PR7.16

Dissecting the role of *Cladosporium fulvum* (secreted) proteases and protease inhibitors

Mansoor Karimi Jashni, Rahim Mehrabi, Harrold A. van den Burg, Pierre J.G.M. de Wit,
Laboratory of Phytopathology, Wageningen University & Research Centre, Droevendaalsesteeg 1, 6708PB,
Wageningen, The Netherlands
mansoor.karimi@wur.nl

In order to facilitate infection, fungal pathogens produce various types of secreted proteases likely to target and perturb important plant proteins that are involved in controlling basal defense. In addition, they secrete several protease inhibitors such as Avr9 of *Cladosporium fulvum* that, based on its structure, is predicted to be a carboxy peptidase inhibitor. Protease inhibitors are potentially able to deactivate or detoxify host target proteases and, therefore, might play an important role during infection. In this study we are investigating the role of *C. fulvum* protease and protease inhibitors in disease establishment by using both functional genomics and biochemical approaches. We mined the genome of *C. fulvum* and found numerous proteases and protease inhibitors of which many are secreted. Expression analyses of these genes were performed using RNA extracted from fungal mycelium grown *in vitro* on liquid media under different conditions as well as from inoculated susceptible tomato plants. Interestingly, many of these genes are highly expressed only *in vitro* and/or *in planta* and based on their expression profiles we selected a number of candidates for further functional analyses. We will generate knock-out mutants of the selected proteases and protease inhibitors to identify their role in virulence. In addition, biochemical approaches will be used to pull-down the host target proteins of some presumably important candidate proteins such as for example the Avr9 protein.

PR7.17

Identification and functional characterization of *Cladosporium fulvum* effectors by genomics, transcriptomics and proteomics approaches

Bilal Okmen[1] Ioannis Stergiopoulos[1] Mattias De Hollander[2] Harrold van den Burg[1] Henriek Beenen[1] Pierre J.G.M. De Wit[1]
[1]Laboratory of Phytopathology, Wageningen University & Research Centre, Droevendaalsesteeg 1, 6708PB, Wageningen, The Netherlands
[2]Applied Bioinformatics, Plant Research International, PO Box 16, 6700AA Wageningen, The Netherlands
bilal.okmen@wur.nl

Cladosporium fulvum is a biotrophic fungal pathogen that causes leaf mold of tomato. During infection *C. fulvum* secretes a number of small proteins into the apoplast of tomato leaves, which are collectively called effectors. So far, ten effector proteins have been characterized that in general show no or limited sequence similarity to other proteins present in public databases. In this study we try to identify and functionally characterize additional *C. fulvum* effector proteins that are involved in fungal pathogenesis. Recently, the genome of *C. fulvum* has been sequenced using the 454 technology. This genome sequence enables the identification of all secreted proteins from the fungus, collectively called the secretome. However, for accurate mining and annotation of the effector secretome, gene calling programs need to be first optimized by analyzing high quality expressed sequence tags (ESTs). Therefore, several cDNA libraries of *C. fulvum* grown under various *in vitro* and *in planta* conditions were constructed and are sequenced to support genome annotation. Initial automated annotation of the genome revealed that the fungus contains approximately 13.000 genes, of which approximately 1200 encode putatively secreted proteins. Bioinformatic analyses identified a subset of 300 putative effectors within the predicted secretome, while additional proteomics analysis from apoplastic fluids of tomato leaves infected by *C. fulvum*, revealed 30 proteins that are specifically produced in the compatible interaction. At this moment we are performing functional profiling of these novel effector proteins by examining their ability to inhibit PAMP triggered Immunity and/or effector-triggered immunity in custom made assays.