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Genetic Characterization of *Pseudomonas syringae* pv. syringae Strains from Stone Fruits in California

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Strains of *Pseudomonas syringae* pv. syringae were isolated from healthy and diseased stone fruit tissues sampled from 43 orchard sites in California in 1995 and 1996. These strains, together with *P. syringae* strains from other hosts and pathovars, were tested for pathogenicity and the presence of the *syrB* and *syrC* genes and were genetically characterized by using enterobacterial repetitive intergenic consensus (ERIC) primers and PCR. All 89 strains of *P. syringae* pv. syringae tested were moderately to highly pathogenic on Lovell peach seedlings regardless of the host of origin, while strains of other pathovars exhibited low or no pathogenicity. The 19 strains of *P. syringae* pv. syringae examined by restriction fragment length polymorphism analysis contained the *syrB* and *syrC* genes, whereas no hybridization occurred with 4 strains of other *P. syringae* pathovars. The *P. syringae* pv. syringae strains from stone fruit, except for a strain from New Zealand, generated ERIC genomic fingerprints which shared four fragments of similar mobility. Of the *P. syringae* pv. syringae strains tested from other hosts, only strains from rose, kiwi, and pear generated genomic fingerprints that had the same four fragments as the stone fruit strains. Analysis of the ERIC fingerprints from *P. syringae* pv. syringae strains showed that the strains isolated from stone fruits formed a distinct cluster separate from most of the strains isolated from other hosts. These results provide evidence of host specialization within the diverse pathovar *P. syringae* pv. syringae.

Bacterial canker and blast of stone fruit trees, caused by *Pseudomonas syringae* pv. syringae, affects all commercially grown *Prunus* species in California including peach (*Prunus persica*), European plum and French prune (*P. domestica*), Japanese plum (*P. salicina*), sweet cherry (*P. avium*), apricot (*P. armeniaca*), and almond (*P. dulcis*). Losses can result from a direct reduction in yield due to cold-induced blast or death of buds and flowers or from tree decline and death due to the development of cankers in branches and major scaffold limbs (20).

P. syringae pv. syringae is unique among most *P. syringae* pathovars in its ability to cause disease in over 180 species of plants in several unrelated genera (1). Strains of *P. syringae* pv. syringae are identified on the basis of biochemical and nutritional tests and symptom expression in host plants. In many cases, strains of *P. syringae* that are found infecting a previously unreported host and are biochemically similar to *P. syringae* pv. syringae strains have been placed in this pathovar without establishment of a common host range (34).

The relationship between *P. syringae* pv. syringae strains infecting *Prunus* species and strains that infect other crops such as tomato, cereals, citrus, and kiwi fruit is unknown and needs to be elucidated. Biochemical tests are not reliable for differentiating strains at or below the pathovar level (12, 25), and pathogenicity tests in greenhouses are not reliable indicators of natural host preferences (2). Peach seedlings (22) and cowpea leaves (14) were found to be susceptible to *P. syringae* pv. syringae strains from various hosts. There is, however, evidence of host specificity among *P. syringae* pv. syringae strains infecting beans (26, 27) and grasses (10) based on the results of pathogenicity tests.

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Molecular analysis of genomic variability has been used to differentiate and classify bacterial strains below the level of species. Analysis of restriction fragment length polymorphisms (RFLP) of the chromosomal DNA of *P. syringae* strains detected differences between and within the pathovars (5, 11, 16). More recently, enterobacterial repetitive intergenic consensus (ERIC) sequences and repetitive extragenic palindromic (REP) sequences, which are short repetitive DNA sequences with highly conserved central inverted repeats that are dispersed throughout the genomes of diverse bacterial species (32), have been used to design universal PCR primers that generate highly reproducible, strain-specific fingerprints that can differentiate bacterial strains below the level of species or subspecies (4, 19).

The objective of this study was to identify and characterize strains of *P. syringae* pv. syringae isolated from various *Prunus* species and other plant hosts by using pathogenicity testing and RFLP and ERIC-PCR analyses.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Many of these strains have been well characterized in previous pathogenicity, biochemical, and genetic studies (6, 9, 23). Strains were maintained in 15% glycerol at −80°C and subcultured on King's medium B (KB) (13) as needed.

Strain isolation. In 1995 and 1996, samples of both healthy and diseased tissues from stone fruit trees were collected from orchard sites in the Sacramento and San Joaquin valleys of California. Samples included healthy flowers, healthy and diseased dormant buds, diseased leaves, twigs, and branches. In addition, samples of weeds were collected during the winter of 1996 from orchards with a history of bacterial canker. Healthy tissues were washed in 0.01 M potassium phosphate buffer (PB) with 0.02% Tween 20 (ca. 3 g of flowers or 5 g of dormant twigs/25 ml of PB; 5 g of weed leaf tissue/100 ml of PB) on a platform shaker at 250 rpm for 30 min, and 100 μl of the wash liquid was spread onto KB plates containing 50 μg of cycloheximide per ml. Three to five healthy buds were ground in 2 ml of PB in a Pyrex tissue grinder, and 100 μl of either undiluted or 1:10-diluted (in PB) wash liquid was plated onto KB or KB with 50 μg of cycloheximide per ml. Diseased tissues were surface sterilized in 0.5% sodium hypochlorite for 1 min, rinsed in sterile water, and ground in a small amount of PB, and the liquid suspension was spread onto KB. The plates were incubated for 3 days, and then blue fluorescent colonies were counted, purified, and tested for

TABLE 1. Bacterial strains used in this study

Strain	Host of origin	Location collected	Source
P. syringae pv. syringae			
61	Wheat	Delaware	D. Cooksey
142	Beet	California	E. Little
321, 408	Tomato	California	E. Little
82-12	Tomato	Georgia	R. Gitaitis
728A	Bean	Wisconsin	S. Lindow
B18	Millet	?	J. DeVay
B37	Rose	California	J. DeVay
B42	Lemon	California	J. DeVay
B39	Corn	Nebraska	J. DeVay
B40	Foxtail grass	?	J. DeVay
84-160	Kiwi fruit	California	K. Conn
B36	Peach	New Zealand	J. DeVay
B3A	Peach	California	J. DeVay
B15	Almond	California	J. DeVay
B301	Pear	England	J. DeVay
B21	Apricot	?	J. DeVay
32 strains	Almond	California	This study
19 strains	Peach	California	This study
13 strains	Cherry	California	This study
12 strains	Prune plum	California	This study
8 strains	Apricot	California	This study
3 strains	Japanese plum	California	This study
072, 073	*Geranium* sp.	California	This study
070, 071	*Malva* sp.	California	This study
P. syringae pv. coriandricola			
269	Cilantro	California	E. Little
P. syringae pv. morsprunorum			
B28	Cherry	?	J. DeVay
048	Cherry	California	E. Little
150	Cherry	California	E. Little
P. syringae pv. tomato			
320	Tomato	California	E. Little

the oxidase reaction, the ability to rot potato slices, the presence of arginine dihydrolase, levan production, and tobacco hypersensitivity (17).

Pathogenicity tests. Bacterial cells grown for 24 h on solid KB at 24°C were suspended in PB to a concentration of $\sim 5 \times 10^7$ CFU/ml. Bacterial suspensions (~0.1 ml) were injected into the stems of 10- to 12-week-old Lovell peach seedlings by using a 22-gauge needle inserted tangentially under the cambium. PB was injected as a control. The plants were maintained in a greenhouse at 28°C and rated after 10 days for disease development on a scale of 0 to 3 as follows: 0, light necrosis associated with wounding at the area of inoculation; 1, dark, water-soaked necrosis confined to the immediate area of inoculation, with some streaking in the cambium; 2, streaking in the cambium extending away from the site of inoculation, necrosis around the wound up to 2 mm above and below the wound with gumming; and 3, necrotic lesion and streaking involving the entire stem, often with girdling and death of distal portions and extensive gumming. Each seedling was inoculated in three places with a strain, and an average pathogenicity rating for each strain was used to determine the mean and standard deviation of the pathogenicity for all strains isolated from a particular host.

DNA preparation. Total genomic DNA was extracted from 10 ml of 24-h shake cultures of bacterial cells. After centrifugation at 10,000 × *g* for 10 min, the bacterial pellet was resuspended in 1.5 ml of buffer (100 mM Tris-HCl [pH 7.5], 100 mM EDTA [pH 8.0]). Freshly prepared lysozyme (Sigma, St. Louis, Mo.) was added to a final concentration of 25 μg/ml, the volume of the solution was brought to 3 ml with sterile distilled water, and the mixture was incubated on ice for 10 min. Sodium dodecyl sulfate and proteinase K (Gibco BRL, Gaithersburg, Md.) were added to final concentrations of 1% and 200 μg/ml, respectively. The suspension was incubated for 1 h at 50°C and extracted four times with 5 ml of phenol-chloroform (1:1). The nucleic acids were precipitated with 0.1 volume of 3 M sodium acetate (pH 5.2)–1 volume of isopropanol, washed in 70% ethanol, and resuspended overnight in 200 μl of TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA [pH 8.0]) with 30 μg of RNase (Amresco, Solon, Ohio) per ml. The DNA solution was extracted again with an equal volume of phenol-chloroform (1:1) followed by an equal volume of chloroform-isoamyl alcohol (24:1). The nucleic acids were precipitated with 0.1 volume of sodium acetate–2 volumes of 100% ethanol, rinsed in 70% ethanol, and resuspended in 200 μl of TE buffer. DNA concentrations were determined with a TKO 100 fluorometer (Hoefer Scientific Instruments, San Francisco, Calif.).

RFLP analysis. Approximately 1 μg of total genomic DNA was digested at 37°C overnight with *Eco*RI (Pharmacia Biotech, Uppsala, Sweden), and nucleic acid fragments were electrophoresed in 1% agarose gels at 45 V for 5 to 6 h with TAE (0.04 M Tris acetate, 0.001 M EDTA). The DNA was transferred to Nytran (Schleicher & Schuell, Keene, N.H.) nylon membranes, and Southern hybridization analysis was performed as previously described (8) with a [^{32}P]dATP-labeled 7-kb *Hin*dIII fragment containing the *syrB* and *syrC* genes from plasmid p601D, which was kindly provided by D. Gross (23). The size of restriction fragment(s) that hybridized with the probe was estimated relative to the mobility of 1-kb DNA standards (Gibco BRL).

Oligonucleotide primers and PCR conditions. ERIC oligonucleotide primers (ERIC1R [5′-ATGTAAGCTCCTGGGGATTCAC-3′] and ERIC2 [5′-AAGTAAGTGACTGGGGTGAGCG-3′]) were purchased from Oligos Etc. (Wilsonville, Oreg.). The PCR conditions were as previously described (21, 32). Bacterial strains were streaked onto plates of KB and incubated for 2 days at 25°C. A small portion of a single colony was transferred to 25 μl of a PCR mixture containing 50 pmol of each primer, 1.25 mM each deoxynucleoside triphosphate, 10% dimethyl sulfoxide, 4 μg of bovine serum albumin (Boehringer Mannheim, Indianapolis, Ind.), 2 U of Ampli*Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.), 16.6 mM ammonium sulfate, 67 mM Tris HCl (pH 8.0), 6.7 mM magnesium chloride, 6.7 μM EDTA, and 30 mM β-mercaptoethanol. The mixture was overlaid with silicone oil (Aldrich Chemicals, Milwaukee, Wis.), and PCR was performed in a no. 480 DNA thermal cycler (Perkin-Elmer Cetus) under the following conditions: 1 cycle at 95°C for 6 min; 35 cycles at 94°C for 1 min, 52°C for 1 min, and 65°C for 8 min; and a final extension cycle at 68°C for 16 min. Aliquots (8 μl) of the reaction mixture were electrophoresed on 1.5% TAE agarose gels at room temperature at 5 V/cm for 4 h. The DNA fragments in the gel were visualized by staining with ethidium bromide.

Data analysis. The amplified fragments of each strain were scored as 1 (present) or 0 (absent), and pairwise comparisons were made of each unique pattern by using the Jaccard similarity coefficient (30) and the NTSYS program (Exeter Software, Setauket, N.Y.). A similarity matrix was generated by using the unweighted pair-group method with averages. Phenograms were constructed with the tree display option (TREE). A cophenetic value matrix was calculated by using the COPH option and compared with the original similarity matrix by using the MXCOMP option to test the goodness of fit of the cluster analysis.

RESULTS

Strain collection and identification. Ninety-one strains of *P. syringae* pv. syringae collected from 43 almond, prune, plum, peach, apricot, and cherry orchard sites in the San Joaquin and Sacramento valleys were used in this study. Each strain was collected from separate tissue samples within an orchard site. The bacterium was detected in diseased samples and as an epiphyte on apparently healthy twigs, flowers, and buds. In addition, *P. syringae* pv. syringae was washed from the leaves of two weeds, a *Geranium* sp. and a *Malva* sp., that were growing in a prune orchard with trees showing symptoms of bacterial canker. All *P. syringae* pv. syringae strains used in this study were negative for oxidase, potato rotting, and arginine dihydrolase and positive for levan production and the hypersensitive response on tobacco.

A total of 76 strains of *P. syringae* pv. syringae isolated in 1995 and 1996 from *Prunus* hosts were tested for pathogenicity on Lovell peach seedlings. In addition, four strains from orchard weeds, nine strains from nine other hosts, and five strains of three other *P. syringae* pathovars were tested. All of the *P. syringae* pv. syringae strains were moderately to highly pathogenic on peach, as evidenced by a pathogenicity rating of 2 or more, except for wheat strain 61, which had a rating of 1.0. The stone fruit strains, together with the bean and lemon strains, had pathogenicity ratings in the range of 2.6 to 3.0, while the grass, millet, pear, tomato, and weed strains had ratings of 2.0. The rose and kiwi strain ratings were 2.5 and 2.3, respectively. *P. syringae* pv. tomato, morsprunorum, and coriandricola were of low virulence on peach (0.5, 1.1, and 1.0 disease rating, respectively), and each incited only a mild necrotic reaction around the site of inoculation.

A total of 23 strains, including 19 strains of *P. syringae* pv. syringae and 4 strains of four other pathovars, were tested for

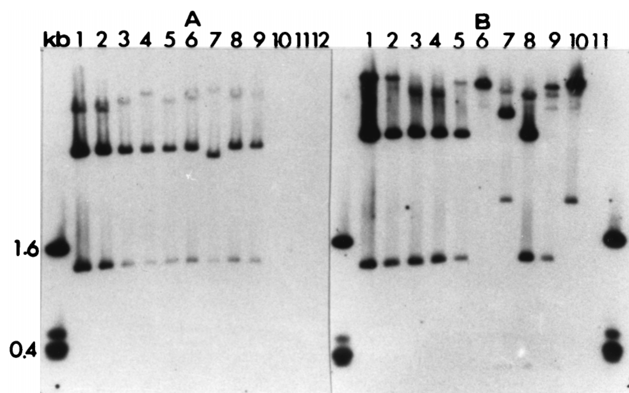

FIG. 1. Southern hybridization of *Eco*RI-digested total genomic DNA of strains of *P. syringae* pv. syringae and other *P. syringae* pathovars probed with a ^{32}P-labeled 7-kb *Hin*dIII fragment containing the *syrB* and *syrC* genes from plasmid p601D. Lanes: kb, the 1-kb molecular marker; A1, B3 peach; A2, B15 almond; A3, 040 almond; A4, B301 pear; A5, 728a bean; A6, B18 millet; A7, B36 peach (New Zealand); A8, 408 tomato; A9, 142 beet; A10, *P. syringae* pv. maculicola 533; A11, *P. syringae* pv. coriandricola 269; A12, *P. syringae* pv. morsprunorum B28; B1, 092 prune; B2, 073 *Geranium* sp.; B3, B21 apricot; B4, 036 peach; B5, 84-160 kiwi; B6, 61 wheat; B7, 321 tomato; B8, B37 rose; B9, B39, corn; B10, B42 lemon; B11, *P. syringae* pv. tomato 320 (A and B in the lane designations refer to panels A and B, respectively).

TABLE 2. Number of strains of *P. syringae* pv. syringae generating 1 of 11 distinct ERIC genomic fingerprint patterns

Host	No. of strains[a] with ERIC pattern[b]:											Total no.
	1	2	3	4	5	6	7	8	9	10	11	
Strains isolated in this study												
Almond		9	2		14	5	1	1				32
Peach		4	9		2	4						19
Prune		8				2				1	1	12
Cherry	1	6	4			1			1			13
Apricot		6	2									8
Plum		3										3
Geranium										2		2
Total	1	36	17	0	16	12	1	1	1	3	1	89
Strains characterized previously												
Peach (B3)	1											1
Almond (B15)		1										1
Pear (B301)				1								1
Apricot (B21)			1									1
Rose (B37)			1									1
Kiwi (84-160)				1								1
Total	1	1	2	2								6

[a] Number of strains tested that generated the banding pattern.
[b] ERIC fingerprint patterns that all share four fragments of similar mobility.

the presence of the *syrB* and *syrC* genes. DNA isolated from all of the *P. syringae* pv. syringae strains, but not the DNA from the other pathovars, hybridized with the *syrB* and *syrC* probe (Fig. 1). The kiwi (84-160), rose (B37), *Geranium* (073), tomato (321), and beet (142) strains and all of the stone fruit strains except for the peach strain from New Zealand (B36) had a similar RFLP pattern.

ERIC analysis. The DNA fingerprints of 104 strains isolated in 1995 and 1996 from 43 orchard sites, including 4 epiphytic weed strains of *P. syringae* and strains obtained from other hosts and/or sources (Table 1), were determined by using ERIC-PCR. The stone fruit strains (except for strain B36, isolated from peach in New Zealand), rose strain B37, kiwi strain 84-160 and pear strain B301 each generated 1 of 11 distinct ERIC genomic fingerprint patterns, which all shared four fragments of similar mobility (Table 2). These 11 patterns could be differentiated by polymorphisms in one or more of the other amplified DNA fragments (Fig. 2). Ninety-three percent of the stone fruit strains isolated in this study produced either pattern 2, 3, 5, or 6 (Table 2). Pattern 10 was represented by the epiphytic *Geranium* sp. weed strains and by an epiphytic strain recovered from a healthy prune bud, each from a different orchard site with a history of bacterial canker disease. The *Malva* sp. weed strains generated a unique pattern, which did not contain the four fragments shared by the stone fruit strain patterns. A strain from a healthy prune flower isolated in the same orchard as the weed strains was the only strain to generate pattern 11. However, 15 other strains isolated from apparently healthy tissues collected in various orchards each generated one of the four most common stone fruit fingerprint patterns.

The occurrence of a particular ERIC fingerprint pattern was not host or location specific. In fact, pattern 2 was common to some strains isolated from all *Prunus* hosts (Table 2). In some cases, strains that generated different patterns were isolated on the same day from separate samples collected in the same orchard. In addition, except for the peach strain from New Zealand, the stone fruit strains from other sources, including B3 and B15, which have been in culture for at least 30 years (6), generated fingerprint patterns similar to those for the strains isolated in this study (Table 2; Fig. 2).

Most *P. syringae* pv. syringae strains (61, 321, 82-12, B18, B39, B40, and B42) from hosts other than stone fruits, together with the New Zealand peach strain (B36), generated patterns that did not contain any of the four DNA fragments shared by the *Prunus* strain patterns (Fig. 3). However, the rose (B37) strain generated stone fruit pattern 3 whereas the pear strain (B301) and the kiwi fruit strain (84-160) generated a unique pattern (pattern 4) that contained the four fragments common to the stone fruit patterns (Fig. 2). The bean strain (728a) contained three of the four common bands.

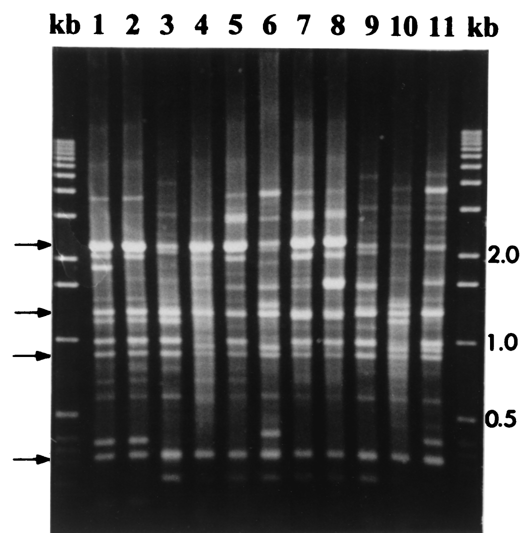

FIG. 2. The 11 ERIC genomic fingerprint patterns which shared four fragments of similar mobilities generated by 95 of the 104 *P. syringae* pv. syringae strains tested. Lanes: kb, the 1-kb molecular marker 1 to 11, ERIC fingerprint patterns 1 to 11, respectively. The arrows on the left indicate the four fragments common to the 11 ERIC patterns.

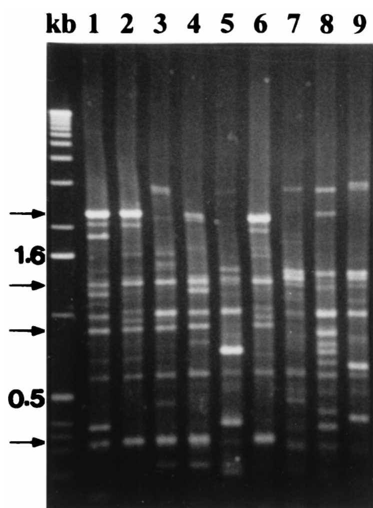

FIG. 3. ERIC fingerprints of *P. syringae* pv. syringae strains isolated from various plant hosts, showing strain variability within the pathovar. Lanes: kb, the 1-kb molecular marker; 1, B3 peach (pattern 1); 2, B301 pear (pattern 4); 3, B728a bean; 4, B37 rose (pattern 3); 5, B42 lemon; 6, 84-160 kiwi (pattern 4); 7, B18 millet; 8, B40 foxtail; 9, 321 tomato. Arrows on the left indicate the four fragments common to 95 of the 104 strains tested.

Sixteen bands were scored for the cluster analysis. The resulting dendrogram (Fig. 4) supported the observation that the genomic fingerprints of *P. syringae* pv. syringae strains from stone fruits had more similarities to each other than to those of most of the strains from other hosts. The *P. syringae* pv. syringae strains tested formed two clusters. One cluster contained the strains with the 10 stone fruit patterns, together with strains B301 and 84-160 (pattern 4), B37, and 728a. The other cluster contained most of the remaining *P. syringae* pv. syringae strains from various hosts, with the *Malva* weed strain 070 being the most divergent strain within this cluster. One tomato strain isolated in Georgia (82-12) and the New Zealand peach strain (B36) were dissimilar from all of the other strains tested and were outliers from the two main clusters. A cophenetic correlation of >0.9 was determined for the similarity matrix, indicating a very high goodness of fit for the cluster analysis.

DISCUSSION

In this study, the *P. syringae* pv. syringae strains isolated from *Prunus* hosts in California generated similar genetic profiles in ERIC-PCR whereas most strains of *P. syringae* pv. syringae isolated from other hosts generated dissimilar patterns. This suggests a host specialization of the stone fruit strains within the heterogeneous pathovar syringae. Specialization of *P. syringae* pv. syringae strains toward a particular host has been observed in previous studies. Saad and Hagedorn (27) used a bean pod pathogenicity assay and found that strains of *P. syringae* pv. syringae isolated from beans or as epiphytes from weeds near bean fields, but not strains isolated from other hosts, caused a pathogenic reaction. The same result was observed in other studies of the strains isolated from beans (2, 7, 26), which led Rudolph (26) to propose designating the bean strains *P. syringae* pv. phaseoli. Legard et al. (16), using RFLP analyses of *P. syringae* pv. syringae strains from various hosts, found that the bean strains formed a separate cluster within the pathovar, substantiating the results of the greenhouse pathogenicity assays. Gross and DeVay (10) found a tendency for grass strains of *P. syringae* pv. syringae to be highly virulent on inoculated maize plants and to reach higher populations in maize leaf tissues than did strains isolated from nongrass hosts. In our study, pathogenicity tests with peach seedlings in the greenhouse failed to distinguish between stone fruit strains and strains from other hosts but were useful in differentiating *P. syringae* pv. syringae strains from strains of other pathovars. Similarly, Otta and English (22) found that *P. syringae* pv. syringae strains from 30 different hosts induced similar cankers on wound-inoculated peach seedling stems.

Syringomycin functions as a nonspecific virulence factor in strains of *P. syringae* pv. syringae (6, 10). Genes for the synthesis and export of the phytotoxin are found in *P. syringae* pv. syringae strains but not in several other related pathovars (23). Some other phytotoxin genes are highly pathovar specific and have been used to develop DNA probes to identify coronatine-producing (3) or phaseolotoxin-producing (28) strains. In addition, the production of syringomycin has been used as a determinative characteristic in identifying strains of *P. syringae* pv. syringae (29, 34). Therefore, the *syrB* and *syrC* genes were used as hybridization probes to confirm the identity of a representative group of the *P. syringae* pv. syringae strains used in this study. The stone fruit strains, except for the New Zealand peach strain (B36), had a similar hybridization pattern to the pear, rose, bean, and kiwi fruit strains (strains which had a similar ERIC pattern), as well as to the strains from millet, beet, and tomato. However, the ubiquitous presence of syringomycin in this pathovar indicates that although strains can be genetically heterogeneous by methods such as ERICs and

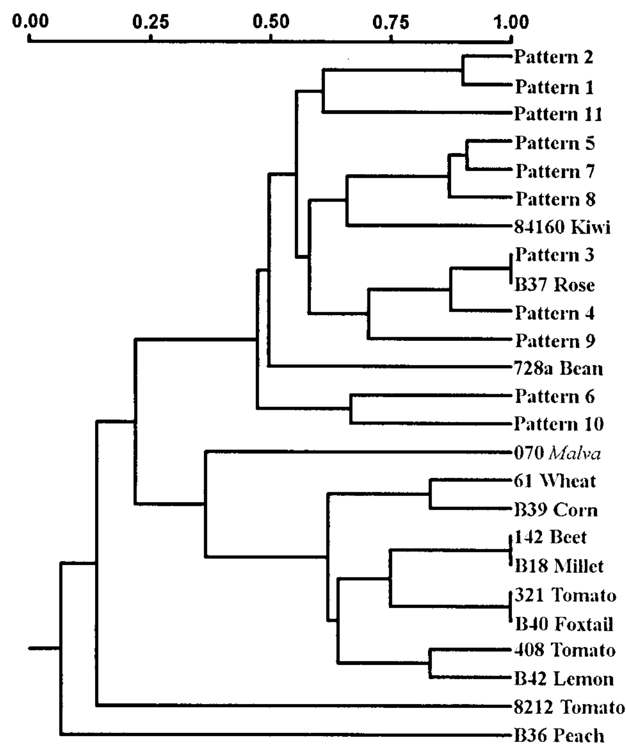

FIG. 4. Dendrogram of genetic relatedness of the ERIC fingerprint patterns generated by 104 strains of *P. syringae* pv. syringae. Cluster analysis was performed by using the Jaccard similarity coefficient (30). Ninety-five of the strains generated 1 of the 11 fingerprint patterns indicated on the dendrogram. The remaining strains are listed with the host from which they were originally isolated. The scale at the top indicates the degree of genetic relatedness between the strains tested.

RFLPs, all of the *P. syringae* pv. syringae strains tested have the genetic potential to produce syringomycin.

Weed hosts within or near orchards or fields have been hypothesized to provide overwintering sites for *P. syringae* pv. syringae and to serve as an inoculum source for disease outbreaks (7, 15, 24). In this study, the ERIC patterns of *P. syringae* pv. syringae strains recovered from weed species were dissimilar to those of strains causing cankers on *Prunus* hosts. Thus, the role played by *P. syringae* pv. syringae epiphytes on weeds in the initiation and development of bacterial canker disease of prune in California remains uncertain. Strains from one of the weed species and two epiphytic strains isolated from healthy prune tissues were the only strains to generate two of the ERIC patterns (patterns 10 and 11). Another 15 epiphytic strains generated the same banding patterns as the strains isolated from diseased tissues. Therefore, healthy tissues appear to harbor a heterogeneous population of epiphytic strains, with at least some of these strains being capable of causing bacterial canker in susceptible tissues.

ERIC and REP PCR has been shown to be a rapid and reliable method to differentiate plant-pathogenic bacteria at or below the pathovar level with highly reproducible results (19). In a study which used REP PCR to compare 100 *P. syringae* pv. syringae strains from ornamental pear trees with 6 strains from peach, wheat, tomato, and maize, all of the ornamental-pear strains clustered into one of two closely related groups while none of the strains from other hosts had any similarities to the pear strains or to each other (31). These results are similar to what was observed in this study when *P. syringae* pv. syringae strains isolated from stone fruits in California were compared to strains isolated from other hosts and support the theory that some, if not all, strains within the heterogeneous pathovar syringae have adapted genetically to a particular host. In addition, similar to what was observed in this study, previous research has demonstrated a close relationship between strains causing disease on pome fruits, such as pear, and stone fruits (9, 25). Weingart and Völksch (33), however, found few similarities in the ERIC banding patterns of five strains of *P. syringae* pv. syringae isolated from pear, apple, and cherry trees in Western Europe. This apparent high diversity might be expected in an area with a long history of cultivating *Prunus* species, where, presumably, the associated microflora would have evolved with and adapted to the various *Prunus* hosts over time. In our study, a peach strain (B36) isolated in New Zealand generated an ERIC pattern unlike those from all of the other *P. syringae* pv. syringae strains tested; this strain may be the result of an evolutionary adaptation separate from North American and European *P. syringae* pv. syringae strains.

Louws et al. (19), using ERIC PCR, found evidence of intrapathovar diversity among strains of *Xanthomonas campestris* pv. vesicatoria and campestris, pathovars which also have more than one host. Other pathovars with a more restricted host range, such as *P. syringae* pv. morsprunorum and tomato, had low or no diversity in their ERIC profiles. Additional studies by other genetic characterization methods support the hypothesis that variation was greater among strains from pathovars with wide host ranges, such as *P. syringae* pv. syringae. Denny et al. (5) used RFLP to analyze six *P. syringae* pv. syringae strains and found that the strains clustered into two groups which contained strains either from monocots or from dicots whereas strains of *P. syringae* pv. tomato were less genetically diverse. In another study involving RFLP and randomly amplified polymorphic DNA analyses (18), strains of *P. syringae* pv. apii, which infect only celery, were more genetically homogeneous than were strains of *P. syringae* pv. maculicola, which infect a wide range of crucifer hosts. Overall, our results suggest that strains of *P. syringae* pv. syringae that are adapted to a specialized niche, such as California stone fruits, may be the result of a recent adaption and/or genetic isolation, resulting in the genetically homogeneous population of *P. syringae* pv. syringae strains from stone fruits observed in this study, which formed a distinct group from strains isolated from other hosts.

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