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Genetic and pathogenic diversity of *Pseudomonas avellanae* strains isolated from *Corylus avellana* trees in north-west of Italy, and comparison with strains from other regions

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Abstract

Forty strains of *Pseudomonas avellanae* isolated from hazelnut (*Corylus avellana* L.) trees in the Langhe district of Italy were compared with 15 strains collected from various geographic areas. All strains were assessed by rep-PCR genomic fingerprinting using ERIC, REP and BOX primer sets. Cluster analysis was performed by means of UPGMA. To check the possible differential virulence of the strains, pathogenicity tests were carried out by inoculating leaf scars of hazelnut trees in early autumn. Cluster analysis indicated that, during a four-year study, at least five groups of strains were isolated from different hazelnut orchards located in the small district of Langhe. Two groups were isolated from the same twig. Such strains showed around 20% similarity with other *P. avellanae* strains collected from northern Greece and central Italy. The strains from Langhe were less aggressive to hazelnut than strains from northern Greece and central Italy. These results and previous genomic characterizations indicate a possible correlation between genomic profile type and regional geographic distribution of *P. avellanae* strains. In addition, the genetic variability found in the strains from Langhe indicates that such populations are older than the more homogenous *P. avellanae* populations from other regions.

Introduction

Pseudomonas avellanae (Psallidas) Janse et al., is the causal agent of bacterial canker of hazelnut (*Corylus avellana* L.), in northern Greece (Psallidas and Panagopoulos, 1979) and in many regions of Italy (i.e. Campania, Latium, Piedmont, Sardinia) (Scortichini and Tropiano, 1994; Janse et al., 1996; Scortichini and Angelucci, 1999). This disease causes economic losses in northern Greece (Psallidas, 1987) and in the Viterbo area of Italy (Scortichini, 1998) killing thousands of trees each year. In other regions (Campania, Piedmont, Sardinia), the disease appears to be endemic and rarely kills trees. Preliminary studies on the population structure of the pathogen using rep-PCR, revealed homogeneity in the genomic profiles of strains isolated over many years from northern Greece and central Italy. However, a small sample of strains from Langhe, Italy, had polymorphic profiles (Scortichini et al., 1998). These differences prompt us to investigate the genetic structure and the pathogenicity of a larger collection of strains of *P. avellanae* isolated from Langhe using rep-PCR genomic fingerprinting with Enterobacterial Repetitive Intergenic Consensus (ERIC), Repetitive Extragenic Palindromic (REP) and Box Elements (BOX) primer sets (Versalovic et al., 1991; Louws et al., 1994).

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Materials and methods

Bacterial cultures

The *P. avellanae* strains are described in Table 1. They were isolated from hazelnut twigs showing symptoms of bacterial canker. Cultures were stored at −20 °C in 523 broth medium (Schaad, 1988) and 15% v/v glycerol added. Strains were cultured on nutrient agar with 5% sucrose (NSA).

Identification of strains

The strains isolated from Langhe were identified according to Psallidas (1993), Scortichini and Tropiano (1994) and Janse et al. (1996). In addition, the method proposed by Young (1991) was adopted to differentiate them from *P. syringae* pv. *syringae* van Hall. Briefly, the following biochemical and nutritional tests were performed according to techniques described by Lelliott and Stead (1987) and by Schaad (1988): metabolism of glucose, aesculin and arbutin hydrolysis, presence of oxidase, urease, tyrosinase, and arginine dihydrolase, ability to liquefy gelatin, nitrate reduction, tolerance to NaCl (4%) and to triphenyltetrazolium chloride (TTC) (0.05%), utilization of inositol, sorbitol, L(+)tartrate, homoserine, amygdalin, D-xylose, alanine, L-aspartic acid, L-histidine, L-leucine and DL-phenylalanine.

Preparation of DNA and rep-PCR analysis

For total genomic DNA preparation, a modification of the technique used by Smith et al. (1995) was used. A loop (i.e. 3 mm) full of a single colony of each strain grown for 24 h on NSA at 25 ± 1 °C was suspended in sterile saline (0.85% of NaCl in distilled water) (SS) and centrifuged at 11,000 rpm for 2 min. After discarding the supernatant, the pellet was suspended in SPS at an optical density corresponding to 1–2 × 10^8 colony forming units (cfu)/ml. The suspension was placed in boiling water for 10 min and then stored at −20 °C. The rep-PCR method used was that of Louws et al. (1994). The ERIC, REP and BOX primer sets were synthesized by Eurogentech (Belgium). Amplification was performed on a MJ Research PTC 100 programmable thermal controller in 25 µl reaction volume containing 200 µM deoxynucleoside triphosphate, 2 mM $MgCl_2$, primers at 60 pmol, Taq polymerase 1.0 U and 4 µl of cell preparation sample. The PCR mixture was overlaid with 25 µl of mineral oil. After thermal cycling (Louws et al., 1994), products of PCR amplifications were separated by gel electrophoresis on 2.0% agarose gel in 1× TAE buffer, at 5 V/cm over 5 h, stained with ethidium bromide, visualized under a UV transilluminator and photographed with a Polaroid film type 55. The PCR amplifications were performed in triplicate. For gel analysis, the method followed by Smith et al. (1995) was adopted. Visual readings of the gels were taken and bands common to all three amplifications were scored and recorded. For each primer 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 coefficient for all pairwise combinations were determined using Dice's coefficients (Dice, 1945) and clustered by unweighted paired-group using arithmetic averages (UPGMA) by means of NTSYS (Exeter Software, New York, USA) software, version 1.80.

Pathogenicity tests

Adult *C. avellana* trees cv. 'Tonda Gentile Romana' and 'Nocchione' located in Latium region (Rome, central Italy) were chosen for the pathogenicity tests. For each group of strains, three representative strains were selected for bioassay tests. The inoculation technique has been previously described (Scortichini and Lazzari, 1996; Scortichini, 1998). At the beginning of October 1998, one-year-old twigs, 30–40 cm long, were chosen on different branches of the trees. For each strain evaluation 15 twigs per cultivar were inoculated. The inocula were obtained from cultures grown for 48 h on NSA at 25 ± 1 °C. For each twig, 10 µl of the suspension in SS (1–2 × 10^5 cfu/ml, i.e. approximately 1000 bacterial cells) were placed by means of a micropipette, on a leaf scar located midway from the tip of the twig immediately after the removal of the leaf at the base of the petiole. The inoculated leaf scars were marked by placing a plastic ring below the corresponding node. A control treatment using only SS was also evaluated. The pathogenicity of strains were assessed in early May 1999 by recording: (i) the number of wilted twigs; and (ii) the length of the internal necrosis after the removal of the epidermis in the un-wilted twigs. The data were statistically analysed by means of ANOVA and the differences among the means assayed with the Student's test. In addition, to confirm the presence of the pathogen inside the inoculated twigs, isolations were carried out as described by Scortichini and Tropiano (1994). Identification of the isolates was done by means of biochemical tests and monodimensional SDS-PAGE of soluble

Table 1. P. avellanae strains used in rep-PCR analysis

Strain	Country	Region	Cultivar	Year isolated
ISPaVe-B-592	Italy	Piedmont	Tonda Gentile Langhe	1995
ISPaVe-B-593	Italy	Piedmont	Tonda Gentile Langhe	1995
ISPaVe-B-595	Italy	Piedmont	Tonda Gentile Langhe	1995
ISPaVe-B-595	Italy	Piedmont	Tonda Gentile Langhe	1995
ISPaVe-B-598	Italy	Piedmont	Tonda Gentile Langhe	1995
ISPaVe-B-599	Italy	Piedmont	Tonda Gentile Langhe	1995
ISF Lan 1	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Lan 2	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Lan 3	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Lan 4	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Lan 5	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Lan 6	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Lan 7	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Lan 8	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Lan 9	Italy	Piedmont	Tonda Gentile Langhe	1997
ISF Cn 1	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cn 2	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cn 3	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cn 4	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cn 5	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cn 6	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cn 7	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 1	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 2	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 3	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 4	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 5	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 6	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 7	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 8	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 9	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF To 10	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cr 1	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cr 2	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cr 3	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cr 4	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cr 5	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cr 6	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cr 7	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF Cr 8	Italy	Piedmont	Tonda Gentile Langhe	1998
ISF C 2	Italy	Campania	Camponica	1997
ISF C 3	Italy	Campania	Camponica	1997
ISF C 4	Italy	Campania	Camponica	1997
ISF Lab 2	Italy	Latium–Rome	Tonda Gentile Romana	1997
ISF Lab 3	Italy	Latium–Rome	Tonda Gentile Romana	1997
ISF Lab 4	Italy	Latium–Rome	Tonda Gentile Romana	1997
ISPaVe-B-037	Italy	Latium–Rome	Tonda Gentile Romana	1993
ISPaVe-B-369	Italy	Latium–Rome	Tonda Gentile Romana	1995
ISPaVe-B-436	Italy	Latium–Rome	Tonda Gentile Romana	1995
ISPaVe-B-690	Italy	Latium–Viterbo	Tonda Gentile Romana	1996
ISPaVe-B-691	Italy	Latium–Viterbo	Tonda Gentile Romana	1996
ISPaVe-B-2059	Italy	Latium–Viterbo	Tonda Gentile Romana	1994
BPIC Fl 3	Greece	Kilkis	Palaz	1976
BPIC 631	Greece	Drama	Palaz	1976
BPIC 703	Greece	Katerini	Palaz	1977

BPIC: culture collection of Benaki Phytopathological Institute, Kiphissia, Athens, Greece.
ISF: culture collection of Istituto Sperimentale per la Frutticoltura, Roma, Italy.
ISPaVe-B-: culture collection of Istituto Sperimentale per la Patologia Vegetale, Roma, Italy.

whole-cell protein extracts with comparison with *P. avellanae* strains according to Janse et al. (1996). The same strains, at 1–2 × 10^8 cfu/ml, were also infiltrated into leaves of lilac (*Syringa vulgaris* L.), peach (*Prunus persica* Batsch.), pear (*Pyrus communis* L.), apricot (*Prunus armeniaca* L.) and Japanese plum (*Prunus salicina* L.) as well as in fruitlets of pear and in ripen fruits of lemon (*Citrus lemon* Osbeck). Leaves, fruitlets and fruits were pricked with a sterile needle and subsequently a drop of the bacterial suspension was placed on the wounds. Leaves were inoculated during spring in open air conditions. Fruitlets and fruits were placed, after the inoculation, in a humid chamber at room temperature for 5–7 days. For each strain, three leaves and one fruitlet and fruit were inoculated.

Results

Biochemical and nutritional tests of the strains isolated from the Langhe district were consistent with those showed by *P. avellanae* and different from those showed by *P. syringae* pv. *syringae* as reported by Young (1991). In particular, the strains showed respiratory metabolism; aesculin and arbutin were not hydrolyzed, nitrates were not reduced and gelatin was not liquefied. They did not produce oxidase, tyrosinase, urease and arginine dihydrolase, whilst they tolerated 4% of NaCl and 0.05% of TTC. The following compounds were utilized: inositol, D-xylose, L-aspartic acid. The following were not utilized: sorbitol, L(+s)tartrate, homoserin, amygdalin, alanine, L-histidine, L-leucine, DL-phenylalanine. In addition, none of the strains induced any lesion on leaves, fruitlets and fruits when artificially inoculated.

ERIC, REP and BOX primers gave reproducible genomic PCR profiles consisting of bands ranging in size from approximately 100 bp to 3 kb. For UPGMA analysis, a total of 39 reproducible clearly resolved bands were scored: 19 for primer ERIC, 14 for primer BOX and 6 for primer REP. ERIC and BOX primers were more discriminative than REP in differentiating *P. avellanae* strains (Figures 1–3). UPGMA analysis indicated that *P. avellanae* strains from Langhe can be differentiated into five groups and that ISF Lan 3 is closely related to strains isolated from distant areas (i.e. Latium, Campania) and from other cultivars (Figure 4). Strains from Langhe had approximately 20% similarity with *P. avellanae* strains isolated from northern Greece and central Italy, whereas strains from within these regions had 90% similarity. Interestingly,

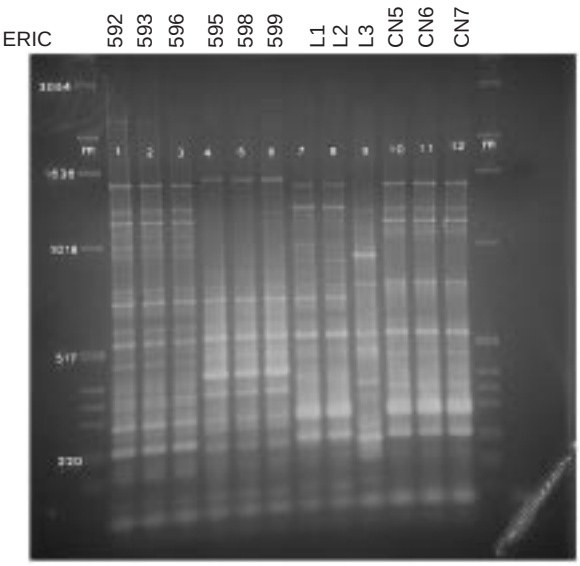

Figure 1. Rep-PCR fingerprinting patterns from genomic DNA of *P. avellanae* strains from the Langhe district (Piedmont, northwest of Italy) obtained by using ERIC primers set. m: DNA molecular size marker (1-kb ladder, Gibco-BRL); the sizes are indicated in base pairs. ISPaVe-B-592 (lane 1); ISPaVe-B-593 (lane 2); ISPaVe-B-596 (lane 3); ISPaVe-B-595 (lane 4); ISPaVe-B-598 (lane 5); ISPaVe-B-599 (lane 6); ISF Lan 1 (lane 7); ISF Lan 2 (lane 8); ISF Lan 3 (lane 9); ISF Cn 5 (lane 10); ISF Cn 6 (lane 11); ISF Cn 7 (lane 12).

strains ISPaVE-B-592, 593 and 596 and ISPaVe-B-595, 598 and 599, isolated from a single hazelnut twig did not group together in the same cluster. By contrast, strains ISF Cn and ISF To, obtained from different orchards clustered within one group. *P. avellanae* strains from other areas clustered in separate groups. Strains ISPaVe-B-037, 369 and 436 isolated from the province of Rome were obtained from an hazelnut orchard built up with propagative material obtained from the province of Viterbo and the strains from the province of Avellino and from the province of Rome (i.e. strains ISF Lab) clustered in the same genomic group even though the orchards were distantly located and the cultivars were different. Strains from northern Greece formed a distinct group closely related to the strains from Viterbo.

The pathogenicity tests towards hazelnut indicated that the *P. avellanae* strains obtained from the Langhe district were significantly less aggressive than those isolated from northern Greece and from the provinces of Viterbo and Rome (i.e. from the orchard built up with propagative material obtained from hazelnuts of

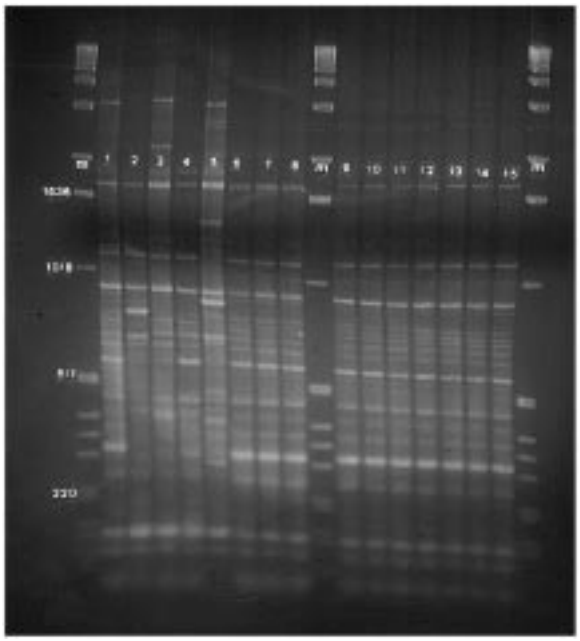

Figure 2. Rep-PCR fingerprinting patterns from genomic DNA of *P. avellanae* strains from the Langhe district (Piedmont, northwest of Italy) obtained by using REP primers set. m: DNA molecular size marker (1-kb ladder; Gibco-BRL); the sizes are indicated in base pairs. ISPaVe-B-592 (lane 1); ISPaVe-B-599 (lane 2); ISF Cn 2 (lane 3); ISF Lan 1 (lane 4); ISF Lan 3 (lane 5); ISF To 1 (lane 6); ISF To 2 (lane 7); ISF To 3 (lane 8); ISF Cr 1 (lane 9); ISF Cr 2 (lane 10); ISF Cr 4 (lane 11); ISF Cr 5 (lane 12); ISF Cr 6 (lane 13); ISF Cr 7 (lane 14); ISF Cr 8 (lane 15).

Viterbo) (Table 2). In fact, strains from such areas caused the wilting of 7–10 inoculated twigs of 'Tonda Gentile Romana' and 'Nocchione', whereas the strains from Langhe induced wilting of 1–6 twigs in both cultivars. The group formed by ISPaVe-B-592, 593 and 596 showed very mild aggressiveness by killing only 1–2 twigs out of 15 in both cultivars. All of the other groups caused the wilting of 3–6 twigs. Also, the mean length of the internal necrosis along the twig was higher for the strains isolated from northern Greece and from the provinces of Viterbo and Rome (i.e. from 10.1–13.7 cm). The *P. avellanae* strains from Langhe produced necrotic lesions 3.0–7.9 cm in length. Strains ISPaVe-B-592, 593 and 596 were the most aggressive and ISPaVe-B-595, 598 and 599 the least. No symptoms were observed in the control treated twigs. Re-isolations from inoculated twigs of both cultivars yielded bacterial colonies on NSA that were confirmed as *P. avellanae* by biochemical tests and whole-cell protein profile comparisons.

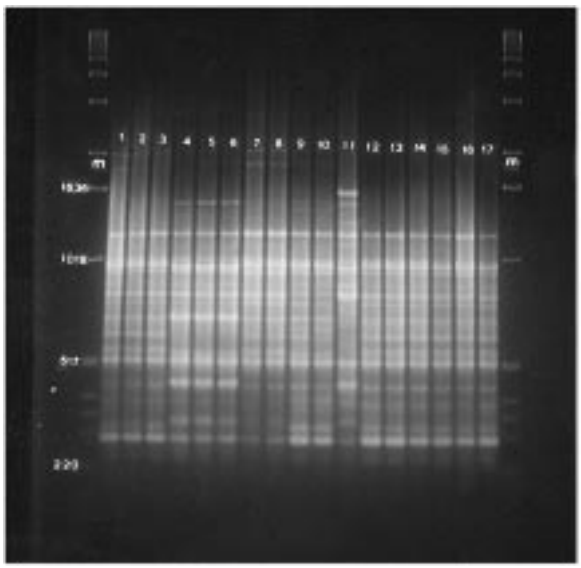

Figure 3. Rep-PCR fingerprinting patterns from genomic DNA of *P. avellanae* strains from the Langhe district (Piedmont, northwest of Italy) obtained by using BOX primer set. m: molecular size marker (1-kb ladder; Gibco-BRL); the sizes are indicated in base pairs. ISPaVe-B-592 (lane 1); ISPaVe-B-593 (lane 2); ISPaVe-B-596 (lane 3); ISPaVe-B-595 (lane 4); ISPaVe-B-598 (lane 5); ISPaVe-B-599 (lane 6); ISF Cn 2 (lane 7); ISF Cn 3 (lane 8); ISF Lan 6 (lane 9); ISF Lan 2 (lane 10); ISF Lan 3 (lane 11); ISF To 1 (lane 12); ISF To 2 (lane 13); ISF To 3 (lane 14); ISF Cn 3 (lane 15); ISF Cn 2 (lane 16); ISF Cn 4 (lane 17).

Discussion

Repetitive extragenic sequences such as ERIC, REP and BOX were present in the genome of *P. avellanae*. Rep-PCR genomic fingerprinting analysis revealed significant heterogeneity among *P. avellanae* strains from Piedmont. Cluster analysis showed that at least five groups of strains were obtained from different hazelnut orchards located in Piedmont. These strains showed 20% similarity to the *P. avellanae* strains isolated from northern Greece and from the province of Viterbo. The latter strains, isolated during many years from different orchards, were highly homogeneous (Scortichini et al., 1998; this study). Interestingly, two groups of strains from Langhe (i.e. ISPaVe-B-592, 593, 596 and ISPaVe-B-595, 598, 599) were isolated from the same hazelnut twig. The strains recently isolated from Rome (i.e. ISF Lab) and Avellino (i.e. ISF C) were grouped in a different cluster from the strains from Langhe though showing a similarity of 80%. These data show a strong correlation between genomic profiles produced with

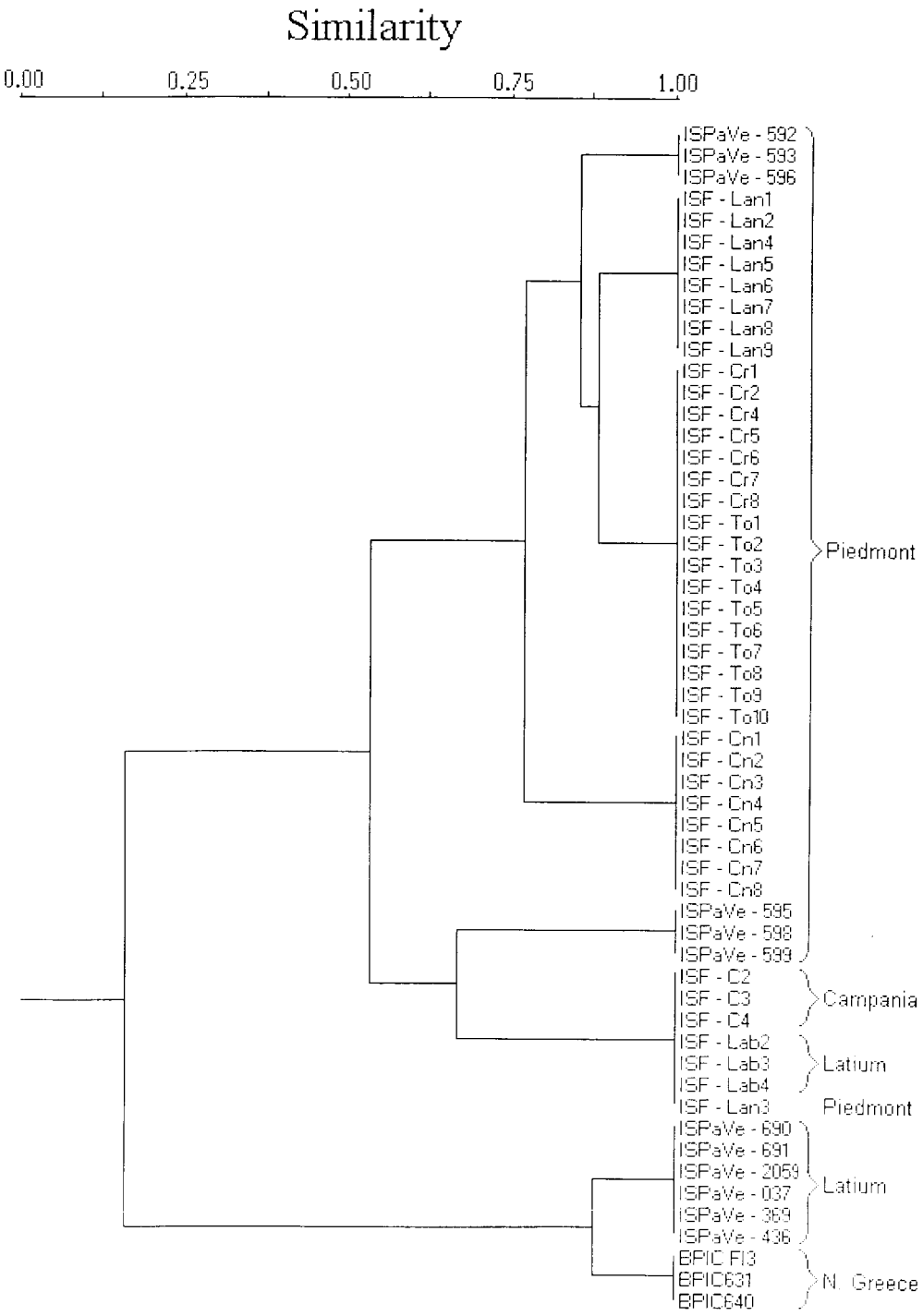

Figure 4. Dendrogram of relationships among *P. avellanae* strains collected from the Langhe district (Piedmont, north-west of Italy) and from other geographic regions. A similarity matrix was produced from analysis of rep-PCR data obtained using ERIC, REP and BOX primer sets. Cluster analysis was performed by UPGMA on matrix calculated with the Dice's coefficients. N. Greece: northern Greece.

Table 2. Pathogenicity tests performed with *P. avellanae* strains towards *C. avellana* cultivars 'Tonda Gentile Romana' (TGR) and 'Nocchione' (N)

Strain	Region	Number of wilted twigs		Mean length of internal necrosis (cm)	
		TGR	N	TGR	N
ISPaVe 595	Piedmont	6/15	6/15	7.9 ± 1.2 abc	6.1 ± 0.8 abc
ISPaVe598	Piedmont	5/15	3/15	6.9 ± 0.4 abc	6.1 ± 0.9 abc
ISPaVe 599	Piedmont	5/15	3/15	7.5 ± 1.2 abc	6.3 ± 0.8 abc
ISPaVe 592	Piedmont	2/15	2/15	5.1 ± 0.7 ab	3.8 ± 0.4 a
ISPaVe 593	Piedmont	2/15	2/15	4.2 ± 0.7 ab	3.9 ± 0.6 a
ISPaVe 596	Piedmont	1/15	1/15	4.1 ± 0.8 ab	3.0 ± 0.5 a
ISF Lan 3	Piedmont	3/15	3/15	3.7 ± 0.6 a	3.1 ± 0.4 a
ISF Lan 4	Piedmont	4/15	3/15	3.8 ± 0.2 a	3.0 ± 0.4 a
ISF Lan 5	Piedmont	3/15	3/15	3.6 ± 0.4 a	3.2 ± 0.3 a
ISF Cn 3	Piedmont	4/15	3/15	5.1 ± 0.8 ab	4.6 ± 0.7 a
ISF Cn 4	Piedmont	6/15	5/15	5.0 ± 0.4 ab	4.8 ± 0.7 a
ISF Cn 5	Piedmont	4/15	3/15	4.5 ± 0.4 ab	4.8 ± 0.6 a
ISF To 4	Piedmont	5/15	4/15	4.1 ± 0.4 a	4.0 ± 0.4 a
ISF To 5	Piedmont	4/15	4/15	4.8 ± 0.6 a	4.1 ± 0.6 a
ISF To 6	Piedmont	4/15	3/15	3.9 ± 0.7 a	3.5 ± 0.2 a
ISF Cr 5	Piedmont	4/15	3/15	4.1 ± 0.4 a	3.6 ± 0.2 a
ISF Cr 6	Piedmont	4/15	4/15	4.0 ± 0.5 a	3.8 ± 0.4 a
ISF Cr 7	Piedmont	4/15	3/15	3.9 ± 0.3 a	3.5 ± 0.3 a
ISF C 2	Campania	4/15	2/15	4.8 ± 0.6 a	3.9 ± 0.4 a
ISF C 3	Campania	3/15	2/15	4.6 ± 0.6 a	3.1 ± 0.5 a
ISF C 4	Campania	4/15	2/15	4.2 ± 0.5 a	3.4 ± 0.3 a
ISF Lab 2	Latium–Rome	4/15	4/15	5.6 ± 0.6 ab	5.1 ± 0.8 ab
ISF Lab 3	Latium–Rome	5/15	5/15	5.8 ± 0.7 ab	4.8 ± 0.8 ab
ISF Lab 4	Latium–Rome	5/15	4/15	5.0 ± 0.8 ab	4.2 ± 0.6 ab
ISPaVe 690	Latium–Viterbo	8/15	7/15	12.1 ± 1.2 d	10.8 ± 0.9 d
ISPaVe 691	Latium–Viterbo	9/15	9/15	11.9 ± 1.1 d	10.7 ± 1.1 d
ISPaVe 2059	Latium–Viterbo	10/15	10/15	13.6 ± 1.0 d	11.0 ± 1.0 d
ISPaVe 037	Latium–Rome	9/15	7/15	11.9 ± 1.2 d	10.7 ± 1.3 d
ISPaVe 369	Latium–Rome	9/15	8/15	12.3 ± 1.4 d	11.1 ± 1.2 d
ISPaVe 436	Latium–Rome	9/15	7/15	11.4 ± 1.2 d	11.0 ± 1.3 d
BPIC Fl 3	Greece	10/15	8/15	12.1 ± 1.0 d	11.1 ± 1.2 d
BPIC 631	Greece	10/15	9/15	13.7 ± 1.3 d	12.1 ± 1.4 d
BPIC 703	Greece	9/15	8/15	11.8 ± 1.1 d	10.9 ± 1.2 d

Data were analyzed by means of ANOVA. Means followed by different letters are significantly different at $P < 0.05$ by using the Student's test.

rep-PCR and the geographic distribution of *P.avellanae* strains.

Pathogenicity tests showed that the strains of *P. avellanae* from central Italy and northern Greece were significantly more aggressive than those from Piedmont. These data confirm previous reports that strains from such areas can kill adult hazelnut trees in eight months when inoculated through leaf scars (Scortichini and Lazzari, 1996; Scortichini, 1998). Strains from Piedmont, when inoculated with the same technique, were much less aggressive and rarely caused inoculated twigs to wilt. The two groups of distantly related strains from Langhe (i.e. ISPaVe-B-592, 593, 596 and ISPaVe-B-595, 598, 599) showed a different level of aggressiveness with the others being more aggressive. In this case, it seems evident that two different strains are capable of infecting a single *C. avellana* twig. It is interesting that no diversity was found among the more aggressive strains within each region. However, a lot of diversity was found among the less aggressive isolates collected from Piedmont. These data would suggest that *P. avellanae* populations of Langhe are older than those currently found in northern Greece and central Italy, even though more data on the population structure of the pathogen are required to validate such an hypothesis. Moreover, the

relevant genetic diversity among *P. avellanae* strains from Langhe, would indicate that such an area is favourable for an adaptation of different population of the pathogen. Genetic diversity in populations of phytopathogenic bacteria has already been reported (Gabriel et al., 1988; Mc Manus and Jones, 1995; Smith et al., 1995; Pooler et al., 1996). However, the strains assessed in such studies were isolated from wide geographic areas where heterogeneity could be expected. A similar situation as that found for *P. avellanae* in Piedmont has been reported for *P. syringae* pv. *persicae* Prunier et al. strains isolated from nectarine (*Prunus persica* Batsch var. *nectarina* Gray) at one site of New Zealand (Young et al., 1996).

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