

## Pathogen profile

**Pathogen profile update: *Fusarium oxysporum***

CAROLINE B. MICHELSE AND MARTIJN REP\*

*Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, the Netherlands***SUMMARY**

**Taxonomy:** Kingdom Fungi; Phylum Ascomycota; Class Sordariomycetes; Order Hypocreales; Family Nectriaceae; genus *Fusarium*.

**Host range:** Very broad at the species level. More than 120 different *formae speciales* have been identified based on specificity to host species belonging to a wide range of plant families.

**Disease symptoms:** Initial symptoms of vascular wilt include vein clearing and leaf epinasty, followed by stunting, yellowing of the lower leaves, progressive wilting, defoliation and, finally, death of the plant. On fungal colonization, the vascular tissue turns brown, which is clearly visible in cross-sections of the stem. Some *formae speciales* are not primarily vascular pathogens, but cause foot and root rot or bulb rot.

**Economic importance:** Can cause severe losses in many vegetables and flowers, field crops, such as cotton, and plantation crops, such as banana, date palm and oil palm.

**Control:** Use of resistant varieties is the only practical measure for controlling the disease in the field. In glasshouses, soil sterilization can be performed.

**Useful websites:** [http://www.broad.mit.edu/annotation/genome/fusarium\\_group/MultiHome.html](http://www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html); <http://www.fgsc.net/Fusarium/fushome.htm>; <http://www.phi-base.org/query.php>

**INTRODUCTION**

The genus *Fusarium*, also known by its teleomorphs *Nectria* and *Gibberella*, harbours notorious plant pathogenic fungi with a wide variety of hosts and infection strategies (Desjardins, 2003; Di Pietro *et al.*, 2003; Goswami and Kistler, 2004). *Fusarium oxysporum* stands out in several ways. It has an apparent long history of predominant, perhaps exclusive, asexual reproduction (Gordon and Martyn, 1997), it invades roots and can cause wilt diseases through colonization of xylem tissue (Tjamos and Beckman, 1989), and it displays apparent gene-for-gene relationships with several hosts. Possibly related to these gene-for-gene relationships

is the large number of host-specific forms, called *formae speciales*, within *F. oxysporum* (Armstrong and Armstrong, 1981; Katan, 1999; Katan and Di Primo, 1999).

Because of its (predominant) asexual reproduction, *F. oxysporum* is generally regarded as a 'species complex'—a collection of clonal lines within the genus *Fusarium*. As such, it constitutes a sister group to the *Fusarium fujikuroi* species complex that harbours sexual species with *Gibberella* teleomorphs, such as *F. verticillioides* and *F. fujikuroi* (Guadet *et al.*, 1989). In 2007, the genome of an *F. oxysporum* isolate was sequenced by the Broad Institute ([http://www.broad.mit.edu/annotation/genome/fusarium\\_group/MultiHome.html](http://www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html)). Ninety per cent of the genome sequence of *F. oxysporum* can be aligned to that of *F. verticillioides*, and the average DNA sequence similarity in the aligned region is 90% (L.-J. Ma, Broad Institute, Cambridge, USA, personal communication). Each *forma specialis* of *F. oxysporum* consists of either one or several clonal lines which correspond to vegetative compatibility groups (VCGs) (Gordon and Martyn, 1997; Katan, 1999; Katan and Di Primo, 1999). When a *forma specialis* harbours several clonal lines, these are generally not monophyletic, giving the appearance that pathogenicity towards a particular plant species has arisen independently several times (Baayen *et al.*, 2000; O'Donnell *et al.*, 1998). Although the focus of most plant pathologists is, naturally, on pathogenic isolates, many isolates found in soil are in fact not pathogenic (Gordon and Martyn, 1997; Recorbet *et al.*, 2003), and a major question is whether these ubiquitous non-pathogenic forms can evolve to become pathogenic and, if so, how that happens.

Although *F. oxysporum* has been found to cause disease in a large number of plant species (with grasses as an outstanding exception), the study of the molecular basis of the pathogenicity of *F. oxysporum* has necessarily involved a limited number of hosts, mainly tomato, melon, bean, banana, cotton, chickpea and, more recently, *Arabidopsis thaliana*. This review is an update to the pathogen profile on *F. oxysporum* by Di Pietro *et al.* (2003), and focuses mainly on new discoveries in the period 2003–2008. New developments in that period primarily involve the discovery and molecular analysis of novel pathogenicity genes, genome sequence analysis, transcriptomics, xylem sap proteomics, avirulence/effector proteins, visualization of the fungus during

\*Correspondence: Tel.: +31-20-5257764; Fax: +31-20-5257934; E-mail: m.rep@uva.nl

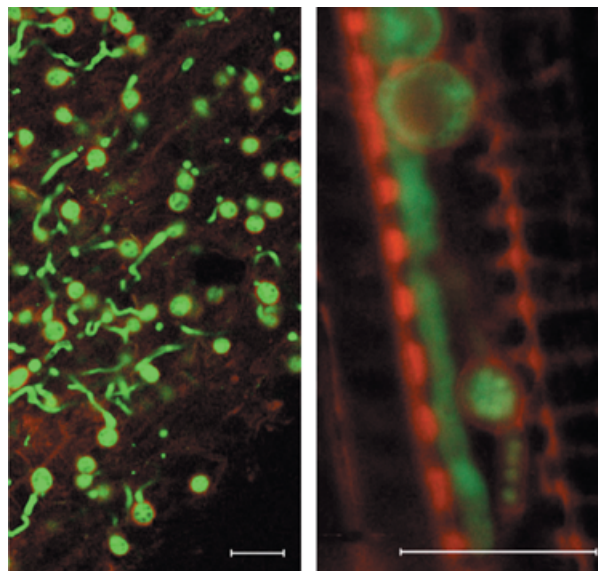
plant colonization using fluorescent proteins and the development of *A. thaliana* as a model host plant.

### VISUALIZATION OF COLONIZATION USING FLUORESCENT PROTEINS

The process of infection by *F. oxysporum* has been studied using light, fluorescence and electron microscopy, and can be divided into several steps: root recognition, root surface attachment and colonization, penetration and colonization of the root cortex and, in the case of wilt-inducing *formae speciales*, hyphal proliferation within the xylem vessels (reviewed in Di Pietro *et al.*, 2003). The first microscopic studies involving fluorescent protein-labelled *F. oxysporum* were by Di Pietro *et al.* (2001b) and Lagopodi *et al.* (2002). Di Pietro *et al.* observed conidial germination on roots, growth in tomato root cortex and colonization of xylem by *F. oxysporum* f. sp. *lycopersici*, and found that the *fmk1* mutant was able to germinate on roots but did not develop any further (Di Pietro *et al.*, 2001a). Lagopodi *et al.* visualized the colonization of tomato roots by the foot and root rot-causing *F. oxysporum* f. sp. *radicis-lycopersici* expressing the fluorescent marker green fluorescent protein (GFP) using confocal laser scanning microscopy (Lagopodi *et al.*, 2002). This study revealed several aspects of tomato root colonization and infection by this *forma specialis*: (i) the first steps of contact between the fungus and the host take place at the root hair zone; (ii) the grooves along the junctions of epidermal cells are the preferential colonization sites; and (iii) there are no specific penetration sites or infection structures (Lagopodi *et al.*, 2002).

In general, a similar pattern of colonization was observed for GFP-labelled *F. oxysporum* f. sp. *lycopersici* colonizing tomato plants in a vermiculite system (R. G. Duyvesteijn and M. Rep, unpublished results). In addition, in this study, no specific penetration sites were observed and hyphae preferentially grew along the grooves of epidermal root cells. A novel finding was the observation of chlamydospores (thick-walled, 'survival' spores) on the root surface, and also within the vascular tissue at a later stage of infection (Fig. 1). In studies using GFP-labelled *F. oxysporum*, neither conidiophores nor microconidia have been observed in xylem vessels of *Arabidopsis* or tomato (Czymmek *et al.*, 2007; van der Does *et al.*, 2008a). This is in apparent disagreement with a model in which microconidia play an important role in the colonization of xylem tissue (Beckman, 1987). A minor role of microconidia in xylem colonization is also supported by a study of Ren1, a putative transcription factor required for micro- and macroconidia development. A *REN1* disruptant produces only chlamydospores and abnormal rod-shaped, conidium-like cells, but is not affected in pathogenesis, suggesting that microconidia and macroconidia are not required for pathogenicity (Ohara *et al.*, 2004).

To understand the biocontrol mechanisms involving non-pathogenic isolates of *F. oxysporum*, the interactions between



**Fig. 1** Confocal images of chlamydospores of green fluorescent protein (GFP)-labelled *Fusarium oxysporum* f. sp. *lycopersici* on and in tomato roots. Left: outside of a tomato root (7 days post-inoculation). Right: in a xylem vessel (22 days post-inoculation). Note the red autofluorescent cell walls of the chlamydospores. Scale bars, 20  $\mu$ m.

different fluorescently labelled pathogenic and non-pathogenic *F. oxysporum* strains during root colonization have also been studied (Bolwerk *et al.*, 2005; Nahalkova *et al.*, 2008; Olivain *et al.*, 2006). Olivain *et al.* (2006) showed that the general pattern of colonization in soil was similar for both a pathogenic strain (*F. oxysporum* f. sp. *lycopersici*) and a non-pathogenic, biocontrol strain (Fo47). However, the biocontrol strain grew faster than the pathogenic strain and, as a consequence, colonized the roots earlier. When the strains were co-inoculated, the biocontrol strain again colonized the roots more extensively, but never excluded the pathogenic strain (Olivain *et al.*, 2006). Similar results were obtained by Bolwerk *et al.* (2005), who studied the interaction between the biocontrol strain Fo47 and *F. oxysporum* f. sp. *radicis-lycopersici* in sand. The biocontrol strain germinated faster than the pathogenic strain on artificial medium containing root exudates. The biocontrol strain reduced the root colonization of the pathogenic strain, but did not exclude it completely from the roots.

Nevertheless, there are some contradictory observations between these studies. Bolwerk *et al.* (2005) reported that the pathogenic strain mainly targeted cellular junctions on roots, similar to the observations reported by Lagopodi *et al.* (2002), who used the same *forma specialis* and inoculation system for colonization studies. This type of colonization behaviour was specific for the pathogenic strain and was not observed for the biocontrol strain (Bolwerk *et al.*, 2005). In contrast, Olivain *et al.* (2006) did not observe any preferential growth of hyphae along

intercellular junctions for either the pathogenic or non-pathogenic strain. This difference may be a result of the different behaviour of the two *formae speciales* or of the experimental design (sand versus soil).

The same pathogenic and non-pathogenic strains used in the study by Olivain *et al.* were also used to observe root colonization in hydroponic cultures (Nahalkova *et al.*, 2008). In this study, no preferential growth of hyphae along cellular junctions was observed. Remarkably, in contrast with the observations of colonization in soil, in hydroponic cultures, the pathogenic strain appeared to be a better and faster colonizer than the biocontrol strain. The patterns of colonization also differed; in the hydroponic system, the elongation zone and root apex were fully colonized—an observation that was never made in soil (Nahalkova *et al.*, 2008; Olivain *et al.*, 2006). Clearly, it should be appreciated that the outcome of colonization studies can be influenced by culture (soil, sand, hydroponic), inoculum concentrations and ratios; moreover, we cannot exclude the potential differences in behaviour between the various *formae speciales*. Nevertheless, from the biocontrol studies, it can be concluded that biocontrol by non-pathogenic *F. oxysporum* does not operate through direct antagonism or exclusion from potential penetration sites, but rather by competition for nutrients and/or induction of defence responses in roots (Nahalkova *et al.*, 2008).

Another recent study has revealed the dynamics of colonization of *Arabidopsis* roots by *F. oxysporum* expressing the fluorescent protein ZsGreen. Colonization was recorded in time and space using time-lapse microscopy and coverglass chambers with an agar-based medium (Czymmek *et al.*, 2007). With regard to the dynamics of colonization, it was observed, for instance, that the fungal growth rate in vascular tissue was up to five times faster than that in the surrounding agar prior to initial penetration of the root. Initial colonization and penetration into the vascular system occurred primarily in the meristematic zone of the root tips and emerging lateral roots, and local development of a mycelial mass appeared to be a precondition for the initiation of the penetration process. As with some studies with tomato, fungal growth on the root surface was preferentially along anticlinal walls. Interestingly, in this study, at high magnification, localized hyphal swellings were observed at the anticlinal interface. These may represent rudimentary appressorium-like penetration structures (Czymmek *et al.*, 2007). In tomato, we have also observed swelling of hyphae at sites of penetration (R. G. Duyvesteijn and M. Rep, unpublished results). After penetration, fungal growth was initially intercellular but, ultimately, became intracellular, and the collapse of plant cells was observed at sites of fungal penetration, presumably as a result of a loss of turgor pressure. Furthermore, changes were observed in plant cells that were not in direct contact with mycelium, such as the loss of autofluorescent vacuolar contents and changes in the appearance of the endoplasmic reticulum (Czymmek *et al.*, 2007).

## SIGNAL TRANSDUCTION

Using forward and reverse genetics approaches, several processes and genes have been identified or implicated as being important for pathogenesis (Table 1). It may be no surprise that signal transduction processes are among these, as, in order to establish disease, fungi need to respond appropriately to the plant environment and adjust their morphology, physiology, metabolism and spectrum of secreted proteins and metabolites. In *F. oxysporum*, cyclic adenosine monophosphate–protein kinase A (cAMP–PKA) and mitogen-activated protein kinase (MAPK) cascades have been implicated in this adaptation; MAPK (*FMK1*) and G-protein subunits  $\alpha$  (*FGA1*) and  $\beta$  (*FGB1*) are required for (full) pathogenicity (reviewed in Di Pietro *et al.*, 2003). More recently, a second gene encoding a G-protein  $\alpha$  subunit (*FGA2*) has been identified and analysed. In contrast with the reduced pathogenicity of *F. oxysporum* f. sp. *cucumerinum* *FGA1* disruptants, deletion of *FGA2* led to a complete loss of pathogenicity on cucumber plants (Jain *et al.*, 2005). In addition, the altered colony morphology and conidiation observed in *FGA1* disruptants were not observed in *FGA2* disruptants. These observations indicate that Fga1 and Fga2 have distinct functions, each being required for adaptation to the plant environment.

The role of signalling pathways in vegetative hyphal fusion (VHF) and the role of VHF in pathogenesis have recently been investigated (Rosales and Di Pietro, 2008). Although it was shown that VHF and pathogenesis share common signalling components, VHF is not required for pathogenesis, as a mutant disrupted for *FSO1*—a gene required for efficient VHF—was fully virulent on tomato plants. As a next step in unravelling the mechanisms of sensing and of adaptation to the plant environment, it will be important to discover the signals or ligands that trigger the cAMP–PKA and MAPK pathways.

## CELL WALL-DEGRADING ENZYMES (CWDEs)

The degree to which CWDEs contribute to the infection process is not yet fully understood. Like most fungi, *F. oxysporum* secretes an array of CWDEs, such as polygalacturonases, pectate lyases, xylanases and proteases, during root penetration and colonization (Beckman, 1987). However, inactivation of individual CWDE- or protease-encoding genes, encoding pectate lyase (*PL1*), xylanase (*XYL3*, *XYL4*, *XYL5*), polygalacturonase (*PG1*, *PG5*, *PGX4*) or a subtilase (*PRT1*), did not have a detectable effect on virulence (reviewed in Di Pietro *et al.*, 2003), possibly because of functional redundancy. A means of assessing the combined contribution of a group of secreted enzymes to virulence is to study the transcriptional regulators involved in the expression of their genes. To explore the role of xylanases in pathogenicity, the *xlnR* gene encoding transcription factor XlnR, a regulator of many xylanolytic and cellulolytic genes, was inactivated in *F. oxysporum*

**Table 1** Gene knock-outs in *Fusarium oxysporum* and their phenotypic effects.

Gene	Product/function	Effect of gene inactivation/deletion	Reference
<i>ARG1</i>	Argininosuccinate lyase	Strongly reduced virulence, arginine auxotrophy	Namiki <i>et al.</i> (2001)
<i>CHS1</i>	Class I chitin synthase	Fully virulent	Martin-Urdiroz <i>et al.</i> (2008)
<i>CHS2</i>	Class II chitin synthase	Reduced virulence	Martin-Urdiroz <i>et al.</i> (2008)
<i>CHS7</i>	Chaperone-like protein	Reduced virulence	Martin-Urdiroz <i>et al.</i> (2008)
<i>CHSV</i>	Class V chitin synthase	Strongly reduced virulence, hypersensitive to $\alpha$ -tomatine and H <sub>2</sub> O <sub>2</sub>	Madrid <i>et al.</i> (2003)
<i>CHSVb</i>	Class VII chitin synthase	Non-pathogenic, hypersensitive to Congo red and Calcofluor white	Martin-Urdiroz <i>et al.</i> (2008)
<i>CMLE1</i>	Carboxy- <i>cis,cis</i> -muconate cyclase	Non-pathogenic, reduced growth on phenolic compounds	L. Reijnen and C. B. Michielse (unpublished results)
<i>CLC1</i>	Chloride channel	Reduced virulence, deficient in laccase activity, increased sensitivity to oxidative stress	Canero and Roncero (2008b)
<i>CTF1</i>	Transcription factor	Fully virulent	Rocha <i>et al.</i> (2008)
<i>CTI6</i>	Transcription factor	Reduced virulence	C. B. Michielse <i>et al.</i> (2009)
<i>DCW1</i>	Cell wall protein	Reduced virulence	C. B. Michielse <i>et al.</i> (2009)
<i>FCD1</i>	Cellobiose:quinone oxidoreductase	Fully virulent	Kawabe <i>et al.</i> (2006)
<i>FGA1</i>	G-protein $\alpha$ -subunit	Markedly reduced virulence, decreased conidiation	Jain <i>et al.</i> (2002)
<i>FGA2</i>	G-protein $\alpha$ -subunit	Non-pathogenic, increase resistance to heat	Jain <i>et al.</i> (2005)
<i>FGB1</i>	G-protein $\beta$ -subunit	Markedly reduced virulence, decreased conidiation	Jain <i>et al.</i> (2003)
<i>FMK1</i>	Mitogen-activated protein kinase	Non-pathogenic, impaired in root attachment and invasive growth	Di Pietro <i>et al.</i> (2001a)
<i>FNR1</i>	Transcription factor	Markedly reduced virulence, reduced ability to use secondary nitrogen sources	Divon <i>et al.</i> (2006)
<i>FOW1</i>	Mitochondrial carrier	Strongly reduced virulence, impaired in plant colonization	Inoue <i>et al.</i> (2002)
<i>FOW2</i>	Transcription factor	Non-pathogenic, impaired in invasive growth, not in root attachment	Imazaki <i>et al.</i> (2007)
<i>FOXG_09487</i>	Hypothetical protein	Reduced virulence	C. B. Michielse <i>et al.</i> (2009)
<i>FPD1</i>	Similar to chloride conductance regulatory protein	Markedly reduced virulence	Kawabe <i>et al.</i> (2004)
<i>FRP1</i>	F-box protein	Non-pathogenic, impaired in root colonization and penetration, impaired growth on various carbon sources	Duyvesteijn <i>et al.</i> (2005); Jonkers <i>et al.</i> (2009)
<i>FSO1</i>	<i>Fusarium so1</i>	Fully virulent, no vegetative hyphal fusion	Rosales and Di Pietro (2008)
<i>FTF1</i>	Transcription factor	Reduced virulence (RNAi silencing)	Ramos <i>et al.</i> (2007); J. M. Diaz-Minguez (University of Salamanca, Salamanca, Spain, personal communication)
<i>GAS1</i>	$\beta$ -1,3-Glucanosyltransferase	Markedly reduced virulence, reduced growth on solid medium	Caracuel <i>et al.</i> (2005)
<i>ICL1</i>	Isocitrate lyase	Fully virulent, no growth on C2 carbon sources and fatty acids	Jonkers <i>et al.</i> (2009)
<i>LCC1</i>	Laccase	Fully virulent	Canero and Roncero (2008a)
<i>LCC3</i>	Laccase	Fully virulent	Canero and Roncero (2008a)
<i>LCC5</i>	Laccase	Fully virulent	Canero and Roncero (2008a)
<i>PACC</i>	Transcription factor	Increased virulence and transcription of acid-expressed genes	Caracuel <i>et al.</i> (2003)
<i>PEX12</i>	Peroxin	Reduced virulence, impaired in growth on fatty acids	C. B. Michielse <i>et al.</i> (2009)
<i>PEX26</i>	Peroxin	Reduced virulence, impaired in growth on fatty acids	C. B. Michielse <i>et al.</i> (2009)
<i>PG1</i>	Endopolygalacturonase	Fully virulent, reduced saprophytic growth on pectin	Di Pietro and Roncero (1998)
<i>PG5</i>	Endopolygalacturonase	Fully virulent	Garcia-Maceira <i>et al.</i> (2001)
<i>PGX4</i>	Exopolygalacturonase	Fully virulent	Garcia-Maceira <i>et al.</i> (2000)
<i>PL1</i>	Pectate lyase	Fully virulent	Huertas-Gonzalez <i>et al.</i> (1999)
<i>PRT1</i>	Serine protease	Fully virulent	Di Pietro <i>et al.</i> (2001b)
<i>REN1</i>	Transcription factor	Fully virulent, no microconidia or macroconidia, but conidium-like cells	Ohara <i>et al.</i> (2004)
<i>RHO1</i>	Monomeric G protein	Markedly reduced virulence, reduced growth on solid media	Martinez-Rocha <i>et al.</i> (2008)
<i>SGE1</i>	Transcription factor	Non-pathogenic, reduced conidiation	C. B. Michielse <i>et al.</i> (unpublished results)
<i>SIX1</i>	Small secreted protein	Reduced virulence, effect more pronounced on 4- to 5-week-old plants	Rep <i>et al.</i> (2005)
<i>SNF1</i>	Protein kinase involved in carbon catabolite repression	Markedly reduced virulence, reduced growth on complex carbon sources	Ospina-Giraldo <i>et al.</i> (2003)
<i>STI35</i>	Functions in thiamine biosynthesis	Fully virulent	Ruiz-Roldan <i>et al.</i> (2008a)
<i>STUA</i>	Transcription factor	Fully virulent, no macroconidia	Ohara and Tsuge (2004)
<i>TOM1</i>	Tomatinase enzyme	Reduced virulence, reduced tomatinase activity, increased sensitivity to $\alpha$ -tomatine	Pareja-Jaime <i>et al.</i> (2008)
<i>WC1</i>	Photoreceptor	Fully virulent	Ruiz-Roldan <i>et al.</i> (2008b)
<i>XLNR</i>	Transcription factor	Fully virulent, reduced extracellular xylanase activity	Calero-Nieto <i>et al.</i> (2007)
<i>XYL3</i>	Family 10 endoxylanase	Fully virulent	Gomez-Gomez <i>et al.</i> (2002)
<i>XYL4</i>	Family 11 endoxylanase	Fully virulent	Gomez-Gomez <i>et al.</i> (2002)
<i>XYL5</i>	Family 11 endoxylanase	Fully virulent	Gomez-Gomez <i>et al.</i> (2001)

*f. sp. lycopersici*. Deletion of *xlnR* reduced the expression of the xylanase genes *XYL3* and *XYL4*, and xylanase activity, without, however, affecting virulence (Calero-Nieto *et al.*, 2007). As not all xylanase activity was lost in the *xlnR* disruptant, it may still be that residual activity was sufficient to cause disease. Alternatively, breakdown of xylan may not be important for the colonization of tomato.

Many CWDEs are subject to carbon catabolite repression which, in yeast, is relieved under non-repressing conditions by the activity of a protein kinase called Snf1. In addition, in *F. oxysporum*, targeted disruption of *SNF1* resulted in reduced growth on several complex and simple carbon sources, other than glucose, and reduced the expression of several CWDEs (*PGX1*, *PGN1*, *PL1*) (Ospina-Giraldo *et al.*, 2003). Concomitantly, virulence towards *A. thaliana* and *Brassica oleracea* was reduced, suggesting that the adaptation of central carbon metabolism and CWDE gene expression plays a role in pathogenicity. Another indication that carbon metabolism and CWDEs play an important role during pathogenesis comes from the analysis of *F. oxysporum f. sp. lycopersici FRP1* disruptants. *FRP1* encodes an F-box protein, implicated in the recruitment of specific proteins to an SCF complex for labelling with ubiquitin, which is usually a signal for degradation by the proteasome. *FRP1* is absolutely required for pathogenicity and the *FRP1* disruptant is impaired in root colonization (Duyvesteijn *et al.*, 2005). Jonkers *et al.* (2009) have recently shown that poor root colonization in the *frp1* mutant can be attributed to reduced assimilation of organic acids, amino acids and/or polysaccharides. External root colonization by the *FRP1* disruptant was restored by the addition of glucose or proline, but virulence was not, as a result of an inability to penetrate the roots. Remarkably, *ICL1* (isocitrate lyase) disruptants, generated in the same study, were able to colonize the roots and were fully virulent, even though *FRP1* and *ICL1* disruptants share a similar reduced growth phenotype on several carbon sources. As the *FRP1* disruptant has additional defects in the expression of CWDE genes, collective secretion of several CWDEs is probably required for superficial root colonization (through the release of cell wall components for nutrition) as well as root penetration (through weakening of cell walls).

Next to carbon regulation, nitrogen regulation has also been shown to be important for the infection process. Inactivation of the global nitrogen regulator, *Fnr1*, resulted in a reduced ability to utilize several secondary nitrogen sources, abolished the expression of nutrition genes normally induced during the early phase of infection, and resulted in reduced pathogenicity towards tomato (Divon *et al.*, 2006). In contrast, inactivation of *CTF1*, encoding a transcriptional activator of cutinase and lipase genes, did not have an effect on pathogenicity. It was shown that Ctf1 regulates the expression of a cutinase gene (*CUT1*) and a lipase gene (*LIP1*), and thus overall extracellular esterase and lipase activities, during growth under inducing

conditions in axenic cultures (Rocha *et al.*, 2008). However, Ctf1 did not seem to be required for the expression of *LIP1* during infection. In addition, *CUT1* was only expressed at low levels during infection. This implies that neither cutinases nor lipases play an important role during root infection, or that Ctf1-independent activation of these genes provides sufficient cutinase and lipase activity.

In summary, as the investigation of the role of individual secreted enzymes in pathogenicity is difficult to establish because of functional redundancy, the focus has turned to the analysis of the role of transcriptional regulators of certain groups of secreted enzymes. Although direct proof of a causal relationship between extracellular enzyme production and pathogenicity has not been obtained, there are now clear indications that the collective action of CWDEs, as well as carbon catabolite repression and nitrogen regulation, is important for the infection process.

## OVERCOMING PLANT DEFENCE RESPONSES

During root invasion and colonization, *F. oxysporum* is exposed to various plant defence mechanisms, such as physical barriers and antifungal compounds (Beckman, 1987; VanEtten *et al.*, 1994). An example is the antifungal compound  $\alpha$ -tomatine in tomato plants (Roddick, 1977), a compound that is able to form complexes with sterols in fungal membranes, thereby causing pores and leakage of fungal cellular content (Keukens *et al.*, 1992, 1995). *Fusarium oxysporum* can secrete a tomatine-degrading enzyme called tomatinase (Lairini *et al.*, 1996). Recent genome analysis has revealed that *F. oxysporum* contains five putative tomatinase genes (Pareja-Jaime *et al.*, 2008). For one of these genes, *TOM1*, its role in tomatine degradation and virulence was investigated. Constitutive overexpression of *TOM1* resulted in high levels of tomatinase activity, independent of the presence of  $\alpha$ -tomatine. In addition, tomato plants infected with this *TOM1*-overexpressing strain showed more rapid development of disease symptoms, whereas targeted disruption of *TOM1* resulted in a 25% decrease in tomatinase activity and a delay in the development of disease symptoms (Pareja-Jaime *et al.*, 2008). These results indicate that the inactivation of tomatine contributes to the virulence of *F. oxysporum f. sp. lycopersici* towards tomato.

A second example of a plant defence mechanism is the chemical modification of the plant cell wall. Soluble and cell wall-bound phenolic compounds accumulate in plants challenged by fungal pathogens or fungus-derived elicitors (de Ascensao and Dubery, 2003; Mandal and Mitra, 2007; von Ropenack *et al.*, 1998). In tomato plants and hairy root cultures, extracts of *F. oxysporum* induce an increase in cell wall strengthening through the deposition of lignin, as well as an increased concentration of phenolic compounds, such as ferulic acid, 4-hydrobenzoic acid and 4-coumaric acid (Mandal and Mitra, 2007, 2008). *Fusarium oxysporum* senses

the presence of these phenolic compounds and responds to them. Wu *et al.* (2008a,b,c) showed that cinnamic acid, coumaric acid or vanillic acid, when applied to *F. oxysporum* f. sp. *niveum* cultures, increased mycotoxin production (probably predominantly fusaric acid) and the activity of hydrolytic enzymes, such as pectinase, cellulose and amylase (only in the presence of cinnamic and vanillic acids). At the same time, these compounds had an inhibitory effect on growth and/or germination of *F. oxysporum* f. sp. *niveum*. Apparently, phenolic compounds produced by plants can have a protective function in reducing fungal growth and thus disease, but can also have a disease-promoting function as mycotoxin levels and hydrolytic enzyme activities are increased.

The degradation of phenolic compounds in fungi is mediated by the  $\beta$ -ketoacid pathway (reviewed in Harwood and Parales, 1996). Through this pathway, lignin monomers, aromatic hydrocarbons and amino aromatics are converted to either protocatechuate or catechol and then to  $\beta$ -ketoacid. In a recent insertional mutagenesis screen, two pathogenicity mutants were identified carrying a T-DNA insertion in the open reading frames of genes coding for catechol dioxygenase and carboxy-*cis,cis*-muconate cyclase (CMLE), two enzymes belonging to the  $\beta$ -ketoacid pathway (Michielse *et al.*, 2009). Targeted inactivation of *CMLE1* verified that this gene is required for pathogenicity (L. Reijnen and C. B. Michielse, unpublished results). In addition, *CMLE1* disruptants showed a strong decrease in growth on medium containing phenolic compounds, such as coumaric acid, ferulic acid and vanillic acid, indicating the importance of aromatic compound degradation for pathogenesis.

Another way for fungi to deal with phenolic compounds is to oxidize them through the action of laccases, which are copper-containing phenol oxidases. Laccases from wood-rotting basidiomycetes, such as *Trametes versicolor*, *Lentinula edodes* and *Pleurotus ostreatus*, are involved in lignin degradation (Baldrian, 2006; Leonowicz *et al.*, 2001). Laccases also play a role in the pathogenesis of the human pathogen *Cryptococcus neoformans* (Zhu *et al.*, 2001; Zhu and Williamson, 2004). In *F. oxysporum*, six laccase genes have been analysed (Canero and Roncero, 2008a). Expression during infection was observed for *LCC1*, *LCC3* and *LCC9*. For the genes *LCC1*, *LCC3* and *LCC5*, disruptants were generated. These mutants displayed decreased laccase activity, increased sensitivity to oxidative stress (not *lcc5*) and increased sensitivity to phenolic compounds, such as chlorogenic acid, caffeic acid and vanillic acid (only *lcc3* was tested). However, *LCC1*, *LCC3* and *LCC5* are individually not required for pathogenicity, as their deletion had no effect on virulence. A decrease in laccase activity and increased sensitivity to oxidative stress were also observed in a chloride channel (*CLC1*) gene disruptant (Canero and Roncero, 2008b). With this mutant, disease development in tomato was delayed. As *Clc1* is required for full laccase activity (probably through the facilitation of metal cofactor insertion into

maturing laccases), laccases may collectively be required for full pathogenicity. However, other defects of the *clc1* mutant may also account for the decrease in virulence.

Adaptation of the fungal cell wall has been shown to play an important role during pathogenicity, possibly by resisting damage by plant enzymes or compounds and/or by reducing the release of cell wall-derived defence elicitors. An early example is the absolute requirement of chitin synthase V (*CHSV*) for pathogenicity (Madrid *et al.*, 2003). More recently, additional chitin synthase genes have been identified and characterized, namely *CHS1*, *CHS2*, *CHSVb* and *CHS7*, encoding for class I, II and VII chitin synthase and a chaperone-like protein, respectively (Martin-Udiroz *et al.*, 2004, 2008). Targeted inactivation of *CHS1* had no effect on virulence, whereas targeted inactivation of *CHS2* and *CHS7* resulted in slightly reduced virulence on tomato plants (Martin-Udiroz *et al.*, 2004). The most pronounced effect on virulence was observed for the *CHSVb* disruptant, which failed to produce any disease phenotypes (Martin-Udiroz *et al.*, 2008). The phenotypes between the various *CHS* mutants with respect to cell wall-disturbing agents varied. Like the inactivation of *CHSV*, inactivation of *CHSVb* confers sensitivity to Calcofluor white and Congo red, but not to sodium dodecylsulphate (SDS), implying similar alterations in cell wall architecture.

Additional genes with a role in cell wall maintenance and pathogenicity are *GAS1*, encoding a  $\beta$ -1,3-glucanosyltransferase, and *RHO1*, encoding a GTPase (Caracuel *et al.*, 2005; Martinez-Rocha *et al.*, 2008). Consistent with alterations in the cell wall, *GAS1* and *RHO1* disruptants displayed enhanced resistance to CWDEs. In addition, increased transcription of *CHSV* and *RHO1* was observed in the *GAS1* disruptant (Caracuel *et al.*, 2005). Both *GAS1* and *RHO1* disruptants were severely reduced in virulence towards tomato.

Taken together, it appears that, for a fungus like *F. oxysporum* to survive and proliferate inside living plant tissue, it needs to be able to control its cell wall composition and to produce enzymes that can deal with plant-derived chemicals.

## PEROXISOMAL FUNCTION

Peroxisomes are single-membrane-bound organelles which, in filamentous fungi, are involved in the  $\beta$ -oxidation of fatty acids, peroxide detoxification and occlusion of septal pores (Jedd and Chua, 2000; Soundararajan *et al.*, 2004; Vandenbosch *et al.*, 1992). More than 20 peroxins (peroxisome biogenesis factors) have been identified in fungi. Mutations in peroxin genes result in the absence of normal peroxisomes, the mislocalization of peroxisomal matrix proteins and the inability to perform specific biochemical reactions, such as fatty acid catabolism (Kiel *et al.*, 2006). Peroxisomal function and fatty acid metabolism have been shown to be required for virulence of the plant-pathogenic fungi *Colletotrichum lagenarium*, *Magnaporthe grisea* and

*Ustilago maydis* (Jeon *et al.*, 2007; Kimura *et al.*, 2001; Klose and Kronstad, 2006; Ramos-Pamplona and Naqvi, 2006; Wang *et al.*, 2007). In *F. oxysporum*, four different *pex* genes, *PEX1*, *PEX10*, *PEX12* and *PEX26*, were identified as potential pathogenicity genes in a recent insertional mutagenesis screen, and the requirement for full pathogenicity was verified for two of them (*PEX12* and *PEX26*) by complementation with the intact genes (C. B. Michielse *et al.*, 2009). These results demonstrate that peroxisomal function is also necessary for the pathogenesis of a root-infecting fungus that lacks appressoria. Possibly, peroxisomal function could be necessary for the utilization of host nutrients during *in planta* growth. Alternatively, or in addition, woronin bodies, a special class of peroxisomes that plug septal pores, could be required for optimal invasive growth by making the fungus less sensitive to cytoplasmic leakage (Jedd and Chua, 2000; Soundararajan *et al.*, 2004; Tenney *et al.*, 2000).

### TRANSCRIPTION FACTORS

Important progress has been made in the identification of transcription factors specifically required for pathogenicity, such as *Fow2* in *F. oxysporum* f. sp. *melonis* and *Sge1* in *F. oxysporum* f. sp. *lycopersici*. For both genes, disruptants completely lost pathogenicity on their host plant (Imazaki *et al.*, 2007) (C. B. Michielse *et al.*, 2009). The mutants were not impaired in either vegetative growth or the utilization of various carbon sources. Although conidiation of the *FOW2* disruptant was similar to that of the wild-type, the *SGE1* disruptant displayed reduced conidiation (about four-fold), although the conidia generated did not differ in germination rate or morphology from the wild-type (C. B. Michielse *et al.*, unpublished results). For both *FOW2* and *SGE1* disruptants, fluorescence microscopy was used to observe their infection behaviour, and both mutants displayed a similar phenotype: no deficiency in root attachment and superficial root colonization, but impaired invasion and/or *in planta* growth (Imazaki *et al.*, 2007) (C. B. Michielse *et al.*, unpublished results).

A third transcription factor gene implicated in virulence is *FTF1*, identified in *F. oxysporum* f. sp. *phaseoli* (Ramos *et al.*, 2007). *FTF1* is a multicopy gene only present in highly virulent strains, and transcribed specifically during the early phases of infection. Interestingly, the multiple copies of *FTF1* in highly virulent strains are all located on the smallest chromosome. Recently, the requirement of *FTF1* for pathogenicity towards bean, as well as tomato, was demonstrated by RNAi experiments (J. M. Diaz-Minguez, University of Salamanca, Salamanca, Spain, personal communication). Clearly, it would be very interesting to identify the target genes of these transcription factors. To this end, the microarrays currently being developed for *F. oxysporum* (C. Kistler, USDA, St. Paul, USA, personal communication), would be of great help.

### INSERTIONAL MUTAGENESIS

Random insertional mutagenesis has been proven to be a useful tool for the identification of the genes involved in fungal pathogenesis (Weld *et al.*, 2006). Relatively small-scale insertional mutagenesis in *F. oxysporum* using restriction enzyme-mediated transformation led to the identification of novel pathogenicity genes, such as *ARG1*, *FOW1*, *FOW2* and *FCD1* (Imazaki *et al.*, 2007; Inoue *et al.*, 2002; Kawabe *et al.*, 2006; Namiki *et al.*, 2001). Additional insertional mutagenesis programmes using plasmid DNA or transposon tagging led to the identification of *CHSV*, *FRP1* and a Velvet-like gene as novel pathogenicity genes (Duyvesteijn *et al.*, 2005; Lopez-Berges *et al.*, 2008; Madrid *et al.*, 2003). Being unbiased, these screens often yield unsuspected links between pathogenicity and other (developmental) processes. For example, two members of the *velvet* gene family, *veA* and *velB*, previously shown to regulate fungal development and secondary metabolism in the model organism *Aspergillus nidulans*, were shown to be required for full virulence in *F. oxysporum* (Lopez-Berges *et al.*, 2008) (A. Di Pietro, University of Cordoba, Cordoba, Spain, personal communication). Recently, a large-scale mutagenesis programme (10 290 transformants), using the T-DNA of *Agrobacterium tumefaciens* as a mutagen, yielded 111 potential pathogenicity genes (C. B. Michielse *et al.*, 2009). In addition to a large group of hypothetical proteins with unknown function, functional distribution of the remaining proteins implicated certain processes, such as amino acid and lipid metabolism, cell wall maintenance and small molecule transport, in pathogenicity. To date, eight of the 11 genes have been verified to be required for (full) pathogenicity by gene knock-out (C. B. Michielse *et al.*, 2009; C. B. Michielse, unpublished results). It is expected that the analysis of additional pathogenicity mutants identified in these insertional mutagenesis programmes will further enhance our understanding of the molecular processes underlying pathogenesis.

### AVIRULENCE AND EFFECTOR PROTEINS SECRETED DURING COLONIZATION

Major resistance (*R*) genes have been found against *F. oxysporum* in several crops, including tomato, melon, cucumber, pea and bean (Table 2). Two of these genes have been cloned: *I-2* from tomato (Simons *et al.*, 1998) and *Fom2* from melon (Joobeur *et al.*, 2004). In cases in which *R* genes have been employed in a crop, the *formae speciales* of *F. oxysporum* that cause disease in that crop usually break up into races, defined by the *R* gene spectrum effective against them. Table 2 lists the *formae speciales* for which this is the case. Although the absence of a sexual cycle under laboratory conditions prevents genetic confirmation of gene-for-gene relationships on the side of the pathogen (Flor, 1971), the existence of major *R* genes in hosts and corresponding races in *F. oxysporum* raised the expectation that *F. oxysporum* should

**Table 2** Monogenic resistance reported against *Fusarium oxysporum*.

Plant species	<i>Forma specialis</i>	<i>R</i> genes	Pathogen races	Reference*
Tomato	<i>lycopersici</i>	<i>I</i> , <i>I-1</i> , <i>I-2</i> , <i>I-3</i>	1, 2, 3†	Hemming <i>et al.</i> (2004); Sela-Buurlage <i>et al.</i> (2001); Simons <i>et al.</i> (1998)
Melon	<i>melonis</i>	<i>Fom-1</i> , <b><i>Fom-2</i></b>	0, 1, 2, 1.2	Joobeur <i>et al.</i> (2004); Oumouloud <i>et al.</i> (2008)
Cucumber	<i>cucumerinum</i>	<i>Foc</i>	1, 2	Vakalounakis (1996)
Pea	<i>pisi</i>	<i>Fw</i>	1, 2A, 2B, 5, 6	Grajal-Martin and Muehlbauer (2002); McClendon <i>et al.</i> (2002)
Chickpea	<i>ciceris</i>	Several, mostly recessive	0, 1A, 1B/C, 2, 3, 4, 5, 6	Sharma and Muehlbauer (2007)
Eggplant	<i>melongenae</i>	(Unnamed)	None reported	Mutlu <i>et al.</i> (2008)
Bean	<i>phaseoli</i>	(Unnamed)	1, 2, 3, 4, 5, 6, 7	Cross <i>et al.</i> (2000); Fall <i>et al.</i> (2001); Salgado <i>et al.</i> (1995)
Cotton	<i>vasinfectum</i>	(Unnamed)	A, 1, 2, 3, 4, 5, 6, 7, 8	Wang and Roberts (2006)
Basil	<i>basilici</i>	(Unnamed)	None reported	Dudai <i>et al.</i> (2002)

Bold, cloned

\*Not exhaustive.

†Sometimes referred to as race 0, 1 and 2, respectively.

contain avirulence genes matching *R* genes in hosts. This was confirmed in 2004, when the first avirulence gene of *F. oxysporum* was identified in *F. oxysporum* f. sp. *lycopersici* (*Fol*) (Rep *et al.*, 2004). Its product, Six1 ('secreted in xylem 1'), was found in xylem sap of infected tomato plants, and was shown to be required for disease resistance mediated by the *R* gene *I-3* ('immunity 3'). Six1 was therefore later renamed Avr3 (Houterman *et al.*, 2008). Similar to avirulence proteins of other fungi (Catanzariti *et al.*, 2007; Fudal *et al.*, 2007; Gout *et al.*, 2006; Rep, 2005; Thomma *et al.*, 2005), Avr3 is a small secreted protein without homologues, even in closely related fungi (Rep *et al.*, 2004). Avr3 is required for full virulence, especially towards older plants (Rep *et al.*, 2005), and is expressed specifically on entry of plant roots or exposure to plant cells in culture (van der Does *et al.*, 2008a).

More recently, a second avirulence gene, *AVR1* (*SIX4*), was identified in *Fol*, which is required for *I*- and *I-1*-mediated disease resistance (Houterman *et al.*, 2008). Its product, Avr1 (*Six4*), was also found in the xylem sap proteome of infected tomato plants, together with several other fungal proteins secreted during colonization (Houterman *et al.*, 2007). Avr1 is not required for virulence towards susceptible tomato plants, but was found to suppress *I-2*- and *I-3*-mediated disease resistance (Houterman *et al.*, 2008), explaining earlier observations that race 1 isolates of *F. oxysporum* f. sp. *lycopersici* can cause disease on tomato lines that carry *I-2* or *I-3* (but not *I* or *I-1*) (Mes *et al.*, 1999; Rep *et al.*, 2005). The avirulence gene matching the remaining *R* gene against *Fol*, *I-2*, has also been identified, and again encodes a small protein secreted in xylem sap during colonization (Houterman *et al.*, in press). Taken together, this shows that the analysis of the xylem sap proteome of infected plants can be an effective approach to identify the avirulence proteins of *F. oxysporum*. It also resulted in the identification of other proteins from *F. oxysporum* secreted during colonization that could play an important role in host colonization (Houterman *et al.*, 2007) (M. Rep, unpublished results). It could be worthwhile,

therefore, to apply this approach to other xylem-colonizing fungi, such as *Verticillium*, or other *formae speciales* of *F. oxysporum*, if the host plant is amenable to the extraction of sufficient amounts of xylem sap. Most importantly, the identification of the molecular targets of effectors should lead to a greater insight into how *F. oxysporum* suppresses or evades primary or secondary immunity in plants.

## DEVELOPMENT OF *A. THALIANA* AS A MODEL HOST PLANT

Over the last 5 years, *A. thaliana* has emerged as a model host for the study of *Fusarium* wilt disease. As detailed above, *Arabidopsis* has been used to investigate the genetic requirements of pathogenicity in the fungus (Ospina-Giraldo *et al.*, 2003) and to visualize root and xylem colonization with fluorescence microscopy (Czymmek *et al.*, 2007). However, the main advantage of the use of *Arabidopsis* is the powerful tools available for *Arabidopsis* to genetically dissect host responses (Berrocal-Lobo and Molina, 2008).

In general, for the infection of *Arabidopsis*, *formae speciales* are used that cause disease on crucifers: f. sp. *conglutinans* (cabbage), f. sp. *matthiolae* (stock) and f. sp. *raphani* (radish). One study (Berrocal-Lobo and Molina, 2004) reports f. sp. *lycopersici*, the tomato pathogen, as also being able to infect *Arabidopsis*, but, in another study, neither f. sp. *lycopersici* nor f. sp. *cubense* (banana) caused disease (Diener and Ausubel, 2005). Elsewhere f. sp. *cubense* was also reported as not being able to cause disease in (wild-type) *Arabidopsis* plants (Van Hemelrijck *et al.*, 2006), suggesting that there is at least some level of host specificity in the *Arabidopsis*-*F. oxysporum* pathosystem.

The main conclusion of infection studies using *Arabidopsis* mutants in various hormone response pathways is that salicylic acid (SA), jasmonate (JA) and ethylene (ET) pathways cooperate to limit disease development (Berrocal-Lobo and Molina, 2004;



Diener and Ausubel, 2005). The involvement of JA signalling in resistance to *F. oxysporum* is also supported by the correlation between JA-responsive gene expression and disease resistance in transgenic plants overexpressing certain transcription factors (McGrath *et al.*, 2005), as well as in mutants lacking a G-protein  $\beta$ -subunit (Trusov *et al.*, 2006) or affected in abscisic acid signalling (Anderson *et al.*, 2004). Interestingly, the *def1* mutant of tomato, which is unresponsive to JA, is also more susceptible to *F. oxysporum* (Thaler *et al.*, 2004). Nevertheless, the application of methyl jasmonate (MeJA) did not enhance resistance, whereas SA application did (Edgar *et al.*, 2006). The latter effect was not accompanied by enhanced *PR-1* expression, which is consistent with the observation that the absence of NPR1, a central regulator of pathogenesis-related (PR) gene expression, does not affect the level of resistance (Diener and Ausubel, 2005). However, PR proteins may still contribute to resistance against *F. oxysporum* under certain conditions; expression of tobacco *PR-1* or *PR-5* increased resistance to *F. oxysporum* in an *Arabidopsis* mutant (*esa1*) that displays enhanced susceptibility (Van Hemelrijck *et al.*, 2006), and repression of PR gene expression by overexpression of the gene for transcription factor ATAF2 correlated with enhanced susceptibility (Delessert *et al.*, 2005). Apparently, the degree of resistance to infection is determined by a complex of factors, each of which by itself has a relatively minor effect. This is also exemplified by an *Arabidopsis* glucosinolate biosynthesis mutant that is slightly, but significantly, more susceptible to *F. oxysporum* f. sp. *matthiola* (Tierens *et al.*, 2001).

Given the many pathways and mechanisms in *Arabidopsis* that contribute to limit infection by *F. oxysporum*, it may be no surprise that there is a large variation between the levels of susceptibility among ecotypes (Czymmek *et al.*, 2007), and that these differences are polygenic in nature (Diener and Ausubel, 2005). Nevertheless, Diener and Ausubel (2005) attributed much of the variation in disease susceptibility between two ecotypes (Col-0 and Ty-0) to a single gene, and were able to isolate this gene, *RFO1* (resistance to *Fusarium oxysporum* 1), by map-based cloning (Diener and Ausubel, 2005). *RFO1* turned out to encode a receptor-like kinase (RLK) with an extracellular WAK (wall-associated kinase) domain. As *RFO1* enhances resistance to several *formae speciales* of *F. oxysporum*, it is unlikely that it recognizes a specific effector, but rather may function as a microbe-associated molecular pattern (MAMP) receptor that is activated by relatively conserved molecules, such as fungal cell wall components or plant cell wall breakdown products.

In conclusion, although not a good model plant for gene-for-gene interactions with *F. oxysporum*, *Arabidopsis* will allow rapid progress in the identification of plant proteins, metabolites and signalling pathways that contribute to basal resistance or susceptibility towards a root-invading fungus. One interesting application of the genetic tools available for *Arabidopsis* is to investigate host versus non-host resistance mechanisms using

different *formae speciales* of *F. oxysporum* (Van Hemelrijck *et al.*, 2006).

## TRANSCRIPTOMICS

A new era for *F. oxysporum* research began when its genome sequence became available (discussed below). The genome sequence enables the development of whole-genome microarrays, and thereby opens up the possibility to identify genes specifically expressed during certain developmental stages, e.g. conidiation, growth in axenic cultures under controlled circumstances and during infection, on a genome-wide scale. Whilst awaiting such experiments, expressed sequence tag analysis has been used to identify genes specifically up-regulated during conidiation and germination (Deng *et al.*, 2006; Iida *et al.*, 2006). Two cDNA libraries corresponding to the expression profile during vegetative growth and conidiation were constructed, and 641 and 626 unique genes, respectively, were identified, 130 of which were common to both libraries (Iida *et al.*, 2006). In addition, Deng *et al.* (2006) constructed a cDNA library containing 6448 cDNA clones, and concluded from expression analysis that *ras* and other signalling pathways cooperatively initiate conidial germination by increasing protein synthesis. The conidiation library was also used to screen for genes regulated by the sporulation-associated transcription factors StuA and Ren1 (Iida *et al.*, 2007). In total, 23 and 12 genes were identified that were positively and negatively regulated, respectively, by Ren1, which is required for micro- and macroconidia development. Only three genes were found to be positively regulated by StuA, a regulator of macroconidia production (Iida *et al.*, 2007; Ohara and Tsuge, 2004).

The sequencing of expressed sequence tags was also used to identify genes associated with pathogenicity and to understand the interaction between *F. oxysporum* f. sp. *vasinfectum* and cotton (Dowd *et al.*, 2004; McFadden *et al.*, 2006). An array was generated containing 4000 cDNA clones from *F. oxysporum*-infected cotton tissue at several time points after infection and 2000 cDNA clones from uninfected cotton tissue (Dowd *et al.*, 2004). This array was used to identify plant genes up- or down-regulated in roots and hypocotyls during a susceptible interaction. In total, 135 and 208 genes differentially expressed during infection were identified in roots and hypocotyls, respectively. Genes related to pathogenesis (PR genes), phytoalexin biosynthesis (gossypol), lignin biosynthesis and ET production were found to be up-regulated in hypocotyls. In contrast, genes related to lignin biosynthesis, stress responses, aquaporins and growth responses were down-regulated in root tissue. No effect on PR gene expression in roots was observed. From these results, it appears that, during infection, *F. oxysporum* may suppress host responses in roots, thereby facilitating infection.

McFadden *et al.* (2006) used the same microarrays to identify *F. oxysporum* genes expressed in root and hypocotyl tissue

during cotton infection. A total of 174 genes was expressed *in planta*, the great majority of which (97%) were found in infected roots. A large proportion of these fungal genes encode hypothetical proteins with an unknown function. The remaining genes are mostly common, highly expressed genes, such as genes for ribosomal proteins, proteins involved in energy or amino acid metabolism, transport and translation. Interestingly, one of the genes found to be up-regulated during cotton infection, *FOXG\_05013*, encoding a putative cell wall protein, was also identified in a recent insertional mutagenesis screen and verified as a gene required for full pathogenicity (C. B. Michielse *et al.*, 2009). In total, 11 genes were found to be preferentially expressed *in planta*. These include a putative ribosomal protein, 6-hydroxy-D-nicotine oxidase, a subunit of ATP synthase, a cytochrome P450 and an oxidoreductase (McFadden *et al.*, 2006). The gene coding for the oxidoreductase (*FOXG\_04087*) shows high similarity to *Agrobacterium tumefaciens AtsC*, a gene involved in attachment and virulence of this bacterium. *FOXG\_04087* expression was higher in plants infected with a pathogenic isolate than in those infected with a non-pathogenic isolate, indicating that this gene might have a role in virulence (McFadden *et al.*, 2006).

As discussed above, these findings constitute the early beginnings of the functional genomics of *F. oxysporum*, and the availability, in the near future, of microarrays based on the genome sequence is expected to lead to the generation of a large amount of information on transcriptional reprogramming during the infection of plants.

## THE GENOME SEQUENCE

The first genome sequence of an *F. oxysporum* isolate, a race 2 strain of *forma specialis lycopersici* (Fol), was produced by the Broad Institute and became available in March 2007. Already, this genome sequence has accelerated genomic approaches to the study of *F. oxysporum*, such as large-scale insertional mutagenesis, transcriptomics and proteomics. One interesting outcome of the ongoing analysis of the *F. oxysporum* genome sequence is that (host-specific) virulence-related genes appear to be non-randomly distributed over the genome. For instance, chromosome 14 contains the *SIX1* (*AVR3*), *SIX2* and *SIX3* effector/avirulence genes in a region that is more conserved between clonal lines of *Fol* than genes on other chromosomes, suggesting that (parts of) chromosome 14 may be subject to horizontal transfer (van der Does *et al.*, 2008b).

Clearly, we are just at the beginning of exploiting the genome sequence of *F. oxysporum* to understand the mechanisms and evolution of the pathogenicity of this fungus. Particular areas of interest are now the nature of the infection-specific transcriptome and the role of transposable elements and horizontal gene transfer in the evolution of the genome structure and host-specific

pathogenicity of *F. oxysporum*. In addition, an exciting prospect will be to gain an insight into the genomic differences between different *formae speciales* and non-pathogenic isolates by sequencing of additional strains (C. Kistler, personal communication). This should also aid efforts in the use of molecular techniques for the identification of *formae speciales* and races, which would be of great assistance in helping to control diseases caused by *F. oxysporum* (Lievens *et al.*, 2008).

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