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AMPLIFIED FRAGMENT LENGTH POLYMORPHISM FINGERPRINTING OF *XANTHOMONAS ARBORICOLA* PV. *PRUNI*

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SUMMARY

The amplified fragment length polymorphism (AFLP) technique was applied to 109 strains of pv. *pruni* and one strain of pv. *juglandis* of the species *Xanthomonas arboricola* and to five strains of other *Xanthomonas* species. Groups of 12 and 41 strains of pv. *pruni* were isolated at the same time from fruit lesions in two peach orchards in the province of Verona (Italy). The other strains of pv. *pruni* were from different geographic areas and/or host plants. The *Eco*RI/*Mse*I and EOO/M02 pairs were used as restriction enzymes and selective amplification primers. Comparison of the AFLP profiles showed that the *X. arboricola* pv. *pruni* profile could be reliably distinguished from those of pv. *juglandis* and the other five species; 108 out of 109 strains of *X. arboricola* pv. *pruni* were included in the main cluster (S_D = 0.93). This was divided into 3 subgroups: I (S_D = 0.982; 73 strains); II (S_D = 0.979; 32 strains); III (S_D = 0.990; 3 strains). Another subgroup, IV, with just one strain clustered with the other 3 subgroups at S_D = 0.89. Isolates from the same peach orchard fell within subgroups I and II, as did strains from other geographic areas or from different host plants. The technique used did not have sufficient resolution to distinguish homogeneous groups of pv. *pruni* of *X. arboricola* on the basis of locality, geographic area or host plant.

RIASSUNTO

La tecnica AFLP è stata applicata a 109 colture della patovar *pruni* e 1 coltura della pv. *juglandis* della specie di *Xanthomonas arboricola* e a 5 ceppi di altrettante specie del genere *Xanthomonas*. Gruppi di 12 e 41 colture della pv. *pruni* sono state isolate contemporaneamente da maculature di frutti in due pescheti in provincia di Verona (Italia); le altre colture della pv. *pruni* avevano diversa origine geografica e/o pianta ospite. Le coppie *Eco*RI/*Mse*I e EOO/M02 sono state usate rispettivamente come enzimi di restrizione e primer di amplificazione selettiva.

La comparazione dei profili AFLP ha mostrato che la pv. *pruni* di *X. arboricola* ha un profilo assai riproducibile e distinguibile da quella della pv. *juglandis* e da quelli delle altre 5 specie; 108 su 109 colture di *X. arboricola* pv. *pruni* erano comprese in un gruppo principale (S_D = 0,93) suddiviso in 4 sottogruppi: I (S_D = 0,982; 73 colture); II (S_D = 0,979; 32 colture; III (S_D = 0,990; 3 colture). Un altro sottogruppo IV con un solo ceppo era associato agli altri tre sottogruppi per S_D = 0,89. Isolati di uno stesso pescheto sono risultati compresi sia nel sottogruppo I che nel sottogruppo II alla pari di ceppi provenienti da aree geografiche diverse o da differenti piante ospite. La tecnica AFLP usata non ha mostrato un potere di risoluzione tale da distinguere nella patovar *pruni* di *X. arboricola* gruppi omogenei per località, area geografica o pianta ospite.

Key words: AFLP, stone fruits, peach, plum, apricot, black spot, bacterial clone.

INTRODUCTION

In the reclassified genus *Xanthomonas*, the causal agent of black spot in stone fruits is designated as pathovar *pruni* of the species *X. arboricola* (Xap) (Vauterin *et al.*, 1995). Correct identification of Xap is essential for diagnosis of latent infections where the number of cells is very low and it is necessary to obtain a pure culture for identification to avoid false positives caused by epiphytic or saprophytic xanthomonads (Zaccardelli *et al.*, 1995)

In the genus *Xanthomonas*, pathovars are currently identified using phenotypic, pathogenic (host range) or biochemical fingerprinting (protein electrophoretograms, fatty acid composition) (Lelliott and Stead, 1987; Schaad, 1988; Vauterin *et al.*, 1990, 1991a, 1991b; Stefani *et al.*, 1994; Zaccardelli *et al.*, 1995). With genomic RFLP fingerprinting it appears possible

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to distinguish between pathovars (Vauterin *et al*., 1990), but this is not used by plant bacteriologists for routine identification.

In a preliminary study of seven species, AFLP was set up for the genus *Xanthomonas* (Janssen *et al*., 1996). When the restriction enzymes *Eco*RI and *Mse*I and the primer pair E00/M02 were used, the technique had a high enough resolution, to justify its use for identification of phytopathogenic xanthomonads, in which the specific and infrasubspecific taxons need to be identified (Janssen *et al*., 1996).

Before routinely using genomic fingerprinting to identify phytopathogenic species or their pathovars, its reliability must be assessed, and measurement of the variability of intraspecific and interstrain fingerprints is essential (Clayton *et al*., 1995). This study examined the intraspecific and intrapathovar variability of fingerprints obtained with the technique of 109 strains of Xap. A comparison was made between the AFLP fingerprints of isolates from the same peach orchard, those from different peach orchards in the same region and between these and authentic Xap strains isolated from other host plants in other Italian regions and abroad.

MATERIALS AND METHODS

Bacterial strains. The Xap strains are listed below according to their origin (host plant and isolation year are in brackets): Australia: NCPPB 1607 (peach, 1964); New Zeland: NCPPB 416 (Japanese plum, 1953), NCPPB 419 (peach, 1956); South Africa: XCP 1 (peach), XCP 3 (peach); XCP 93 (plum), XCP 96 (plum), NCPPB 923 (plum, 1960); USA: X120 (plum, 1989), X121 (peach, 1989), X122 (peach, 1987), X123 (apricot, 1987), X124 (nectarine, 1986), NCPPB 273 (1949); Campania: IPV-NA 45 (plum, 1996); Emilia Romagna: IPV-BO 3015 (peach, 1996), 1196 (plum, 1979), 1192 (plum, 1979), 2959 (plum, 1996), OMP-BO 576/91 (peach, 1991), 628/91 (plum, 1991), 691.1/91 (apricot, 1991); Lazio: I-461(plum, 1995), I-462 (peach, 1995), I-463 (peach, 1995), I-464 (peach, 1995), I-465 (peach, 1995), I-514 (peach, 1995), I-515 (peach, 1995), I-516 (peach, 1995); Marche: PV-BO 3022 (plum, 1996); Piemonte: IPV-BO 2286 (plum, 1993); Veneto: VR69^{V} (peach, 1989), 85^{P} (peach, 1989), 87^{S} (peach), 92 (peach), 93^{P}, 95 (peach), 98^{P} (peach), 110^{P} (peach), 110/B^{P} (peach), IPV-BO 2293^{Z} (peach, 1993), 2616^{S} (peach, 1992), 2617^{S} (peach, 1992), 2618^{S} (peach, 1992), 2619^{S} (peach, 1992), 2620^{S} (peach, 1992), 2623^{S} (peach, 1992), 2624^{S} (peach, 1992), 2625^{VL} (peach, 1992), 2626^{VL} (peach, 1992), 2629^{S} (peach, 1992), 2630^{S} (peach, 1992), 2633^{S} (peach, 1992), 2634^{S} (peach, 1992), 2635^{R} (peach, 1992), 2636^{R} (peach, 1992), 2638^{R} (peach, 1992), 2639^{R} (peach, 1992), 2640^{R} (peach, 1992), 2641^{R} (peach, 1992), 2642^{R}, (peach, 1992), 2643^{R} (peach, 1992), 2644^{R} (peach, 1992), 2645^{R} (peach, 1992), 2646^{R} (peach, 1992), 2647^{R} (peach, 1992), 2649^{R} (peach, 1992), 2650^{R} (peach, 1992), 2651^{R}, (peach, 1992), 2652^{R} (peach, 1992), 2835^{R} (peach, 1992), 2836^{R} (peach, 1992), 2837^{R} (peach, 1992), 2838^{R} (peach, 1992), 2839^{R} (peach, 1992), 2840^{R} (peach, 1992), 2841^{R} (peach, 1992), 2842^{R} (peach, 1992), 2843^{R} (peach, 1992), 2844^{R} (peach, 1992), 2845^{R} (peach, 1992), 2846^{R} (peach, 1992), 2847^{R} (peach, 1992), 2848^{R} (peach, 1992), 2849^{R} (peach, 1992), 2850^{R} (peach, 1992), 2851^{R} (peach, 1992), 2852^{R} (peach, 1992), 2853^{R} (peach, 1992), 2854^{R} (peach, 1992), 2855^{R} (peach, 1992), 2856^{R} (peach, 1992), 2857^{R} (peach, 1992), 2858^{R} (peach, 1992), 2859^{R} (peach, 1992), IS.1a (peach, 1993), IS.1b (peach, 1993), IS.3b (peach, 1993), IS.4a (peach, 1993), IS.4b (peach, 1993), IS.5 (peach, 1993), IS.6 (peach, 1993), IS.7 (peach, 1993), IS.8 (peach, 1993), IS.9 (peach, 1993), C3-3 (peach, 1993), C3-4 (peach, 1993).

The letters at the top right of the strain codes indicate the locality in the province of Verona (P = Pescantina; R = Rosegaferro; S = Sommacampagna; VL = Valeggio sul Mincio; V = Villafranca; Z = Zevio). The strains marked with the code IPV-BO IS were isolated from bud or leaf scars during the winter in peach orchards at Valeggio sul Mincio (Zaccardelli *et al*., 1995).

The strains were routinely grown at 27°C on YDC agar slants (Stolp and Starr, 1964) and stored for medium and long periods at -70°C in nutrient media with 15% glycerol or freeze-dried at 4°C. The strains from Rosegaferro and Sommacampagna were isolated at the same time from fruit spots in two orchards in the summer of 1992.

Cultures of the isolates from the peach orchards in the Veneto region were identified as Xap on the basis of colony morphology, pathogenicity on green fruitlets, total cell protein profiles and sensitivity to phage F_8. Authentic cultures of the same pathovar and of other species were included as positive and negative controls for the AFLP protocol (see below).

Other species or pathovars were used (name according to Vauterin *et al*., 1995, in brackets): OMP-BO 588/90 *X. campestris* pv. *campestris* (*X. campestris* pv. *campestr*is); IPV-BO 2535 *X. campestris* pv. *pelargoni* (*X. hortorum* pv. *pelargonii*); IPV-PG 32 *X. campestris* pv. *vesicatoria* (*X. axonopodis* pv. *vesicatoria*); IPV-BO 796 *X. campestris* pv. *juglandis* (*X. arboricola* pv. *juglandis*); IPV-BO 1921 *X. campestris* pv. *phaseoli* (*X. axonopodis* pv. *phaseoli*); NCPPB 1632 *X. oryzicola*

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(*X. oryzae* pv. *oryzicola*); NCPPB 1150 *X. oryzae* (*X. oryzae* pv. *oryzae*); OMP-BO 543/95, OMP-BO 619/96 *X. fragariae* (*X. fragariae*).

Abbreviations: IPV-BO = Istituto di Patologia Vegetale, Bologna; VR = Osservatorio per le Malattie delle Piante, Verona; OMP-BO = Osservatorio per le Malattie delle Piante, Bologna; IPV-PG = Istituto di Patologia Vegetale, Perugia; IPV-NA = Istituto di Patologia Vegetale, Napoli-Portici; I = Istituto Sperimentale per la Patologia Vegetale, Roma; NCPPB = National Collection of Plant Pathogenic Bacteria, United Kingdom.

Isolation and purification of DNA. The miniprep protocol of bacterial genomic DNA described in Ausubel *et al.* (1987-1988) was applied.

The concentration of the DNA samples was determined on a Uvidec-610 Jasco spectrophotometer by measuring A_{260} (1 absorbance unit = 50 µg ml^{-1}) and the quality was checked by measuring A_{280}. The DNA was stored at 4°C.

DNA restriction and ligation. The method of Janssen *et al.* (1996) was used with a few modifications. Restriction was done at 37°C for 2 h using a Perkin Elmer 2400 thermocycler. Each reaction contained 5 µl of 5 x reaction buffer (50 mM Tris-acetate, pH 7.5; 50 mM Mg-acetate; 250 mM K-acetate) 2.5 U each of *Eco*RI and *Mse*I enzymes and 250 ng of DNA. The final volume was 25 µl. At the end of digestion, the enzymes were denatured at 70°C for 15 min. The restriction enzymes were purchased from New England Biolabs.

Ligation. This was done at 20°C for 2 h using the thermocycler. To the digested DNA were added 12 µl of 2 x ligase buffer (20 mM Tris-acetate, pH 7.5; 20 mM Mg-acetate; 100 mM K-acetate; 0.8 mM ATP), 2 picomol of *Eco*RI adapter, 20 picomol of *Mse*I adapter, 1 U of T4 DNA ligase. The final volume was 50 µl. At the end of ligation, the enzyme was denatured at 70°C for 15 min. Adapters and ligase enzyme (Cat: no. 15224) were purchased from Gibco Brl.

DNA amplification (PCR). Each ligated sample was diluted 1:10 in filtered sterile double distilled water (SDDW) and 5 µl of the dilution were amplified in a thermocycler adding 5 µl of 10 x PCR buffer, 4 µl of 2.5 mM dNTPs, 80 ng each of selective primers E00 (for *Eco*RI: sequence 5'-GTAGACTGCGTACCAATTC-3') and M02 (for *Mse*I: sequence 5'-GATGAGTCCTGAGTAAC-3') and 1 U of Taq DNA polymerase; the final volume was 50 µl. There were 30 amplification cycles: 30 s at 94°C, 60 s at 55°C and 60 s at 72°C. Amplification was checked on 2% agarose gel. An equal volume of loading buffer (98% formamide, 10 mM EDTA, 1 mg ml^{-1} bromophenol blue) was added to each amplification sample, then stored at -20°C. The nucleotides were purchased from Boehringer Mannheim (Cat. no. 1277049), the primers from Gibco Brl, the Taq DNA-polymerase and 10 x reaction buffer from New England Biolabs (Cat. no. F5015).

Electrophoresis of amplicons. 5% denaturating polyacrylamide sequencing gel was used according to the method of Janssen *et al.* (1996). The electrophoretic cell was the S2S model (77 wells, 35 x 45 cm gel dimension) from Nugeneration™. The samples were denaturated at 95°C for 5 min before transfer to the gel; the marker was the 100 bp DNA ladder of Gibco Brl. The run was done at 70 W for 150 min.

Silver staining. The gel fixed on the glass was gently shaken for 30 min in 2 litre of 10% acetic acid solution (fixing step). The acetic acid solution was then recovered, the gel was washed twice in double distilled water (DDW) (5 min/time) and then gently shaken in 2 litre of staining solution (0.1% silver nitrate, 0.15% formaldehyde) for 30 min (staining step). After staining, the gel was immersed in DDW for 8 s and immediately shaken in 1 litre of developing solution (development step). When the first stained band appeared, the gel was immersed in another 1 litre of developing solution and shaken until staining was complete. Staining was blocked by adding the acetic acid solution used in the fixing step (stop step). The gel fixed on the glass was air dried or transferred to 3 MM paper by immersion in 2% NaOH.

Numerical analysis of banding patterns. For each group of bacteria with an identical pattern, a rectangular binary matrix (1 = presence of band, 0 = absence of band) was constructed to assess the gels. Using the software program Numeric Taxonomy Ntsys-pc (Numerical Taxonomy and Multivariate Analysis System) version 1.80, a similarity triangular matrix was created from the rectangular matrix using the band-based Dice similarity coefficient (S_D). The S_D was equal to the ratio of twice the number of common bands in two compared patterns and the sum of all bands in both patterns (Sneath and Sokal, 1973). Once the similarity matrix was constructed, the unweighted-pair group method with average linkages (UPGMA) (Sneath and Sokal, 1973) was used to cluster the patterns (Vauterin and Vauterin, 1992).

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RESULTS

The stability of the AFLP genomic profiles of Xap were determined before this study in strains 69, 95, 98 VR and in the strain NCPPB 923. The culture 69 VR used as a reference had an AFLP profile consisting of 50 bands. The sum of the molecular weight of the bands analysed between 700 bp and approximately 50 bp was 16,200 bp. Visual inspection of each gel revealed that the profiles of different Xap strains were quite similar to the reference (Fig. 1). Numeric analysis of the profiles, placed the 109 fingerprints in 12 homogeneous groups, each containing cultures with indistinguishable profiles.

The most numerous group A included 65 cultures, 51 of which came from peach orchards in the province of Verona, 5 isolates from Lazio (1 from plum and 4 from peach), 3 isolates from the Emilia Romagna region (from plum), 5 isolates from South Africa (2 from peach and 3 from plum) and 1 isolate from New Zealand (peach). The 51 cultures from peach in the province of Verona came from peach orchards in different localities: Rosegaferro (30); Sommacampagna (7); Pescantina (5); Valeggio sul Mincio (4); Villafranca (1). The original orchard of 2 cultures (92 and 95 VR) was unknown and the same for 2 strains (IPU-BO C3-3 and C3-4) isolated from dormant buds during the winter.

The second most numerous group B included 29 cultures, 19 isolated from peach orchards in the province of Verona, 3 in Emilia Romagna (2 from peach and 1 from plum), 3 in Lazio (from peach), 1 in the Marches (from plum), 1 in Piemonte (from plum), 1 in Campania (from plum) and 1 in the United States (from plum). The 19 strains from the province of Verona were isolated from different peach orchards: Rosegaferro (10), Sommacampagna (5), Valeggio sul Mincio (3), Zevio (1).

Groups C and D included 4 and 2 cultures isolated from dormant buds during the winter in peach orchards in the province of Verona. Group E included the profiles of 2 cultures isolated in the United States from peach and nectarine. Groups F and G included one strain each isolated from peach and from apricot in the USA.

The remaining 5 groups included profiles of individual cultures: one from New Zealand from Japanese plum (H); one from Emilia Romagna from apricot (I); one from Verona from peach (L), one from USA from peach (M), one from Australia from peach (N).

After building a linear rectangular matrix for the 12 groups, a dendrogram was obtained using the numeric taxonomy program NTSYS (Fig. 2). Nine groups out of 12, equal to 105 strains out of 109 were included in

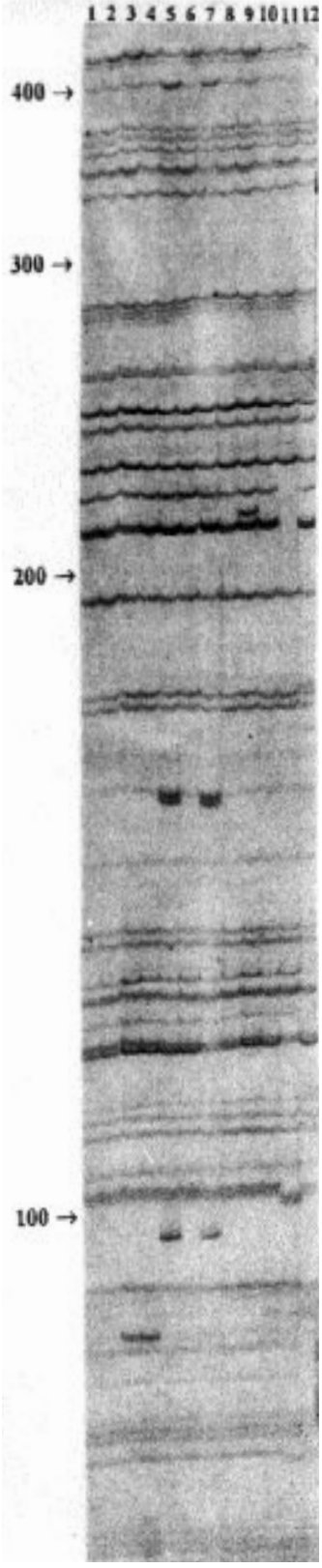

Fig. 1. AFLP fingerprints of 12 *X. arboricola* pv. *pruni* strains. On the left the numbers indicate the molecular weight expressed in base pairs. 1) IPV-BO 2618 (group A) isolated from peach in Veneto Region (Italy); 2) I-461 (group A) plum, Lazio (Italy); 3) IPV-BO IS.5 (group C) peach bud, Veneto (Italy); 4) IPV-BO IS.6 (group D) peach bud, Veneto; 5) X121 (group E) peach, USA; 6) IPV-BO 1192 (group A) plum, Emilia-Romagna; 7) X 123 (group G) apricot, USA; 8) NCPPB 923 (group A) plum, South Africa; 9) OMP-BO 691.1/91 (group I) apricot, Emilia Romagna (Italy); 10) NCPPB 419 (group A) peach, New Zealand; 11) NCPPB 273 (group M) USA; 12) NCPPB 1607 (group N) peach, Australia.

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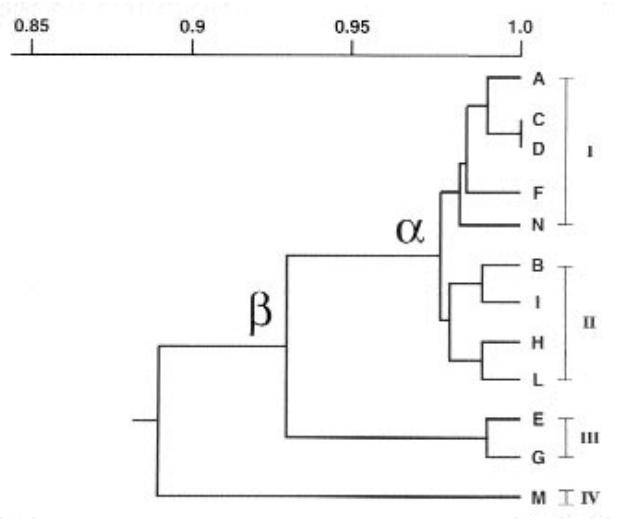

Fig. 2. Dendrogram obtained from AFLP analysis of the 109 *X. arboricola* pv. *pruni* strains. Each letter corresponds to one group of strains with an identical profile (Table 1). The numbers on the horizontal axis express the similarity index values. α and β are clusters of strains with similarity coefficients of 0.976 and 0.930 respectively.

the same cluster α with a correlation index of 0.976. A cluster β with a correlation index of 0.930 included 108 strains out of 109. Finally, all the 109 strains were included in a single cluster with S_D = 0.890. The 12 groups of profiles could be divided into four quite homogeneous subgroups: I (A, C, D, F, N; S_D = 0.982; 73 cultures); II (B, I, H, L; S_D =0.979; 32 cultures); III (E, G; S_D = 0.990; 3 cultures); IV (M; S_D = 0.89; 1 culture).

As compared to group A of Xap, the pathovar *juglandis* of *X. arboricola* had a S_D = 0.42; the other *Xanthomonas* species had the following correlation coefficients: *X. fragariae* (S_D = 0.38); *X. hortorum* (S_D = 0.37); *X. axonopodis* pv. *phaseoli* (S_D = 0.37); *X. axonopodis* pv. *vesicatoria* (S_D = 0.34); *X. oryzae* pv. *oryzicola* (S_D = 0.28); *X. campestris* pv. *campestris* (S_D = 0.22); *X. oryzae* pv. *oryzae* (S_D = 0.05).

Table 1. Twelve groups of Xap strains with AFLP profiles distinguishable with numeric analysis. The letters at the top right of the numbers indicate the locality in the province of Verona.

Groups	Cultures
A	IPV-BO: 2618^S, 2620^S, 2623^S, 2626^{VL}, 2629^S, 2630^S, 2633^S, 2635^R, 2636^R, 2638^R, 2639^R, 2640^R, 2646^R, 2650^R, 2651^R, 2652^R, 2835^R, 2836^R, 2838^R, 2839^R, 2840^R, 2841^R,2842^R, 2843^R, 2844^R, 2845^R, 2846^R, 2847^R, 2848^R, 2849^R, 2850^R, 2851^R, 2852^R, 2853^R, 2854^R, 2858^R, 2859^R, C3-3, C3-4, 1192, 2959, IS.1a, IS.3a, IS.4a; I: 461, 462, 463, 465, 516; NCPPB: 923, 419; OMP-BO 628/91; XCP: 1, 3, 93, 96; VR: 69^V, 85^P, 87^S, 92, 93^P, 95, 98^P, 110^P, $110B^P$.
B	IPV-BO: 2630^S, 2616^S, 2617^S, 2624^S, 2625^{VL}, 2634^S, 2641^R, 2642^R, 2643^R, 2644^R, 2645^R, 2647^R, 2649^R, 2855^R, 2856^R, 2857^R, 2293^Z, 3022, IS.3b, IS.3, IS.8, 1196, 3015; OMP-BO 576/91; I: 464, 514, 515; IPV-NA 45; X 120.
C	IPV-BO: IS.1b, IS.4b, IS.5, IS.7.
D	IPV-BO: IS.6, IS.9.
E	X: 121, 124.
F	X 122.
G	X 123.
H	NCPPB 416.
I	OMP-BO 691.1/91.
L	IPV-BO 2837^R.
M	NCPPB 273.
N	NCPPB 1067.

(P: Pescantina; R: Rosegaferro; S: Sommacampagna; VL: Valeggio sul Mincio; V: Villafranca; Z: Zevio).
Strains with numbers between IPV-BO 2635^R and IPV-BO 2859^R were isolated at the same time from fruit spots from the same orchard (Rosegaferro).

DISCUSSION

Numeric analysis of the AFLP fingerprints grouped 108 Xap strains out of 109 in a single main cluster β (S_D = 0.93) including 3 subgroups: I with 73 strains (S_D = 0.98); II with 32 strains (S_D = 0.979) and III with 3 strains (S_D = 0.990). The remaining strain, NCPPB 273 (group IV) isolated in the USA (unknown host) was however very similar (S_D = 0.89) to the main group. These high correlation index values indicate high DNA/DNA homology amongst the strains of the *X. arboricola* pv. *pruni* pathovars. On the other hand, the AFLP fingerprints of all the Xap strains were similar and were easy to distinguish even by visual inspection from those of 6 pathovars of 5 species of the *Xanthomonas* genera [*X. axonopodis* (S_D = 0.34 and 0.37); *X. hortorum* (S_D = 0.37); *X. fragariae* (S_D = 0.38);

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X. oryza (S_D = 0.28 and 0.05); *X. campestris* (S_D = 0.22)]. This indicates that the AFLP fingerprints obtained here can be used to identify Xap isolates at a species and pathovar level. For a full assessment of their reliability it is however necessary to compare analogous AFLP fingerprints of a wide collection of pathovars and species and also of saprophytic xanthomonads possibly associated with stone fruits.

Most of the strains produced AFLP fingerprints within subgroups I and II. Subgroup I (S_D = 0.982) included strains from five countries [Italy (65); South Africa (5); New Zealand (1); USA (1); Australia(1)] and from two host plants [peach (66); plum (7)]; the original Italian strains were from the Veneto (57), Lazio (5) and Emilia Romagna regions (3).

Subgroup II (S_D = 0.979) included strains from 3 countries [Italy (30); USA (1) and New Zealand (1)] and three host plants [peach (25); plum (5); Japanese plum (1); apricot (1)]; the Italian strains came from the Veneto (20), Emilia Romagna (4), Lazio (3), Piemonte (1), Marche (1), Campania (1) regions. These results show that the Xap strains from different host plants and from quite different geographic areas have indistinguishable AFLP genomic fingerprints using the *Eco*RI/*Mse*I pairs as restriction enzymes and E00/M02 as selective amplification primers.

The AFLP fingerprints of the 41 isolates from spots on peaches from the Rosegaferro orchard were included as part of subgroup I (30 isolates) and part of subgroup II (11 isolates); similarly 12 isolates from peaches from the Sommacampagna orchard (7 in subgroup I, 5 in subgroup II). In Subgroups I and II these showed type A and type B profiles which could not be distinguished from the authentic strains of Xap in one and in the other subgroup, isolated from peach or from other host plants, in other Italian regions or abroad. Clearly the quite low degree of variability between the isolates from the same orchard was also noted with the strains from different geographic areas and host plants.

AFLP fingerprints with a correlation index of approximately 0.98 can distinguish a bacterial clone (Huys *et al.*, 1996). Consequently the two subgroups I and II can be interpreted as separate clones. While it is easy to think that in each of the two orchards there might be two clones of Xap, it is unlikely that other strains heterogeneous for geographic origin and host plant belong to the same clones. This suggests that the AFLP technique used here does not have sufficient resolution to distinguish between Xap clones or strains of different geographic origin or host plants. Other restriction enzymes and adapters designed *ad hoc* could possibly enhance the resolution and identify groups at a lower hierarchical level to the Xap pathovar.

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