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MEETING ABSTRACTS

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Thursday April 1

Parallel session 7: Fungal and Oomycete Effectors

PS7.1

Fungal secondary metabolites as effectors of pathogenicity: role in the complex interplay between rice and *Magnaporthe grisea*

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Functional analyses of fungal genomes are expanding our view of the metabolic pathways involved in the production of secondary metabolites. These genomes contains a significant number of genes encoding key biosynthetic enzymes such as polyketides synthases (PKS), non-ribosomal peptide synthases (NRPS) and their hybrids (PKS-NRPS), as well as terpene synthases (TS). *Magnaporthe grisea* has the highest number of such key enzymes (22 PKS, 8 NRPS, 10 PKS-NRPS, and 5 TS) among fungal plant pathogens, suggesting that this fungal species produce a large number of secondary metabolites. In particular, it has 10 hybrid PKS-NRPS that likely produce polyketides containing a single an amino-acid. Three of them (*ACE1, SYN2* and *SYN8*) have the same expression pattern that is specific of early stages of infection (appressorium-mediated penetration), suggesting that the corresponding metabolites are delivered to the first infected cells. *M. grisea* mutants deleted for *ACE1* or *SYN2* by targeted gene replacement are as pathogenic as wild type Guy11 isolate on susceptible rice cultivars. Such a negative result could result from a functional redundancy between these pathways. However, *ACE1* null mutants become specifically pathogenic on resistant rice cultivars carrying the *Pi33* resistance gene compared to wild type Guy11 isolate that is unable to infect such rice cultivars. Introduction of a Guy11 wild type *ACE1* allele in *Pi33* virulent *M. grisea* isolates restore their avirulence on Pi33 resistant rice cultivars, showing that *ACE1* behaves as a classical avirulence gene (AVR). *ACE1* differs from other fungal *AVR* genes (proteins secreted into host tissues during infection) as it likely controls the production of a secondary metabolite specifically recognized by resistant rice cultivars. Arguments toward this hypothesis involve the fact that the protein Ace1 is only detected in the cytoplasm of appressoria and is not translocated into infectious hyphae inside epidermal cells. Furthermore, *Ace1-ks0*, an *ACE1* allele obtained by site-directed mutagenesis of a single amino acid essential for the enzymatic activity of Ace1, is unable to confer avirulence. According to this hypothesis, resistant rice plants carrying *Pi33* are able to recognize its fungal pathogen *M. grisea* through the perception of one fungal secondary metabolite produced during infection. The map based cloning of the *Pi33* rice gene was initiated and this gene maps at a locus rich in classical NBS-LRR resistance genes. Further work is ongoing to identify which gene is *Pi33*. In order to characterize the secondary metabolite produced by *ACE1*, this gene was expressed in a heterologous fungal host such as *Aspergillus oryzae* under the control of an inducible promoter. The removal of the three introns of *ACE1* allowed the expression of the enzyme in *A. oryzae*. Characterization of the novel metabolite produced by *Ace1* is in progress.

PR2.7

What large-scale T-DNA insertional mutagenesis tells us about pathogenicity? a functional genomics analysis in the dothideomycete *Leptosphaeria maculans*

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The Dothideomycete phytopathogen *Leptosphaeria maculans* is capable to alternate saprophytic, hemibiotrophic, endophytic and necrotrophic life styles during a single infectious cycle on its host plant, *Brassica napus*. However, little is known about the determinants of such plasticity. A reverse genetic strategy was developed, and a large-scale T-DNA insertional mutagenesis project was conducted resulting in: i) a collection of 5000 transformants phenotyped when interacting with plants, ii) a collection of 170 pathogenicity altered mutants, and iii) a set of 400 T-DNA flanking sequences. In addition, the *L. maculans* genome was sequenced and annotated, and whole genome Gene Ontology (GO) analysis was performed. Here we present combined analyses of the genomic pattern of 318 T-DNA insertion events (T-IEs), and the functional pattern of 279 T-DNA targeted genes (T-TGs). T-IEs analyses showed that: i) T-IEs favoured regulatory regions of gene-rich euchromatic genomic regions, and ii) T-IEs density correlated with CG skew near the transcription initiation site. These results are consistent with the T-DNA intranuclear targeting model, relying on gene expression machinery. T-TGs analyses showed that: i) T-IEs targeted 48.9% of the biological processes that were identified by whole-genome GO analysis, and that ii) T-IEs favoured biological processes that are consistent with the cellular state of a germinating conidia. Functional analysis of T-TGs in pathogenicity altered mutants will be presented.

PR2.8

Host-parasite interaction in the *Brassica napus/Verticillium longisporum* system

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The first leaky auxotrophic mutant for aromatic amino acids of the near-diploid fungal plant pathogen *Verticillium longisporum* (VL) has been generated. VL enters its host *Brassica napus* through the roots and colonizes the xylem vessels. The xylem contains little nutrients including low concentrations of amino acids. We isolated the gene *Vlaro2* encoding chorismate synthase by complementation of the corresponding yeast mutant strain. Chorismate synthase produces the first branch point intermediate of aromatic amino acid biosynthesis. A novel RNA-mediated gene silencing method reduced gene expression of both isogenes by 80% and resulted in a bradytrophic mutant, which is a leaky auxotroph due to impaired expression of chorismate synthase. In contrast to the wild type, silencing resulted in increased expression of the cross-pathway regulatory gene *VlcpcA* (similar to *cpcA/ GCN4*) during saprotrophic life. The mutant fungus is still able to infect the host plant *B. napus* and the model *Arabidopsis thaliana* with reduced efficiency. *VlcpcA* expression is increased in planta in the mutant and the wild-type fungus. We assume that xylem colonization requires induction of the cross-pathway control, presumably because the fungus has to overcome imbalanced amino acid supply in the xylem.

Poster Category 7:

Fungal and Oomycete Effectors

PR7.1

GEMO: Evolutionary Genomics of *Magnaporthe oryzae*

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Developing integrated control methods against pests of cultivated plants can significantly contribute to increasing food production while reducing inputs threatening the environment. The durability of a control method can be improved by a better knowledge of the pathogen's genetic determinants that are responsible for this adaptation. We were granted by the French National Research Agency for a project that aims at sequencing the genomes of several strains of the phytopathogenic model species *Magnaporthe oryzae* and at exploiting these complete sequences to characterize the repertoire of genes involved in pathogenicity and host specificity, and study their evolution. We will sequence 7 strains of the species *M. oryzae* representing different genetic groups pathogenic of different species of Poacees and one strain of the sister species *M. grisea*. ESTs produced during the infection by two strains pathogenic of rice and wheat on their respective host will also be sequenced. Different available annotation pipelines will permit to list and do comparative analyses of different gene families known or speculated to be involved in pathogenicity. Transcriptomic data of the two strains with different host specificities will be compared to identify key genes in specialization to the host. Genome fluidity will be characterized by synteny analyses and by the identification and localization of repeated elements. The impact of these rearrangements on pathogenicity genes and host specificity genes will be tested. Molecular signatures of positive or purifying selection in coding and regulatory sequences will be searched for by different methods. The whole set of data will be integrated in a database that will be designed to be accessible publicly.

PR7.2

Molecular characterisation of effector proteins from the fish pathogenic oomycete *Saprolegia parasitica*

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Water molds (oomycetes) are destructive pathogens of aquatic animals and terrestrial plants. *Saprolegnia* species cause Saprolegniosis, a disease that is characterized by visible white or grey patches of filamentous mycelium on the body or fins of freshwater fish. *Saprolegnia parasitica* is economically one of the most important fish pathogens, especially on catfish, salmon and trout species, causing millions of dollar losses to the aquaculture business worldwide. Several *Saprolegnia* species have also been linked to declining wild fish stocks and amphibian populations around the world. Currently, the genome of *S. parasitica* (isolate CBS223.65) is being sequenced by combined Sanger, 454 and Illumina data. The annotation is supported by paired-end EST sequences. Analysis of the preliminary genome sequence and EST libraries resulted in the identification of putative effector proteins. Electron microscope analysis showed that *Saprolegnia* interacts with fish cells by forming haustoria-like structures. Detailed expression studies of the genes encoding the putative effectors were performed during the biotrophic and necrotrophic infection stages of *S. parasitica*. Also localization and uptake studies were performed to show a role of the effector proteins in the interaction of *S. parasitica* with a rainbow trout cell-line.

PR7.18

Identification and analysis of secreted proteins from the maize pathogen *Colletotrichum graminicola*

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The filamentous Ascomycete *Colletotrichum graminicola* is the causal agent of stem rot and leaf anthracnose on *Zea mays*. After germination and penetration of epidermal cells, this hemibiotrophic fungus enters a short biotrophic phase that is followed by a destructive necrotrophic phase resulting in the production of conidia. Secreted fungal proteins are believed to play important roles in the progress of both phases of pathogenesis. The Yeast Secretion Signal Trap (YSST) was used to identify cDNAs encoding peptides containing signal sequences, starting from mRNA from *in vitro*-grown mycelium, induced with a corn leaf-extract. Of the 94 obtained sequences, 45 showed significant similarities to genes with a reported function, 24 were similar to genes annotated in fungal genome projects and 27 showed no similarity to database entries. Macroarray hybridisation showed that most of these genes are expressed *in planta*. Transcript abundance of most genes peaks at specific periods during pathogenesis, while some are expressed constantly. A minor set exhibits two peaks and a minimum during the biotrophy-necrotrophy transition phase. Expression patterns of several genes from each set were confirmed by qRT-PCR.

To test possible roles of secreted proteins as pathogenicity or virulence factors, knock-out strains were generated for 18 genes identified in the screen, encoding proteins of various classes: enzymes, small cysteine-rich proteins and proteins of unknown function. Results from infection assays will be presented.

PR7.19

Crystal structure of the avirulence gene AvrLm4-7 Of *Leptosphaeria* maculans illuminates its evolutionary and functional characteristics

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Leptosphaeria maculans, a phytopathogenic ascomycete causing stem canker of oilseed rape, develops "gene-for-gene" interactions with its host plant where fungal avirulence (*AvrLm*) genes are the counterpart of plant resistance (*Rlm*) genes. *AvrLm4-7* encodes a 143 amino-acid cysteine-rich protein, secreted outside of the fungus cells and strongly induced during the early stages of plant infection. AvrLm4-7 can bind to phosphoinositides and is translocated within plant cells. AvrLm4-7 crystal structure was determined following heterologous production in *Pichia pastoris*. The protein shows the presence of 4 disulfide bridges, and is strongly positively charged, suggesting interaction with minus charged molecules such as DNA or phospholipids. *AvrLm4-7* confers a dual specificity of recognition by *Rlm7* or *Rlm4* resistance genes and occurs as three alleles only: the double avirulent (A4A7), the avirulent towards *Rlm7* only (a4A7), or the double virulent (a4a7). Sequencing of diverse alleles coupled with targeted point mutagenesis strongly suggested that one single base mutation, leading to the change of a glycine residue to an arginine, was responsible for the A4A7 to a4A7 phenotype change. This amino acid being located on an external loop of the protein, its change is unlikely to alter the 3-D structure of the protein, but rather must correspond to a specific recognition target for the plant cell. In contrast, multiple mechanisms are responsible for the complete loss of avirulence (a4a7 phenotype), mostly drastic events leading to inactivation or complete deletion of the gene. The few single point mutations found targeted amino acids essential for the 3-D structure of the protein.