. persica</i>	Brazil	1987	A
CFBP 5723 ^d	...	<i>P. persica</i>	Uruguay	Unknown	M
CFBP 5724 ^d	...	<i>P. amygdalus</i> (ornamental)	North Carolina, United States	Unknown	N
CFBP 5725 ^d	...	<i>P. persica</i>	South Carolina, United States	1993	A
CFBP 6653 ^d	...	<i>P. persica</i>	Gard, France	2000	A

^a ATCC = American Type Culture Collection, Manassas, VA, USA; CFBP = Collection Française de Bactéries Phytopathogènes, INRA, Angers, France; ICMP = International Collection of Microorganisms from Plants, Manaaki Whenua Landcare Research, Auckland, New Zealand; IPV-BO = Istituto di Patologia Vegetale, Bologna, Italia; LMG = Laboratorium Microbiology Gent Culture Collection, Rijksuniversiteit, Belgique; LNPV = Laboratoire National de la Protection des Végétaux, Angers, France; NCPBP = National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Harpenden, Hertfordshire, UK; OMP-BO = Osservatorio per le Malattie delle piante, Bologna, Italia; VR = Osservatorio per la Malattie delle Piante, Verona, Italia.

^b FAFLP = fluorescent amplified fragment length polymorphism.

^c Strains numbers used in Zaccardelli et al. (50).

^d Strains provided by: D. F. Ritchie, North Carolina State University, Department of Plant Pathology.

from the precedent predigestion step was mixed in a total volume of 15 μ l with 3.6 U of each of the restriction enzymes *Eco*RI and *Msp*I (Eurogentec), 2 μ l of 0.5 M NaCl, 0.2 μ l of 100 $\times$ BSA solution (Biolabs), 1.5 μ l of 10 $\times$ T4 ligase buffer (Biolabs), 1 μ l (2 μ M) of *Eco*RI adaptor (Cybergène) (5'-CTC GTA GAC TGA GTA CC-3'), and 1 μ l (20 μ M) of *Msp*I adaptor (Cybergène) (5'-GAC GAT GAG TCC TGA G-3'). The second mixture was prepared in a total volume of 5 μ l, with 0.5 μ l of 10 $\times$ T4 ligase buffer and 100 U of T4 DNA ligase (Biolabs). The two reaction mixtures were combined and incubated for 3 h at 37°C. After incubation, the reaction mixture was diluted 10-fold with distilled water. Two successive amplification reactions then were performed. A preselective amplification was performed in a total volume of 20 μ l containing 4 μ l of diluted DNA from the restriction-ligation step, 2 μ l of 10 $\times$ Goldstar buffer (Eurogentec), 5 mM MgCl₂, 2 mM dNTPs, 0.25 μ M each of *Eco*RI and *Msp*I primer (5'-GAC TGC GTA CCA ATT C-3' and 5'-CGA TGA GTC CTG AGC GG-3', respectively), and 0.5 U of *Taq* DNA polymerase (Goldstar red; Eurogentec). The mixture was incubated for 2 min at 72°C followed by 20 cycles with the following cycle profile: a 20-s DNA denaturation step at 94°C, 30-s annealing step at 56°C, and 2-min extension step at 72°C. PCR products then were diluted 10-fold with distilled water. A selective amplification was performed in a 20- μ l reaction mixture containing 1.5 μ l of diluted pre-amplified DNA from the previous PCR step, 2 μ l of 10 $\times$ Goldstar buffer (Eurogentec), 2.5 mM MgCl₂, 2 mM dNTPs, 50 nM 5'-JOE-labeled selective *Eco*RI primer (5'-GAC TGC GTA CCA ATT CG-3'), 250 nM unlabeled selective *Msp*I+TG primer (5'-CGA TGA GTC CTG AGC GGT G-3') or *Msp*I+TA primer (5'-CGA TGA GTC CTG AGC GGT A-3'), and 0.5 U of *Taq* DNA polymerase (Goldstar red; Eurogentec). The selective amplification consisted of a 2-min denaturation step at 94°C, followed by 33 cycles of a DNA denaturation step at 94°C for 20 s, and a 30-s annealing step and 2-min extension step at 72°C. The annealing temperature was 66°C for the first cycle, then decreased by 1°C per cycle for the next nine cycles, and was 56°C for the last 23 cycles. A final extension step was performed at 60°C for 30 min. One microliter from the selective amplification product was added to 18.2 μ l of formamide and 0.3 μ l of GENESCAN TAMRA-500 ladder as an internal standard (Applied Biosystems) and denatured at 95°C for 3 min. Samples then were submitted to capillary electrophoresis for 35 min (15 kV) in an ABI-310 Genetic Analyzer (Applied Biosystems) with performance-optimized polymers POP-4. The FAFLP fingerprints were superimposed and compared visually using GENESCAN software (Applied Biosystems). The threshold for assigning a peak was set to 100 relative fluorescence.

Statistical analysis of FAFLP data. The presence and absence of fragments were scored in a binary matrix. A similarity matrix using the Jaccard coefficient (17) was calculated (NTSYS 2.0) and an unweighted neighbor-joining tree (11,36) was built using DARWIN 4.0 software (28). The robustness of the tree was assessed by bootstrap analysis with the same software (1,000 repeated samplings). Current genome misparing (CGM) values were calculated as described by Mougél et al. (26). CGMs are obtained from the mathematical transformation of Jaccard coefficients into genetic distances, which takes into account the number of nucleotide sites that constrain the AFLP technique. MultiLocus V1.2.2 software (2) was used to calculate the genetic diversity (H) of Nei (27) based on the haplotype frequency and defined as $H = [n/(n-1)](1 - \sum p_i^2)$, where n is the number of individuals, $n/(n-1)$ is a small sample correcting term, and p_i is the frequency of the i th haplotype. An exact test of population differentiation based on haplotype frequency was performed using the Arlequin version 2.2 software (38). The hypothesis of a random distribution of the different haplotypes among the populations was tested as described by Raymond and Rousset (31). This software also was used to calculate the Tajima's D (43,44) to

test selective neutrality. A split decomposition analysis was conducted using the SplitsTree software (16). The Jaccard matrix constructed with DARWIN software was used for the split decomposition analysis.

RESULTS

Pathogenicity tests. Approximately 7 days after inoculation, the 23 *X. arboricola* pv. *pruni* strains isolated in France from *Prunus* spp. and the 41 strains obtained from different collections induced watersoaked angular leaf spots. These spots turned into brown lesions during the next 7 days. Symptoms caused by all *X. arboricola* pv. *pruni* strains were similar on detached peach leaves, thus confirming pathogenicity of all isolates of the pathogen previously identified as *X. arboricola* pv. *pruni*. The negative control strain, *X. arboricola* pv. *corylina* strain CFBP 1846, did not cause any symptom on peach.

Molecular characterization. The ITS sequence obtained in our study for *X. arboricola* pv. *pruni* strain CFBP 5530 was identical to the one found in GenBank (accession no. AF035441 on NCBI). The sequences of genes *atpD*, *dnaK*, *efp*, and *glnA* and of the ITS region were identical for all *X. arboricola* pv. *pruni* strains. In all, 3,897 bases per strain were sequenced and, after alignment, none of the sequences contained gaps or insertions. The average nucleotide identity of the housekeeping genes was $94.32 \pm 0.82\%$ between an *X. axonopodis* pv. *citri* str.306 strain and *X. campestris* pv. *campestris* strain ATCC 33913. It was $95.74 \pm 1.3\%$ between all *X. arboricola* pv. *pruni* strains and *X. axonopodis* pv. *citri* str.306 strain. It was $95.11 \pm 1.16\%$ between all *X. arboricola* pv. *pruni* strains and *X. campestris* pv. *campestris* strain ATCC 33913. The mean amino acid identity was $98.67 \pm 0.90\%$ between all *X. arboricola* pv. *pruni* strains and *X. axonopodis* pv. *citri* strain, and $98.66 \pm 1.54\%$ between all *X. arboricola* pv. *pruni* strains and *X. campestris* pv. *campestris* strain.

FAFLP analysis. The reproducibility of the FAFLP patterns of *X. arboricola* pv. *pruni* was assessed with six different runs of strain CFBP 6653. Two nonreproducible band positions were identified and discarded for the polymorphism analysis among 87 bands obtained with the two selective primers *Msp*I+TA and *Msp*I+TG. Size of bands varied between 50 and 440 bp, 15 of which were polymorph (17.7%). In all, 47 fragments were obtained with the selective primer *Msp*I+TA, and 7 were polymorphic (14.9%). The use of selective primer *Msp*I+TG yielded 38 fragments, with 8 being polymorphic (21%).

Fourteen genotypes were identified by comparison of the genetic profile of 64 strains of *X. arboricola* pv. *pruni* (Fig. 1; Table 1). One genotype (group A) was represented by 46 strains collected in five countries representing four continents. Most of strains from France (20/23) and Italy (18/23), almost half the U.S. strains (6/14), and one strain each from Australia and Brazil belonged to genotype A. American strains of this genotype were collected in at least three states where the disease occurred. The oldest American strain of our collection (CFBP 411, stored in 1963) also belonged to genotype A. Other genotypes were represented by a few strains. One strain for genotypes G, H, I, J, K, L, M, and N and two strains for genotypes B, C, D, and E originated from one country, with the exception of group F, which included one strain from Italy and one from France.

Genotypes F, G, H, K, and L differed from genotype A by only one band and originated from France or Italy. Only two European strains of genotype D differed from genotype A by two bands. The others genotypes differing from genotype A by more than two mutations did not originate in Europe. Strains having the most genotypic diversity were supported by high bootstrap values (≈ 60).

Unlike the differences detected among the strains based on the locations from which they were isolated, the host from which they

were isolated did not influence the genotype. The strains of *X. arboricola* pv. *pruni* originated from five host species, but *Prunus persica* is represented by 52 of 64 strains within which no host-genotype association was identified.

Mean CGM values between *X. arboricola* pv. *pruni* strains and the *X. arboricola* pv. *corylina* CFBP 1846 outgroup strain were $3.39 \pm 0.09\%$. CGM values between *X. arboricola* pv. *pruni* strains ranged from 0.1% between strains of genotype A and strains of the closely related genotypes B, E, F, G, H, K, and L; between strains of genotypes C and I; and between strains of genotypes D and G, to 0.6% between strains of genotypes C and N, which were two genotypes with strains from the United States. Mean CGM values were $0.29 \pm 0.11\%$ among all *X. arboricola* pv. *pruni* strains. Within the Western European (W-European) strains, CGM values ranged from 0.1% between strains of genotype A and genotypes F, G, H, K, and L and between strains of genotypes D and G, to 0.3% between strains of genotype D and of genotypes F, H, K, and L, with a mean value of $0.19 \pm 0.07\%$. Within the American strains, CGM values ranged from 0.1% between strains of genotype A and strains of genotypes B and E and between strains of genotypes C and E, to 0.6% between strains of genotypes C and N, with a mean value of $0.3 \pm 0.14\%$. A significant difference measured by variance analysis ($P = 0.001$; F test, threshold 0.05) was observed between the W-European population and the American population.

Total genotypic diversity (H) was 0.48. For American strains, H was 0.8 and, for French and Italian strains, H was 0.25 and 0.39, respectively.

Differentiation based on haplotype frequency among French, Italian, and American populations was tested and no differentiation was found between the French and the Italian populations ($P = 58.6\%$). Therefore, these two populations were considered to be similar, comprising a single, W-European population. In contrast, the American population was differentiated from both the French and Italian populations ($P = 0.08\%$ 0.42%, respectively) as well as from the W-European population ($P < 0.001\%$).

Selective neutrality measured by Tajima's test was nonsignificant for the American population ($P = 24\%$; comparison of randomized Tajima's D to the observed D). It was significant for W-European, Italian, and French populations. Tajima's D value was -1.78 ($P = 2.7\%$) for a W-European population, -1.73 ($P = 3.2\%$) for a French population, and -1.65 ($P = 4.1\%$) for an Italian population.

Interrelationships among all *X. arboricola* pv. *pruni* strains were determined by splitree analysis (split decomposition method) for parallel changes (Fig. 2). Two parallelograms were identified and the fit was 98, indicating that most of the information is consistent with the analysis.

Fig. 1. Dendrogram obtained by fluorescent amplified fragment length polymorphism analysis of 64 *Xanthomonas arboricola* pv. *pruni* strains. Bold capital letters correspond to genetic groups of strains with an identical profile. The origin of the strains (Aus = Australia, Bra = Brazil, Fr = France, Ita = Italy, N-Z = New Zealand, USA = United States of America, Uru = Uruguay) and the number of strains of each genotype are in parenthesis. Only bootstrap values higher than 50 are shown. Root branching of the outgroup CFBP 1846 is indicated by an arrow on the main branch.

TABLE 2. Primers used in this study

Coding region	Primer	Tm (°C) ^a	Length (bp)	Sequence (5'–3')
ITS	P-X-16S-F ^b	60	21	GTT CCC GGG CCT TGT ACA CAC
	P-X-23S-R ^b	60	21	GGT TCT TTT CAC CTT TCC CTC
atpD	P-X-ATPD-F	56	18	GGG CAA GAT CGT TCA GAT
	P-X-ATPD-R	56	18	GCT CTT GGT CGA GGT GAT
efp	P-X-EFP-F	56	18	TCA TCA CCG AGA CCG AAT
	P-X-EFP-R	56	17	TCC TGG TTG ACG AAC AG
glnA	P-X-GLNA-F	56	19	ATC AAG GAC AAC AAG GTC G
	P-X-GLNA-R	56	18	GCG GTG AAG GTC AGG TAG
dnaK	P-X-DNAK-F	60	20	GTG ACC AAC CCG AAG AAC AC
	P-X-DNAK-R	60	19	GGT TCC TTG CCG AAG AAA T

^a Tm = melting temperature.

^b Primers designed by Honeycutt et al. (15).

TABLE 3. Sequenced gene fragments

Gene	Length (bp)	Gene product or inferred function	GenBank accession no.
Intergenic transcribed spacer and partial 16S and partial 23S	1,005	Intergenic spacer	AJ936965
atpD partial sequence	756	F ₁ -F ₀ -ATPase β subunit	AJ936969
dnaK partial sequence	759	70-kDa heat shock protein (Hsp70)	AJ936967
efp partial sequence	417	Elongation factor P	AJ936966
glnA partial sequence	960	Glutamine synthetase I (GSI)	AJ936968

DISCUSSION

Structure and diversity of *X. arboricola* pv. *pruni* populations. No genetic diversity among the *X. arboricola* pv. *pruni* was found within the ITS and housekeeping gene sequences, even between strains that showed the higher AFLP polymorphism. Different results were obtained in studies conducted with human pathogens for which the biology and ecology is very different from those of plant pathogens. In *H. pylori*, nucleotide identity of the *efp* gene was $\approx 85\%$ for strains from different geographical regions (1). Nucleotide identity was $\approx 81\%$ for the *atpD* gene and 78% for the *glnA* gene (42) from a southern Switzerland population. These data revealed sequence polymorphism for a bacterium whose minimal age, compared with the common ancestor, was estimated to be between 2,500 and 11,000 years (9). Nucleotide identity was $\approx 99\%$ for the *atpD* gene of subspecies of *Salmonella enterica* (7). It was $\approx 95\%$ among *X. arboricola* pv. *pruni*, *X. axonopodis* pv. *citri*, and *X. campestris* pv. *campestris*, which was enough to conduct phylogenetic studies within the genus *Xanthomonas*.

AFLP analyses carried out with different bacterial species revealed different levels of polymorphism. Detected polymorphism varied with the combination of selective primers from 20 to 75% (mean 43%) for 47 strains of *X. axonopodis* pv. *manihotis* from Colombia (32). It was 63% for *X. axonopodis* pv. *allii* strains causing bacterial blight of onion (BBO) (35) and even higher (92%) for *X. arboricola* pv. *juglandis* (22). For *X. arboricola* pv. *pruni*, FAFLP using two pairs of primers generated 85 useful fragments and 15 (17.7%) were polymorphic. The polymorphism was 10% for the American population and 7.7% for the European population and, as for other *Xanthomonas* population studies, polymorphism revealed by FAFLP was sufficient to identify different genotypes. FAFLP diversity of *X. arboricola* pv. *pruni* was lower when compared with other species. However, the comparison with *X. axonopodis* pv. *allii* is more appropriate because the same primers were used, but comparison of results obtained with other species are less informative because of the use of different primers. A very low diversity among 46 isolates of *X. arboricola* pv. *pruni* also was observed by rep-PCR, and this diversity also was lower than the one found for *X. campestris* pv. *campestris* (23).

The CGM value is known to provide an accurate measure of the genetic distance between genomes (26). Mougél et al. (26) defined a threshold of 13.6% that corresponds to the 70% hybridization

threshold defined by Wayne et al. (49) as the species limit. First, the CGM values between the *X. arboricola* pv. *corylina* strain and all *X. arboricola* pv. *pruni* strains (3.4 ± 0.09) confirmed that *X. arboricola* pv. *corylina* and *X. arboricola* pv. *pruni* belong to the same species. Roumagnac et al. (35) found a mean CGM value of $3.6 \pm 0.6\%$ between *X. axonopodis* pv. *allii* (BBO) strains, and $5.5 \pm 1.5\%$ between *X. axonopodis* strains. This value was close to the mean CGM value between *X. arboricola* pv. *corylina* and *X. arboricola* pv. *pruni* strains. CGM values among all *X. arboricola* pv. *pruni* strains were very low ($0.29 \pm 0.11\%$), suggesting that all the *X. arboricola* pv. *pruni* strains were very close and belong to a population with a low genetic diversity. Mean CGM values also confirmed that the *X. arboricola* pv. *corylina* strain used in our study does not belong to the genetic population of *X. arboricola* pv. *pruni*, confirming that *X. arboricola* pathovars are different genetic entities (39).

Origin of *X. arboricola* pv. *pruni*. Different data can be used to establish the origin of *X. arboricola* pv. *pruni*. Historical data which consider that *X. arboricola* pv. *pruni* first was described in North America (40) are helpful, even if first reports of a disease could be correlated to the progress of knowledge and expertise but not with the origin of the pathogen. In our study, a major genotype (group A) was identified by FAFLP analysis. This major genotype contained 46 of the 64 strains. It included most strains from W-Europe (38/45), half of isolates from different states in the United States (6/13), a strain from Brazil (1/1), and one from Australia (1/1). The occurrence of this genotype in different countries can be explained only by migration. In our case, we can hypothesize that humans were the migration support of *X. arboricola* pv. *pruni* (4).

CGM values of the American population of the pathogen were significantly higher than CGM values of the W-European population. This result confirmed those obtained by the population differentiation test, which indicated that the W-European population was different from the American one. In contrast, no differentiation was found between French and Italian populations ($P = 61\%$) which, therefore, can be considered as a single population. The W-European population ($H = 0.32$) also is less diversified than the American population ($H = 0.8$). Additionally, it should be noted that the highest CGM value (0.6%) was observed between two American genotypes (C and N) and, therefore, two genotypes of the same region were more different than any two genotypes from different continents.

Fig. 2. Split graph constructed with Splitstree and based on Jaccard distance matrix. Bold capital letters correspond to genetic groups of strains with an identical profile. The origin of the strains (Aus = Australia, Bra = Brazil, Fr = France, Ita = Italy, N-Z = New Zealand, USA = United States of America, Uru = Uruguay) and the number of strains of each genotype are in parenthesis.

Such a scenario of recent introduction of a narrow bacterial population in W-Europe inferred a bottleneck for the *X. arboricola* pv. *pruni* W-European population. This was confirmed by a significant negative Tajima's D for the W-European population ($DE = -1.78$, $P = 2.7\%$), which is a characteristic of populations which have experienced a recent bottleneck. The American population Tajima's D was not significant, suggesting that this population was in selective neutrality, characteristic of populations which have reached equilibrium. These data reinforce the hypothesis that the W-European population is a new and recently introduced population when compared with the older American population.

The diversity analysis, the polymorphism analysis, and historical data all led to the conclusion that *X. arboricola* pv. *pruni* originated from North America before spreading to W-Europe. Because the most frequent genotype, A, was common in the United States and dominant in Italy and France, introduction of *X. arboricola* pv. *pruni* to France may have occurred directly from the United States to France or as a second step from Italy. Our population analysis did not differentiate French and Italian populations; therefore, it is more likely that *X. arboricola* pv. *pruni* was introduced to France via Italy. Because genotype A was the only genotype from the United States present to W-Europe, it is possible that the introduction from North America occurred only once. Anyway, we cannot exclude the possibility that genotype A introduction to W-Europe occurred more than once because it is highly frequent in the United States.

W-European genotypes not collected in the United States differed from genotype A by only one or two AFLP bands, which probably are mutations that may have occurred after introduction of genotype A into W-Europe. This result was visually represented by the global star shape of the dendrogram (Fig. 2), which is due to the fact that other genotype differ from genotype A by one or two different markers. One or two mutations revealed by FAFLP markers, rarely common to different strains, are sufficient to explain evolution of nongenotype A strains. This dendrogram structure can illustrate a short-time evolution of the *X. arboricola* pv. *pruni* population after a unique event of pathogenicity adaptation to a new host group, including genetically closed species. Genotype A, therefore, appears to be the ancestor genotype, and probably was the genotype introduced into W-Europe, where the pathogen population seems to be in a diversification process.

ACKNOWLEDGMENTS

This work was supported by Languedoc-Roussillon region associated to the Institut National de la Recherche Agronomique (INRA) funds. We thank P. Rott and C. Bull for presubmission reviews of the manuscript and helpful suggestions and D. F. Ritchie for providing most of the American strains.

LITERATURE CITED

- Achtman, M., Azuma, T., Berg, D. E., Ito, Y., Morelli, G., Pan, Z. J., Suerbaum, S., Thompson, S. A., Van der Ende, A., and Van Doorn, L. J. 1999. Recombination and clonal groupings within *Helicobacter pylori* from different geographical regions. *Mol. Microbiol.* 32:459-470.
- Agapow, P.-M., and Burst, A. 2000. MultiLocus 1.2 Logiciel d'Analyse de données. Department of Biology, Imperial College, Silwood Park-Ascot, Berkshire, UK.
- Ahmed, N., Alam, M., Rao, K. R., Kauser, F., Kumar, N. A., Qazi, N. N., Sangal, V., Sharma, V. D., Das, R., Katoch, V. M., Murthy, K. J. R., Suneetha, S., Sharma, S. K., Sechi, L. A., Gilman, R. H., and Hasnain, S. E. 2004. Molecular genotyping of a large, multicentric collection of tubercle bacilli indicates geographical partitioning of strain variation and has implications for global epidemiology of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* 42:3240-3247.
- Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., and Daszak, P. 2004. Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol. Evol.* 19:535-544.
- Aoki, H., Dekany, K., Adams, S. L., and Ganoza, M. C. 1997. The gene encoding the elongation factor P protein is essential for viability and is required for protein synthesis. *J. Biol. Chem.* 272:32254-32259.
- Battilani, P., Rossi, V., and Saccardi, A. 1999. Development of *Xanthomonas arboricola* pv. *pruni* epidemics on peaches. *J. Plant Pathol.* 81:161-171.
- Christensen, H., and Elmerdahl Olsen, J. 1998. Phylogenetic relationships of *Salmonella* based on DNA sequence comparison of atpD encoding β subunit of ATP synthase. *FEMS Microbiol. Lett.* 161:89-96.
- Du Plessis, H. J. 1988. Differential virulence of *Xanthomonas campestris* pv. *pruni* to peach, plum, and apricot cultivars. *Phytopathology* 78:1312-1315.
- Falush, D., Kraft, C., Taylor, N. S., Correa, P., Fox, J. G., Achtman, M., and Suerbaum, S. 2001. Recombination and mutation during long-term gastric colonization by *Helicobacter pylori*: Estimates of clock rates, recombination size, and minimal age. *Proc. Natl. Acad. Sci. USA* 98:15056-15061.
- Feavers, I. M., Gray, S. J., Urwin, R., Russell, J. E., Bygraves, J. A., Kaczmarek, E. B., and Maiden, M. C. J. 1999. Multilocus sequence typing and antigen gene sequencing in the investigation of a meningococcal disease outbreak. *J. Clin. Microbiol.* 37:3883-3887.
- Gascuel, O. 1997. Concerning the NJ algorithm and its unweighted version UNJ. Pages 149-171 in: *Mathematical Hierarchies and Biology*, Vol.37. B. Mirkin, F. R. McMorris, F. Roberts, and A. Rzhetsky, eds. American Mathematical Society, Providence, RI.
- Gonçalves, E. R., and Rosato, Y. B. 2002. Phylogenetic analysis of *Xanthomonas* species based upon 16S-23S rDNA intergenic spacer sequences. *Int. J. Syst. Evol. Microbiol.* 52:355-361.
- Gupta, R. S., and Golding, G. B. 1993. Evolution of Hsp70 gene and its implications regarding relationships between archaeobacteria, eubacteria and eukaryotes. *J. Mol. Evol.* 37:573-582.
- Hauben, L., Vauterin, L., Swings, J., and Moore, E. R. B. 1997. Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. *Int. J. Syst. Bacteriol.* 47:328-335.
- Honeycutt, R. J., Sobral, B. W. S., and McClelland, M. 1995. tRNA intergenic spacers reveal polymorphisms diagnostic for *Xanthomonas albilineans*. *Microbiology* 141:3229-3239.
- Huson, D. H. 1998. SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* 14:68-73.
- Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaudoise Sci. Nat.* 44:223-270.
- Janse, J. D., Rossi, M. P., Gorkink, R. F. J., Derks, J. H. J., Swings, J., Janssens, D., and Scortichini, M. 2001. Bacterial leaf blight of strawberry (*Fragaria* (x) *ananassa*) caused by a pathovar of *Xanthomonas arboricola*, not similar to *Xanthomonas fragariae* Kennedy & King. Description of the causal organism as *Xanthomonas arboricola* pv. *fragariae* (pv. nov., comb. nov.). *Plant Pathol.* 50:653-665.
- Janssen, P., Coopman, R., Huys, G., Swings, J., Bleeker, M., Vos, P., Zabeau, M., and Kersters, K. 1996. Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. *Mycobiology* 142:1881-1893.
- Kotetishvili, M., Stine, O. C., Kreger, A., Morris, J. G., and Sulakvelidze, A. 2002. Multilocus Sequence typing for characterization of clinical and environmental *Salmonella* strains. *J. Clin. Microbiol.* 40:1626-1635.
- Kumada, Y., Benson, D. R., Killemann, D., Hosted, T. J., Rochefort, D. A., Thompson, C. J., Wohlleben, W., and Taten, Y. 1993. Evolution of the glutamine synthetase gene, one of the oldest existing and functioning genes. *Proc. Natl. Acad. Sci. USA* 90:3009-3013.
- Loreti, S., Gallelli, A., Belisario, A., Wajnberg, E., and Corazza, L. 2001. Investigation of genomic variability of *Xanthomonas arboricola* pv. *juglandis* by AFLP analysis. *Eur. J. Plant. Pathol.* 107:583-591.
- Louws, F. J., Ritchie, D. F., and Shoemaker, P. B. 2001. Genetic diversity of selected bacterial populations in north Carolina. Pages 124-127 in: *Plant Pathogenic Bacteria: Proceedings of the 10th International Conference on Plant Pathogenic Bacteria*. S. H. De Boer, ed. Kluwer Academic Publishers, Norwell, MA.
- Maiden, M. C. J., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Cagant, D. A., Feavers, I. M., Achtman, M., and Spratt, B. G. 1998. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic micro-organisms. *Proc. Natl. Acad. Sci. USA* 95:3140-3145.
- McGowan, C. C., Cover, T. L., and Blaser, J. 1997. Analysis of F₁F₀-ATPase from *Helicobacter pylori*. *Infect. Immun.* 65:2640-2657.
- Mougel, C., Thioulouse, J., Perrière, G., and Nesme, X. 2002. A mathematical method for determining genome divergence and species delineation using AFLP. *Int. J. Syst. Evol. Microbiol.* 52:573-586.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distances from a small number of individuals. *Genetics* 89:583-590.
- Perrier, X., Flori, A., and Bonnot, F. 1999. Les méthodes d'analyse des données. Pages 43-76 in: *Diversité génétique des plantes tropicales cultivées*. P. Hamon, M. Seguin, X. Perrier, and J. X. Glaszmann, eds. CIRAD, Montpellier, France.

29. Pezole, G., Bozzetti, M. P., Lanave, C., Preparata, G., and Saccone, C. 1991. Glutamine synthetase gene evolution: A good molecular clock. *Proc. Natl. Acad. Sci. USA* 88:522-526.
30. Pezole, G., Gissi, C., Lanave, C., and Saccone, C. 1995. Glutamine synthetase gene evolution in bacteria. *Mol. Biol. Evol.* 12:189-197.
31. Raymond, M., and Rousset, F. 1995. An exact test for population differentiation. *Evolution* 49:1280-1283.
32. Restrepo, S., Duque, M., Tohme, J., and Verdier, V. 1999. AFLP fingerprinting: An efficient technique for detecting genetic variation of *Xanthomonas axonopodis* pv. *Manihotis*. *Microbiology* 145:107-114.
33. Restrepo, S., Vélez, C. M., and Verdier, V. 2000. Measuring the genetic diversity of *Xanthomonas axonopodis* pv. *manihotis* within different fields in Colombia. *Phytopathology* 90:683-690.
34. Ritchie, D. F. 1995. Bacterial spot. Pages 50-52 in: Compendium of Stone Fruit Diseases. J. M. Ogawa, E. I. Zehr, G. W. Bird, D. F. Ritchie, K. Uriu, and J. K. Uyemoto, eds. The American Phytopathological Society, St. Paul, MN.
35. Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, R. E., Jones, J. B., Rott, P., and Pruvost, O. 2004. Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. *Int. J. Syst. Evol. Microbiol.* 54:15-24.
36. Saitou, N., and Nei, M., 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406-425.
37. Savulescu, A., Hulea, A., Bucur, E., Savescu, A., and Ionescu, M. 1960. Pages 187-191 in: *Protectia Plantelor in Sprijinul Zonarii Productiei Agricole* in R.P.R. Academiei Republicii Populare Romine, Bucurest, Romania.
38. Schneider, S., Rosseli, D., and Excoffier, L. 2000. Arlequin ver. 2.000: A software for population genetics data analysis. *Genetics and Biometry Laboratory*, University of Geneva, Switzerland.
39. Scortichini, M., and Rossi, M.-P. 2003. Genetic diversity of *Xanthomonas arboricola* pv. *fragariae* strains and comparison with some other *X. arboricola* pathovars using repetitive PCR genomic fingerprinting. *J. Phytopathol.* 151:113-119.
40. Smith, E. F. 1903. Observations on a hitherto unreported bacterial disease, the cause of which enters the plant through ordinary stomata. *Science* 17:456-457.
41. Smith, N. H., Dale, J., Inwald, J., Palmer, S., Gordon, S. V., Hewinson, R. G., and Smith, J. M. 2003. The population structure of *Mycobacterium bovis* in Great Britain: Clonal expansion. *Proc. Natl. Acad. Sci. USA* 100:15271-15275.
42. Solcà, N. M., Bernasconi, M. V., Valsangiacomo, C., Van Doorn, L.-J. V., and Piffaretti, J. C. 2001. Population genetics of *Helicobacter pylori* in the southern part of Switzerland analyzed by sequencing of four housekeeping genes (*atpD*, *glnA*, *scoB* and *recA*), and by *vacA*, *cagA*, *iceA* and IS605 genotyping. *Microbiology* 147:1693-1707.
43. Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585-595.
44. Tajima, F. 1996. The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. *Genetics* 143:1457-1465.
45. Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22:4673-4680.
46. Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. *Int. J. Syst. Bacteriol.* 45:472-489.
47. Vauterin, L., Hoste, B., Yang, P., Alvarez, A., Kersters, K., and Swings, J. 1993. Taxonomy of the genus *Xanthomonas*. Pages 157-192 in: *Xanthomonas*. J. G. Swings and E. L. Civerolo, eds. Chapman & Hall, London.
48. Vauterin, L., Rademaker, J., and Swings, J., 2000. Synopsis on taxonomy of the genus *Xanthomonas*. *Phytopathology* 90:677-682.
49. Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E., Stackebrandt, E., Starr, M. P., and Trüper, H. G. 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37:463-464.
50. Zaccardelli, M., Ceroni, P., and Mazzucchi, U. 1999. Amplified fragment length polymorphism fingerprinting of *Xanthomonas arboricola* pv. *pruni*. *J. Plant Pathol.* 81:173-179.
51. Zaccardelli, M., Malaguti, S., and Bazzi, C. 1998. Biological and epidemiological aspects of *Xanthomonas arboricola* pv. *pruni* on peach in Italy. *J. Plant Pathol.* 80:125-132.