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Evolutionary relationships between *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* isolates inferred from mating type, elongation factor-1α and exopolygalacturonase sequences

Bart LIEVENS[a,b], *Peter VAN BAARLEN*[c], *Christel VERRETH*[a,b], *Stefan VAN KERCKHOVE*[a,d], *Martijn REP*[e], *Bart P. H. J. THOMMA*[f,*]

[a]*Scientia Terrae Research Institute, 2860 Sint-Katelijne-Waver, Belgium*
[b]*Process Microbial Ecology and Management, Lessius Hogeschool, Campus De Nayer, KULeuven Association, 2860 Sint-Katelijne-Waver, Belgium*
[c]*Host–Microbe Interactomics, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands*
[d]*Laboratory of Plant Ecology, KULeuven, 3001 Leuven, Belgium*
[e]*Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, 1098 SM Amsterdam, The Netherlands*
[f]*Laboratory of Phytopathology, Wageningen University, PO Box 8025, 6700 EE Wageningen, The Netherlands*

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ABSTRACT

Fusarium oxysporum is a ubiquitous species complex of soilborne plant pathogens that comprises many different *formae speciales*, each characterized by a high degree of host specificity. In this study, the evolutionary relationships between different isolates of the *F. oxysporum* species complex have been examined, with a special emphasis on the *formae speciales lycopersici* and *radicis-lycopersici*, sharing tomato as host while causing different symptoms. Phylogenetic analyses of partial sequences of a housekeeping gene, the elongation factor-1α (EF-1α) gene, and a gene encoding a pathogenicity trait, the exopolygalacturonase (*pgx4*) gene, were conducted on a worldwide collection of *F. oxysporum* strains representing the most frequently observed vegetative compatibility groups of these *formae speciales*. Based on the reconstructed phylogenies, multiple evolutionary lineages were found for both *formae speciales*. However, different tree topologies and statistical parameters were obtained for the cladograms as several strains switched from one cluster to another depending on the locus that was used to infer the phylogeny. In addition, mating type analysis showed a mixed distribution of the *MAT1-1* and *MAT1-2* alleles in the *F. oxysporum* species complex, irrespective of the geographic origin of the tested isolates. This observation, as well as the topological conflicts that were detected between EF-1α and *pgx4*, are discussed in relation to the evolutionary history of the *F. oxysporum* species complex.

Introduction

Fusarium oxysporum Schlechtend:Fr is a ubiquitous, asexually reproducing fungal species complex that occurs in soil and includes morphologically indistinguishable plant pathogenic strains, as well as strains for which no host(s) have been identified (yet). Collectively, *F. oxysporum* strains can cause wilt or root rot in a very broad range of host plants, among which are

* Corresponding author. Tel.: +31 317 484536; fax: +31 317 483412.
E-mail address: bart.thomma@wur.nl
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many economically important crops (Gordon & Martyn 1997). Therefore, *F. oxysporum* strains have been grouped into *formae speciales* based on host specificity, and further into races based on cultivar specificity (Armstrong & Armstrong 1981). In addition, *F. oxysporum* strains have been subdivided into vegetative compatibility groups (VCGs) (Puhalla 1985) based on the ability to establish hyphal anastomosis and form stable heterokaryons. Molecular and genetic analyses have shown that strains within a VCG are genetically similar. In addition, it has been suggested that each VCG may represent a clonal population (Correll 1991; Koenig *et al.* 1997; Kistler *et al.* 1998; Katan & Katan 1999). Nevertheless, examples of molecular variability between strains of the same VCG exist, indicating they do not belong to the same clonal lineage (Leslie 1993; Appel & Gordon 1995, 1996; Kawabe *et al.* 2007).

F. oxysporum is considered a monophyletic, diverse complex of evolutionary lineages (O'Donnell & Cigelnik 1997). An understanding of the evolutionary history of the *formae speciales* and races within *F. oxysporum* requires knowledge of the phylogenetic relationships among isolates (Appel & Gordon 1996). Phylogenetic analyses based on DNA sequences of housekeeping genes such as, for example, the mitochondrial small subunit (mtSSU) ribosomal RNA gene, the rDNA intergenic spacer (IGS) region, and the translation elongation factor (EF)-1α gene have helped to reveal the genetic and evolutionary relationships within and among *formae speciales* of *F. oxysporum* (Lievens *et al.* 2008). Such studies showed that a limited number of *F. oxysporum formae speciales* is monophyletic while many were found to be polyphyletic, including the *formae speciales asparagi*, *cubense*, *cucumerinum*, *dianthi*, *gladioli*, *lini*, *lactucae*, *lycopersici*, *melonis*, *opuntiarum*, *phaseoli*, *radicis-lycopersici* and *vasinfectum* (O'Donnell *et al.* 1998; Alves-Santos *et al.* 1999; Baayen *et al.* 2000; Kistler 2001; Skovgaard *et al.* 2001; Abo *et al.* 2005; Kawabe *et al.* 2005; Wong & Jeffries 2006; Lievens *et al.* 2007; Mbofung *et al.* 2007; van der Does *et al.* 2008), suggesting that pathogenicity towards a specific crop has evolved several times independently. In addition to housekeeping genes, DNA sequences encoding pathogenicity factors are increasingly used to study genetic relationships between isolates within a species (Lievens & Thomma 2005). For example, *endo*polygalacturonase (*pg1*) and *exo*polygalacturonase gene (*pgx4*; Garcia-Maceira *et al.* 2000) sequences have been used to study the genetic diversity of *F. oxysporum* isolates (Kawabe *et al.* 2005; Hirano & Arie 2006).

Recently, the mating type locus, *MAT1*, which regulates sexual reproduction in ascomycete fungi, was cloned from *F. oxysporum* and found to be present in all tested isolates (Arie *et al.* 2000). Each isolate had either of the two idiomorphs (*MAT1-1* or *MAT1-2*). Previous studies have demonstrated that, in addition to phylogenetic analyses based on the previously mentioned genes, *MAT*-based phylogenetic analyses can be useful for studying the evolution of closely related fungi (Pöggeler 1999; Barve *et al.* 2003), including *formae speciales* of *F. oxysporum* (Kawabe *et al.* 2005, 2007).

F. oxysporum forma specialis (f. sp.) *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* are two *formae speciales* that infect tomato. While *F. oxysporum* f. sp. *lycopersici* is the causal agent of vascular wilt only on tomato, *F. oxysporum* f. sp. *radicis-lycopersici* causes crown and root rot on tomato and several other hosts (Menzies *et al.* 1990). For *F. oxysporum* f. sp. *lycopersici*, three races and five VCGs (0030–0033 and 0035) have been reported so far (Katan 1999; Katan & Di Primo 1999; Cai *et al.* 2003); though VCG 0030 was found to include VCG 0032 (Cai *et al.* 2003; Kawabe *et al.* 2005). For *F. oxysporum* f. sp. *radicis-lycopersici* nine VCGs (0090–0094 and 0096–0099) were identified, but no races have been reported (Katan 1999; Katan & Di Primo 1999). Based on the intensity of hyphal complementation, each of the VCGs 0090, 0091 and 0094 can be subdivided into at least two subgroups.

Remarkably, topological discrepancies between IGS and EF-1α or mtSSU trees have been reported for *F. oxysporum*, a species that is often thought of as only occurring in clonal populations (Mbofung *et al.* 2007; O'Donnell *et al.* 2008). We hypothesize that not all genes in the different *F. oxysporum formae speciales* have the same evolutionary history. The objective of this study was to evaluate this hypothesis and, at the same time, determine the evolutionary relationships among isolates that belong to the two *F. oxysporum formae speciales* of tomato, using a phylogenetic analysis of both a housekeeping gene (EF-1α) and a gene encoding a cell wall-degrading enzyme (*pgx4*), as well as by the distribution of the mating type locus (*MAT1*). Together with the random distribution of the mating type idiomorphs MAT1-1 and MAT1-2 the different tree topologies that were obtained are discussed in relation to the evolutionary history of the *F. oxysporum* species complex.

Materials and methods

Fungal isolates and DNA extractions

A worldwide collection of 52 *Fusarium oxysporum* strains, including 16 of *F. oxysporum* f. sp. *lycopersici*, 20 of *F. oxysporum* f. sp. *radicis-lycopersici* and 16 of other *formae speciales*, was used in this study. Isolates were mainly from North America, Asia and Europe, but also from Australasia, Africa and South America (Table 1). For most of these isolates pathogenicity and vegetative compatibility have been assessed in previous studies (e.g. Katan *et al.* 1991; Marlatt *et al.* 1996; O'Donnell *et al.* 1998; Katan & Katan 1999; Cai *et al.* 2003; Balmas *et al.* 2005; Kawabe *et al.* 2005; van der Does *et al.* 2008). Isolates were grown on potato dextrose agar containing 100 ppm streptomycin sulphate, in the dark, at 22 °C. Genomic DNA was extracted using the phenol–chloroform extraction method as described previously (Lievens *et al.* 2003) and the yield was determined spectrophotometrically. As a check for DNA quality, all samples were successfully subjected to PCR analysis using the universal primers ITS5 and ITS4, which anneal to conserved regions of the 18S and 28S ribosomal RNA genes, respectively (White *et al.* 1990; Table 2).

Mating type determination

The mating type of each isolate was determined using a *MAT1*-specific PCR assay as described (Arie *et al.* 1999, 2000). Isolates for which an approximately 370-bp fragment was generated using primers Falpha1 and Falpha2 (Table 2) were classified as MAT1-1, and those for which a ca. 190-bp fragment was obtained using primers FHMG11 and FHMG12