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Investigation of genomic variability of *Xanthomonas arboricola* pv. *juglandis* by AFLP analysis

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Abstract

Xanthomonas arboricola pv. *juglandis* is the causal agent of walnut blight, one of the most important and widespread diseases of Persian (English) walnut (*Juglans regia* L.), causing severe damage to leaves, twigs and nuts. To investigate the genomic variability of *X. arboricola* pv. *juglandis*, 66 isolates obtained from different countries (England, France, Italy, The Netherlands, Romania, Spain, USA, and New Zealand) were analysed using the Amplified Fragment Length Polymorphism (AFLP) technique. *Eco*RI and *Mse*I were used as restriction endonucleases. Primers with a core sequence including endonuclease recognition sites and a 3′-terminal cytosine selective base for *Mse*I primer, or no selective base for *Eco*RI primer, were used. Data were analysed by means of a multiple correspondence analysis. A total of 76 amplified polymorphic DNA fragments were used to compute relationships among isolates. The AFLP profiles of *X. arboricola* pv. *juglandis* isolates appeared to be reliably distinguishable from *X. arboricola* pv. *pruni* and *X. arboricola* pv. *corylina*, and from other *Xanthomonas* species, i.e. *X. campestris* pv. *campestris*, *X. fragariae*, *X. hortorum, X. axonopodis* pv. *vesicatoria*. Though this pathogen is associated with one single host genus, a high level of genomic diversity was found. This diversity might be partly explained by the geographic origin. Nevertheless, isolates with different patterns were collected within one country, and similar molecular patterns were found in isolates collected at different sites. However, genetic diversity might be influenced by exchanging vegetative material from different countries. Mixing of *X. arboricola* pv. *juglandis* isolates might have partly concealed the influence of the geographic location from which the bacteria were isolated.

Introduction

Xanthomonas arboricola pv. *juglandis* (Vauterin et al., 1995), also known as *Xanthomonas campestris* pv. *juglandis* (Pierce) Dye, is the causal agent of walnut blight, one of the most important diseases of Persian (English) walnut (*Juglans regia* L.). The disease has been known since the end of the 19th century (Ferraris, 1927) and is widespread in walnut growing areas. It causes severe damage to leaves, twigs, buds, petioles, rachides, male and female catkins, nutlets and kernels, and it is considered a major cause of reductions in fruit yield and tree vigour (Belisario et al., 1999). All succulent new growth is very susceptible to the disease, becoming more resistant when maturing (Belisario et al., 1997). A different susceptibility to walnut blight within *Juglans* species (Belisario et al., 1999) and *J. regia* cultivars has been observed, though no resistant cultivars have been discovered (Tamponi and Donati, 1990; Woeste et al., 1992).

The broad geographical distribution of the pathogen, for so long associated with walnut cultivation, could have induced genotypic heterogeneity. Investigations of genomic variability could provide useful information to aid identification of isolates to be used as inoculum, in breeding programmes for resistance of English walnut against blight.

In this research, genomic variability was assessed by Amplified Fragment Length Polymorphism (AFLP) analysis, a powerful tool for taxonomic studies as well as for DNA fingerprinting of bacteria, fungi, and many other organisms (Vos et al., 1995; Janssen et al., 1996; Majer et al., 1996; Semblat et al., 2000). The aim was to assess the molecular diversity of 66 isolates of *X. arboricola* pv. *juglandis* collected in different countries worldwide. Results were analysed by means of multiple correspondence analysis (MCA).

Materials and methods

Bacterial cultures

All the isolates used in this study are listed in Table 1. Each isolate was analysed by SDS-PAGE of whole-cell proteins for pathovar identification. Cultures were grown on nutrient agar (Oxoid LTD, Hampshire, England, UK) containing 0.25% D-glucose (NAG), at 27–28 °C. Stock cultures were lyophilized and stored at 4 °C.

Genomic DNA extraction

Total genomic DNA was extracted from 1.5 ml broth cultures (Ausubel et al., 1995). DNA concentration was determined spectrophotometrically at A_{260} (1 absorbance unit = 50 µg/ml), and DNA quality was checked by measuring the ratio between A_{260}/A_{280}.

DNA restriction and ligation

DNA restriction and ligation were performed following the method of Janssen et al. (1996) with minor modifications (Zaccardelli et al., 1999). DNA digestion was performed for 2 h at 37 °C; each reaction contained 5 µl of 5× 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 (New England Biolabs, Inc., USA) and 250 ng DNA. Enzyme denaturation was performed at 70 °C for 15 min. DNA ligation was performed by the addition of 2 µl of 5× reaction buffer, 0.1 µl of 100 mM ATP, 2.5 pmol of *Eco*RI adapter, 25 pmol of *Mse*I adapter (Gibco-BRL, Life Technology, UK), 1 U of T_4 ligase (Gibco-BRL, Life Technology, UK), and incubation for 3 h at 37 °C.

DNA amplification

PCR reactions were carried out as described by Zaccardelli et al. (1999) with minor modifications. PCR reactions (25 µl) contained 5 µl of ligation mixture diluted ten fold as template, 1 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech, UK) and 50 ng of selective primers E00 (5′-GACTGCGTACCAATTC-3′) and M02 (5′-GATGAGTCCTGAGTAAC-3′) (Gibco-BRL, Life Technology, UK). The primer E00 was constituted by a core sequence which included an *Eco*RI recognition site without any selective base, M02 primer included the *Mse*I recognition site in its core sequence with a 3′-terminal cytosine as selective base (Janssen et al., 1996). The thermal profile consisted of an initial denaturation step at 94 °C (30 s), followed by 30 cycles at 94 °C (30 s), 56 °C (30 s), 72 °C (1 min). PCR reactions were performed in Perkin Elmer 2400 and 480 thermocyclers. The amplification products were detected by electrophoresis on 2% agarose gel (Gibco-BRL, Life Technology, UK) in Tris-borate-EDTA (TBE) buffer, and stained with ethidium bromide (EtBr 1.25 mg/l), visualized under UV light. An equal volume of loading buffer (98% formamide, 10 mM

Table 1. Bacterial isolates used in this study, their geographic origin, host and date of isolation

Species	Isolate	Source[1]	Origin	Host	Date
X. a. pv. *juglandis*	411	NCPPB	New Zealand	*Juglans regia*	1956
	412	NCPPB	New Zealand	*Juglans regia*	11/1956
	413	NCPPB	New Zealand	*Juglans regia*	01/1957
	1447	NCPPB	Romania	*Juglans regia*	1962
	362	NCPPB	England	*Juglans regia*	1955
	1659	NCPPB	England	*Juglans regia*	1964
	1013	ISPaVe	Italy (Roma)	*Juglans regia*	06/1995
	1014	ISPaVe	Italy (Caserta)	*Juglans regia*	11/1996
	1017	ISPaVe	Italy (Cuneo)	*Juglans regia*	08/1996
	1018	ISPaVe	Italy (Caserta)	*Juglans regia*	1996
	1020	ISPaVe	Italy (Perugia)	*Juglans regia*	1993

Table 1. Continued

Species	Isolate	Source[1]	Origin	Host	Date
	1027	ISPaVe	Italy (Roma)	*Juglans regia*	1996
	1083	ISPaVe	Italy (Venezia)	*Juglans regia*	07/1998
	1086	ISPaVe	Italy (Venezia)	*Juglans regia*	07/1998
	1087	ISPaVe	Italy (Rovigo)	*Juglans regia*	1993
	1096	ISPaVe	Italy (Latina)	*Juglans regia*	02/1998
	1123	ISPaVe	Italy (Rovigo)	*Juglans regia*	1996
	1124	ISPaVe	Italy (Roma)	*Juglans regia*	1996
	1134, 1148, 1149	ISPaVe	France (Grenoble)	*Juglans regia*	07/1999
	1157, 1131	ISPaVe	France (Bassanne)	*Juglans regia*	07/1999
	1158	ISPaVe	France (Martel)	*Juglans regia*	07/1999
	1159	ISPaVe	France (Anhliac)	*Juglans regia*	07/1999
	1160	ISPaVe	France (Fougeres)	*Juglans regia*	07/1999
	1161, 1162	ISPaVe	France (Chatte)	*Juglans regia*	07/1999
	1178	ISPaVe	France (St Etienne)	*Juglans regia*	07/1999
	1163, 1177	ISPaVe	France (Labretoigne)	*Juglans regia*	07/1999
	1164	ISPaVe	Italy (Rovigo)	*Juglans regia*	07/1999
	1165, 1166, 1167	ISPaVe	Italy (Roma)	*Juglans regia*	09/1999
	34, 9, 10, 43	IPVNA	Italy (CE)	*Juglans regia*	—
	9508	S. Lindow	USA (California)	*Juglans regia*	—
	9509	S. Lindow	USA (California)	*Juglans regia*	—
	9525EXTL	S. Lindow	USA (California)	*Juglans regia*	—
	9608	S. Lindow	USA (California)	*Juglans regia*	—
	9609C	S. Lindow	USA (California)	*Juglans regia*	—
	9610D	S. Lindow	USA (California)	*Juglans regia*	—
	9501	S. Lindow	USA (California)	*Juglans regia*	—
	97-424B2L2R, 4-7-2, 2-7	S. Lindow	USA (California)	*Juglans regia*	—
	97-1Un18	S. Lindow	USA (California)	*Juglans regia*	—
	10-15-2	S. Lindow	USA (California)	*Juglans regia*	—
	2-15-N2	S. Lindow	USA (California)	*Juglans regia*	—
	97-1-Un4, 97-6/24B3n1	S. Lindow	USA (California)	*Juglans regia*	—
	4-5-S1, 4-5-S2	S. Lindow	USA (California)	*Juglans regia*	—
	189	PD	The Netherlands	*Juglans regia*	09/1979
	165	PD	France	*Juglans regia*	11/1987
	878	CFBP	France	*Juglans regia*	—
	176	CFBP	France	*Juglans regia*	—
	L121-2	L. Gardan	France	*Juglans regia*	—
	L119	L. Gardan	France	*Juglans regia*	1986
	G105-1	L. Gardan	France	*Juglans regia*	1986
	1317.bef	E. Montesinos	Spain (Benavidez)	*Juglans regia*	—
	1317-4df	E. Montesinos	Spain (Benavidez)	*Juglans regia*	—
	1330.3b	E. Montesinos	Spain (Daroca)	*Juglans regia*	—
X. a. pv. *pruni*	2588	NCPPB	South Africa	*Prunus armeniaca*	1973
X. a. pv. *corylina*	935	NCPPB	USA (Oregon)	*Corylus avellana*	1939
X. hortorum	250.1	—	Portogallo	*Pelargonium hortorum*	02/1992
X. c. pv. *campestris*	1032	ISPaVe	Italy (Roma)	*Brassica* spp.	10/1998
	35	CDF	Italy (Verona)	*Brassica oleracea*	—
X. fragariae	438	BPIC	—	*Fragaria* spp.	—
X. a. pv. *vesicatoria*	351	ISPaVe	Italy (Viterbo)	*Lycopersicon esculentum*	07/1993

[1]NCPPB: National Collection of Plant Pathogenic Bacteria, York, UK; ISPaVe: Istituto Sperimentale per la Patologia Vegetale, Rome, Italy; IPVNA, Istituto di Patologia Vegetale, Università degli Studi Federico II, Naples, Italy; S. Lindow, Department of Plant and Microbial Biology, University of California, Berkeley, USA; PD: Plantenziektenkundige Dienst, Bacteriology Department, Wageningen, The Netherlands; CFBP: Collection Francaise de Bactéries Phytopathogènes, Angers, France; L. Gardan, INRA, Station de Pathologie Végétale, Beaucouzé, France; E. Montesinos, Institut de Tecnologia Agralimentaria, Universitat de Girona, Spain; CDF: Centro Diagnostico Fitopatologico, Lugo – Ravenna, Italy; BPIC: Benaki Phytopathological Institute, Athens, Greece.

EDTA, 1 mg/ml bromophenol blue) was added to each amplification product and stored at −20 °C.

Electrophoresis of PCR products

The amplified fragments were analysed on 5% denaturing polyacrylamide gels according to the method of Janssen et al. (1996), using a 100 bp DNA ladder (Gibco-BRL, Life Technology, UK) as a size marker. Gels were routinely run at a constant power of 60 W for 200 min. Finally, the gel was fixed on the glass and silver stained (Zaccardelli et al., 1999).

Data analysis

Experiments were repeated at least once, and DNA fingerprints from each isolate were first converted to a 0–1 matrix (i.e. presence (1) or absence(0)) directly from the fixed gel. These data were analysed by a multiple correspondence analysis (MCA) (Benzecri, 1973; Greenacre and Hastie, 1987), using the PROC CORRESP procedure in the SAS/STAT package (SAS Institute Inc., 1990). This statistical method is a descriptive/exploratory technique designed to analyse simple two-way or multi-way tables containing some measure of correspondence between the rows (here the different isolates) and columns (here the presence/absence of the different bands). More accurately, this procedure corresponds to a weighted principal component analysis of a multi-way contingency table. In the present study, it has been used to find a low-dimensional graphical representation of the association between bacterial isolates and AFLP data in an Euclidian space. For this, the matrix to be analysed was first standardized, so that the relative frequencies across all cells sum to 1.0. This step enabled the data to be expressed in terms of distances between isolates (i.e. according to their respective AFLP band pattern) in a low-dimensional space. In the present case, the analysis produced the coordinates of the 66 isolates onto successive axes with decreasing importance. The first axes were by definition those that explained most of the information available in the data. These first axes were thus combined two by two in order to produce factorial planes that were more easy to read. The planes combining axes 1 and 2, and axes 3 and 4 were by definition those presenting the better graphical representation of all distances between the different bacterial isolates studied. Two isolates sharing close coordinates on these planes could be considered as being closely related based on their AFLP profiles. This method is widely considered to be appropriate for the analysis of categorical data and to produce a visual representation of the relationships between the row categories and the column categories in the same space. It is used, successfully, to compare presence/absence data in electrophoretic band patterns by several authors with different species (e.g., Lakrodi et al., 2000; Mahuku et al., 2000; Semblat et al., 2000). In order to increase the legibility of the factorial planes obtained, all 66 isolates were not plotted individually on each plane. Instead, ellipses representing 5% confidence intervals of average locations were computed and drawn on each plane in order to describe isolates on the basis of their geographic origin. Coordinates of all isolates on the first five axes only (representing 54.83% of the whole information of the data) were then used to compute an Euclidian distance between all isolate combinations. These distances were graphically described by a dendrogram (hierarchical ascending clustering) by PROC CLUSTER (option: CENTROID) of the SAS/STAT package (SAS Institute Inc., 1990).

Results

The reproducibility of the AFLP pattern of *X. arboricola* pv. *juglandis* was assessed using four isolates (NCPPB411, ISPaVe1163, 9619D, 9509) which were processed three times. No differences were observed between repeated experiments. Visual inspection of the gels revealed differences among banding patterns of *X. arboricola* pv. *juglandis* isolates (Figure 1), which appeared to be quite different from other *Xanthomonas* species (*X. arboricola* pv. *pruni*, *X. arboricola* pv. *corylina*, *X. campestris* pv. *campestris*, *X. fragariae*, *X. hortorum*, *X. axonopodis* pv. *vesicatoria*) (data not shown). AFLP fingerprints of *X. arboricola* pv. *juglandis* isolates were compared after identifying a total of 83 bands, between 200 and 600 bp, 76 of which were polymorphic. The presence/absence of these bands was used to analyse proximities between patterns of the isolates by means of a MCA, leading to the dendrogram shown in Figure 2.

Though identical profiles were observed for isolates coming from the same geographic location (2-7 and 10-15-2; ISPaVe1013 and ISPaVe1014; 97-424B2L2R and 4-7-2; 4-5-S1 and 4-5-S2; NCPPB411 and NCPPB412; ISPaVe1086 and ISPaVe1083; 97-1Un18

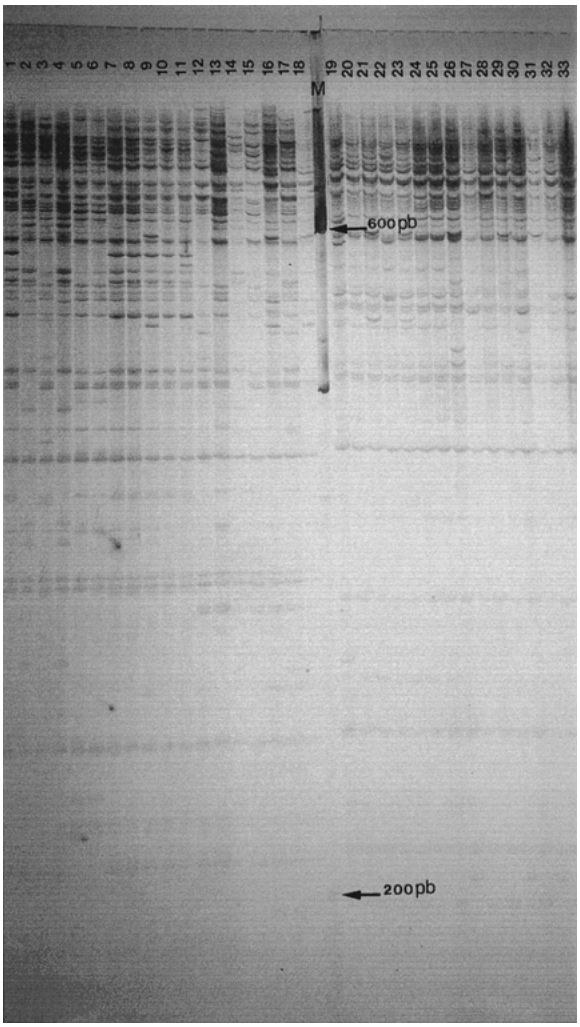

Figure 1. AFLP fingerprints of *X. arboricola* pv. *juglandis* isolates. AFLP was performed on genomic DNA using primer E00, constituted by a core sequence which includes *Eco*RI recognition site without any selective base, and M02 primer which includes the *Mse*I recognition site in its core sequence with a 3′-terminal cytosine as selective base. M = 100 bp ladder (Gibco-BRL, Life Technology, UK). Arrows indicate the molecular weight expressed in base pairs. Isolate number in lanes: 1, G105-1; 2, ISPaVe1013; 3, NCPPB1659; 4, ISPaVe1014; 5, IPVNA10; 6, IPVNA9; 7, CFBP878; 8, L121-2; 9, 97-6/24B3n1; 10, ISPaVe1027; 11, ISPaVe1149; 12, 1330.3b; 13, NCPPB362; 14, PD165; 15, 1317.bef; 16, 97-1Un18; 17, NCPPB413; 18, NCPPB412; 19, 97-424B2L2R; 20, ISPaVe1083; 21, 4-7-2; 22, L119; 23, NCPPB411; 24, ISPaVe1096; 25, ISPaVe1018; 26, ISPaVe1017; 27, PD189; 28, IPVNA43; 29, CFBP176; 30, NCPPB1447; 31, ISPaVe1087; 32, ISPaVe1086; 33, 10-15-2.

and 97-1Un4; IPVNA9 and IPVNA10; ISPaVe1131, ISPaVe1163, and ISPaVe1178), this could not be taken as a rule, since the profile of ISPaVe1087 from Italy was identical to the profile of NCPPB1447 from Romania, and the profiles of ISPaVe1131, ISPaVe1163 from France were identical to 1317.4df from Spain.

Information on banding patterns obtained was used to determine distances between isolates and to construct a dendrogram (Figure 2) revealing groups organized in clusters.

Five distinct groups (from G1 to G5) were delineated at a distance of 0.78 and are shown in detail in Table 2. Sub-groups were created where obvious divisions within the main group were present. Groups G2 and G3, which separated at a distance of 1.03, were included in cluster I. G4 merged with cluster I at a distance of 1.06, delineating cluster II which included G2, G3, G4. Isolates included in G5 were clearly distant (1.32) from cluster II, constituting cluster III. Finally, G1 merged with the other groups at a distance of 1.34.

The distribution of the 66 isolates of *X. arboricola* pv. *juglandis* in the factorial planes [1,2] and [3,4] is presented in Figure 3. Each of the five axes explained 21.42, 12.42, 8.28, 6.98, and 5.73% of the total variability respectively, resulting in an assessment of almost 55% of the total variability. Because the first four axes already explained 49% of the total variability, the analysis was limited to these axes. *X. arboricola* pv. *juglandis* populations could be divided into different groups on the basis of their geographic location in the factorial plane [1,2], which explained almost 34% of the total variability (Figure 3a). In the factorial plane [3,4], which explained more than 15% of the total variability, most of the populations were concentrated around the origin of both axes (Figure 3b).

Discussion

The purpose of the present study was to investigate the genomic variability of *X. arboricola* pv. *juglandis* and the potential role of geographic location as an origin of genetic diversity. Results revealed the presence of a considerable genomic variability in *X. arboricola* pv. *juglandis*. This heterogeneity differentiated this pathovar from *X. arboricola* pv. *pruni*, the variability of which has already been investigated by other authors (Zaccardelli et al., 1999). These two pathovars were reported to have some similarities, namely (1) they belonged to the same DNA homology group 6, (2) they

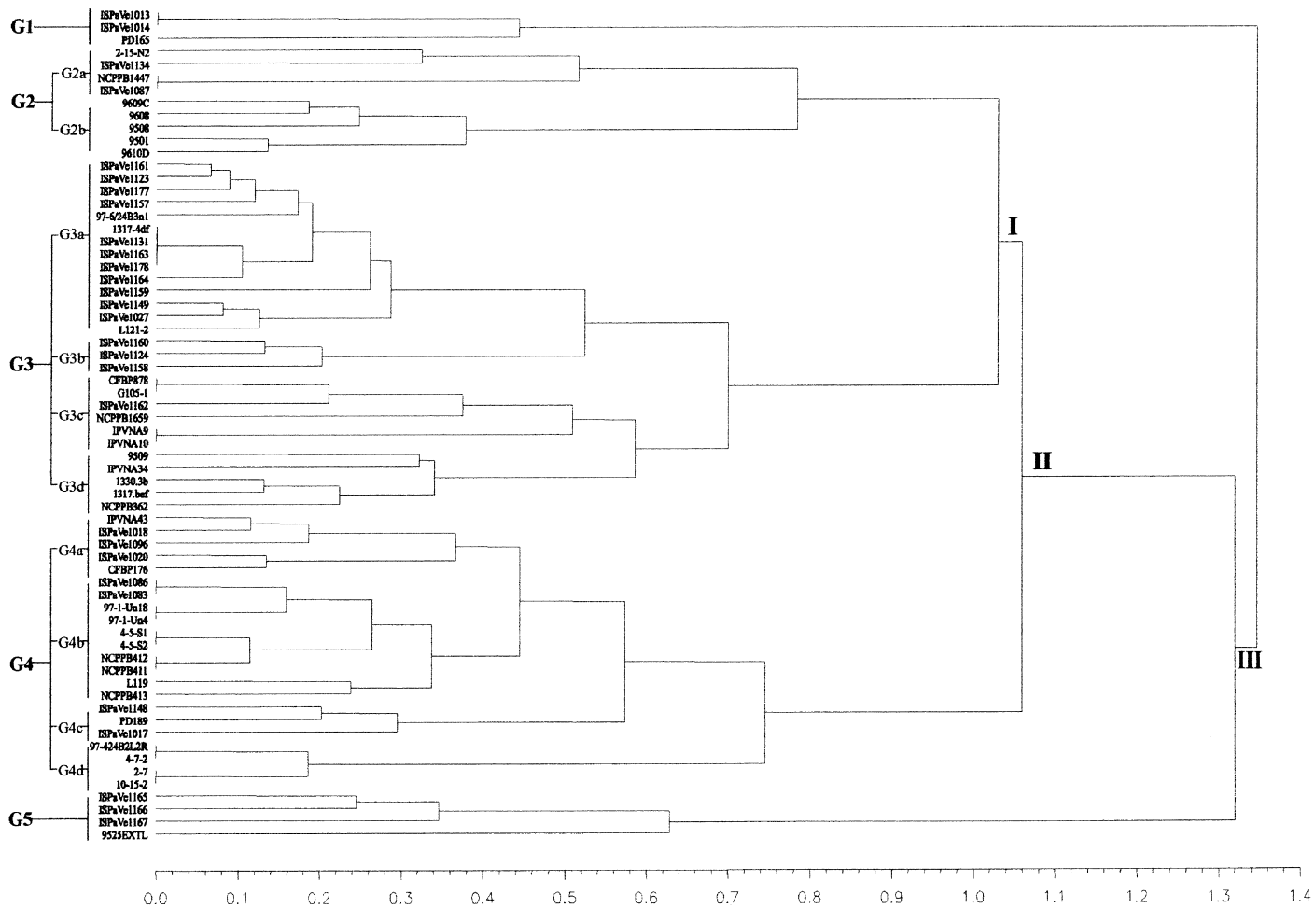

Figure 2. Dendrogram constructed from the coordinates of the 66 isolates of *X. arboricola* pv. *juglandis* studied on the first five axes of a multiple correspondence analysis. The dendrogram shows the distances between isolates according to AFLP profiles. Groups G1 to G5 were delineated at a distance of 0.78 and are shown with the respective subgroups that are detailed in Table 2. Groups were organized in clusters (I, II, III).

Table 2. Groups, subgroups and bacterial isolates of *X. arboricola* pv. *juglandis* distinguishable by multiple correspondence analysis

Groups	Subgroups	Isolates	Origin
G1		ISPaVe1013, 1014	Italy (2)
		PD165	France (1)
G2	a	ISPaVe1087	Italy (1)
		ISPaVe1134	France (1)
		2-15-N2	USA (1)
		NCPPB1447	Romania (1)
	b	9501, 9610D, 9609C, 9608, 9508	USA (5)
G3	a	ISPaVe1123, 1164, 1027	Italy (3)
		ISPaVe1161, 1177, 1157, 1131, 1163, 1178, 1159, 1149 L121-2	France (9)
		97-6/24B3n1	USA (1)
		1317-4df	Spain (1)
	b	ISPaVe1124	Italy (1)
		ISPaVe1160, 1158	France (2)
	c	IPVNA9, 10	Italy (2)
		CFBP878, G105-1, ISPaVe1162	France (3)
		NCPBB1659	England (1)
	d	IPVNA34	Italy (1)
		9509	USA (1)
		1330.3b, 1317.bef	Spain (2)
		NCPPB362	England (1)
G4	a	IPVNA43, ISPaVe1018, 1096, 1020	Italy (4)
		CFBP176	France (1)
	b	ISPaVe1086, 1083	Italy (2)
		97-1Un18, 97-1-Un4, 4-5-S1, 4-5-S2	USA (4)
		L119	France (1)
		NCPPB411, 412, 413	New Zealand (3)
	c	ISPaVe1017	Italy (1)
		ISPaVe1148	France (1)
		PD189	The Netherlands (1)
	d	97-424B2L2R, 4-7-2, 2-7, 10-15-2	USA (4)
G5		ISPaVe1165, 1166, 1167	Italy (3)
		9525EXTL	USA (1)

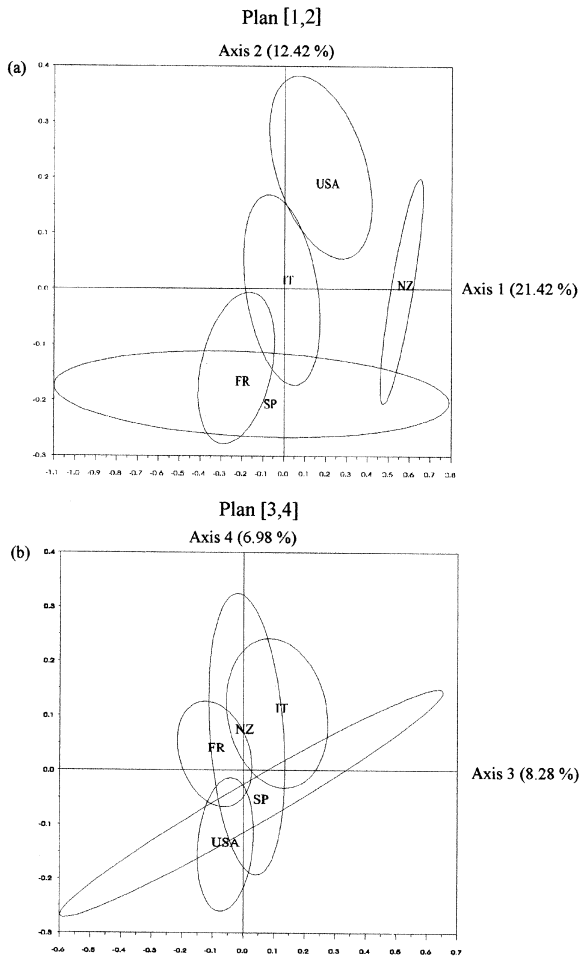

Figure 3. Distribution of *X. arboricola* pv. *juglandis* isolates according to a multiple correspondence analysis performed on AFLP data. Both factorial planes [1,2] (a) and [3,4] (b) are represented. Each ellipsis represents a 2D 5% confidence interval of average location of all isolates of a given geographic origin.

were both identified by the quinate metabolism test (Lee et al., 1992), and (3) they were grouped in the same cluster by SDS-PAGE analysis (Vauterin et al., 1991). Besides these similarities, AFLP analysis of *X. arboricola* pv. *pruni* revealed quite a lower degree of variability between isolates from different geographic areas and host plants (Zaccardelli et al., 1999). On the other hand, *X. arboricola* pv. *juglandis* heterogeneity has already been observed by SDS-PAGE analysis of whole-cell protein (Vauterin et al., 1991), and by DNA restriction fragment banding patterns of 15 *X. arboricola* pv. *juglandis* South African isolates (Du Plessis et al., 1995).

The genomic heterogeneity could be partly explained by differences between geographic origin. The factorial plane [1,2], which explained almost 34% of the total variability, showed that such genetic

variation is, at least partly, related to geographic location. In this graphical representation, European isolates (i.e. from France, Italy, and Spain) showed a greater overlap with one another. As can be seen from the enlarged ellipses, isolates from Spain and New Zealand seemed to be loosely clustered together, in comparison with the bacteria from Italy, France, and the USA. This fact could be related to the lower number of isolates representing those populations. Groups obtained from the dendrogram (Figure 2) did not strictly correspond to the geographic origin of the isolates (Table 2). Nevertheless, European isolates clustered in G1 (3 out of 3), G3 (26 out of 28), G4a (5 out of 5), G4c (3 out of 3); in particular French isolates clustered in G3 (14 out of 19). Although all the USA isolates were collected in California, they were distributed throughout the groups (except G1), being mainly in G2 (6 out of 17) and G4 (8 out of 17). Italian isolates showed the highest heterogeneity since they were present in all groups. In particular, they were present in the two groups, G5 and G1, which showed the highest distance values found. At the same time they tended to cluster frequently with French isolates (i.e. G1, G3a, b, c, G4a, c), but also with American isolates (i.e. G3d, G4b, G5). This last aspect is well represented on the factorial plane [1,2], on which Italian isolates were located on average in between the French and the USA bacteria, with a greater overlap with French isolates. It could be speculated that the cultivation of French (i.e. Lara) and American (i.e. Chandler, Serr) walnut cultivars in Italy from vegetative material imported directly from the original countries, might have contributed to the high heterogeneity of *X. arboricola* pv. *juglandis* in Italy. Conversely, exchange of vegetative material could have favoured the spread of *X. arboricola* pv. *juglandis* isolates worldwide. Moreover, the fact that the bacterium overwinters in dormant buds (Mulrean and Schroth, 1981, 1982) can facilitate the spread of this pathogen, even if apparently healthy material is commercialized. Mixing of *X. arboricola* pv. *juglandis* isolates might have partly concealed the influence of the geographic location from which the bacteria were isolated.

Nevertheless, the high variability observed in *X. arboricola* pv. *juglandis* seems to be a peculiar aspect of this pathovar. It is currently thought that bacterial evolution can be influenced by the characteristics and interactions of chromosome, plasmids and transposable elements (Coplin, 1989; Vivian et al., 2001). Transposable elements induce genomic rearrangement and can be considered as a major source of mutation (Haren et al., 1999) also in prokaryotic organisms. For instance, insertion elements have been identified in *X. arboricola* pv. *juglandis* and they were also found highly distributed in most pathovars of *X. campestris* (Lee and Chiu, 1998). These factors might be involved in increasing the genetic variability of *X. arboricola* pv. *juglandis*, even though they are general features occurring in prokaryotes.

The indications provided on the genetic variability observed in *X. arboricola* pv. *juglandis* could be useful for choosing isolates to be used for selection of walnut germplasm in breedings programmes for resistance against blight.

In conclusion, AFLP analysis proved to be reproducible, reliable, and sufficiently sensitive to reveal the individual genomic variability within *X. arboricola* pv. *juglandis* isolates used in this study. Results indicate that geographic location could be partly responsible for the genomic heterogeneity.

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References

Ausubel FM, Brent R, Kinston RE, Moore DD, Seidman JD, Smith JA and Struhl K (1995) Short Protocols in Molecular Biology (pp. 2–15) John Wiley & Sons Inc., New York

Belisario A, Palangio C and Zoina A (1997) Suscettibilità e fattori predisponenti a *Xanthomonas campestris* pv. *juglandis* in vivaio di noce comune. Informatore Fitopatologico 4: 60–64

Belisario A, Zoina A, Pezza L and Luongo L (1999) Susceptibility of species of *Juglans* to pathovars of *Xanthomonas campestris*. European Journal of Forest Pathology 29: 75–80

Benzecri JP (1973) L'analyse des Données. L'analyse des correspondances, Vol. 2 (pp. 619) Dunod, Paris

Coplin DL (1989) Plasmids and their role in the evolution of plant pathogenic bacteria. Annual Review of Phytopathology 27: 187–212

Du Plessis HJ and Van der Westhuizen TJ (1995) Identification of *Xanthomonas campestris* pv. *juglandis* from (Persian) English walnut nursery trees in South Africa. Journal of Phytopathology 143: 449–454

Ferraris T (1927) Trattato di patologia e terapia vegetale. Vol. II (pp.1019–20) Hoepli, Milano

Greenacre MJ and Hastie T (1987) The geometric interpretation of correspondence analysis. Journal of American Statistical Association 82: 437–447

Haren L, Ton Huang B and Chandler M (1999) Integrating DNA: transposase and retroviral integrases. Annual Review of Microbiology 53: 245–281

Janssen P, Coopman R, Huys G, Swings J, Blecker MD, Vos P and Zabeau M (1996) Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. Microbiology 142: 1881–1893

Lakrodi K, Chaisrisook C, Yongsmith B and Skinner DZ (2000) RAPD analysis of genetic variation within a collection of *Monascus* spp. isolated from red rice (ang-kak) and sofu. Mycological Research 104: 403–408

Lee YA and Chiu SP (1998) IS*1403* and IS*1404*: analysis and distribution of two new insertion sequences in *Xanthomonas campestris*. Botanical Bullettin of Academia Sinica 39: 231–239

Lee YA, Hildebrand DC and Schroth MN (1992) Use of quinate metabolism as a phenotypic property to identify members of *Xanthomonas campestris* DNA homology group 6. Physiological Biochemestry 82: 971–973

Mahuku G, Peters RD, Platt HW and Daay F (2000) Random amplified polymorphic DNA (RAPD) analysis of *Phytophthora infestans* isolates collected in Canada during 1994 to 1996. Plant Pathology 49: 252–260

Majer D, Mithen R, Lewis BG, Vos P and Oliver RP (1996) The use of AFLP fingerprinting for the detection of genetic variation in fungi. Mycological Research 100: 1107–1111

Mulrean EM and Schroth NM (1981) A semiselective medium for the isolation of of *Xanthomonas campestris* pv. *juglandis* from walnut buds and catkins. Phytopathology 71: 336–339

Mulrean EM and Schroth NM (1982) Ecology of *Xanthomonas campestris* pv. *juglandis* on Persian (English) walnuts. Phytopathology 72: 434–438

SAS Institute, Inc. (1990) SAS/STAT User's Guide, Release 6.07. Vol. 1 SAS Institute Inc., Cary, North Carolina

Semblat JP, Bongiovanni M, Wajnberg E, Dalmasso A, Abad P and Castagnone-Sereno P (2000) Virulence and molecular diversity of parthenogenetic root-knot nematodes, *Meloidogyne* spp. Heredity 84: 81–89

Tamponi G and Donati GP (1990) Walnut cultivars susceptible to *Xanthomonas juglandis*. Acta Horticolturae 284: 301

Vauterin L, Swings J and Kersters K (1991) Grouping of *Xanthomonas campestris* pathovars by SDS-PAGE of proteins. Journal of General Microbiology 137: 1677–1687

Vauterin L, Hoste B, Kersters K and Swings J (1995) Reclassification of *Xanthomonas*. International Journal of Systematic Bacteriology 45: 472–489

Vivian A, Murillo J and Jackson R (2001) The roles of plasmids in phytopathogenic bacteria: mobile arsenal? Microbiology 147: 763–780

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot Jerina, Peleman J, Kuiper M and Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407–4414

Woeste KE, McGranahan GH and Schroth NM (1992) Variation among Persian walnuts in response to inoculation with *Xanthomonas campestris* pv. *juglandis*. Journal of the American Society of Horticultural Science 117: 527–531

Zaccardelli M, Ceroni P and Mazzucchi U (1999) Amplified fragment length polymorphism fingerprinting of *Xanthomonas arboricola* pv. *pruni*. Journal of Plant Pathology 81: 173–179