Repetitive Genomic Sequences for Determining Relatedness Among Strains of *Fusarium oxysporum*

H. C. Kistler, E. A. Momol, and U. Benny

Assistant professor, graduate research assistant, and biological scientist, Plant Pathology Department, University of Florida, Gainesville 32611.

We thank G. Benny for assistance and guidance in using the PAUP program. Florida Agricultural Experimental Station Journal Series R-00622.

Accepted for publication 18 September 1991.

ABSTRACT

Kistler, H. C., Momol, E. A., and Benny, U. 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. Phytopathology 81:331-336.

Polymorphic restriction fragments containing moderately repetitive DNA sequences have been identified in the fungus *Fusarium oxysporum*. Arbitrarily chosen genomic clones pEY1, pEY2, pEY7, and pEY10, containing 1.1, 1.1, 2.3, and 1.2 kb of fungal DNA, respectively, were used to identify the repetitive sequences. When used as probes in DNA/DNA hybridization analyses of restriction endonuclease-digested DNAs from various strains of *F. oxysporum*, these probes identified distinctive banding patterns for each strain. These probes were used to infer the phylogenetic relationships among crucifer-infecting strains of *F. oxysporum*. Parsimony analysis suggests a common ancestry for strains representing different races of *F. o.* f. sp. *conglutinans*, but a distinct ancestry between these and strains of *F. o. raphani* and *F. o. matthioli*. A geographical subpopulation of *F. o. conglutinans* was identified by pEY2. Clone pEY10 was useful to fingerprint individual strains *F. o. cubense*, causal agent of Fusarium wilt of banana.

Fusarium oxysporum Schlect. emend. Snyd. & Hans. has one of the broadest host ranges of any plant pathogenic fungus. However, individual pathogenic strains within the species normally have a highly circumscribed host range. Strains with similar or identical host ranges often are assigned to common subspecific groupings called formae speciales. Scores of formae speciales have been described for *F. oxysporum* (1). In addition, numerous nonpathogenic strains of the fungus are known to exist (5,8). The pathogenic diversity of *F. oxysporum* has been interpreted as suggesting extreme genetic divergence within the species despite its highly conserved morphology (13).

The exact relationship among the various strains of the fungus within a forma specialis has been questioned. If host specialization has arisen by convergent evolution, classification of fungi by host range may lead to erroneous assumptions of relatedness (13). In many cases, strains within a forma specialis are quite similar, if not identical, based on various genetic evidence. For example, little variation has been detected among individual strains of the cabbage yellows pathogen (*F. o. conglutinans*) with respect to restriction fragment length polymorphisms (RFLPs) in nuclear, mitochondrial, or plasmid DNA (10–13), or with respect to elec-

© 1991 The American Phytopathological Society

trophoretic patterns of isozymes (2). Likewise, all members of this forma specialis belong to the same vegetative compatibility group (VCG) and thus may have the potential for genetic exchange only with other members of the group (2). Thus, the potential for genetic variation may be limited in this subpopulation.

In other cases, however, genetic differences among individuals within a forma specialis are more extensive. For example, strains of the pathogen, *F. o. cubense*, causal agent of Fusarium wilt of banana, may belong to any of at least 11 VCGs (17) and show considerable DNA polymorphism (16; H. C. Kistler, *unpublished*). Still, little DNA polymorphism has been noted within strains of the same VCG in this forma specialis.

The purposes of this study were to develop a sensitive method for detecting genetic differences among strains of *F. oxysporum*; to use this technique to examine the genetic relationships among members of an individual forma specialis or VCG; to compare genetic variation within these groups to variation between the groups; and to develop a method to identify specific strains of the fungus by DNA fingerprinting.

TABLE 1. Strains of *Fusarium oxysporum* used in this study[a]

Strain	Forma specialis	Race	VCG	Origin	Source
002	*conglutinans*	1	0101	Florida	PHW
081	*conglutinans*	1	0101	Wisconsin	PHW
684	*conglutinans*	2	0101	California	PHW
689	*conglutinans*	2	0101	California	PHW
694	*conglutinans*	2	0101	California	PHW
719	*conglutinans*	1	0101	Hungary	PHW
722	*conglutinans*	1	0101	ATCC 9990	PHW
723	*conglutinans*	1	0101	ATCC 16600	PHW
752	*conglutinans*	1	0101	Wisconsin	PHW
768	*conglutinans*	1	0101	Wisconsin	PHW
777	*conglutinans*	1	0101	Japan	PHW
808	*conglutinans*	2	0101	California	PHW
809	*conglutinans*	2	0101	California	PHW
811	*conglutinans*	2	0101	California	PHW
242	*raphani*	...	0102	Florida	PHW
699	*raphani*	...	0102	Wisconsin	PHW
724	*raphani*	...	0102	ATCC 16601	PHW
760	*raphani*	...	0102	Germany	PHW
779	*raphani*	...	0102	Japan	PHW
795	*raphani*	...	0102	California	PHW
796	*raphani*	...	0102	CBS488.67	PHW
815	*raphani*	...	0102	France	PHW
821	*raphani*	...	0102	Taiwan	PHW
1088	*raphani*	...	0102	Japan	PHW
725	*matthioli*	1	0103	ATCC 16602	PHW
781	*matthioli*	1	0103	Japan	PHW
793	*matthioli*	1	0103	Germany	PHW
806	*matthioli*	1	0103	Italy	PHW
1094	*matthioli*	1	0103	Japan	PHW
726	*matthioli*	2	0103	ATCC 16603	PHW
247	*pisi*	5	unknown	...	HDV
S1	*cubense*	1	0126	Honduras	RCP
3S1	*cubense*	1	0120	Honduras	RCP
8606	*cubense*	1	0125	Australia	RCP
F9129	*cubense*	1	0123	Taiwan	RCP
O1222	*cubense*	1	0120	Australia	RCP
C1	*cubense*	4	0120	Canaries	RCP
8604	*cubense*	4	0129	Australia	RCP
N5331	*cubense*	4	0129	Australia	RCP
F9127	*cubense*	4	0120	South Africa	RCP
22410	*cubense*	4	0120	Australia	RCP

[a] Strains of *F. o. conglutinans*, *F. o. raphani*, and *F. o. matthioli* are described in (2); strains of *F. o. cubense* are described in (16). Abbreviations are as follows: VCG = Vegetative compatibility group; ATCC = American Type Culture Collection number; CBS = Centraalbureau voor Schimmelcultures number; PHW = Paul H. Williams, Dept. Plant Pathology, University of Wisconsin, Madison. HDV = Hans D. VanEtten, Dept. Plant Pathology, University of Arizona, Tucson. RCP = Randy C. Ploetz, Tropical Research and Experimental Center, University of Florida, Homestead.

MATERIALS AND METHODS

Fungal strains. Strains of *F. oxysporum* used for this study are listed in Table 1. Strain T-2 of *Nectria haematococca* Berk. & Br. was obtained from H. D. VanEtten. All strains were preserved as conidia and hyphal fragments in sterile 50% glycerol and kept at −80 C.

DNA extraction. Cultures from single spores were grown on potato-dextrose broth (PDB) plus 1.5% agar. A small cube of agar containing mycelium from these cultures was transferred to 15 ml of PDB and grown in still culture for 3 days. The mycelium was filtered through cheesecloth and lyophilized. Lyophilized mycelium was ground to a powder in a conical 1.5-ml microcentrifuge tube with a wooden stick and then 500 μl of extraction buffer (100 mM Tris, pH 8.0; 50 mM ethylenediaminetetraacetic acid [EDTA]; 100 mM NaCl; 10 mM *p*-mercaptoethanol, and 1% sodium dodecyl sulfate [SDS]) was added and mixed with the powder. Suspensions were incubated at 65 C for 10 min, after which 250 μl of ice-cold 5 M potassium acetate was added. The suspension then was mixed and incubated on ice for 20 min. After centrifugation at 4 C for 10 min, the supernatant was collected and treated with 5 μl of a solution containing 10 mg of RNase (Sigma Chemical Co., St. Louis, MO) per milliliter for 30 min; 10 μl of a solution containing 10 mg of proteinase K (Sigma) per milliliter then was added and incubated at 37 C for 10 min. Samples were extracted with 500 μl of a solution of phenol/chloroform/isoamyl alcohol (25:24:1). Nucleic acids were precipitated by the addition of 1 ml of absolute ethanol to 500 μl of sample; the sample was incubated at room temperature for 5 min. The samples were centrifuged at 5,000 *g* in a microcentrifuge for 15 min at ambient temperature, and the pellets were washed once with 70% ethanol. The ethanol was removed and the pellets were air dried for 30 min; 50 μl of TE (10 mM Tris, 1 mM EDTA, pH 7.4) was added to each sample and the samples were incubated at room temperature for 60 min. DNA was stored at −20 C. The technique for the separation of the nuclear and mitochondrial DNA fractions was described previously (6).

Clones of genomic and ribosomal DNA. Nuclear DNA from *F. oxysporum* strain 722 (ATCC 9990) was digested to completion with restriction enzyme *Eco*RI according to the manufacturer's recommendations (Bethesda Research Laboratories [BRL], Gaithersburg, MD). Similarly digested *Escherichia coli* plasmid pUC119 (20) was mixed with a threefold molar excess of the *F. oxysporum* DNA, allowed to anneal, and ligated with T4 DNA ligase (BRL). DNA was transformed to competent cells of *Escherichia coli* DH5α (BRL) and plated on LB medium containing ampicillin and X-gal at specified concentrations (18). White, ampicillin-resistant colonies were isolated and plasmid DNA from these strains was extracted by the alkaline lysis method (18).

Hybridization analysis. Fungal DNAs were digested with restriction enzymes and restriction fragments were separated in 0.7% agarose by electrophoresis in TBE buffer (18) at 1.25 V/cm for 16 hr. DNA fragments from bacteriophage lambda DNA digested with *Hin*dIII were used as molecular weight markers. Gels were incubated in 0.25 N HCl for 15 min at room temperature, and DNAs denatured in the presence of 1.5 M NaCl, 0.5 M NaOH for 30 min, then neutralized in 3 M NaCl, 0.5 M Tris, pH 7.0, for 30 min. Capillary transfers of DNA to nylon supports (Gene Screen Plus, DuPont Canada Inc., Lachine, Quebec) were accomplished by methods described by the manufacturer. The nylon screens were prehybridized in 20 ml of an aqueous solution (5 ml 20× SSC [20× SSC = 3 M NaCl, 0.3 M sodium citrate, pH 7.0], 10 ml of formamide, and 5 ml 4% SDS), contained in a heat sealable bag. Prehybridization was for at least 15 min at 42 C. After removal of the prehybridization solution, 20 ml of an identical hybridization solution was added. Probes were radioisotope-labeled by nick translation (18) and denatured with 0.4 volume of 2 N NaOH. After addition of the probe, the mixture was incubated with constant agitation for 16–24 hr at 42 C. Filters then were washed twice with 2× SSC for 5 min, twice with 2× SSC, 1% SDS at 65 C for 30 min, and once briefly at room

temperature with 0.2× SSC. Filters were exposed to Kodak X-Omat-AR film at −80 C with two Cronex Lightning-plus intensifying screens for 1–7 days.

Phylogenetic analysis. Banding patterns of hybridization on autoradiograms were used to compare the relatedness of strains. Each band with a different electrophoretic mobility was assigned a position number. If the DNA from a strain contained a band of this mobility it was assigned the value "1" for this position. If the DNA lacked a band with this mobility it was assigned the value "0" for this position. The values for each position were compared for various strains of the fungus. The relatedness of strains was estimated from these values by Wagner parsimony with PAUP program version 2.4.1 (19). Trees were rooted by using a data set containing the value "0" for all numbers.

RESULTS

Ten arbitrarily chosen genomic clones of DNA from strain ATCC 9990 were used as hybridization probes to detect restriction fragments with sequence similarity from the DNA of other strains of the fungus. Six of the 10 clones hybridized to single major restriction fragments of genomic DNA. Clone pEY4, which contained 4.8 kb of genomic DNA (Fig. 1), was typical of this type. Hybridization was primarily to one major DNA fragment for all strains when the DNA was digested with *Bgl*II (Fig. 1) or *Eco*RI. Several minor bands were detected and DNA from strain 699 showed additional polymorphism.

Four of the 10 arbitrarily chosen clones hybridized to multiple restriction fragments in genomic DNA of all strains. Clone pEY1, which contained a 1.1-kb fragment of DNA, hybridized to multiple bands of genomic DNA digested with *Hin*dIII (Fig. 2). Strains from three crucifer-infecting formae speciales of *F. oxysporum* were examined and several polymorphic bands were identified in DNA from these strains. In fact, the electrophoretic banding pattern for each strain was different. The polymorphisms detected by this clone and a similar clone (pEY7) were used to create dendrograms of the relationships among members of the various formae speciales. Nine members of *F. o. conglutinans, F. o. matthioli*, and *F. o. raphani* were compared at a total of 44 restriction fragment positions.

The PAUP program was used for phylogenetic analysis (19) and the presence or absence of hybridizing bands at certain sites was employed to construct trees representing the most parsimonious interpretation of the relationships among the strains. A data subset containing null values at all sites was used as a designated ancestor to root the trees. The complete data set is shown in Table 2. A total of nine most parsimonious trees were found by this analysis and all had the same general form as the example shown in Figure 3. The trees differed in the relative relationships among the representative strains of *F. o. conglutinans* race 1. In general, however, the strains clustered according to forma specialis (regardless of race) and corresponding VCG. The greatest differences detected were between the *F. o. conglutinans* (VCG 0101) strains and all others. The one isolate of *F. o. conglutinans* race 2 (strain 808) was more distant from race 1 representatives of this forma specialis than the race 1 strains

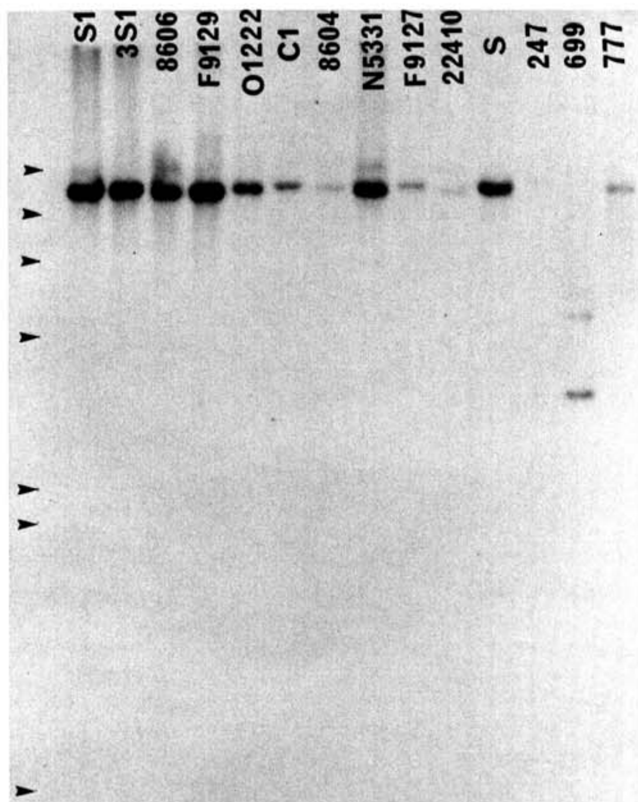

Fig. 1. Hybridization of pEY4 to genomic DNA from 14 strains of *F. oxysporum*. Total DNA from the strains indicated was digested with *Bgl*II, fractionated by agarose electrophoresis, transferred to nylon supports, and hybridized to labeled pEY4. Lane S contains DNA from strain ATCC 16603. Arrows denote positions of the restriction fragments of bacteriophage lambda digested with *Hin*dIII with the sizes, from top to bottom, 23.1, 9.4, 6.7, 4.4, 2.3, 2.0, and 0.56 kb.

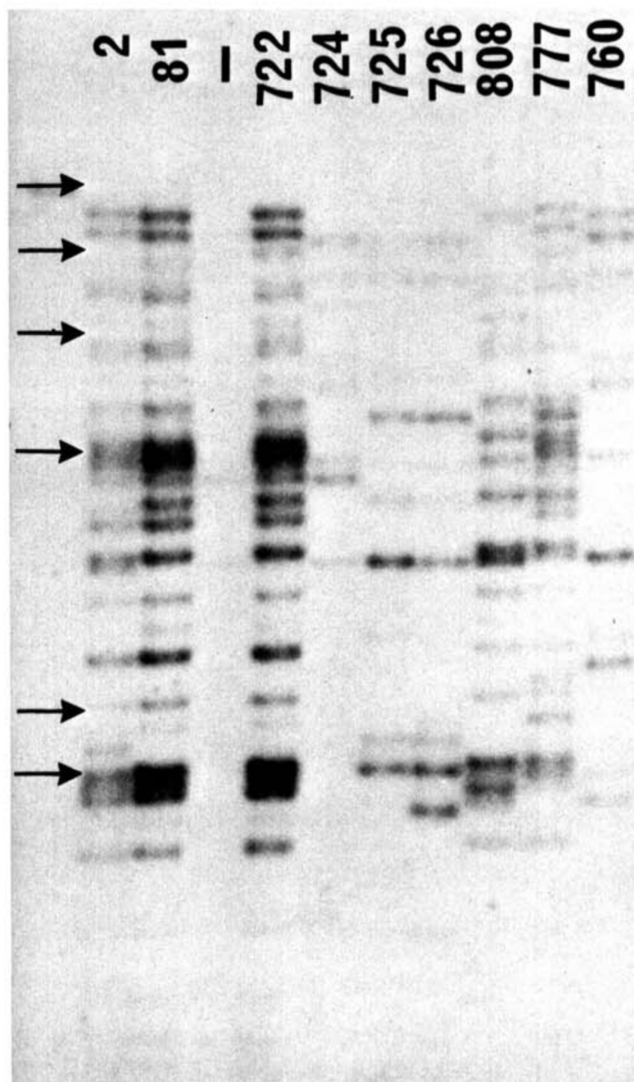

Fig. 2. Hybridization of pEY1 to DNA from nine crucifer-infecting strains of *Fusarium oxysporum*. Total DNA from the strains indicated was digested with *Hin*dIII, fractionated on a 0.7% agarose gel, transferred to a nylon membrane and hybridized to pEY1. Arrows denote the position of DNA size markers that are, from top to bottom, 23.1, 9.4, 6.7, 4.4, 2.3, and 2.0 kb.

were to each other. However, compared to other strains of *F. oxysporum*, the members of *F. o. conglutinans* appear monophyletic.

To test the consistency of this type of analysis, another genomic clone (pEY2) that hybridized to multiple, polymorphic restriction fragments was used to analyze DNA from 30 crucifer-infecting strains. A different DNA banding pattern was present in nearly every strain (Table 3). When these data were analyzed using the PAUP program, similar, but not identical groupings were noted among strains (Fig. 4). All strains of *F. o. conglutinans* race 2 appeared to have similar banding patterns and clustered more closely with some strains of *F. o. conglutinans* race 1 than with strains of other formae speciales. Likewise, strains of *F. o. matthioli* also seemed to group together as a unit. In general, pEY2 hybridized to few DNA bands for strains of *F. o. raphani*. Additionally, very few DNA bands from three Wisconsin strains of *F. o. conglutinans* race 1, hybridized to pEY2. Included among these was strain 722 (ATCC 9990), the strain from which the DNA of pEY2 was derived.

Another arbitrarily chosen genomic clone, pEY10, containing 1.2 kb of fungal DNA, hybridized to highly variable regions of the *F. oxysporum* genome. Genomic DNA from 10 representatives of *F. o. cubense* were tested along with individual strains of *F. o. matthioli, F. o. pisi, F. o. raphani*, and *F. o. conglutinans* (Fig. 5). Each strain had a distinct DNA hybridization pattern, even individuals within the same forma specialis and vegetative compatibility group (cf. 3S1, 01222, C1, F9127, and 22410, all strains of *F. o. cubense* within VCG 0120). The only two strains (8604 and N5331) that shared a number of hybridizing bands were members of the same VCG and were isolated from banana cultivar Cavendish, in Queensland, Australia.

DISCUSSION

Until recently, repetitive DNA sequences in the chromosomes of filamentous fungi were thought to be rare (14) and restricted to well-characterized elements such as genes for ribosomal RNAs. In the past 3 yr, several novel dispersed repetitive DNAs have been reported, many from plant pathogenic fungi. One repetitive element called *tad* has been associated with spontaneous mutation and high frequency reversion at the *am* locus of *Neurospora crassa* (9) and is the first described transposable element isolated from a filamentous fungus. Other described repetitive elements have unknown function but have been useful for taxonomic purposes (7,15). These repetitive elements apparently are highly variable and are detected as numerous RFLPs in genomic DNA. They have been used to identify genetic subpopulations within morphologically identical members of a species. These subpopulations can be related to the pathogenic specialization of the fungus (7,15). The variable nature of these repetitive elements is helpful for detecting subtle genetic differences between closely related fungal strains.

The results obtained here are broadly consistent with the results obtained on repetitive DNAs from other fungi. A large proportion of arbitrarily chosen clones of small fragments of DNA (four of 10 in this study) detected repetitive elements in the *Fusarium oxysporum* genome. The repetitive elements appear highly variable and detect many RFLPs among strains. When used as hybridization probes, three of the four clones of repetitive DNA showed a similar result. This result was that DNA from strains with the same host specialization as the strain from which the cloned DNA was derived (ATCC 9990) had a greater number and intensity of hybridizing bands than DNA from strains infecting other hosts. This is similar to the case of the "MGR" repeat isolated from a rice pathogen of the species *Magnaporthe grisea*. MGR is more highly conserved (i.e., detected in greater abundance, with greater hybridization signal in Southern hybridizations) among rice-infecting strains of the fungus, than in strains infecting other grass species (7).

However, an exception to this pattern exists for clone pEY2 in *F. oxysporum*. In this case, hybridization is weak to DNA from the strain of *F. o. conglutinans* from which the cloned DNA was derived (and two closely related strains) and few bands hybridize. DNA from other members of *F. o. conglutinans* hybridize to a greater extent and to a greater number of bands for this same clone; DNAs from *F. o. matthioli* and *F. o. raphani*, respectively, show a decreasing number of hybridizing bands (Fig. 4).

The repetitive DNAs described in this paper have been used to infer genetic relationships and phylogeny among strains of the crucifer-infecting *F. oxysporum* by using parsimony analysis. Our interpretation of this analysis is that a close relationship exists among members of common forma specialis and that there is a monophyletic origin of members. In doing so we assume that the presence of bands hybridizing to the cloned repeated sequence is a derived trait. This assumption is justified by the observations that the clones containing repetitive DNA hybridize neither to genomic DNA from the distantly related fungi *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (data not shown) nor to DNA from the more closely related fungus *Nectria haematococca* (anamorph = *F. solani*). Thus, data subsets having null values for each site are appropriate outgroups representing primitive states. However, in the case of clone pEY2, we suggest that an extreme loss of hybridizing bands for strains 81, 722, and 768, may be an advanced trait. This interpretation would be consistent with the observation that a greater number of hybridizing bands exist in geographically more distantly related strains of *F. o. conglutinans*. Because all clones used for analysis were obtained from the fungal strain ATCC 9990 and since strains within the same VCG (0101) as this strain usually display the

TABLE 2. Data set showing presence (1) or absence (0) of restriction fragments at 44 positions hybridizing to genomic clones pEY1 and pEY7

Strain	Probe pEY1	pEY7
002	10010111100100111110111010110111110010	101101
081	10010101100101101101111010110111110010	101101
722	10010101100101101101111010110111101010	101101
724	00100000001010010000100001001000100100	010110
725	00001010000000100100001001000000100100	001101
726	01001011000000100100001001000000100100	001101
760	00100000001010010000010001001000100101	010110
777	10001101110101111101111110110011111010	101101
808	10110100100101111001011010010111100001	001101
null	00000000000000000000000000000000000000	000000

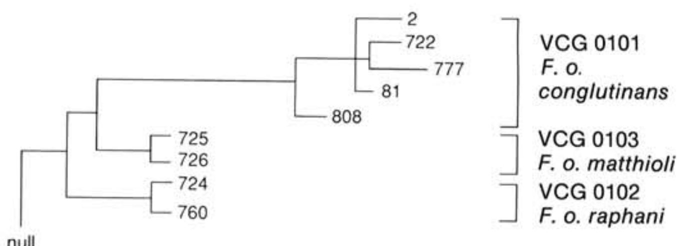

Fig. 3. Dendrogram showing the inferred relationships of crucifer-infecting strains of *Fusarium oxysporum*. Shown is one of the nine most parsimonious patterns given by PAUP, derived from the data given in Table 2.

TABLE 3. Data set showing presence (1) or absence (0) of restriction fragments at 41 positions hybridizing to genomic clone pEY2

Strain	Restriction fragments
002	11100101000111101100100111101101110101000
081	00000000000000000000000000000011000010001
242	00000000000000000000000010000001000000100
684	11100101001101000100001111001001010101001
689	10100101001101000100001111001001010101001
694	11110101010101000000101111001001010101001
699	00000010000000000000000010000101000000100
719	10100101000101001100101011101000110000010
722	00000000000000000000000000000011000010001
723	10100001000111001100101011101001000000010
724	00000010000000000000000010000101000000100
725	11000000000010010000000010010000001000000
726	11001000100010010000000010010000001000000
752	11100101000101001100101011101000110001000
760	00000000010000000001010010000000100000101
768	00000000000000000000000000000011000010001
777	11000101000101001110101111101101110101000
779	00000000000000000000000010000001000000100
781	11000000000010010000000010010000001000000
793	11001000100010100000000010010000001000000
795	00000000000000000101001000000000000000100
796	00000000000000000000000010000001000000100
806	00001000100000100000000010010000001000000
808	11110101010111000100101111001001010101001
809	11110101010101000100101111001001010101001
811	11110101010101000100101111001001010101001
815	00000000000000000000000010000101000000100
821	00000000000000000100001001000100000000100
1088	00000000000000000100000100000001000000100
1094	00000000000000100100000010000110001000100
T-2	00000000000000000000000000000000000000000

greatest number of hybridizing bands to these clones, a greater amount of information is obtained for comparing relationships among the strains of VCG 0101. This type of analysis thus would be most appropriate for comparing strains within this VCG or, more to the point, comparing differences from ATCC 9990. Still, members of other vegetative compatibility groups and formae speciales also cluster according to their own kind using this analysis. We hope to analyze these DNAs further by using clones of repetitive elements obtained from strains within VCG 0102 and VCG 0103 to test if similar results are obtained.

The distribution of repetitive sequences supports the concept that there is a common ancestry for members of the same forma specialis and VCG. This view is consistent with previously published data on plasmid typing (11,12), mitochondrial RFLP (10,11), and isozyme analysis (2). These three sets of characters revealed little or no variation within the pathogenically defined subpopulations in *F. o. conglutinans, F. o. matthioli,* and *F. o. raphani* (along with their corresponding VCGs), but considerable variation was seen in these traits between the subpopulations.

The interpretation of common ancestry is directly opposed to a notion repeatedly put forth by Buddenhagen (3,4) that members of *F. o. conglutinans* race 1 and race 2 are very different and independently evolved pathogens of cabbage (race 2 is called race 5 in the older literature; see reference 2). The idea of independent descent, unsubstantiated by experimental evidence of any kind, is inconsistent with all aforementioned genetic data. The genetic data support the idea that race 1 and race 2 are closely related, even when compared to strains of *F. o. raphani* and *F. o. matthioli* that have host ranges overlapping that of *F. o. conglutinans*. Until experimental evidence appears to the contrary, the simplest and most congruous interpretation of existing data is that race 1 and race 2 of *F. o. conglutinans* are monophyletic and closely related.

The use of hypervariable repetitive DNA recently has been suggested for DNA fingerprinting strains of a particular pathogenic type or geographic locale (7). We have identified a genomic clone (pEY10) that, when used for Southern hybridization analysis of genomic DNA, yields highly individual hybridization patterns (Fig. 5). These patterns are characteristic for strain type and geographic location. Only members of the

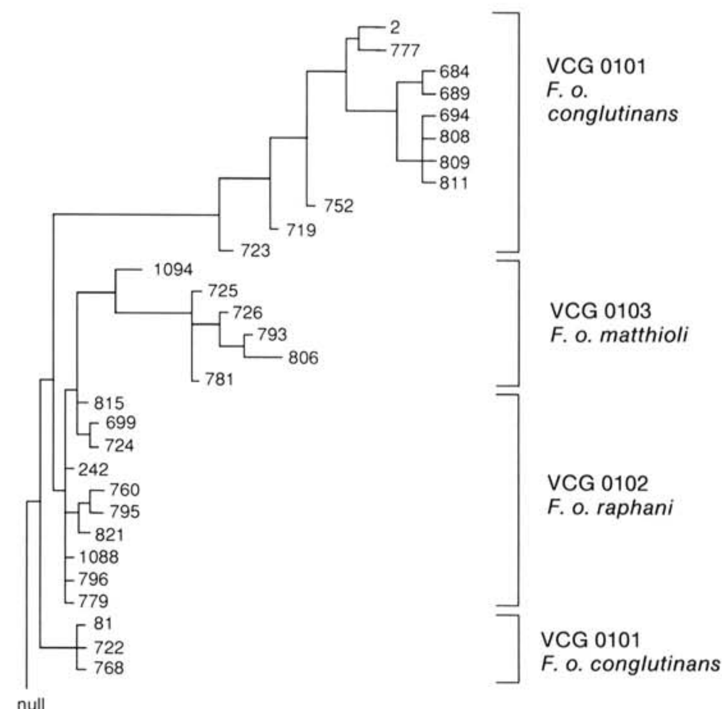

Fig. 4. Dendrogram showing the inferred relationships of 30 crucifer-infecting strains of *Fusarium oxysporum*. Shown is one of 50 most parsimonious patterns given by PAUP, derived from the data given in Table 3.

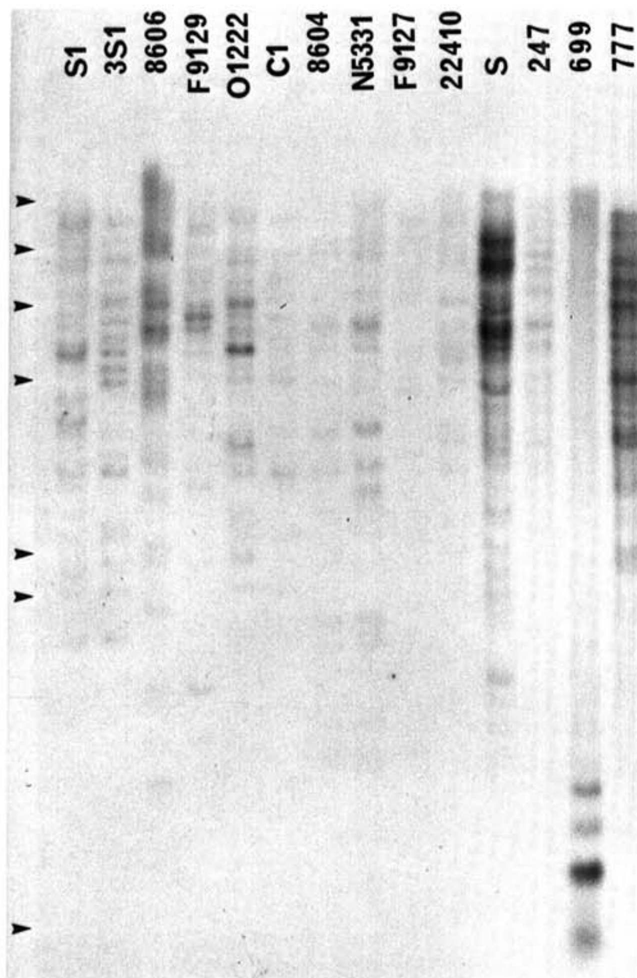

Fig. 5. Hybridization of pEY10 to genomic DNA from 14 strains of *Fusarium oxysporum*. Total DNA from the strains indicated was digested with *Hin*dIII, fractionated by agarose electrophoresis, blotted, and probed with radiolabeled pEY10. Lane S contains DNA from strain ATCC 16603. Arrows, from top to bottom, indicate molecular size markers for 23.1, 9.4, 6.7, 4.4, 2.3, 2.0, and 0.56 kb.

same pathogen forma specialis, VCG, and race from the same area of the world show similar banding patterns. This pattern is similar to the variation detected both between and within formae speciales of the powdery mildew pathogen, *Erysiphe graminis*, upon examination of repetitive DNA (15). Three sequences from barley-infecting *E. graminis* DNA were cloned and used as hybridization probes. Variation was detected in repetitive sequences among strains of the pathogen affecting different hosts, but no variation was seen for five strains of the oat powdery mildew pathogen that were collected from the same variety of oat, from the same field at the same time.

Given a sufficiently large data base, DNA fingerprint information could be used to trace the origins of newly introduced pathogen races or species. For example, *F. o. cubense* race 4, virulent toward Cavendish clones of banana, has not been reported to date in the Western Hemisphere (16). If and when the presence of race 4 is noted in the West, DNA fingerprinting using clone pEY10 may give information on the origin of the introduction or whether the race arose de novo from endemic *F. oxysporum* populations. This could prove to be valuable information for development of plant material resistant to this particular biotype of the pathogen and for the proper deployment of resistant varieties in the field.

LITERATURE CITED

1. Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute/The Eastern Press, Ltd., London. 237 pp.
2. Bosland, P. W., and Williams, P. H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility and geographic origin. Can. J. Bot. 65:2067-2073.
3. Buddenhagen, I. W. 1987. Disease susceptibility and genetics in relation to breeding of bananas and plantains. Pages 95-109 in: Banana and Plantain Breeding Strategies. G. H. Persley and E. A. DeLangh, eds. Australian Centre for International Agricultural Research.
4. Buddenhagen, I. W. 1990. Banana breeding and Fusarium wilt. Pages 107-113 in: Fusarium Wilt of Banana. R. C. Ploetz, ed. American Phytopathological Society, St. Paul, MN.
5. Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Vegetative compatibility groups among non-pathogenic root-colonizing strains of *Fusarium oxysporum*. Can. J. Bot. 64:2358-2361.
6. Garber, R. C., and Yoder, O. C. 1983. Isolation of DNA from filamentous fungi and separation into nuclear, mitochondrial, ribosomal, and plasmid components. Anal. Biochem. 135:416-422.
7. Hamer, J. E., Farrall, L., Orbach, M. J., Valent, B., and Chumley, F. G. 1989. Host species-specific conservation of a family of repeat DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. USA 86:9981-9985.
8. Katan, T., and Katan, J. 1988. Vegetative-compatibility groupings of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. Phytopathology 78:852-855.
9. Kinsey, J. A., and Helber, J. 1989. Isolation of a transposable element from *Neurospora crassa*. Proc. Natl. Acad. Sci. USA 86:1929-1933.
10. Kistler, H. C., and Benny, U. 1989. The mitochondrial genome of *Fusarium oxysporum*. Plasmid 22:86-89.
11. Kistler, H. C., Bosland, P. W., Benny, U., Leong, S., and Williams, P.H. 1987. Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. Phytopathology 77:1289-1293.
12. Kistler, H. C., and Leong, S. 1986. Linear plasmid-like DNAs in the plant pathogenic fungus *Fusarium oxysporum* f. sp. *conglutinans*. J. Bacteriol. 167:587-593.
13. Kistler, H. C., and Momol, E. A. 1990. Molecular genetics of plant pathogenic *Fusarium oxysporum*. Pages 49-54 in: Fusarium Wilt of Banana. R. C. Ploetz, ed. American Phytopathological Society, St. Paul, MN.
14. Michelmore, R. W., and Hulbert, S. H. 1987. Molecular markers for genetic analysis of phytopathogenic fungi. Annu. Rev. Phytopathol. 25:383-404.
15. O'Dell, M., Wolfe, M. S., Flavell, R. B., Simpson, C. G., and Summers, R. W. 1989. Molecular variation in populations of *Erysiphe graminis* on barley, oats and rye. Plant Pathol. 38:340-351.
16. Ploetz, R. C. 1990. Population biology of *Fusarium oxysporum* f. sp. *cubense*. Pages 63-76 in: Fusarium Wilt of Banana. R. C. Ploetz, ed. American Phytopathological Society, St. Paul, MN.
17. Ploetz, R. C., and Correll, J. C. 1988. Vegetative compatibility among races of *Fusarium oxysporum* f. sp. *cubense*. Plant Dis. 72:325-328.
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning. A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
19. Swofford, D. L. 1985. PAUP. Phylogenetic analysis using parsimony. Version 2.4.1. Illinois Natural History Survey. Champaign, IL.
20. Vieira, J., and Messing, J. 1987. Production of single-stranded plasmid DNA. Methods Enzymol. 153:3-11.