Functional characterization of avirulence genes in Leptosphaeria maculans: linking 3-D structure, functional characteristics and evolution

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Leptosphaeria maculans is an ascomycete causing stem canker of oilseed rape. To date, four *L. maculans* avirulence genes were cloned by our team and our objective is to elucidate their involvement in pathogenicity through their structural and functional characterization and the determination of their interactants. *AvrLm4-7* confers a dual specificity of recognition by two resistance genes (*Rlm4* and *Rlm7*) and is strongly involved in fungal fitness. The AvrLm4-7 protein was produced in *Pichia pastoris* and its crystal structure determined. It revealed the presence of four disulfide bridges and no close structural analogs could be identified. Translocation assays in oilseed rape roots and transient expression in tobacco leaves showed that AvrLm4-7 is translocated into plant cells in the absence of the pathogen and targeted to the cytoplasm. Translocation necessitates the presence of a RAWG motif located in a loop as part of a positively charged region and also the presence of a well-conserved stretch of amino acids (R/N)(Y/F)(R/S)E(F/W) in the C-terminal part of the protein. Loss of recognition of *AvrLm4-7* by *Rlm4* is due to mutation of a single glycine to arginine residue located in a loop of the protein. Loss of recognition by *Rlm7* is governed by three point mutations targeting residues either located in the (R/N)(Y/F)(R/S)E(F/W) motif or close to the glycine involved in *Rlm4*-mediated recognition. Using bombardment assays, we determined that recognition by Rlm4 and Rlm7 occurred into the cytoplasm of plant cells. Finally, a yeast two hybrid screen was performed and possible plant targets will be discussed.