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Randomly Amplified Polymorphic DNA Analysis of *Xylella fastidiosa* Pierce's Disease and Oak Leaf Scorch Pathotypes

J. CHEN,[1]* O. LAMIKANRA,[1] C. J. CHANG,[2] AND D. L. HOPKINS[3]

Center for Viticultural Science, Florida A&M University, Tallahassee, Florida 32307[1]; Department of Plant Pathology, University of Georgia, Griffin, Georgia 30223[2]; and Central Florida Research and Education Center, University of Florida, Leesburg, Florida 34784[3]

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Randomly amplified polymorphic DNA analysis was conducted with 14 primers to 17 strains of *Xylella fastidiosa*. There was a high degree of similarity among the seven Pierce's disease (PD) strains ($S_{xy} > 0.93$) and the seven oak leaf scorch (OLS) strains ($S_{xy} > 0.96$). However, the two groups were different, with a similarity index of 0.67, confirming the presence of a PD DNA cluster and suggesting the presence of a new OLS cluster. The control plum leaf scald strains (two strains) together with the periwinkle wilt strain had a much smaller similarity index (0.44) compared with the PD and OLS clusters.

Xylella fastidiosa is a newly described and nutritionally fastidious plant pathogen (22). Only a few population analyses of this bacterium have been conducted because of difficulties in strain isolation and long-term maintenance. Among the many diseases caused by *X. fastidiosa*, Pierce's disease (PD) of grapevine is the limiting factor preventing the commercial production of bunch grapes in the southeastern United States (10), and recently the disease incidence has been increasing in California. Oak leaf scorch (OLS) is commonly found in the southeastern to northeastern United States (5, 15), and its potential impact on urban forestry is high (11).

Presently, all strains, though varying in pathogenicity, are classified under one species, *X. fastidiosa*. However, this can be inconvenient for scientific research and in disease control. We are interested in investigating the relationships among pathogenically distinct *X. fastidiosa* groups, or pathotypes, and accumulating evidence for future establishment of a pathovar or subspecies system. Previous studies to differentiate *X. fastidiosa* strains by using cultivation media (11), cellular protein profiles (4), DNA homology (14), restriction fragment length polymorphisms (RFLPs) (6), and PCR (18) have been successful. The randomly amplified polymorphic DNA (RAPD) technique is unique compared with the methods mentioned above in that no preknown sequences are required, and it is the simplest and quickest way to study strain differentiations of microorganisms (3, 9, 16).

In both RFLP and RAPD analyses, DNA banding profiles of different bacterial strains are quantified and similarity indices are calculated to generate a multidimensional data set. The interrelationships of the bacterial strains are then elucidated by cluster analysis with a discrete dendrogram and/or by principal component analysis with a two- or three-dimensional diagram. Although less commonly used than cluster analysis in bacterial population study, principal component analysis is useful for the examination of entities whose relationships might constitute a continuum, as probably occurs with bacteria (1). The central idea of principal component analysis is to reduce the dimensionality of a data set, which consists of a large number of interrelated variables, while retaining as much as possible of the variation present in the data set. This is achieved by transforming, to a new set of variables, the principal components so that the first few retain most of the variation present in all of the original variables. The variation of a principal component is indicated by a statistic, called eigenvalue (13).

Alderson (1) had discussed a number of successful studies that used principal component analysis to describe different microbial populations. Some recent examples include analyses of data generated from cellular protein profiles (20), fatty acid profiles (12), DNA RFLP (6), and DNA pulsed-field gel electrophoresis (2) of different groups of prokaryotes.

In a previous work, the presence of a PD DNA cluster and the possible occurrence of a plum leaf scald (PLS) cluster were clearly demonstrated through RFLP analysis (6). In this paper, we report the results of RAPD analysis of PD and OLS strains of *X. fastidiosa*.

MATERIALS AND METHODS

PD strains of *X. fastidiosa* were grown in PD2 medium (8), whereas all other strains were grown in PW medium (7) (Table 1). Bacterial cells (100 ml) were harvested by centrifugation from cultures incubated at 30°C for 7 to 10 days with agitation and lysed in 400 μl of lysozyme buffer (Tris-HCl, 50 mM; EDTA, 62.5 mM; LiCl, 2.5 M; Triton X-100, 0.4%; lysozyme, 0.1%) at 37°C for 2 h. Further lysis was done by the addition of 50 μl of 20% Triton X-100 and incubation at room temperature for 30 min. Cell debris was removed by chloroform extraction, and DNA was isolated by sodium acetate-ethanol precipitation (17). RAPD primers were purchased from the University of British Columbia, Vancouver, British Columbia, Canada. The 14 randomly selected primers used were as follows: UBC-215 (tca cac gtg c), UBC-232 (cgg tga cat c), UBC-234 (tcc acg gac g), UBC-240 (atg ttc cag g), UBC-241 (gcc cga cgc g), UBC-248 (gag taa gcg g), UBC-250 (cga cag tcc c), UBC-269 (cca gtt cgc c), UBC-271 (gcc atc aag a), UBC-281 (gag agt gga a), UBC-282 (ggg aaa gca g), UBC-283 (cgg cca ccg t), UBC-286 (cgg agc cgg c), and UBC-287 (cga agc gcg g). For the PCR procedure, the reaction mixture (25 μl) contained the following: deoxynucleoside triphosphate, 250 mM; $MgCl_2$, 10 mM; primer, 15 mM; *Taq* DNA polymerase (Promega Corp., Madison, Wis.), 0.1 U; and bacterial DNA, 30 to 70 ng. Amplification was performed in 1× reaction buffer with an MJ Research thermal cycler (model PTC-100) for 40 cycles. Each cycle consisted of the following: denaturing at 94°C for 1 min, annealing at 35°C for 2 min, and extension at 72°C for 2 min. The last cycle of extension was extended to 8 min before the reaction products were stored at 4°C for use. The amplified DNAs were subjected to 1.5% agarose gel electrophoresis and viewed under UV light after ethidium bromide staining. To test reproducibility, primers UBC-234, UBC-248, and UBC-283 were randomly selected to repeat the DNA amplification experiment under the same conditions two to three times.

DNA profiles were recorded in the binary form; i.e., 1 = presence of a band and 0 = absence of a band. A similarity index (S_{xy}) was calculated on the basis of the formula: $S_{xy} = 2N_{xy}/(N_x + N_y)$, where N_{xy} is the number of bands of strains x and y in common; N_x is the number of bands of strain x; and N_y is the number of bands of strain y (19). Statistical analyses were performed with a SAS package

* Corresponding author. Phone: (904) 599-3996. Fax: (904) 561-2617. Electronic mail address: jchen@vm.cc.famu.edu.

TABLE 1. Names, sources, and pathotypes of *X. fastidiosa* strains

Isolate	Pathotype	Location	Source
OLS92-3	OLS	Florida	D. Hopkins
OLS92-4	OLS	Florida	D. Hopkins
OLS92-7	OLS	Florida	D. Hopkins
OLS92-8	OLS	Florida	D. Hopkins
Stucky-I	OLS	Georgia	C. J. Chang
Stucky-IV	OLS	Georgia	C. J. Chang
Stucky-V	OLS	Georgia	C. J. Chang
PCE-FG (ATCC 35881)	PD		ATCC[a]
R112v2#1	PD	Georgia	C. J. Chang
R116v11	PD	Georgia	C. J. Chang
R118v3-4#12	PD	Georgia	C. J. Chang
PD1-FD	PD	Florida	D. Hopkins
PD92-8	PD	Florida	D. Hopkins
PD92-9	PD	Florida	D. Hopkins
Plum2#9	PLS	Georgia	L. Pusey[b]
Plum4#5	PLS	Georgia	L. Pusey
PWT-22 (ATCC 35878)	PW[c]		ATCC

[a] ATCC, American Type Culture Collection, Rockville, Md.
[b] SE Fruit and Tree Nut Research Laboratory, Agriculture Research Service, U.S. Department of Agriculture, Byron, Ga.
[c] PW, periwinkle wilt.

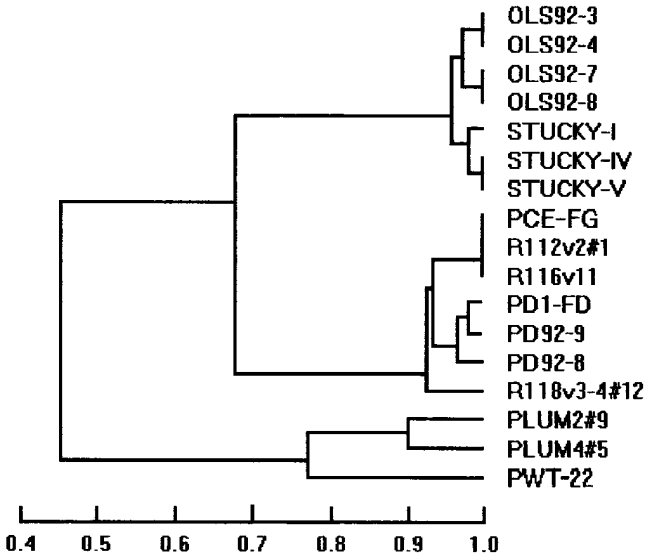

FIG. 2. Dendrogram using the UPGMA algorithm to group the 17 *Xylella* strains on the basis of their similarity indices.

(20a) through an IBM 4381 mainframe computer hosted by Florida A&M University, Tallahassee. An UPGMA (unweighted pair group method with arithmetic averages) algorithm was used to perform cluster analysis and construct a dendrogram. Principal component analysis was also applied to depict the relationships among the different strains.

RESULTS AND DISCUSSION

A total of 90 scorable characters were generated from the 14 RAPD primers with the 17 *X. fastidiosa* strains. A representative RAPD profile is shown in Fig. 1. There were two types of DNA bands, strong and weak in intensity. In this experiment, we scored every possible DNA band to obtain the maximum number of scorable characters and minimize the statistical errors. Primers UBC-238, UBC-248, and UBC-283 consistently generated the same RAPD profiles, indicating the good reproducibility of this experiment.

Similar to a previous report of RFLP analysis (6), all PD strains subjected to RAPD analysis demonstrated a high degree of similarity ($S_{xy} > 0.93$; Fig. 2), confirming the existence of a PD cluster in *X. fastidiosa*. OLS strains were compared, and the seven strains (four from Florida and three from Georgia) appear to be very similar ($S_{xy} > 0.96$; Fig. 2). In contrast, PD and OLS strains are distantly related at a similarity of 0.67 (Fig. 2), suggesting the presence of an OLS cluster. Although only two PLS strains and one periwinkle wilt strain were used, results of this RAPD analysis agree with those of the RFLP analysis (6) in that PLS strains are clearly removed from PD strains ($S_{xy} = 0.44$; Fig. 2) and the periwinkle wilt strain is closer to PLS ($S_{xy} = 0.76$; Fig. 2).

Results of principal component analysis confirm those of cluster analysis (Fig. 2 and 3). The first and second principal components accounted for 98.7% of the total eigenvalue, or variation. Principal component analysis directly illustrates the relationships between individual strains, whereas cluster anal-

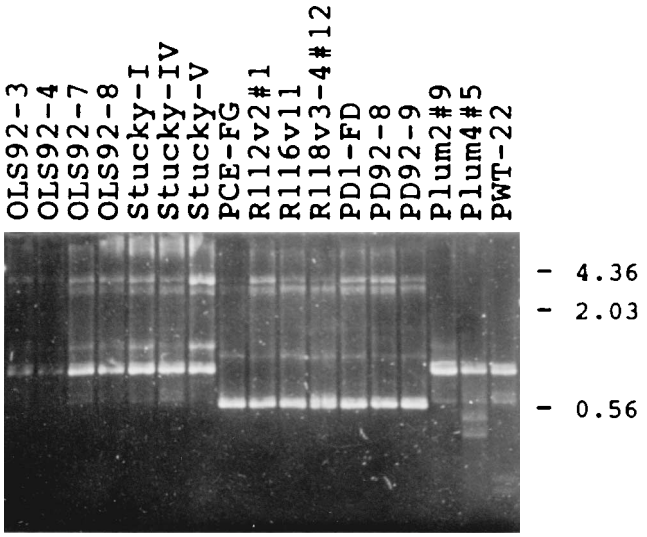

FIG. 1. Representative RAPD profile of *Xylella* strains with primer UBC-287. Values on the right are molecular weight markers (in kilobases).

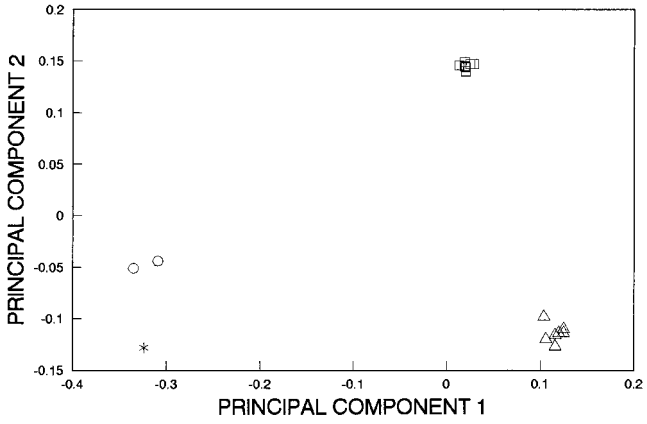

FIG. 3. Two-dimensional visualization of relationships among the 17 *Xylella* strains from principal component analysis. The seven squares represent strains OLS92-3, OLS92-4, OLS92-7, OLS92-8, Stucky-I, Stucky-IV, and Stucky-V. The seven triangles represent strains PCE-FG, R112v2#1, R116v11, R118v3-4#12, PD1-FD, PD92-8, and PD92-9. The two circles represent strains Plum2#9 and Plum4#5. The star represents strain PWT-22.

ysis with UPGMA makes it easy to interpret similarities between groups of strains (Fig. 2 and 3). Sneath and Sokal (21) reported that cluster analysis gave useful summaries of the bacterial relationships, whereas principal component analysis was especially valuable for understanding the population structure in more detail. The two methods are complementary.

The seven OLS strains (Table 1) shared highly similar RAPD profiles with all primers used except one, UBC-248, giving rise to an extra strong band in the three OLS strains from Georgia but not the four strains from Florida (data not shown). In fact, OLS strains from Georgia were isolated from red oak (*Quercus rubra*) and the OLS strains from Florida were isolated from Turkey oak (*Q. laevis*). However, more study is needed to conclude that geographic or host specialization exists among strains of the same pathotype in *X. fastidiosa*. A similar phenomenon was not observed with PD strains, which all were isolated from *Vitis vinifera* in this experiment.

As pointed out by many other researchers, the main advantage of RAPD analysis is its simplicity and rapidity (3, 9, 16). Starting from DNA, which can be easily isolated with the method mentioned, a RAPD experiment can be finished within 1 working day. Therefore, the RAPD technique is very suitable for analysis of large numbers of strains. We also reused (remelted and recasted) agarose up to four times to reduce the experimental expense and still had satisfactory results (Fig. 1). Since maintenance of many *X. fastidiosa* strains is usually a very challenging task, DNA can be extracted and stored at −20°C for future use once a strain is isolated and cultivated. The requirement of a small amount of sample DNA (at the nanogram level) makes RAPD particularly useful for population analysis of the difficult-to-isolate and slow-growing *X. fastidiosa* strains. Furthermore, RAPD also can be used for strain identification purposes, or fingerprinting, by running the undetermined *X. fastidiosa* strains side by side with the known *X. fastidiosa* strains for comparison.

In summary, the results (Fig. 2 and 3) of this RAPD analysis strongly indicate the presence of PD and OLS clusters in *X. fastidiosa* strains and add more evidence to demonstrate the existence of a PLS cluster. PD, OLS, and PLS strains also are pathogenically distinct (5, 11). The good association between RAPD clusters and pathogenicity groups of *X. fastidiosa* and the simplicity of the RAPD experiment suggest that RAPD analysis can play an important role in the future for the establishment of a pathovar, or subspecies, classification system for *X. fastidiosa*.

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