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Genetic Diversity of *Xanthomonas arboricola* pv. *fragariae* Strains and Comparison with some other *X. arboricola* Pathovars using Repetitive PCR Genomic Fingerprinting

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With 6 figures

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Abstract

The genetic relationship within 26 *Xanthomonas arboricola* pv. *fragariae* strains and between this pathovar and 20 strains of *X. arboricola* pv. *corylina*, 22 strains of *X. arboricola* pv. *juglandis* and 16 strains of *X. arboricola* pv. *pruni* has been assessed by means of repetitive polymerase chain reaction (rep-PCR) using Enterobacterial Repetitive Intergenic Consensus), BOX (BOXA subunit of the BOX element of *Streptococcus pneumoniae*) and repetitive extragenic palindromic primer sets. Cluster analysis was performed by means of unweighted paired group method using arithmetic average (UPGMA). Upon rep-PCR and UPGMA cluster analysis, a relevant genetic diversity was found within the strains. The overall similarity, however, was high (i.e. 80%). The four *X. arboricola* pathovars showed similar but clearly different genomic patterns and clustered into four different groups, with *X. arboricola* pv. *corylina* and *X. arboricola* pv. *juglandis* more closely related to *X. arboricola* pv. *fragariae*. Representative strains of *X. arboricola* pv. *fragariae* and the putative xanthomonads isolated from strawberry leaves showing leaf blight symptoms underwent pathogenicity tests. After artificial inoculation, *X. arboricola* pv. *fragariae* induced necrotic spots accompanied, sometimes, by a chlorotic halo. The blackening of the leaf veins and peduncle was, sometimes, also observed. The four putative xanthomonads isolated from diseased strawberry leaves and not inducing symptoms after artificial inoculation, clustered apart from *X. arboricola* pathovars.

Introduction

Xanthomonas arboricola is a bacterial species (Vauterin et al., 1995) with restricted pathogenicity and genetically different from other *Xanthomonas* species. It includes pathovars *celebensis*, *corylina*, *juglandis*, *poinsetticola* type C strains, *populi* and *pruni* (Vauterin et al., 1995). Recently, Janse et al. (2001) described a new *X. arboricola* pathovar pathogenic to strawberry [*Fragaria vesca* L. x *ananassa* (Duch.) Guedes.], namely *X. arboricola* pv. *fragariae*, the causative agent of bacterial leaf blight of strawberry. Until now, the pathogen appears more frequently in the open-air cultivations than in the protected cultures. Within this new pathovar, DNA : DNA hybridization studies pointed out homology values among the studied strains ranging from 83 to 100%. The same three strains showed homology values with *X. arboricola* pv. *juglandis* NCPPB 411, the pathotype strain, ranging from 71 to 79% (Janse et al., 2001). In addition, fatty acid methyl ester and amplified fragment length polymorphism analysis revealed some variability among the 12 *X. arboricola* pv. *fragariae* strains tested that were isolated in Italy on November 1993 (Janse et al., 2001). In order to enlarge the collection of strains of this new pathogen, isolations were performed in the years 1999 and 2000. Over this period, other 18 putative *X. arboricola* pv. *fragariae* isolates were obtained from strawberry plants cultivated in northern Italy. Then, a study aimed to assess the genetic diversity of this new pathovar was performed by using repetitive polymerase chain reaction (rep-PCR) with Enterobacterial Repetitive Intergenic Consensus (ERIC), BOX (BOXA subunit of the BOX element of *Streptococcus pneumoniae*) and repetitive extragenic palindromic (REP) primer sets. A comparison with some other economically important pathovars of *X. arboricola*, namely *corylina*, *juglandis* and *pruni*, was also carried out by means of rep-PCR. The pathogenicity of some *X. arboricola* pv. *fragariae* strains was also tested. The results of this study are reported herein.

Materials and Methods

Bacterial cultures and isolation

The strains utilized in this study are listed in Table 1. They were cultured on glucose-yeast extract-calcium carbonate medium (GYCA) at 25–27°C. Isolations were performed from visibly infected strawberry leaves showing necrotic lesions on the leaflet blades. Pieces of leaf tissue adjacent to necrotic areas as well as portions of midrib, major veins and petioles showing symptoms resembling those induced by *X. arboricola* pv. *fragariae*, were crushed in sterile mortars containing sterile saline (0.85% of NaCl in distilled water). Tenfold dilutions were also performed. Aliquots of 0.1 ml of the suspensions were spread on GYCA medium and incubated for 3–4 days at 25–27°C. The mucoid and yellow colonies were transferred to nutrient agar (Oxoid, Basingstoke, UK) for purification.

Biochemical and physiological tests

With isolates suspected to belong to *X. arboricola* pv. *fragariae*, the following tests were performed according to the techniques reported by Lelliott and Stead (1987): oxidative and fermentative metabolism of glucose, oxidase reaction, presence of arginine dihydrolase and catalase, nitrate reduction, hydrolysis of esculin, urea, starch, gelatin and Tween 80, tobacco hypersensitivity.

Whole-cell protein extracts comparison

To confirm the identity of the isolates, sodium dodecyl sulphate-polyacrylanide gel electrophoresis (SDS-PAGE) of soluble whole-cell protein extracts were performed. Cultures were grown on GYCA for 48 h at 25–27°C. Protein harvesting and extraction were carried out by following the method of Van den Mooter et al. (1987). The profiles of the isolates were compared with those of *X. arboricola* pv. *fragariae* PD 2695, 2696 and 2780. Each strain was examined in duplicate.

Repetitive PCR genomic fingerprinting and UPGMA analysis

With all strains listed in Table 1, rep-PCR was performed by using ERIC, BOX and REP primer sets according to the procedures of Louws et al. (1994). The ERIC, BOX, and REP primer sets were synthesized by Eurogentech (Seraing, Belgium). Amplification was performed in an MJ Research PTC 100 programmable thermal controller (Watertown, MS, USA). Each 25 μl reaction mixture contained 200 μM deoxynucleoside triphosphate, 2 mM $MgCl_2$, primers at 60 pmol, *Taq* polymerase 1.0 U and 4 μl of mineral oil. After thermal cycling (Louws et al., 1994), the PCR products were separated by vertical gel electrophoresis on 6% acrylamide gels in 1X TBE buffer, at 160 V, 4°C for 30 min, in a Bio Rad Mini Protein apparatus (Hercules, CA, USA) (Scortichini et al., 2001, 2002). The PCR amplifications were performed in triplicate. The gels were stained with ethidium bromide and visualized under a UV transilluminator (Spectroline, Westburg, NY, USA) and photographed with a Polaroid film type 55. Gel analysis was made as described by Smith et al. (1995). The gels were recorded and bands common to all three amplifications were recorded. For each primer sets and for each strain, bands were scored as present (1) or absent (0) and the readings were entered in a computer file as a binary matrix. Similarity coefficients for all pairwise combinations were determined using Dice's coefficients (Dice, 1945) and clustered by UPGMA by means of NTSYS (Numerical Taxonomy Systematic) (Exeter Software, New York, NY, USA), pc-version, 1.80.

Pathogenicity tests

With *X. arboricola* pv. *fragariae* PD 2695, PD 2780 (pathotype strain), ISF F 23, ISF F 26, ISF F 30 and ISF F 99 representing the groups obtained upon rep-PCR and UPGMA analysis, pathogenicity tests were performed. Briefly, pot-cultivated strawberry cultivar Chandler plants were cultivated, in autumn, in glasshouse at 20–25°C day and 8–15°C night temperatures. Bacterial suspensions in sterile physiological saline (0.85% of NaCl in distilled water, SPS) were prepared from 48-h-old colonies grown on GYCA. A concentration of $1–2 \times 10^7$ cells/ml was used. Single leaflets were inoculated by puncturing major veins and peduncles and by placing 10 μl of the suspension on the wound. Six leaflets and six peduncles per each strain were inoculated. Control leaves were inoculated with SPS only. Symptom development was assessed up to 60 days after the inoculations. Re-isolations were carried out after the appearance of symptoms.

Results

Isolations, biochemical tests and SDS-PAGE of protein extracts

The GYCA medium frequently allowed the isolation of mucoid and yellow-pigmented bacteria from infected strawberry leaves. However, only 18 isolates showed the biochemical features showed by *X. arboricola* pv. *fragariae*: oxidative metabolism of glucose, oxidase reaction-negative, presence of catalase, absence of arginine dihydrolase and nitrate reduction, esculin, starch and gelatin hydrolysis-positive, urease and Tween 80 hydrolysis-negative, tobacco hypersensitivity-positive. This last test resulted in a delay of 48 h for ISF F 26, ISF F 29, ISF F 30 and ISF F 31. The visual comparison of protein extracts, upon SDS-PAGE, revealed an identical profile between *X. arboricola* pv. *fragariae* PD 2695, PD 2696, PD 2780 isolated in 1993 and the isolates obtained in 1999 and 2000, with the exception of ISF F 26, ISF F 29, ISF F 30 and ISF F 31 which showed qualitative diversity in the profile pattern.

Pathogenicity test

Xanthomonas arboricola pv. *fragariae* strains PD 2780 and PD 2695 as well as ISF F 23 and ISF F 99 induced discoloration of the veins 10–15 days after the inoculation, whereas the peduncle started to become

Table 1
List of strains used in this study

Strain	Host plant	Country	Year of isolation
Xanthomonas arboricola pv. *fragariae*			
PD 2694	*Frag. x an.*	Italy	1993
PD 2695	*Frag. x an.*	Italy	1993
PD 2696	*Frag. x an.*	Italy	1993
PD 2697	*Frag. x an.*	Italy	1993
PD 2698	*Frag. x an.*	Italy	1993
PD 2699	*Frag. x an.*	Italy	1993
PD 2780^{T}	*Frag. x an.*	Italy	1993
PD 2782	*Frag. x an.*	Italy	1993
PD 2803	*Frag. x an.*	Italy	1993
PD 2804	*Frag. x an.*	Italy	1993
PD 2805	*Frag. x an.*	Italy	1993
PD 2806	*Frag. x an.*	Italy	1993
ISF F23	*Frag. x an.*	Italy	1999
ISF F99	*Frag. x an.*	Italy	1999
ISF F113	*Frag. x an.*	Italy	2000
ISF F115	*Frag. x an.*	Italy	2000
ISF F116	*Frag. x an.*	Italy	2000
ISF F124	*Frag. x an.*	Italy	2000
ISF F126	*Frag. x an.*	Italy	2000
ISF F128	*Frag. x an.*	Italy	2000
ISF F129	*Frag. x an.*	Italy	2000
ISF F130	*Frag. x an.*	Italy	2000
ISF F135	*Frag. x an.*	Italy	2000
ISF F136	*Frag. x an.*	Italy	2000
ISF F137	*Frag. x an.*	Italy	2000
ISF F139	*Frag. x an.*	Italy	2000
Xanthomonas arboricola pv. *corylina*			
NCPPB 2896	*C. avellana*	UK	1976
NCPPB 3037	*C. avellana*	UK	1977
NCPPB 3339	*C. avellana*	France	1984
PD 1896	*C. avellana*	The Netherlands	1991
PD 1897	*C. avellana*	The Netherlands	1991
PD 3657	*C. avellana*	Germany	1999
Fil 6	*C. avellana*	USA (Oregon)	Unknown
Fil 19	*C. avellana*	USA (Oregon)	Unknown
Fil 29	*C. avellana*	USA (Oregon)	Unknown
Fil 85	*C. avellana*	USA (Oregon)	Unknown
ISF F Nc1	*C. avellana*	Italy	1996
ISF F Nc2	*C. avellana*	Italy	1996
ISF F Nc4	*C. avellana*	Italy	1997
ISF F Nc5	*C. avellana*	Italy	1997
ISF F Nc6	*C. avellana*	Italy	1998
ISF F Nc7	*C. avellana*	Italy	1998
ISF F Nc 9	*C. avellana*	Italy	1999
ISF F Nc10	*C. avellana*	Italy	1999
ISF F Nc 14	*C. avellana*	Italy	2000
ISF F Nc 15	*C. avellana*	Italy	2000
Xanthomonas arboricola pv. *juglandis*			
NCCPB 411^{T}	*J. regia*	New Zealand	1957
NCPPB 412	*J. regia*	New Zealand	1957
NCPPB 413	*J. regia*	New Zealand	1957
NCPPB 362	*J. regia*	UK	1955
NCPPB 1659	*J. regia*	UK	1964
NCPPB 1447	*J. regia*	Romania	1963
NCPPB 2927	*J. regia*	Iran	1977
NCPPB 3340	*J. regia*	France	1984
PD 130	*J. regia*	The Netherlands	1978
PD 157	*J. regia*	The Netherlands	1987
PD 2365	*J. regia*	The Netherlands	1994
PD 2277	*J. regia*	The Netherlands	1993
BPIC 279	*J. regia*	Greece	1970
BPIC 281	*J. regia*	Greece	1970
BPIC 349	*J. regia*	Greece	1971
BPIC 733	*J. regia*	Greece	1979
IVIA 1317.3	*J. regia*	Spain	1993
IVIA 1325. 2b	*J. regia*	Spain	1993
ISF FN1	*J. regia*	Italy	1991
ISF FN2	*J. regia*	Italy	1995
ISF FN3	*J. regia*	Italy	1999
ISF FN12	*J. regia*	Italy	1999

Table 1 (*Continued*)

Strain	Host plant	Country	Year of isolation
Xanthomonas arboricola pv. *pruni*			
NCPPB 416^T	*P. salicina*	New Zealand	1957
NCPPB 417	*P. salicina*	New Zealand	1957
NCPPB 926	*P. domestica*	South Africa	1961
NCPPB 1607	*P. persica*	Australia	1964
NCPPB 2587	*P. armeniaca*	South Africa	1964
NCPPB 2588	*P. persica*	South Africa	1964
NCPPB 3156	*P. persica*	Italy	1981
NCPPB 3877	*P. salicina*	Italy	1990
NCPPB 3878	*P. salicina*	Italy	1991
ISF FXP1	*P. salicina*	Italy	1995
ISF FXP2	*P. salicina*	Italy	1995
ISF FXP3	*P. persica*	Italy	1995
ISF FXP11	*P. salicina*	Italy	2000
ISF FXP12	*P. salicina*	Italy	2000
ISF FXP13	*P. salicina*	Italy	2000
ISF FXP14	*P. salicina*	Italy	2000
Unknown xanthomonads			
ISF F26	*Frag. x an.*	Italy	1999
ISF F29	*Frag. x an.*	Italy	1999
ISF F30	*Frag. x an.*	Italy	1999
ISF F31	*Frag. x an.*	Italy	1999

^{T}: Pathotype strain; *Frag. x an.* : *Fragaria vesca* x *ananassa*; *C. avellana*: *Corylus avellana*; *J. regia*: *Juglans regia*; *P. armeniaca*: *Prunus armeniaca*; *P. domestica*: *Prunus domestica*; *P. salicina*: *Prunus salicina*; *P. persica*: *Prunus persica*.
NCCPB: National Collection of Plant Pathogenic Bacteria, York, United Kingdom; PD: Plant Protection Service, Wageningen, The Netherlands; BPIC: Culture Collection of Benaki Phytopathological Institute, Kiphissia-Athens, Greece; IVIA: Instituto Valenciano de Investigaciones Agrarias, Moncada-Valencia, Spain; ISF: Culture Collection of Istituto Sperimentale per la Frutticoltura, Roma, Italy.

reddish 15–18 days after the inoculation. Blackening of the veins and a subsequent necrosis of the leaf tissue was observed 1 month after the inoculation. Afterwards, a chlorotic halo was, sometimes, noticed around the necrotic tissue. The leaf blight symptom was observed only in some symptomatic leaves showing necrosis and chlorotic halo, approximately 2 months after the inoculation. *Xanthomonas arboricola* pv. *fragariae* strain PD 2780 (pathotype strain) was the most aggressive, causing blight in four leaflets, whereas ISF F 23 inciting blight only on one leaflet was the least aggressive. The blackening of the peduncle was observed only in some cases and mainly with strain PD 2780 that induced such a symptom on three of the six inoculated peduncles. Isolates ISF F 26 and ISF F 30 induced only a slight discoloration around the inoculation site that never developed into a necrotic tissue. Re-isolations on GYCA performed from symptomatic leaflets and peduncles yielded yellow-pigmented colonies identical to *X. arboricola* pv. *fragariae* upon SDS-PAGE.

On the basis of biochemical tests, SDS-PAGE comparison of protein extract profiles with type and reference strains of *X. arboricola* pv. *fragariae* and pathogenicity tests, we conclude that the 14 isolates listed in Table 1 belong to *X. arboricola* pv. *fragariae*. ISF F 26, ISF F 29, ISF F 30 and ISF F 31 could not be classified, although, probably, they are xanthomonads.

Repetitive PCR genomic fingerprinting

ERIC, BOX and REP primer sets gave reproducible genomic PCR profiles consisting of bands of approximately 100–1700 bp. The bands were clearly differentiated by PAGE. For UPGMA analysis, a total of 41 reproducible, clearly resolved bands were scored: 18 for primers ERIC, 14 for primer BOX and nine for primer REP. ERIC and BOX primers were more discriminative than REP in differentiating the *X. arboricola* pv. *fragariae* strains. Representative genomic patterns are shown in Fig. 1–4. *UPGMA analysis

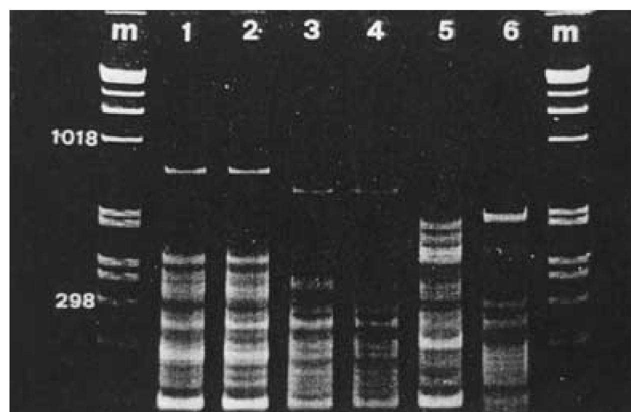

Fig. 1 Polymerase chain reaction fingerprinting patterns from genomic DNA of *Xanthomonas arboricola* pv. *fragariae* and putative xanthomonads strains, obtained by using enterobacterial repetitive intergenic consensus primer sets. m: molecular size marker (1-kb ladder, Gibco BRL, Life Technologies, Italy); the sizes are indicated in base pairs. Lane 1: ISF F 29; lane 2: ISF F 30; lane 3: *X. arboricola* pv. *fragariae* PD 2697; lane 4: *X. arboricola* pv. *fragariae* PD 2696; lane 5: *X. arboricola* pv. *fragariae* PD 2782; lane 6: *X. arboricola* pv. *fragariae* 2695

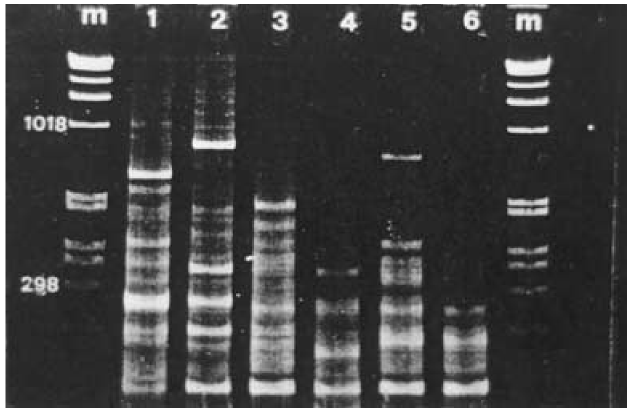

Fig. 2 Polymerase chain reaction fingerprinting patterns from genomic DNA of *Xanthomonas arboricola* pv. *fragariae* and putative xanthomonads strains, obtained by using enterobacterial repetitive intergenic consensus primer sets. m: molecular size marker (1-kb ladder, Gibco BRL, Life Technologies, Italy); the sizes are indicated in base pairs. Lane 1: *X. arboricola* pv. *fragariae* ISF F 128; lane 2: *X. arboricola* pv. *fragariae* ISF F 124; lane 3: *X. arboricola* pv.*fragariae* ISF F 116; lane 4: *X. arboricola* pv. *fragariae* ISF F 115; lane 5: ISF F 26; lane 6: ISF F 23

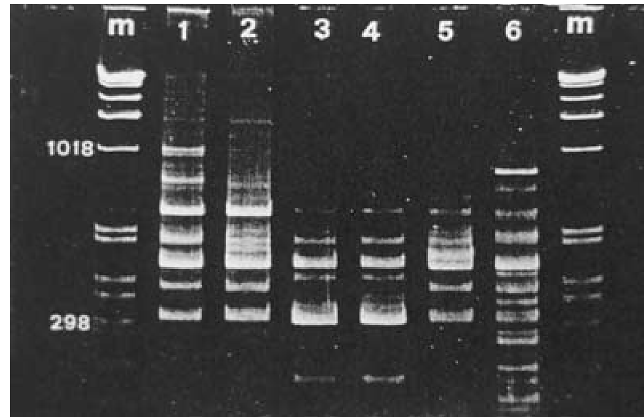

Fig. 4 Polymerase chain reaction fingerprinting patterns from genomic DNA of *Xanthomonas arboricola* pv. *corylina*, *X. arboricola* pv. *juglandis, X. arboricola* pv. *pruni* and putative xanthomonad strains, obtained by using BOX primer set. m: molecular size marker (1-kb ladder, Gibco BRL, Life Technologies, Italy); the sizes are indicated in base pairs. Lane 1: *X. arboricola* pv. *juglandis* NCPPB 483; lane 2: *X. arboricola* pv. *juglandis* 411^T; lane 3: *X. arboricola* pv. *pruni* NCPPB 416^T; lane 4: *X. arboricola* pv. *pruni* NCPPB 417; lane 5: *X. arboricola* pv. *corylina* Fil 6; lane 6: ISF F 31. Note the different genomic pattern of ISF F 31

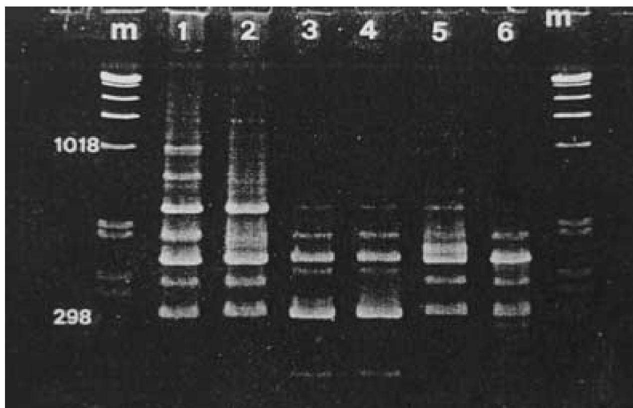

Fig. 3 Polymerase chain reaction fingerprinting patterns from genomic DNA of *Xanthomonas arboricola* pv. *fragariae*. *X. arboricola* pv. *corylina, X. arboricola* pv. *juglandis* and *X. arboricola* pv. *pruni* strains, obtained by using BOX primer set. m: molecular size marker (1-kb ladder, Gibco BRL, Life Technologies, Italy); the sizes are indicated in base pairs. Lane 1: *X. arboricola* pv. *juglandis* NCPPB 483; lane 2: *X. arboricola* pv. *juglandis* 411^T; lane 3: *X. arboricola* pv. *pruni* NCPPB 416^T; lane 4: *X. arboricola* pv. *pruni* NCPPB 417; lane 5: *X. arboricola* pv. *corylina* Fil 6; lane 6: *X. arboricola* pv. *fragariae* PD 2780^T

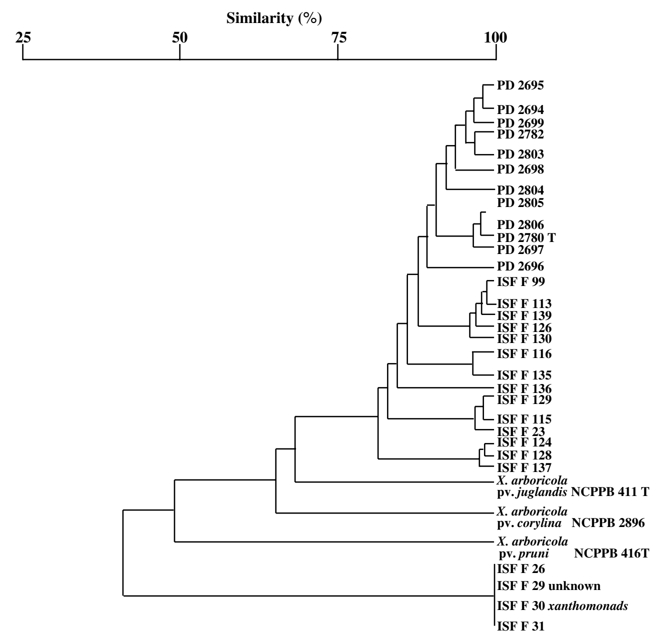

Fig. 5 Dendrogram showing relationship between *Xanthomonas arboricola* pv. *fragariae* strains, representative and type-strains of *X. arboricola* pvs. *corylina, juglandis* and *pruni*, and putative xanthomonads isolated from diseased strawberry leaves

revealed a relevant diversity among the 26 *X. arboricola* pv. *fragariae* strains because each strain showed a unique profile. The overall similarity, however, is high (i.e. $>80\%$) (Fig. 5). Each of the representative strains of *X. arboricola* pv. *juglandis, X. arboricola* pv. *corylina* and *X. arboricola* pv. *pruni* showed a similar but clearly different profile from *X. arboricola* pv. *fragariae* (Figs 3 and 4) and clustered apart (Fig. 5). When UPGMA analysis was carried out with all the strains listed in Table 1, the four pathovars studied formed four different clusters with *X. arboricola* pv. *juglandis* and *X. arboricola* pv. *corylina* the most closely related (Fig. 6). Also ISF F 26, ISF F 29, ISF F 30 and ISF F 31, the strains that induced a delayed hypersensitivity reaction of the tobacco leaves, showed a distinctive pattern and clustered apart from all the other *X. arboricola* strains (Fig. 5). In addition, all of the four strains showed the same genomic profile.

Discussion

This study showed that genetic variability is present in *X. arboricola* pv. *fragariae* strains isolated in Italy.

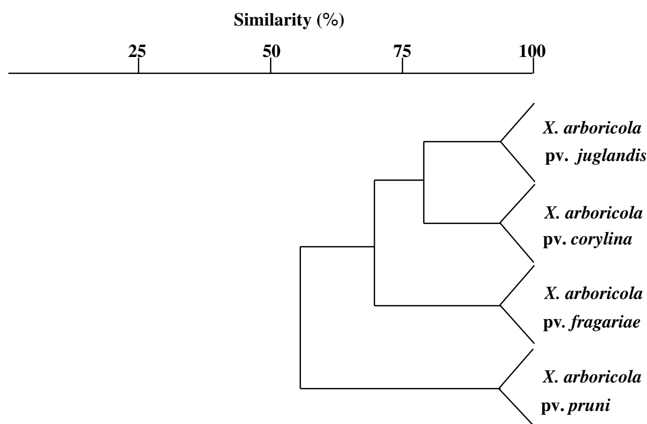

Fig. 6 Simplified dendrogram showing relationships between *Xanthomonas arboricola* pvs. *corylina, fragariae, juglandis* and *pruni*. Cluster analysis obtained by means of unweighted paired group method using arithmetic average with Dice's coefficients after repetitive polymerase chain reaction using enterobacterial repetitive intergenic consensus, BOX and repetitive extragenic palindromic primer sets

Rep-PCR and UPGMA analysis indicated that each strain has a distinct genomic profile, although the overall similarity of the patterns is high (i.e. around 80%). Further studies, taking into account the population structure of the pathogen (Maynard Smith et al., 1993), are necessary to assess if such a relevant genetic diversity is related to a real panmictic structure of the pathovar. In addition, the present study, performed with more strains than previously (Janse et al., 2001), confirms that such a new pathovar represents a distinct group within *X. arboricola*, with *X. arboricola* pv. *corylina* and *X. arboricola* pv. *juglandis* as the closest pathovars. Rep-PCR, based on primers targeting the highly conserved DNA sequences present in bacterial species, once more confirms its capability to easily discriminate among closely related phytopathogenic bacteria (Louws et al., 1999) and its used as a rapid and discriminatory technique to determine taxonomic diversity (Rademaker et al., 2000). The presence of these xanthomonads would be worthy of consideration also outside Italy as strawberry cultivation, nowadays, is based on an extensive exchange of propagative material on a world-wide scale. Genetic assessment by means of rep-PCR also ascertained that we isolated other xanthomonads of uncertain identity that exhibit substantial pathogenic and genetic diversity from *X. arboricola* pv. *fragariae*. Interestingly, this group did not induce any significant symptom to strawberry. These findings indicate that pathogenic and putative non-pathogenic xanthomonads can be present at the same time in/on strawberry leaves. Non-pathogenic xanthomonads have been repeatedly isolated from various plant material such as apple explants (Maas et al., 1985), vegetables and fruits (Liao and Wells, 1987), mixed infections in tomato and pepper transplants (Gitaitis et al., 1987), bean debris (Gilbertson et al., 1990) weeds (Angeles-Ramos et al., 1991). In an extensive study carried out with non-pathogenic xanthomonads, Vauterin et al. (1996) showed that none of the 70 strains tested induced symptoms on the plants from which they were originally isolated. Moreover, the identification of such strains was rather ambiguous and they could not be classified in the known pathovar system (Vauterin et al., 1996). The ecological role of such non-pathogenic xanthomonads is unknown, and Vauterin et al. (1996) argued that they could represent pathogenic *Xanthomonas* strains with still unknown host plant(s), being able to live as resident or epiphytes on many plants or that they can become pathogenic only under certain specific conditions. Our experience with *X. arboricola* pv. *fragariae* strains indicate that this micro-organism incites the leaf blight symptom mainly in open-field cultivation and during mid-autumn weather conditions characterized by a very high humidity content of the air. In fact, it is not always possible to artificially reproduce in glasshouse the symptoms observed in the field and the leaf blight symptoms are visible many weeks after the inoculation. Ecological and/or edaphic factors, possibly favouring the phytopathogenic activity of *X. arboricola* pv. *fragariae* are still unknown.

In conclusion, the isolation of yellow-pigmented bacteria from strawberry leaves is not rare, whereas the recovery of putative *X. arboricola* pv. *fragariae* would seem less frequent (R. Gozzi and A. Calzolari, personal communication). In addition, *X. arboricola* pv. *fragariae* and *X. fragariae* can be, sometimes, contemporaneously isolated either from strawberry plants with symptoms of angular leaf spot or with leaf blight (Scortichini, 1996). These findings indicate that mixed infections due to the contemporaneous presence of the two pathogens can occur on strawberry plants. Moreover, it has been ascertained that, within *X. fragariae*, different populations of the pathogen do exist either in the United States and Canada or Europe (Opgenorth et al., 1996; Pooler et al., 1996; Roberts et al., 1998; Janse et al., 2001). Consequently, an update of the techniques aiming to detect the pathogenic xanthomonads in the strawberry propagative material would seem important.

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