



## GENOMIC FINGERPRINTING OF SOME *PSEUDOMONAS SYRINGAE* PV. *PISI* STRAINS FROM SICILY

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### SUMMARY

*Pseudomonas syringae* pv. *pisi* strains were characterized by pathogenicity tests on eight differential pea genotypes and by two genomic fingerprinting methods: PCR with REP and ERIC primers, and pulsed-field gel electrophoresis (PFGE) of genomic DNA digested by rare-cutting restriction endonucleases. The study comprised reference type strains of all 7 known races and 21 strains obtained from field pea crops in Sicily. PFGE was the most discriminatory method, delineating 6 pulsed-field gel profiles not coinciding with races, whereas REP and ERIC PCR identified 2 profile types. All the *P. syringae* pv. *pisi* isolated in Sicily were identified as race 6 on the basis of pathogenicity tests, and their genomic fingerprints by PFGE and REP and ERIC PCR were identical and more closely related to the type strains of races 3 and 6 than to those of the other races. The results are discussed in relation to improvement of detection methods and race-identification protocols.

### RIASSUNTO

**ANALISI GENOMICA DI CEPPI SICILIANI DI *PSEUDOMONAS SYRINGAE* PV. *PISI*.** Ceppi di *Pseudomonas syringae* pv. *pisi*, provenienti da collezioni internazionali e da coltivazioni di pieno campo in Sicilia, sono stati caratterizzati mediante saggi di patogenicità su cultivar differenziali e mediante due tipi di analisi del genoma: amplificazione enzimatica del DNA (PCR) con primer REP ed ERIC, ed elettroforesi in campo pulsato (PFGE) di macroframmenti di restrizione del DNA. L'elettroforesi in campo pulsato ha portato alla identificazione di 6 distinti profili di restrizione non coincidenti con le razze, mentre la tecnica PCR ha consentito di identificarne due. Tutti i ceppi locali, identificati come razza 6 tramite i saggi di patogenicità, possedevano

identici profili, a loro volta uguali o simili ai ceppi tipo delle razze 3 e 6, mentre differivano dalle altre razze tipo del patogeno. I risultati ottenuti vengono discussi per la loro utilità nella diagnosi e nella identificazione delle razze del batterio.

Key words: *Pseudomonas syringae* pv. *pisi*, rare-cutting enzymes, PFGE, REP-PCR, ERIC-PCR.

### INTRODUCTION

Bacterial blight of pea (*Pisum sativum* L.) caused by *Pseudomonas syringae* pv. *pisi* (Sackett) Young, Dye and Wilkie, first described by Sackett (1916) in the USA, is a disease that occurs in most pea growing area of the world, occasionally causing serious reduction in yield and seed quality following epidemics (Bradbury, 1986; Schmit, 1991; Halloway and Bretag, 1995; Roberts et al., 1995). The most typical symptoms are water-soaked lesions on leaves, petioles and pods, spreading into the stems and often accompanied by necrosis.

*P. syringae* pv. *pisi* is seedborne and can be carried internally or externally. Bacteria from contaminated seeds can multiply and survive on seedlings, spreading to neighbouring plants in the field. Under conditions suitable for bacterial penetration in plant tissues (most frequently frost, hail, or any kind of injury), dissemination can occur throughout the field.

Occurrence of races of *P. syringae* pv. *pisi* was first studied in New Zealand by Taylor (1972). At present, seven races have been identified on the basis of their reaction on a set of 8 differential pea cultivars, and the genetics of the cultivar-race combination in terms of a gene-for-gene relationship has been explained (Bevan et al., 1995). Taylor et al. (1989) found widespread resistance in different commercial pea cultivars to races carrying different avirulence genes, whereas race 6 carries no avirulence genes and is able to attack all pea cultivars.

The disease has been increasingly reported in Europe since 1985 (Stead and Pemberton, 1987), following expansion in the area under cultivation and international exchanges of seed. The pathogen was first ob-

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served in Italy in 1969 (Cirulli and Ercolani, 1969). Since then, up to 1996, the disease has never been reported again, and no research was carried out on the presence of different races in Italy.

In 1996, *P. syringae* pv. *pisi* was isolated from pea leaves and pods in several field crops in Sicily (Cirvilleri and Caldarera, 1997). Even though there was a high level of inoculum in a crop survey, only a few plants showed symptoms of bacterial blight infection. The bacteria have already been reported to have a resident phase on their host without inducing any (or only cryptic) symptoms (Grondeau et al., 1996). The occurrence of the pathogen and environmental conditions favourable to an outbreak of the disease in winter crops represent a potential threat to field production if susceptible cultivars are used or are introduced.

An understanding of the genetic diversity and the identification of locally prevalent races and strains of *P. syringae* pv. *pisi* is necessary to determine the choice of resistant cultivars in different growing areas and possibly also to improve detection protocols and control measures.

Existing techniques for detection and identification of pathogen are based on isolation using semi-selective media (Mohan and Schaad, 1987) and/or serology with polyclonal and monoclonal antisera (Taylor, 1972; Candler et al., 1988; Grondeau et al., 1992). In addition to identification tests at pathovar level, pathogenicity testing on 8 differential pea cultivars is the only method of identifying the seven currently known races of *P. syringae* pv. *pisi* (Taylor et al., 1989) and to distinguish *P. syringae* pv. *pisi* from *P. syringae* pv. *syringae*, *P. viridiflava* and other organisms that often cross-react serologically (Grondeau et al., 1992). Recently, two pairs of oligonucleotide primers permitted the amplification of DNA fragments potentially unique to *P. syringae* pv. *pisi* (Arnold et al., 1996).

In recent years, improved genomic fingerprinting protocols have revealed high levels of genetic diversity within populations of bacteria. Consensus sequences derived from highly conserved palindromic inverted repeat regions found in enteric bacteria have also been used to fingerprint gram-negative species (Versalovic et al., 1991; de Bruijn, 1992). These conserved regions have been called repetitive extragenic palindromic (REP) sequences (Stern et al., 1984) and enterobacterial repetitive intergenic consensus (ERIC) sequences (Hulton et al., 1991). PCR primers corresponding to REP and ERIC sequences have been shown to yield genomic fingerprints specific to pathovars and strains of gram-negative bacteria (de Bruijn, 1992; Louws et al., 1994; Frey et al., 1996). Methods that rely on DNA restriction profiles generated by rare-cutting restriction endonu-

cleases, and resolution of macrofragments by pulsed-field gel electrophoresis (PFGE), yield particularly sensitive restriction profiles readily amenable to analysis for typing bacterial strains (Cirvilleri et al., 1991; Egel et al., 1991; Rainey et al., 1994; Catara, 1995; Frey et al., 1996; Davis et al., 1997)

The objective of this study was to determine the race of *P. syringae* pv. *pisi* strains isolated in Sicily during recent cases of bacterial blight of pea (Cirvilleri and Caldarera, 1997) using the pathogenicity tests. REP and ERIC PCR and the PFGE technique of contour-clamped homogeneous electric fields (CHEF) were utilized to generate genomic fingerprints to support the race determination.

## MATERIALS AND METHODS

**Bacterial isolates.** The isolates of *P. syringae* pv. *pisi* used in this study are listed in Table 1. Twenty-one isolates were obtained from symptomatic pea plants during a crop survey in 1996 conducted in Sicily. Isolates were from pods, stems, petioles and leaves. The identity of isolates as *P. syringae* pv. *pisi* was confirmed by biochemical and pathogenicity tests and serology (Cirvilleri and Caldarera, 1997). Type strains of seven races previously described (Taylor et al., 1989), obtained from the National Collection of Plant Pathogenic Bacteria (NCPBB), Harpenden, England, and from the Collection Française de Bactéries Phytopathogènes (CFBP), Station des Phytobactériologie, Angers, France, were included in all experiments.

Bacteria were routinely grown and maintained in the short term on King B medium (KB) plates (King et al., 1954) and stored long term in 15% glycerol at -80°C.

**Race determination.** Identification of races of *P. syringae* pv. *pisi* was based on their interaction with eight differential pea genotypes (Taylor et al., 1989). The resistance or susceptibility of each cultivar to the Sicilian isolates was recorded and compared with typical resistance-susceptibility patterns of the 7 type races. Pure lines of each of the differential cultivars obtained from the John Innes Pea Germplasm Collection and their designated accession numbers are given in Table 2. Seeds of the eight cultivars were germinated in covered trays on moist cellulose wadding at about 20°C. Three days later, seedlings were selected for uniformity and planted 2.5 cm deep in 10 cm pots. Plants were grown in a glasshouse at temperatures of 18-20°C, and were inoculated approximately 14 days after sowing using the stem inoculation procedure described by Taylor et al. (1989). Bacterial cells from 48 h cultures grown at 25°C were suspended in sterile phosphate buffer, pH 7.0, to give a

**Table 1.** Sources and origins of type strains of *P. syringae* pv. *pisii*.

Strain	Source	Country	Race	PFGE profile	PCR profile
299A	NCPPB 3430	New Zealand	1	1a	I
202	NCPPB 3429	USA	2	2	II
870A	NCPPB 3496	USA	3	3a	II
895A	NCPPB 3500	USA	4	4	II
974B	NCPPB 3433	USA	5	1a	I
1704B	CFBP 2709	France	6	3a	II
SL5560	CFBP		7	1b	I
B2, B3, B4, B5, B6, B7	pod	Sicily	6	3b	II
St1, St2, St3, St4, St5, St6	stem	Sicily	6	3b	II
P1, P2, P3, P4	petiole	Sicily	6	3b	II
F1, F2, F3, F4, F5, F6	leaf	Sicily	6	3b	II

NCPPB: National Collection of Plant Pathogenic Bacteria, Harpenden, England.

CFBP: Collection Française de Bactéries Phytopathogènes, Angers, France.

**Table 2.** Accession numbers and abbreviations for cultivars of *Pisum sativum*.

Cultivars	JI accession number	Abbreviation
Kelvedon Wonder	JI 2430	KW
Early Onward	JI 2431	EO
Belinda	JI 2432	B
Hurst's Greenshaft	JI 2435	HGS
Partridge	JI 2438	P
Vinco	JI 2436	V
Sleaford Triumph	JI 2437	ST
Fortune	JI 2439	F

JI: John Innes Pea Germoplasm Collection, Norwich, UK.

concentration of approximately  $5 \times 10^6$  cfu ml<sup>-1</sup>. Suspensions were injected with a hypodermic syringe into the main stem at the junction with the stipules at the two youngest nodes. Each strain was inoculated three times into each of three plants. Individual plants were kept in plastic bags for 2 days following inoculation, then plants were kept separated on greenhouse benches and incubated at temperatures of 25°C. Symptom development was followed until the seventh day after inoculation.

**REP and ERIC PCR analysis.** The type strains of the seven races of *P. syringae* pv. *pisii* obtained from international collections and 21 strains of *P. syringae* pv. *pisii* isolated in Sicily were characterized by a genomic fingerprinting method based on PCR using the REP and ERIC primers previously described (Versalovich et al., 1991). Primers corresponding to REP [REP1R (5'-ICGICGICATCIGGC3') and REP2I (5'-ICGICTTATCIGGCCTA3')] and to ERIC [(ERIC1R 5'-ATGTAAGCTCCTGGGGATTAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3')] were manufactured by Genenco M- Medical -S.r.l. Firenze. PCR reactions were carried out as described by de Bruijn (1992) in a 25 µl volume containing 50 pmol each of the two primers, 1.25 pmol deoxynucleoside triphosphates, and 2 U of Taq DNA polymerase recombinant (Gibco BRL). Cell suspensions in sterile water ( $10^9$  cfu ml<sup>-1</sup>) from 24 h bacterial cultures grown on KB were used for amplifications. Amplifications were performed in a thermal cycler (480 Perkin-Elmer Cetus) as described by de Bruijn (1992): an initial cycle at 95°C for 7 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 40 and 52°C for 1 min with REP and ERIC primers, respectively, and extension at 65°C for 8 min with a single final extension cycle at 65°C for 16 min and final soak at 4°C. After the reaction, 8 µl of the PCR products were separated on 1.5% agarose gels in 1x TAE (Sambrook et al., 1989) for 5 h at 5 V cm<sup>-1</sup>. Gels were stained with

ethidium bromide and photographed with type 55 Polaroid film. Fingerprints were read by eye and by a photoanalyzer (Image Master VDS Pharmacia Biotech).

**CHEF analysis.** Genomic fingerprints were obtained by CHEF electrophoresis from DNA of *P. syringae* pv. *pisii* strains digested with rare-cutting restriction endonucleases *Xba*I and *Spe*I as described by Egel et al. (1991) with minor modifications (Catara, 1995). *P. syringae* pv. *pisii* strains were grown in Luria Bertani broth (LB) (Miller, 1972) to mid-log phase. Cells were harvested, washed once with SE (75 mM NaCl; 25 mM EDTA, pH 8.0), and resuspended at about  $10^9$  cfu ml<sup>-1</sup> in 0.5 ml of SE buffer. The cell suspension was mixed with an equal volume of 2% low-melting-point agarose solution [10 mM Tris, pH 8.0; 10 mM MgCl<sub>2</sub>; 10 mM EDTA, pH 8.0; 2% LMP agarose (Bio-Rad Laboratories, Richmond, Ca)]. The mixture was allowed to solidify in a plastic plug mold (Bio-Rad). The embedded cells were lysed by incubating the blocks in lysing solution (0.5 mg ml<sup>-1</sup> proteinase K, 1% [w/v] N-lauryl Sarkosyl, 0.5 mM EDTA, pH 9.5) at 50°C in a water bath overnight. Prior to restriction digestion, gel blocks were treated with 10 mM phenylmethylsulfonyl fluoride (PMSF) in TE buffer (10 mM Tris, 1 mM EDTA) at 50°C for 1 h and at room temperature for 1 h to inactivate proteases. The blocks were then washed twice in TE buffer. *Xba*I and *Spe*I endonucleases (Boehringer Mannheim, Indianapolis, Ind.) were utilized for digestion and the manufacturer's buffers were used throughout. DNA blocks were equilibrated for 15 min in H buffer, 10-30 U of restriction enzymes were added in microcentrifuge tubes containing 200 µl of fresh H buffer, and incubated overnight at 37°C. The restriction buffer was replaced with 1 ml TE and plugs were stored at 4°C. PFGE was performed with a CHEF DR II system (Bio Rad). A 1% agarose gel was subjected to electrophoresis in 0.5x TBE buffer (Sambrook et al., 1989) at 16 V cm<sup>-1</sup> and 14°C. Pulse times were 2 s for 2 h and subsequently 12 s for 22 h for DNA restricted with *Xba*I, and 2 s for 2 h and 14 s for 22 h for DNA restricted with *Spe*I. Bacteriophage lambda DNA concatamers (Boehringer Mannheim) were used as molecular size standards. Gels were stained with ethidium bromide and photographed with type 55 Polaroid film.

Gels were read by eye and by a photoanalyzer. The presence or absence of a particular DNA fragment larger than 100 kb was converted into binary data.

Similarity coefficients for all profiles in pairwise combinations were determined by Dice's coefficient (Dice, 1945) and clustered by the unweighted pair-group method using arithmetic average (UPGMA) (Sneath and Sokal, 1973).

## RESULTS

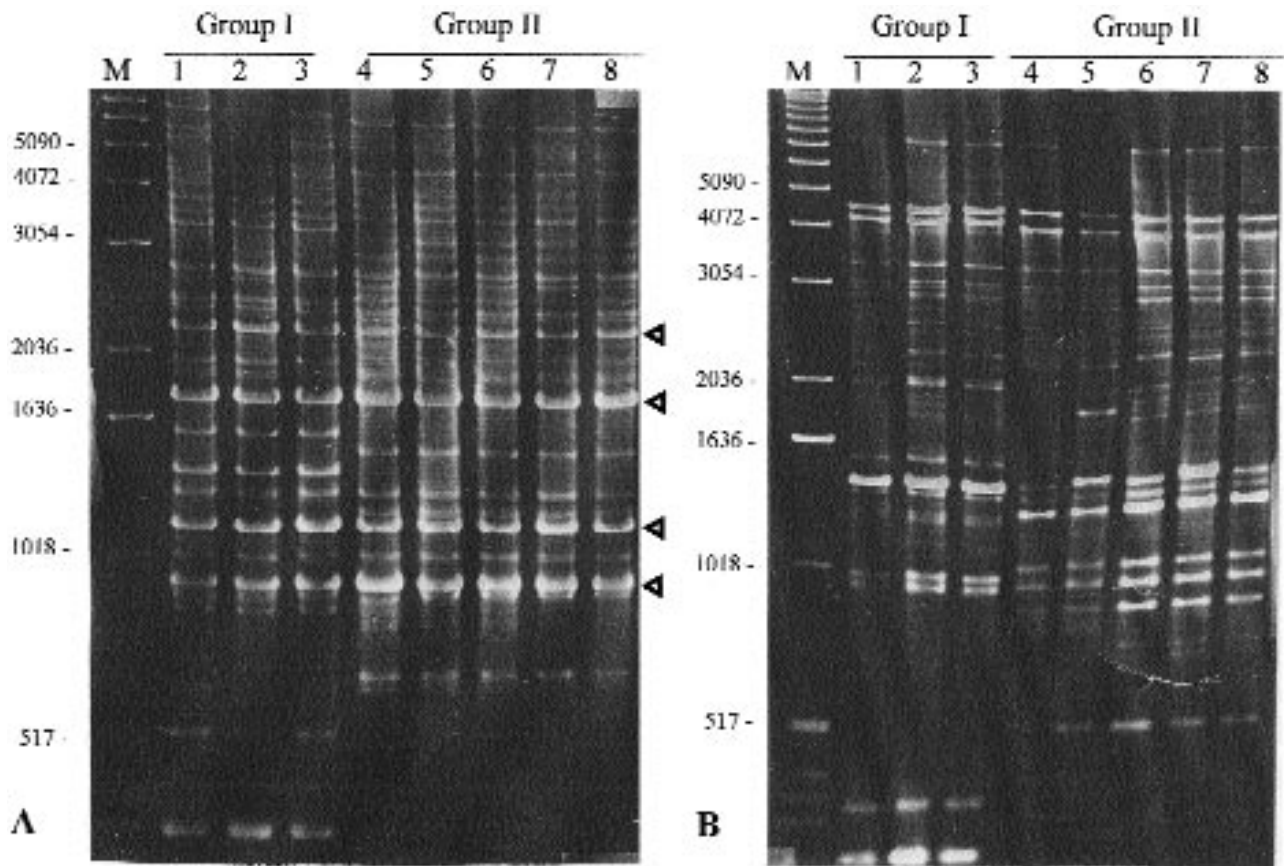
**Race determination.** The separation of *P. syringae* pv. *pisii* isolates into races was based on their interaction with eight differential pea cultivars (Table 2) (Taylor et al., 1989). Water-soaking, the typical pea blight reaction, was observed 6-8 days after inoculation. A resistance response, characterized by localized necrosis at the inoculation site, was visible within 24-48 h. The 21 *P. syringae* pv. *pisii* strains isolated in Sicily induced water-soaked lesions on stems of all the eight differential pea lines. On present evidence, all the 21 Sicilian strains tested were race 6. Reference strains of the seven type races induced water-soaking and/or localized necrosis in the different cultivars as expected.

**Genomic fingerprinting by REP and ERIC-PCR.** REP and ERIC primers, in combination with PCR, generated genomic fingerprint patterns consisting of about 30 DNA bands, ranging in size from approximately 100 bp to over 5 kb. Comparison of fingerprint patterns after analysis of different experiments identified reproducible profiles.

Seven type races of *P. syringae* pv. *pisii* obtained from international collections and 21 Sicilian strains were classified into two distinct genotypes based on their fingerprint patterns. REP-PCR (Fig. 1A) and ERIC-PCR (Fig. 1B) were equally effective in delineating the two different genotypes. The first genotype comprised type races 1, 5, and 7 (group I), and the second genotype included type races 2, 3, 4, 6 and the 21 local strains (group II). All 21 field isolates, identified as race 6 in pathogenicity tests, produced identical REP or ERIC banding patterns (data not shown).

A few bands common to both genotypes were generated by REP and ERIC-PCR. For example, in the REP-PCR experiment four bright bands (indicated by arrows in Fig. 1A on the right) and about ten minor bands appeared to be similar to strains of groups I and II.

In contrast to the different fingerprint patterns between genotypes, REP and ERIC-PCR fingerprint profiles within each genotype were highly similar (Figs 1A and 1B). Type races 2, 3, 4 and 6 and the Sicilian strains had identical REP-PCR fingerprint patterns (Fig. 1A, lanes 4-8) as well as type races 1, 5, and 7 (Fig. 1A, lanes 1-3). ERIC-PCR did not differentiate between type races 1, 5 and 7 (Fig. 1B, lanes 1-3) whereas it discriminated among strains within group II. Fingerprint patterns of races 2, 3, 4, 6 and the Sicilian strains were highly similar (Fig. 1B, lanes 4-8), with differences limited to the presence or absence of one to three bands.



**Fig. 1.** REP-PCR (A) and ERIC-PCR (B) fingerprint patterns generated from cultures of *P. syringae* pv. *psidi* strains. Lanes: 1: 299A (race 1); 2: 974B (race 5); 3: SL5560 (race 7); 4: 202 (race 2); 5: 870A (race 3); 6: 895A (race 4); 7: 1704B (race 6); 8: P1 (representative of Sicilian strains). M: DNA molecular size marker (1-kb ladder; Gibco-BRL). Sizes (in base pairs) are indicated on the left.

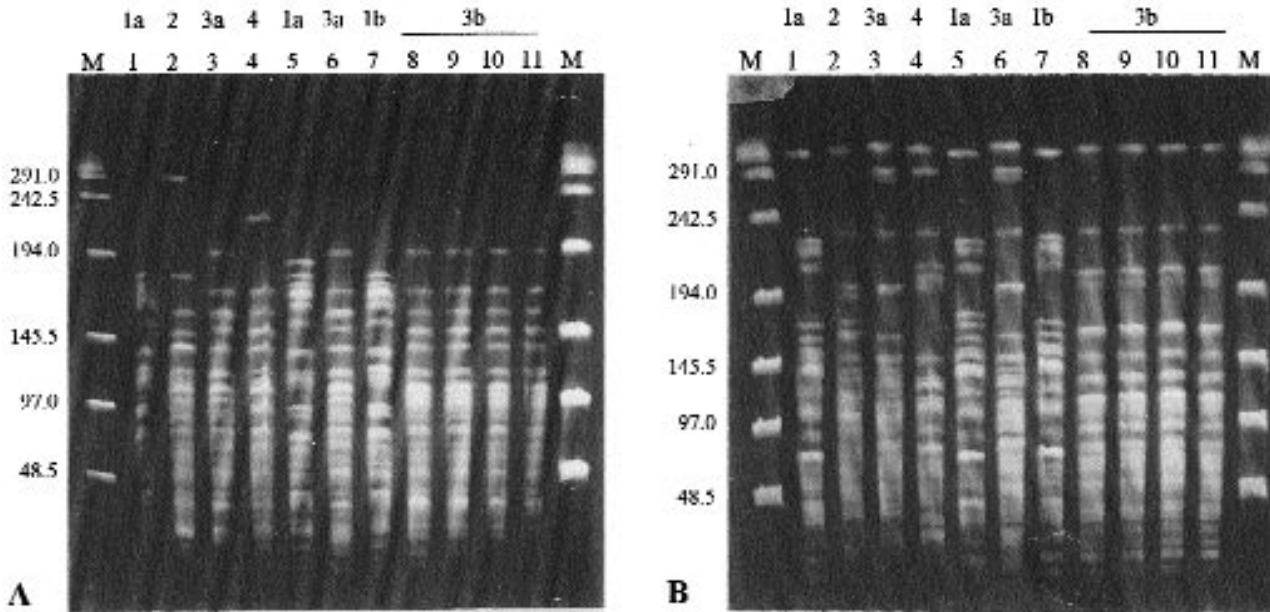
**Genomic fingerprinting by rare-cutting restriction enzymes and CHEF.** Genomic DNAs from seven races obtained from international collections and 21 strains isolated in Sicily were digested with the rare-cutting restriction endonucleases *Xba*I and *Spe*I and their DNA fingerprints analyzed by CHEF. With both enzymes six distinct pulsed-field gel (PFG) profile types were identified (Figs 2A, 2B, and Table 1, profiles 1a, 1b, 2, 3a, 3b, 4).

After digestion with *Xba*I, DNA fingerprints yielded 8-10 fragments between 100 and 200 kb and several minor fragments <100 kb (Fig. 2A). Type races 3 and 6 generated the same fingerprint (PFG 3a), as did type races 1 and 5 (PFG 1a). Race 7 (PFG 1b) generated a fingerprint similar to those of races 1 and 5. Type races 2 and 4 yielded distinct DNA fingerprints (PFG 2 and 4, respectively). The 21 Sicilian strains of *P. syringae* pv. *psidi* (PFG 3b) yielded the same fingerprint (Fig. 2A, lanes 8-11). These fingerprints were identical to the fin-

gerprint of races 3 and 6 (PFG 3a) except for one band, and were distinct from those of all the other type races examined (Fig. 2A).

After digestion with *Spe*I, fingerprints contained fragments of higher molecular weight when compared to the *Xba*I fingerprint (Fig. 2B). Approximately 4-6 DNA fragments larger than 200 kb and 10 to 15 fragments between 100 and 200 kb were detected. Type races 3 and 6 (PFG 3a) showed the same fingerprint, as well as races 1 and 5 (PFG 1a). Race 7 (PFG 1b) generated a fingerprint similar to those of races 1 and 5. The 21 strains isolated in Sicily (PFG 3b) yielded the same fingerprint (Fig. 2B, lanes 8-11). Using *Spe*I, the Sicilian strains generated a fingerprint that was similar to the fingerprint of races 3 and 6 (PFG 3a), but with the exception of 4 bands. *Xba*I and *Spe*I digestions were performed for each isolate a minimum of three times, and fingerprints were reproducible.

The resolution of individual fragments permitted a



**Fig. 2.** XbaI (A) and SpeI (B) macro-restriction fragments resolved by CHEF of *P. syringae* pv. *pisii*. Lanes: 1: 299A (race 1); 2: 202 (race 2); 3: 870A (race 3); 4: 895A (race 4); 5: 974B (race 5); 6: 1704B (race 6); 7: SL5560 (race 7); 8, 9, 10, 11: Sicilian strains P1, F2, St3, and B4 respectively. M: molecular size marker (50- to 1000-kb concatamer ladder; Boehringer). Sizes (in kilobases) are indicated on the left.

numerical analysis of similarities in patterns between strains. With pulse times that allowed resolution of fragments larger than 100 kb (Figs 2A and 2B), 8-15 fragments larger than 100 kb were resolved. Similarity coefficients were calculated for each pair of strains, and these data were used to perform cluster analysis.

The dendrograms derived from XbaI and SpeI fingerprints analysis showed two distinct groups of strains (Figs 3A and 3B). Both formed clusters of relatively closely related bacteria. The genetic distance of the first group (races 1, 5, and 7) from the second group (race 2, 3, 4, 6, and 21 Sicilian isolates) was substantial, with a similarity coefficient of about 0.4 with both enzymes. The first group comprised races with similarity coefficient of 0.88. In the second group the Sicilian strains were more similar to race 3 and 6 (similarity coefficient of 0.88 with XbaI and 0.75 with SpeI) than to races 2 and 4 (Figs 3A and 3B).

## DISCUSSION

In the present study all the 21 *P. syringae* pv. *pisii* previously isolated in Sicily (Cirvilleri and Caldarera, 1997) were identified as race 6 on the basis of the differential pathogenicity tests. Previously, the only indica-

tion of pea blight in Italy was provided by Cirulli and Ercolani (1969), who identified the pathogen (NCPPB 2222) later referred as race 1. Seven races identified by their differential pathogenic potential have been reported in Europe, and this specificity is postulated to reside in recognition between host and pathogen involving single resistance genes in the host matching with single avirulence genes in the pathogen (Flor, 1956; Keen, 1990; Cournoyer et al., 1995; Gibbon et al., 1997; Vivian and Gibbon, 1997). As a consequence, knowledge on the distribution of races is of basic importance for choosing the most suitable varieties in each growing area. Surveys conducted in the UK (Taylor et al., 1989) and France (Schmit, 1991) indicated that race 2 was predominant. Race 4, which is not widely distributed in the world, was also exported from the UK (Taylor et al., 1989) and France (Schmit, 1991). Race 6, unique in its ability to cause disease in all commercial pea cultivars (Taylor et al., 1989) constitutes only a small proportion of all the isolates in the UK (Taylor et al., 1989), in France (Schmit, 1991), and in Australia (Hollaway and Bretag, 1995).

In this study, all strains of isolated from peas in Sicily in 1996 were identified as race 6, the race which, as noted, is pathogenic for all cultivars evaluated so far, thus limiting control options.



**Fig. 3.** Dendrogram showing relationship between *P. syringae* pv. *pisii* strains isolated in Sicily and type strains based on CHEF analysis of genomic fingerprints generated with *Xba*I (A) and *Spe*I (B). Similarities were calculated by using Dice's coefficient and clustering with UPGMA.

Detection methods based on serology and on PCR have been developed (Grondeau et al., 1992; Arnold et al., 1996) but, up to now, a pathogenicity test on differential pea cultivars is the only way to identify the different races (Taylor et al., 1989). The length of pathogenicity tests, and the fact that such analysis can be influenced by many environmental variables, stress the need for improved rapid methods for race recognition.

CHEF analysis and REP and ERIC-PCR of the 21 Sicilian strains, identified as race 6 in pathogenicity tests, generated identical genomic DNA fingerprints, which were similar (CHEF) or identical (REP and ERIC-PCR) to the fingerprints from type races 3 and 6. Cluster analysis of CHEF fingerprints showed that the Sicilian strains were more closely related to type races 3 and 6 both with *Xba*I and *Spe*I than to the other races. Rare-cutting restriction enzymes, such as *Xba*I and *Spe*I, proved to be suitable for genome identification of the GC-rich *Pseudomonas* chromosome (Grotheus et al., 1988; Cirvilleri et al., 1996). As the bacterial DNA is analyzed for the presence of a specific enzyme-recognition site, this technique may not detect certain variations between strains, and then it may be necessary to use more than one enzyme to clearly distinguish between strains. Using *Xba*I, the CHEF fingerprints of Sicilian strains were quite similar to the fingerprints of type races 3 and 6 (similarity coefficient of 0.88). However, when digested with *Spe*I, the fingerprints of Sicilian strains were distinguishable from races 3 and 6 (similarity coefficient of 0.88). However, when digested with *Spe*I, the fingerprints of Sicilian strains were distinguishable from races 3 and 6 (similarity coefficient of 0.75) Genomic fingerprinting by rare-cutting restriction profiles resolved by CHEF was the most discriminatory protocol employed, identifying 6 distinct profile types, whereas REP-ERIC-PCR distinguished only 2 profile types. With REP-PCR the Sicilian strains were indistinguishable from type races 2, 3, 4, and 6, and with ERIC-PCR the differences were limited to one to three DNA bands.

Cluster analysis of genomic fingerprints by CHEF of race 1, 2, 3, 4, 5, 6, 7 obtained from CFBP and NCPPB showed a clear division between races 1, 5, 7 (group I), and 2, 3, 4, 6 (group II), with these groups exhibiting a high level of genetic diversity. The same division was found in this work by using REP and ERIC-PCR, and was previously observed when the strains were characterized by classical physiological tests (Cirvilleri and Calderera, 1997). The same groups (I and II) were also identified by the presence of unique fragments of DNA sequences generated by RAPD-PCR in each group (Arnold et al., 1996). Nevertheless, the authors observed that a few strains of race 3 and 4 (group II)

showed the DNA fragments of group I (Arnold et al., 1996).

DNA fingerprinting analysis by CHEF and REP- and ERIC-PCR obtained here revealed the existent heterogeneity within the pathovar *pisii* of *P. syringae*. Since these DNA fingerprinting methods were not suitable for race identification, race-specific DNA bands should be exploited to develop differential probes for dot blot or PCR-based protocols.

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