European Journal of Plant Pathology **109:** 565–576, 2003.

Pseudomonas syringae pv. *avii* (pv. nov.), the causal agent of bacterial canker of wild cherries (*Prunus avium*) in France

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Accepted 14 March 2003

Key words: Prunus avium, bacterial canker, *Pseudomonas syringae*, phenotypic characteristics, numerical taxonomy, DNA–DNA hybridization and rep-PCR

Abstract

Bacterial strains isolated from cankers of wild cherry trees (*Prunus avium*) in France were characterized using numerical taxonomy of biochemical tests, DNA–DNA hybridization, repeat sequence primed-PCR (rep-PCR) based on REP, ERIC and BOX sequences, heteroduplex mobility assay (HMA) of internal transcribed spacer (ITS) as well as pathogenicity on wild cherry trees and other species of *Prunus*. They were compared to reference strains of *Pseudomonas syringae* pathovars isolated from wild and sweet cherry and various host plants. Wild cherry strains were closely related to *P. syringae* (*sensu lato*) in LOPAT group Ia (+ - - - +). Wild cherry strains were pathogenic to wild cherry trees and produced symptoms similar to those observed in orchards. They were pathogenic also, but at a lesser extent, to sweet cherry trees (cv. Napoléon). The wild cherry strains were collected from five different areas in France and appeared to constitute a very homogeneous group. They showed an homogenous profile of a biochemical and physiological characteristics. They were closely related by DNA–DNA hybridization and belonged to genomospecies 3 '*tomato*'. Rep-PCR showed that wild cherry strains constitute a tight group distinct from *P. s.* pv. *morsprunorum* races 1 and 2 and from other *P. syringae* pathovars. HMA profiles indicated that the ITS of all wild cherry strains were identical but different from *P. s.* pv. *persicae* strains since the two heteroduplex bands with reduced mobility were generated by hybridization with the *P. s.* pv. *persicae* pathotype strain CFBP 1573. The 8 genomospecies of Gardan et al. (1999) have not been converted into formal species as they cannot be differentiated by biochemical tests. Therefore, the pathovar system within *P. syringae* was currently used. *P. syringae* pv. *avii* is proposed for this bacterium causing a wild cherry bacterial canker and strain CFBP 3846 (NCPPB 4290, ICMP 14479) is designated as the pathotype.

Abbreviations: HMA – Heteroduplexes Mobility Assay; ITS – Internal Transcribed Spacer; rep-PCR – repeat sequence primed PCR.

Introduction

Bacterial canker of sweet (*Prunus cerasus*) and wild (*Prunus avium*) trees may be caused by *Pseudomonas syringae* pv. *morsprunorum*, *Pseudomonas viridiflava* or *P. syringae* pv. *syringae* (Prunier and Cotta, 1985). Bacterial canker of sweet cherry is widespread in commercial orchards and is of economic importance throughout the world (Prunier and Cotta, 1985; Prunier et al., 1985; Bradbury, 1986; Garrett, 1988). Bacterial canker of wild cherry tree due to *P. s.* pv. *morsprunorum* race 1 and 2 (Freigoun and Crosse, 1975) and *P. s.* pv. *syringae* occurs in the UK, Italy, New Zealand and France (Crosse, 1966).

In northern France, a disease was observed first in 1989 on seven-year-old (ca.) wild cherry trees. Symptoms included longitudinal bark cankers along the trunk and branches, and gum exudation was usually present. Extensive browning of the xylem was observed and the cankers could girdle trunk or branch causing dieback of the extremities. Year old shoots were also destroyed and death of trees was observed.

Bacterial strains belonging to *P. syringae* group (LOPAT) Ia (+ - - - +) were isolated from cankers and necrotic tissues in 1991. These were similar to *P. s.* pv. *persicae* but different from strains isolated from wild cherry in the UK (Luz and Shaw, 1996) and Italy. Wild cherry strains are atypical for the *syringae* group since they failed to produce fluorescent pigment on King's medium B (Freigoun and Crosse, 1975; Scortichini et al., 1995).

The objective was to characterize the causal agent of bacterial canker of wild cherries in France with a polyphasic taxonomy approach including numerical taxonomy of phenotypic characteristics, DNA–DNA hybridization, rep-PCR (REP, ERIC and BOX), HMA and pathogenicity tests. The description of a new pathovar, *P. syringae* pv. *avii*, resulted from this research.

Materials and methods

Bacterial strains

Nine strains from a collection of 19 strains isolated from bacterial canker of wild cherry trees were selected as representative of the different geographical areas of the disease in France (Table 1). Eleven strains of *P. syringae* pv. *morsprunorum*: 4 of race 1 and 7 of race 2, isolated from wild cherry bacterial canker (Table 1) and 62 type and pathotype strains belonging to *P. syringae* group were included as reference strains (Figure 1) (Gardan et al., 1999). In addition, 5 strains of the recently described pathovars of *P. syringae*

Table 1. Origin of the strains isolated from wild cherry, sweet cherry tree and other *Prunus* spp.

CFBP number*	Country (area)	Source	Year of isolation
Wild cherry strains			
3846	France (Picardy)	This study	1991
3847	France (Picardy)	This study	1991
3848	France (Picardy)	This study	1995
3849	France (Normandy)	This study	1995
3850	France (Normandy)	This study	1995
3851	France (Ardennes)	This study	1995
3852	France (Normandy)	This study	1995
3853	France (Normandy)	This study	1995
3854	France (Normandy)	This study	1995
P. s. pv. *morsprunorum* race 1			
2116	France	J. P. Prunier, JP	1974
3802	UK	Garrett, CME	1957
3840	France	Ménard, M	1996
4701	France	Ménard, M	1998
P. s. pv. *morsprunorum* race 2			
3799	UK	Garrett, CME	
3800	UK	Garrett, CME	
6411	UK	Luz, JP	
6412	UK	Luz, JP	
6413	UK	Luz, JP	
6414	UK	Luz, JP	
6415	UK	Luz, JP	
P. s. pv. *persicae*			
1573	France	Prunier, JP	1974
P. amygdali			
3205	Greece	Panagopoulos, CG	1967

*Collection Française des Bactéries Phytopathogènes, INRA Angers, France.

(Young et al., 1996) were used: *P. s.* pv. *cunninghamiae* CFBP 4218, *P. s.* pv. *coriandricola* CFBP 5008, *P. s.* pv. *actinidiae* CFBP 4909, *P. s.* pv. *daphniphylli* CFBP 4219 and *P. s.* pv. *raphiolepidis* CFBP 4220.

Biochemical and physiological tests

Gram reactions were performed with 3% KOH solution (Suslow et al., 1982). Twenty biochemical tests were used (Sutra et al., 1997): arginine dihydrolase activity, oxidase reaction, gelatin liquefaction, nitrate reduction, levan production, fluorescence on King's medium B (King's et al., 1954), hypersensitive reaction (HR) on tobacco, esculin hydrolysis, pectolytic, Tween esterase and Dnase activities, polypectate hydrolysis at pH 5 and 8.3, and utilization as sole source of carbon of sucrose, lactate, L-(+)-tartrate, D-(−)-tartrate, erythritol, mannitol and sorbitol added at a concentration of 5% (w/v) to a sterile Ayers's medium (Ayers et al., 1919). Each test was replicated three times. Assimilation of 99 carbon sources was determined using the API Biotype 100 (bio-Mérieux) which were inoculated with a suspension prepared from bacteria grown on YPGA (yeast extract 0.3% w/v, peptone 0.5% w/v, dextrose 0.5% w/v and agar 1.5% w/v) and growth was recorded after 6 days of incubation at 25 °C (Gardan et al., 1997).

Numerical taxonomy

A total of 119 characteristics including the 20 conventional biochemical tests and the 99 carbon sources were used for the numerical taxonomy analysis. A distance matrix was calculated using the Jaccard coefficient (Sneath and Sokal, 1973). Cluster analysis was done using the unweighted pairgroup method with arithmetic mean (UPGMA). Diagnostic ability coefficient (DAC) was calculated according to Descamps and Véron (1981). All calculations were done with the original TAXONUM program (G. Hunault, Faculté des Sciences, and L. Gardan, INRA, Angers, France).

Pathogenicity tests

Five wild cherry strains, 1 strain of *P. s.* pv. *morsprunorum* race 1, 1 strain of *P. s.* pv. *morsprunorum* race 2 and 1 strain of *P. s.* pv. *persicae* were selected for comparative inoculation of two years old wild and sweet (cv. Napoléon) cherries grown in the Picardie area in November 1996. In addition five apricot (*P. armeniaca*), peach (*P. persica*) and plum (*P. domestica*) trees were inoculated with five wild cherry strains. In 1997, three wild cherry strains were inoculated in three-year-old wild cherry trees and inoculations were performed every month from September 1997 to October 1998 to determine the optimum susceptibility. Transversal incisions made into the xylem (Ridé and Ridé, 1978) of trunk and two-year-old branches. Five trees were used to inoculate each strain. Bacterial suspensions were prepared from a 48-h-old culture, adjusted to ca. 10^8 CFU ml^{-1} and 25 µl were introduced with manual dispenser Multipette Eppendorf™ in each wound. Sterilized distilled water was used as the control.

Canker severity was determined by longitudinal and lateral lesions recorded as a girdling index which varied from 0 to 5, with 0 as no infection and 5, where the stem was completely girdled (Ridé and Ridé, 1978).

Ice nucleation activity

Bacterial suspensions were prepared in distilled water adjusted to ca. 10^9 CFU ml^{-1} with 24-h-old culture grown on King's medium B at 16 °C. Suspensions were then diluted 10-fold in distilled water (five replicates) and kept 1 h at 5 °C before immersing them in a cryostat for 5 min at each temperature from −3 to −9 °C. The test was considered as positive if the suspension froze at −7 °C and above.

DNA–DNA hybridizations

Bacterial DNA was extracted and purified according to Brenner et al. (1982). Native DNA of strain CFBP 3846 was labelled *in vitro* by random priming with tritium-labelled nucleotides (Amersham, UK). For DNA–DNA hybridization, the S1 nuclease procedure was used as described by Crosa et al. (1973). The reassociation temperature was 70 °C. ΔTm was determined using the method of Crosa et al. (1973). DNA–DNA hybridization experiments were repeated twice.

Rep-PCR

Strains were analysed by rep-PCR with REP, ERIC and BOX primer sets (Versalovic et al., 1991). PCR conditions were those described by De Bruijn (1992) and Louws et al. (1994). Cell suspensions in sterile water (10^9 CFU ml^{-1}) from 24-h bacterial cultures grown on YPGA were used as templates. Amplification was performed on a PTC-100 Thermocycler (MJ Research,

Inc.) as described by Marques et al. (2000) under a 25-µl reaction volume. PCR products were separated by 1.5% agarose gel electrophoresis in TAE buffer (Sambrook et al., 1989) at 5 V cm^{-1} over 5 h, stained with ethidium bromide, and visualized under a UV transilluminator. PCR amplifications were performed at least twice from fresh sample preparations and PCR master mix. The patterns were analysed using the Bio-Profil software (Vilber Lourmat, France). Bands sizes were assigned by direct comparison to currently DNA standards (1 kb). For each strain, the presence and the absence of a band were encoded as 1 and 0, respectively. A distance matrix between strains was calculated using the Jaccard coefficient and a dendrogram was constructed by the UPGMA method using TAXONUM Software. We performed numerical analysis of REP, ERIC and BOX bands alone and REP, ERIC and BOX bands together.

DNA base composition

The guanine-plus-cytosine (G + C) content of strain CFBP 3846 was determined by the thermal denaturation temperature (Mamur and Doty, 1962) and was calculated by using the equation of Owen and Lapage (1976).

Analysis of ITS by HMA

The internal transcribed spacer (ITS) between 16S and 23S rRNA genes was amplified using primers D21 and D22 (Manceau and Horvais, 1997) and amplification was checked by migration of PCR products in 1.5% agarose gel in TAE buffer. The polymorphism of ITS were studied using a HMA (Delwart et al., 1993). PCR-amplified ITS of wild cherry strains were compared with those of pv. *morsprunorum* races 1 and 2 and *P. s.* pv. *persicae*. For this comparison, PCR products were studied by direct migration in polyacrylamide gels and by cross-hybridization before polyacrylamide gel electrophoresis (Delwart et al., 1993).

Results

Genus and species identification of the strains from wild cherry

Bacterial cells were Gram-negative rods. On YPGA plates, colonies were creamy with a diameter of ca. 1.5 cm after 3 days of incubation with a viscous when taken from agar plate with a platinum needle. Bacteria did not fluoresce on King's medium B, were obligate aerobic and oxidized glucose. They produced levan and were negative in arginine dihydrolase and oxidase tests; they did not liquefy pectate gel and gave a HR on tobacco leaves. Thus, they belonged to the LOPAT Ia group (+ - - - +) according Lelliott et al. (1966). These characteristics led us to assign this bacterium to the species *P. syringae*, *sensu lato* (Palleroni, 1984).

Numerical analysis of phenotypical properties

The dendrogram of the 9 strains from wild cherry and 77 strains of *P. syringae* pathovars and related bacteria is shown in Figure 1. At a distance of 0.14, 10 phenons and 44 isolated strains were observed. All wild cherry strains were clustered in phenon 10 at a low distance and they clustered with *P. s.* pv. *persicae* CFBP 1573 and *P. amygdali* CFBP 3340^T at the distance of 0.22 and 0.33, respectively. On the opposite, they were very distantly related to the other strains. The other strains pathogenic to wild or sweet cherry tree were clustered in phenon 1 (*P. s.* pv. *morsprunorum* race 2) and 8 (*P. s.* pv. *morsprunorum* race 1). Phenons 2, 3, 4, 5, 6, 7 and 9 clustered either two or four reference strains of pathovars of *P. syringae*.

Based on DAC values, 23 biochemical or physiological tests were selected that differentiated wild cherry strains from the strains clustered in the different phenons and from some isolated strains (Table 2). Wild cherry strains were differentiated from other strains pathogenic on wild and sweet cherry trees clustered in phenons 1 and 8 by their ability to utilize 11 and 10 carbon sources, respectively. The pathotype strain of *P. s.* pv. *morsprunorum* CFBP 2351 was distantly related to the typical pathogenic strains of *P. s.* pv. *morsprunorum* either clustered in phenon 8 (race 1) or 1 (race 2).

Pathogenicity tests

Typical symptoms of canker were obtained after inoculations of wild cherry strains on wild cherry trees (Table 3). The inoculation sweet cherry tree (cv. Napoléon) with the wild cherry strains induced similar symptoms (Table 3). Peach, plum, and apricot trees, inoculated under the same conditions exhibited restricted canker without subsequent development. Symptoms were observed only for inoculations performed from September to March, but the widest

Figure 1. Dendrogram of distances between 9 wild cherry strains and strains of *P. syringae* pathovars and related species tested for 119 phenotypic characteristics.

Table 2. Discriminating biochemical characteristics between wild cherry strains and pathovars of *P. syringae* and related bacteria

Bacteria	Differential sources[a]																
	Ery	Tri	Bet	Cap	L + T	LHi	Esc	m-I	Cpr	Glu	DGa	KB	Gel	Put	α-K	DGl	DX
P. s. pv. *avii* (phenon 10)	−[b]	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	+
P. s. pv. *morsprunorum* race 2 (phenon 1)	+	+	+	+	−	−	83	+	+	+	+	83	+	+	−	50	+
CFBP 2351	+	+	−	−	−	+	+	−	+	+	−	+	−	−	−	+	+
P. s. pv. *morsprunorum* race 1 (phenon 8)	+	+	+	+	+	25	−	+	+	−	+	75	−	−	50	−	+
P. s. pv. p*ersicae* (CFBP 1573)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
P. amygdali (CFBP 3840)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+
Phenon 2	+	+	+	+	+	75	75	+	+	+	+	+	−	+	+	75	+
Phenon 3	+	+	+	+	−	+	+	+	+	+	+	+	50	50	+	75	+
Phenon 4	+	−	+	75	−	+	+	+	75	50	+	+	50	−	+	−	+
Phenon 5	+	50	50	+	+	+	+	+	+	−	+	+	−	−	+	−	+
Phenon 6	50	+	+	−	50	+	+	+	−	−	+	+	50	−	+	+	+
Phenon 7	−	+	+	+	50	+	+	+	+	50	+	+	−	−	50	+	+
Phenon 9	−	+	75	+	+	+	−	+	+	50	+	+	25	−	+	−	+

[a]Tests are classified by decreasing values of diagnostic ability coefficient: Ery, erythritol; Tri, trigonelline; Bet, betaine; Cap, caprate; L+T, L(+) tartrate; LHi, L-histidine; Esc, esculin; m-I, myo-inositol; Cpr, caprylate; Glu, glutarate; DGa, D-galacturonate; KB, fluorescence on King B; Gel, hydrolysis of gelatine; Put, putrescine; α-K, α-ketoglutarate; DGl, D-gluconate; DX, D(+) xylose.
[b] −, >90% of negative strains; +, ≥90% of positive strains; numbers correspond to the percentages of positive strains.

Table 3. Pathogenicity on wild and sweet cherry trees of wild cherry strains (*P. s.* pv. *avii*) and *P. s.* pvs. *morsprunorum* and *persicae* expressed as the average of girdling index (0–5) with 0 as no infection and 5, where the stem completely girdled (Ridé and Ridé, 1978)

Strains	Pathogenicity on	
	Wild cherry tree	Sweet cherry tree
P. s. pv. *persicae* CFBP 1573	0.2	
P. s. pv. *morsprunorum* race 2 CFBP 3800	0.3	
P. s. pv. *morsprunorum* race 1 CFBP 3840	1.2	1.8
Wild cherry strain CFBP 3846	3.6	1.5
Wild cherry strain CFBP 3849	1.3	1.0
Wild cherry strain CFBP 3850	3.5	4.2
Wild cherry strain CFBP 3851	1.6	0.7
Wild cherry strain CFBP 3852	2.2	1.2
Control	0.0	0.0

cankers were obtained for inoculations in November and December (Figure 2).

Ice nucleation activity

Ice nucleation activity was negative for the 9 wild cherry strains tested.

DNA relatedness and G + C content

All the wild cherry strains tested were 87–100% related to strain CFBP 3846 from wild cherry (Table 4). The pathotype strains of *P. syringae* pv. *persicae* CFBP 1573, *P. s.* pv. *tomato* CFBP 2212 and 1323 and *P. s.* pv. *morsprunorum* CFBP 2351, 6415 and 3800 were 63–84% related to strain CFBP 3846. For the lowest DNA reassociation values (63–67%), ΔTm values were ranged from 1.3 to 1.7 °C. The remaining 12 type and pathotype strains tested, belonging to genomospecies 1, 2 and 4–9, were 26–60% related to the strain CFBP 3846. For the highest DNA reassociation value of 60% (*P. avellanae*), ΔTm value was 5.2 °C. Thus, wild cherry strains constituted an homogeneous genomic group and belong to genomospecies 3 '*tomato*' which included also *P. s.* pv. *morsprunorum* race 2 strains. The G+C content of strains CFBP 3846 was 59.0 mol.%.

Rep-PCR fingerprints

Reproducible DNA fingerprints were generated from total DNA of the wild cherry strains, *P. s.* pv. *morsprunorum* race 1 and race 2, *P. s.* pv. *persicae* strains and of other reference strains of *P. syringae*.

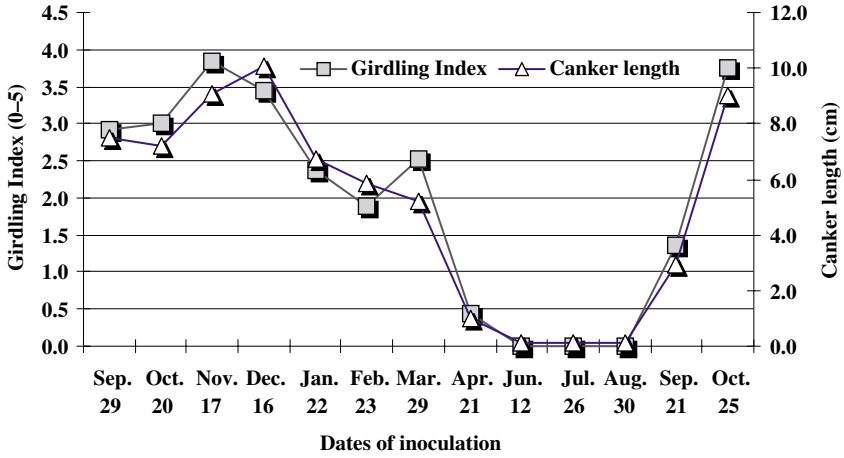

Figure 2. Average canker severity determined with girdling index (0 as no infection to 5, where the stem completely girdled (Ridé and Ridé, 1978)) and canker length produced after inoculation of 3 wild cherry strains (CFBP 3846, 3850 and 3851) from September 29th 1997 to October 25th 1998.

Table 4. DNA relatedness of five wild cherry strains (*P. s.* pv. *avii*) and type or pathotype strains of the different genomospecies of *P. syringae*

Unlabelled DNA from CFBP strains	Genomospecies[a]	Relative reassociation at 70 °C with labelled DNA of the wild cherry strain CFBP 3846 (%)
Wild cherry strains:		
3846	3	100
3849	3	97
3850	3	94
3852	3	88
3851	3	87
P. s. pv. *persicae* 1573	3	84
P. s. pv. *persicae* 3970	3	80
P. s. pv. *tomato* 1323	3	84
P. s. pv. *tomato* 2212	3	74
P. s. pv. *morsprunorum* race 2 6415	3	67 (1.3)[b]
P. s. pv. *morsprunorum* race 2 3800	3	63 (1.7)
P. s. pv. *morsprunorum* 2351	3	66 (1.8)
P. s. pv. *syringae* 1392^T	1	36
P. s. pv. *morsprunorum* race 1 3840	2	51
P. s. pv. *morsprunorum* race 1 2116	2	45
P. savastanoi 1670^T	2	45
P. s. pv. *porri* 1908	4	42
P. tremae 3229^T	5	45
P. viridiflava 2107^T	6	26
P. s. pv. *helianthi* 2067	7	42
P. s. pv. *tagetis* 1694	7	38
P. avellanae 4060^T	8	60 (5.2)
P. s. pv. *theae* 2353	8	59
P. cannabina 2341^T	9	40

[a]After Gardan et al. (1999).
[b]Numbers in parenthesis represent ΔTm (°C).

Complex fingerprinting patterns consisting of 35 BOX-PCR bands ranging from 0.2 to 2.5 kb, 29 ERIC-PCR bands ranging from 0.2 to 3.1 kb and 33 REP-PCR bands ranging from 0.2 to 3.3 kb were obtained for all strains tested. From a visual analysis of the profiles, ERIC-, BOX- and REP-PCR bands examined separately allow to differentiate wild cherry strains from *P. s.* pv. *morsprunorum* (race 1 and race 2) strains pathogenic to wild cherry (Figure 3). Strains from wild cherry displayed distinct profiles to that of *P. s.* pv. *persicae* CFBP 1573 (Figure 3).

The dendrogram issued from the analysis of combined data of ERIC-, BOX- and REP-PCR experiments revealed that wild cherry strains were grouped in a unique cluster different at the distance of 0.41, from those of *P. s.* pv. *morsprunorum* race 1, *P. s.* pv. *morsprunorum* race 2 and other *P. syringae* pathovars strains (Figure 4). *P. s.* pv. *persicae* was differentiated from the wild cherry strains using BOX and ERIC-PCR but not REP-PCR.

HMA profiles of ITS

As shown in Figure 5A, a HMA profile with multiple bands was obtained by direct migration of PCR products for strain of pv. *morsprunorum* race 1 CFBP 2116, whereas the pathotype strains of pv. *morsprunorum* race 2 CFBP 2351 and pv. *persicae* CFBP 1573 and all wild cherry strains had a HMA profile with a unique band. Based on distance migration, the ITS of pv. *morsprunorum* race 2 CFBP 2351 strain was smaller than those of pv. *persicae* CFBP 1573 and of the wild cherry strains (Figure 5A). HMA profiles obtained after hybridization of amplified ITS of all wild cherry strains with that of strain 3846 were characterized by a unique band corresponding to a homoduplex (Figure 5B).

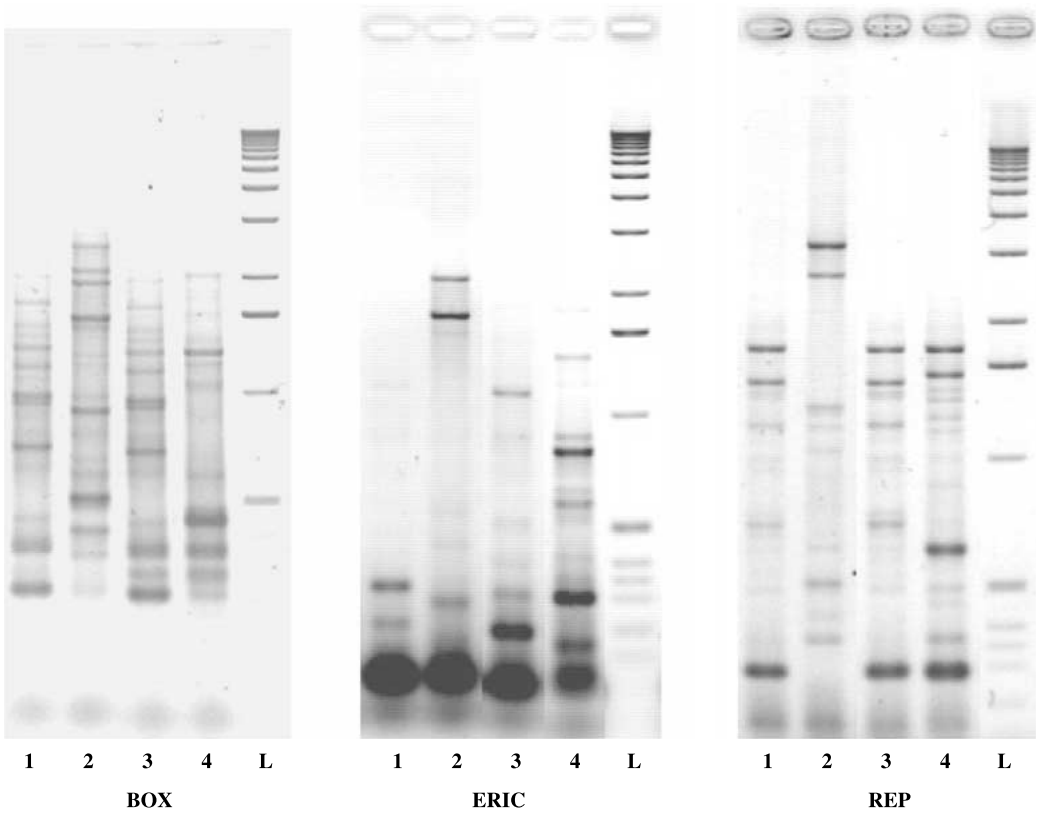

Figure 3. Composite gel showing representative rep-PCR profiles. Direct migration; 1, pv. *persicae* CFBP 1573; 2, pv. *morsprunorum* race 1 CFBP 2116; 3, wild cherry strain CFBP 3846; 4, pv. *morsprunorum* race 2 CFBP 3799; L, 1-kb ladder.

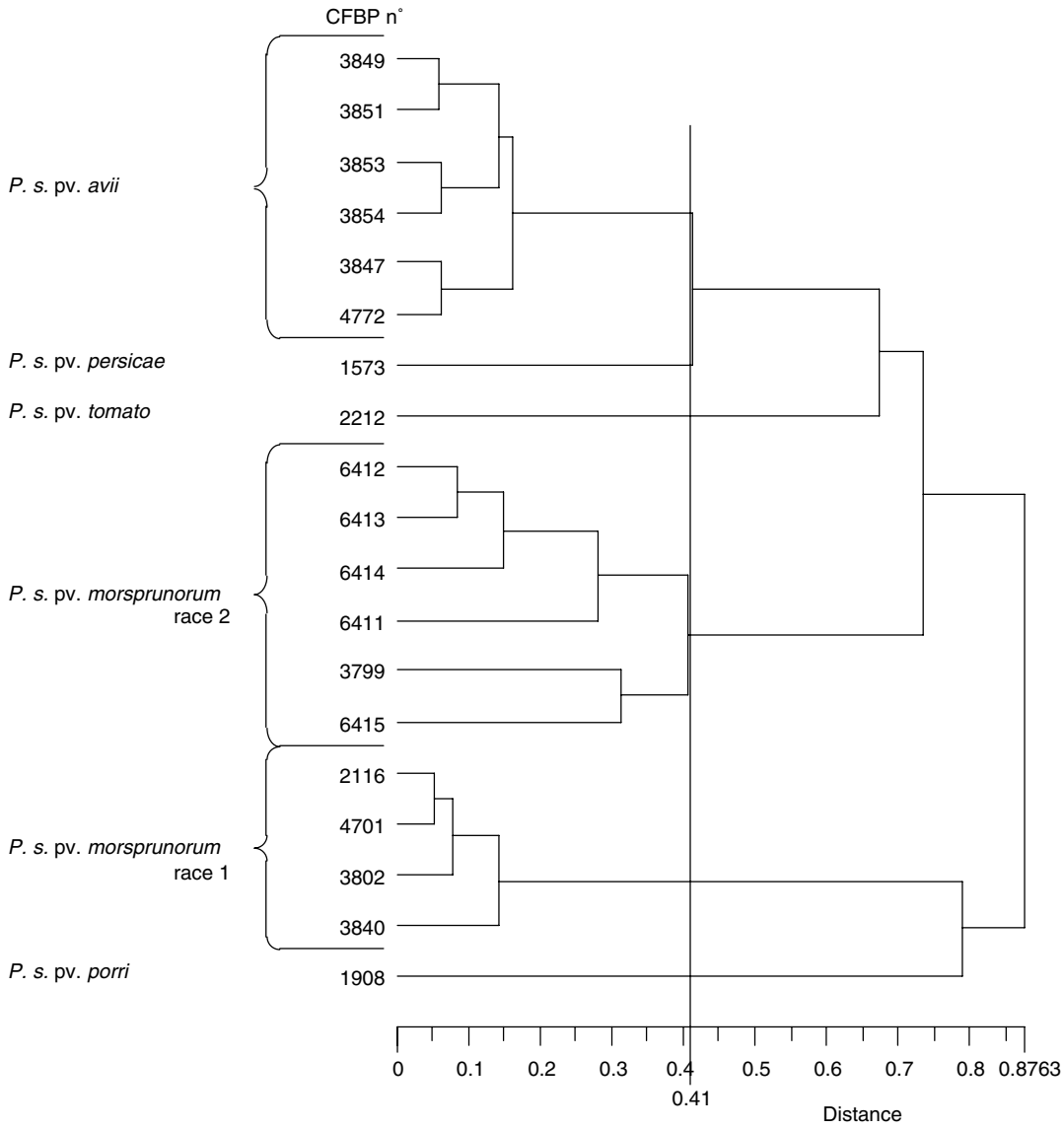

Figure 4. Dendrogram obtained by comparison of REP-PCR fingerprinting patterns from 6 *P. s.* pv. *avii* (wild cherry strains) and strains of *P. syringae* pathovars and related species.

In contrast, HMA profiles obtained after hybridization with pv. *persicae* strain CFBP 1573 generated two bands with reduced mobility that corresponded to heteroduplexes (Figure 5C).

Discussion

The wild cherry strains collected from five different areas in France, formed a very homogeneous group. They displayed a unique biochemical and physiological profile since all strains were clustered in a unique phenon when compared to 77 other reference strains representing 68 pathovars of *P. syringae* or related species. Moreover, utilization of some carbon sources discriminated wild cherry strains from other strains pathogenic to wild cherry tree, such as *P. s.* pv. *morsprunorum* race 1 and 2, from *P. s.* pv. *persicae* and *P. amygdali* strains, the most closely related strains, and from all other reference strains of *P. syringae* pathovars.

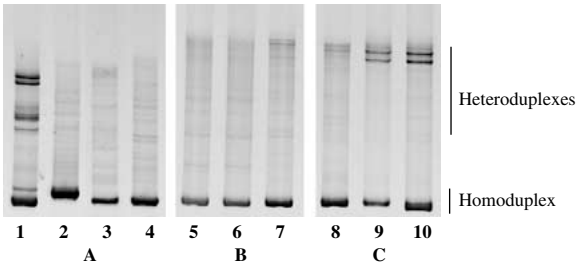

Figure 5. Composite gel showing representative profiles of PCR-amplified ITS obtained by HMA. A: Direct migration; 1, pv. *morsprunorum* race 1 strain CFBP 2116; the same profile with multiple bands was obtained with strains CFBP 3801, 3840 and 4701; 2, pv. *morsprunorum* race 2 CFBP 2351; 3, pv. *persicae* CFBP 1573; 4, strain CFBP 3846; the same profile was obtained with all other wild cherry strains. B: Profiles obtained after hybridization with strain CFBP 3846; 5, strain CFBP 3847; 6, strain CFBP 3851; 7, strain CFBP 3850; the same profile was obtained with all other strains from wild cherry. C: Profiles obtained after hybridization with pv. *persicae* strain CFBP 1573; 8, pv. *persicae* strain CFBP 1573 (homoduplex); 9, strain CFBP 3846; 10, strain CFBP 3849; the same profile was obtained with all other wild cherry strains.

By artificial inoculation, all wild cherry strains were confirmed to be pathogenic to wild cherry tree and to a lesser extent to sweet cherry tree. This new pathovar is very severe in comparison of those previously described.

The results of DNA–DNA hybridization indicated that wild cherry strains belonged to the genomospecies 3 '*tomato*' of Gardan et al. (1999), together with pv. *persicae* and pv. *morsprunorum* race 2, whereas *P. s.* pv. *morsprunorum* race 1 is included in genomospecies 2. The G+C content value of 59.0 mol.% for strain CFBP 3846 is in the range of values of 55.2–61.1 mol.% characterizing *P. syringae* pathovars and related species (De Vos et al., 1985).

Pathotype strain of pv. *morsprunorum* CFBP 2351 that should belong to genomospecies 2 and to race 1 is effectively classed in genomospecies 3 and is atypical for phenotypic characteristics. This result is in accordance with Gardan et al. (1999) and we propose to replace this nonpathogenic strain by a typical pathogenic one CFBP 2116.

Rep-PCR has already been used to study the genetic diversity of the pathovars of *P. syringae* group (Louws et al., 1994; Weingart and Völksh, 1997; Marques et al., 2000). Louws et al. (1994) have shown that *P. s.* pv. *morsprunorum* and *P. s.* pv. *syringae* isolated from sweet cherry could be distinguished on the basis of rep-PCR profiles. Similar results were obtained by Marques et al. (2000) for *P. s.* pv. *phaseolicola*, *P. s.* pv. *glycinea* and *P. s.* pv. *tabaci* included in genomospecies 2 of Gardan et al. (1999). In this study, rep-PCR profiles generated with REP-, BOX- and ERIC primers showed that wild cherry strains constitute a unique genetic group, clearly separated from other *P. syringae* pathovars pathogenic to wild or sweet cherry tree, pv. *morsprunorum* race 1 and 2. However, wild cherry strains and pv. *persicae* both belong to genomospecies 3 of Gardan et al. (1999) were closely related; identical REP-PCR profiles were obtained for wild cherry strains and *P. s.* pv. *persicae* but these bacteria could be differentiated by BOX and ERIC-PCR profiles. Thus, rep-PCR was well adapted to distinguished wild cherry strains from other pathovars isolated from wild cherry trees.

Because it is variable both in length and nucleotide sequence, ITS has been previously used to identify bacteria at specific or infraspecific levels, for example, *Pseudomonas stutzeri* genomovars (Guasp et al., 2000) and pathovars of *P. syringae* genomospecies (Manceau and Horvais, 1997) by enzymatic restriction fragment length and sequence analyses and *Salmonella* serovars (Jensen and Hubner, 1996) by HMA. HMA is based on the fact that heteroduplexes formed between PCR products which contained homologous sequences and regions with sequence divergence, had a reduced mobility in polyacrylamide gels with respect to homoduplex fragments (Jensen et al., 1993; Jensen and Hubner, 1996). HMA profiles with multiple bands obtained for strains of pv. *morsprunorum* race 1 indicated that the ITS of the different copies of the rRNA operons within each of these strains were different in sequence (Jensen and Hubner, 1996). In contrast, a unique band that corresponded to an homoduplex was obtained for strains of pv. *morsprunorum* race 2 CFBP 2351 and pv. *persicae* CFBP 1573 and for all wild cherry strains isolated; this would mean that no sequence divergence existed between the different copies of ITS for these bacteria. Moreover, the ITS of all wild cherry strains are identical since no heteroduplex bands were detected after hybridization with strain CFBP 3846, but are different from that of pv. *persicae* since two heteroduplex bands were generated after hybridization with pv. *persicae* strain CFBP 1573.

According to our results based on phenotypic traits, genomic patterns generated by rep-PCR, HMA profiles of ITS and pathogenicity, the strains from wild cherry should be considered as a new taxon. Since

the genomospecies of Gardan et al. (1999) have not been converted into formal species, wild cherry strains should be included in *P. syringae* as a new pathovar according to the international standards for naming new phytopathogenic bacteria (Young et al., 1996). The name *P. syringae* pv. *avii* pv. nov. is proposed and strain CFBP 3846 is designated as the pathotype strain.

Description of P. syringae *pv.* avii*, pv. nov*

P. syringae pv. *avii*, L. gen. n. of *avium*, the Latin name of wild cherry (*Prunus avium*). Cells are Gram-negative, motile with several polar flagella, and obligate aerobes with oxidative metabolism of glucose. Slow growth occurs on YPDA and King's medium B, producing colonies of 1–1.5 mm in diameter, after 3 days of incubation. Bacteria hydrolyse gelatin and do not produce any fluorescent pigment on King's medium B. They are levan positive, oxidase negative, nonpectolytic, arginine dihydrolase negative, and give a HR on tobacco (LOPAT Ia (+ - - - +) of Lelliot et al. (1966)).

Differential growth on the following carbon sources separates *P. syringae* pv. *avii* from the others *P. syringae* pathovars and related bacteria: no assimilation of erythritol, trigonelline, betaine, caprate, L-(+)-tartrate, L-histidine, esculine, myo-inositol, caprilate, glutarate, putrescine, α-ketoglutarate and D-glucuronate and assimilation of D-galacturonate and D-xylose.

Based on DNA–DNA hybridization, pv. *avii* belongs to genomospecies 3 of Gardan et al. (1999).

The pathotype strain of *P. syringae* pv. *avii* was deposited in the Collection Française des Bactéries Phytopathogènes as CFBP 3846, in the National Collection of Plant Pathogenic Bacteria as NCPPB 4290 and the International Collection of Microorganisms from Plants as ICMP 14479. The G+C content of the DNA of the type strain is 59.0 mol.%.

Acknowledgements

We wish to thank Sophie Bonneau and Joël Bénard for technical assistance and Alain Huard for designing the dendrograms. We are grateful to the French Agricultural Ministry, Direction de l'Espace Rural et de la Forêt (DERF) for their financial contribution.

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