

MEETING

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A DNA Sequence-Based Phylogenetic Structure for the *Fusarium oxysporum* Complex

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Fusarium oxysporum Schlecht. is one of the most successful plant pathogens, as reflected in the abundance and diversity of the 150 host-specific *formae speciales* described for this vascular wilt pathogen. A *forma specialis* is distinguished by a unique host range and may consist of one or more vegetative compatibility groups (VCGs) and pathogenic races. Considerable genetic diversity within pathogenic and putatively nonpathogenic strains of *F. oxysporum* has been documented in numerous studies. Furthermore, strains of *F. oxysporum* are increasingly isolated from immunosuppressed and immunocompromised humans. Most researchers use Snyder and Hansen's emendation of *F. oxysporum*, which includes all of the species recognized in the infrageneric group called Section Elegans. We have generated DNA sequences from the mitochondrial small subunit (mtSSU) ribosomal DNA and translation elongation factor EF-1 introns and exons to infer phylogenetic relationships of 332 strains within the *F. oxysporum* complex and to investigate its near relatives. A clade containing the *F. oxysporum* and *Gibberella fujikuroi* complexes and an Asian grass-associated clade formed an unresolved trichotomy in the molecular phylogeny. Although the *F. oxysporum* complex is strongly supported as monophyletic, several *F. oxysporum*-like strains representing phylogenetically distinct species were nested outside of this complex. Therefore, strains identified as *F. oxysporum* morphologically may exhibit either a para- or polyphyletic set of relationships. Within the *F. oxysporum* complex four clades were resolved comprising the 332 ingroup strains sequenced. Approximately 80% of the *formae speciales* sequenced that have two or more VCGs appear to be either para- or polyphyletic. Although isolates from human patients have multiple evolutionary origins, 19 of the 41 clinical strains sequenced formed a single clade. Ongoing studies are directed at extending this database to every *forma specialis* and VCG and towards developing a robust phylogeographic hypothesis for this complex. In addition, plans are in progress to post the aligned *F. oxysporum* DNA sequence database on the NCAUR World Wide Web site [<http://nrrl.ncaur.usda.gov/>] by the end of 1999 as an electronically portable diagnostic tool for fungal biologists interested in determining the phylogenetic placement of newly isolated strains within this complex.

Development of Specific Primers for PCR Detection of *Fusarium oxysporum* f.sp. *basilici*

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Wilt and crown rot of sweet basil, caused by *Fusarium oxysporum* f.sp. *basilici* (*Fob*), represents a major problem with this crop. *Fusarium* wilt management relies on the integration of different control measures, such as soil and substrate disinfestation, raised bench cultivation, seed dressing and the use of antagonistic *Fusarium* spp. However, soil contamination through infected seed and airborne propagules makes soil disinfestation only partially effective against *Fob*. Low efficacy of chemical control, the limited availability of resistant cultivars and the unsatisfactory level of control through commercial formulations of biocontrol agents, boost the urgency for seed and transplant certification procedures with sweet basil. The aim of this research was to design a reliable and rapid method for the unequivocal recognition of *Fob* isolated from contaminated basil seed, plants and infested soil. Fifty-two isolates of *Fusarium oxysporum*, obtained from infected basil plants, seeds, flower residues and soil from different growing areas in Italy and Israel, were analyzed by random amplification of polymorphic DNA (RAPD-PCR), coupled to a DNA extraction protocol from colonies grown on a *Fusarium*-selective medium. In a pathogenicity assay, 35 isolates determined 32–92% of diseased seedlings on the highly susceptible basil cultivar 'Fine Verde' 21 days after sowing in an artificially infested substrate, whereas 17 isolates were nonpathogenic on basil. All *Fob* isolates gave identical amplification patterns by using 31 different random primers. All tested primers allowed the clear differentiation of *Fob* from representatives of other *formae speciales* or from non-pathogenic strains of *F. oxysporum*. Several amplification products were eluted from gel, digoxigenin-labeled and tested for *Fob*-specificity in a dot blot assay. A 1 kb amplicon hybridized only to DNA from all *Fob* isolates but not to DNA from other *F. oxysporum* isolates or from representatives of *F. redolens*, *F. tabacinum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *S. minor* and *Pythium ultimum*. This DNA fragment was cloned in pGEM-T, subcloned in pBS-SK and sequenced. Two pairs of *Fob*-specific primers were designed based on these sequences, giving rise to amplification products of 381 and 331 bp, respectively. The two primer sets allowed PCR identification of the pathogen in infected plants, in naturally and artificially infected seeds and in infested soil samples.

Clonal Lineages of *Fusarium oxysporum* f.sp. *cubense* in Kenya as Determined through Vegetative Incompatibility and Growth on Copper Sulfate

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Fusarium oxysporum f.sp. *cubense* (*Foc*), a soilborne fungus, is causing significant crop losses to banana growers in Kenya. As a prerequisite to development of sustainable control measures, the genetic variability of *Foc* in Kenya was studied by determining the vegetative incompatibility groupings of over 200 *Foc* isolates. Compatibility was recorded with testers from six existing vegetative compatibility groups, VCGs (0124, 0125, 0128, 01220, 01222 and 01212), but several isolates were compatible with two or more testers from the 0124/5/8/20/22 complex. Isolates that belonged to VCG 01212, however, were distinct and did not anastomose with any other VCG testers.

Since vegetative incompatibility is a genetically controlled trait, isolates belonging to the VCGs 0124, 0125, 0128, 01220 and 01222 were considered to form one clonal lineage (CL1) and those in the VCG 01212 to belong to a different clonal lineage (CL2). This result was further confirmed by differential reactions of these isolates on medium amended with copper sulfate. On Czapek agar medium amended with 150 mg of copper (II) sulfate per liter of the medium, isolates of *Foc* could be separated into two groups: a small group (22% of the tested isolates) that formed copper complexes below the mycelium in the medium; and a major group that did not form copper complexes in the medium. On this medium colonies of the copper-complexing group (CCG) appeared pale mouse gray to dark mouse gray or black, whereas those of the copper-non-complexing group (CNG) appeared pale purplish gray to white. Within the CCG and the CNG were subgroups which correlated with geographic location and/or host cultivar. Isolates belonging to the CL2 (VCG 01212) were within the CCG group, whereas isolates within the CL1 (VCG complex group 0124/5/8/20) were in the CNG group. Although the CNG was widespread in the country and was isolated from a wide range of cultivars, the majority of the isolates within the CCG were found mainly on cv. 'Wang'ae' (=Ney poovan: AB) and a few on cv. 'Bluggoe' (ABB). Within the CCG and the CNG were isolates whose VCGs could not be determined, suggesting the existence of at least one more novel clonal lineage. Breeding or deployment of banana cultivars resistant to these clonal lineages would be the best approach to the control of Fusarium wilt in Kenya.

Phylogeny of VCGs and *Formae Speciales* of *Fusarium oxysporum* and *F. redolens*

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Fusarium oxysporum is divided into *formae speciales*, races, and clonal lineages (vegetative compatibility groups, VCGs). Knowledge of the phylogenetic relationships among such entities is essential for developing proper diagnostic tools as well as for predicting the pathogenic behavior and potential of strains. By comparing nuclear (EF-1 α) and mitochondrial (mtSSU rDNA) sequences, O'Donnell *et al.* (1998) were able to demonstrate that *F. oxysporum* comprises three major clades. Isolates of *F. oxysporum* f.sp. *cubense* fell into five phylogenetically distinct lineages within two of the three major clades. The present study was undertaken to test whether other *formae speciales* also consist of fungi with independent evolutionary origins. EF-1 α and mtSSU rDNA sequences were analyzed for *F. oxysporum* ff.spp. *dianthi*, *gladioli*, *lilii*, *tulipae* and *opuntiarum*, as well as for a number of nonpathogenic isolates. Among this series, members were found of all three major clades. Only a single nonpathogenic isolate from lily fell in major clade 1. Clade 2 comprised *F. oxysporum* f.sp. *dianthi*, f.sp. *lilii*, and three VCGs of f.sp. *gladioli*. Clade 3 comprised *F. oxysporum* f.sp. *tulipae*, f.sp. *opuntiarum*, and three other VCGs of f.sp. *gladioli*. Biocontrol strains from carnation were not related in any way to f.sp. *dianthi*, nor were nonpathogenic isolates from lily related to f.sp. *lilii*. AFLP analyses supported the distinction of *F. oxysporum* f.sp. *opuntiarum* as a monophyletic group, and supported the resolution of *F. oxysporum* f.sp. *gladioli* into two distinct major clades. The results indicate that *F. oxysporum* f.sp. *dianthi* and f.sp. *gladioli* have a paraphyletic origin.

Pathogenic isolates associated with wilt and rot diseases have also been identified in *F. redolens*, a species closely related to *F. oxysporum*. Contrary to the commonly accepted view of conspecificity of both species involved, *F. redolens* can be differentiated from *F. oxysporum* in several ways including gene genealogies, isozyme polymorphisms, and AFLP fingerprints. In several cases, however, the disease symptoms caused by both fungi on a given host are indistinguishable. Within *F. redolens*, also an asexual fungus, clonal lineages have been identified in a similar manner as in *F. oxysporum*. Besides six VCGs in *F. oxysporum* f.sp. *dianthi*, wilt-inducing isolates belonging to four VCGs

(corresponding to four unique races) have been identified in *F. redolens* f.sp. *dianthi* (Baayen *et al.*, 1997). Next to *F. oxysporum* f.sp. *asparagi*, causal agent of asparagus root rot, similarly pathogenic isolates occur in *F. redolens* that should be classified as a distinct *forma specialis*, *F. redolens* f.sp. *asparagi*. Similarly, pathogenic isolates of *F. redolens* ff.spp. *spinaciae* and *pisi* exist next to *F. oxysporum* ff.spp. *spinaciae* and *pisi* proper. Gene genealogies indicate that three major clades exist within *F. redolens*, harboring VCGs belonging to distinct *formae speciales* in a similar complex pattern as for *F. oxysporum*. Horizontal gene transfer may be involved in the evolution of pathogenicity to the same host in distinct (lineages within) species.

Population Genetic Analysis of a Lineage of *Fusarium oxysporum* f.sp. *radicis-lycopersici* Found in Florida and Northwestern Europe

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Tomato crown and root rot is caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. The disease was first reported from Japan in 1969 and now commonly occurs in many tomato-growing regions. Despite its recent identification, *F. oxysporum* f.sp. *radicis-lycopersici* displays considerable genetic diversity. So far, seven vegetative compatibility groups (VCGs) have been described among a collection obtained from ten countries. Two VCGs (designated 0090 and 0091) were widely distributed. In Florida, tomato crown rot was reported as early as 1975, and currently is present in all tomato-growing counties of central and southern Florida. Previous to this investigation, isolates of *F. oxysporum* f.sp. *radicis-lycopersici* had not been examined for VCGs. Several hundred *F. oxysporum* samples from tomato plants displaying crown rot symptoms were collected in central and southern Florida. VCG 0094, previously reported only from northwestern Europe, was predominant in the collection. Two additional, newly described VCGs (0098 and 0099) were detected at low frequency in several counties in Florida. Isolates of VCG 0094 from Florida and Europe (Netherlands, Belgium, UK) were examined using homologous RFLP probes. Using one repetitive and three low-copy probes, we constructed multilocus haplotypes and arranged isolates according to their geographic origin (Florida county or Europe). Pairwise population comparisons were conducted using analysis of molecular variance (AMOVA). No significant difference was found between the European isolates and the population from Palm Beach County, Florida, with a pairwise Φ_{ST} of 0.0049. In addition, gene diversity among Palm Beach isolates was five times greater than for European isolates. These data are typical of population bottlenecks, when small groups of emigrants found new subpopulations. We therefore propose that the European population was established by inter-continental migration of isolates from Palm Beach County to Europe, perhaps by way of infected tomato seeds.