Studies on fungal secreted proteins that activate plant immunity in *Colletotrichum* species

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SUMMARY

CHAPTER I: Comparative transient expression of two conserved *Colletotrichum orbiculare* effectors reveal their distinct cell death-inducing activities between *Nicotiana benthamiana* and melon

Colletotrichum orbiculare infects cucurbits, such as cucumber and melon, as well as the model Solanaceae plant *Nicotiana benthamiana,* by secreting an arsenal of effectors that suppress the immunity of these distinct plants. Two conserved effectors of *C. orbiculare*, called NLP1 and NIS1, induce cell death responses in *N. benthamiana*, suggesting that these secreted effectors activate the plant immunity in *N. benthamiana*. However, it is unclear whether they exhibit the same activity in Cucurbitaceae plants. In this chapter, I newly established a transient expression system to investigate the cell death-inducing activity of NLP1 and NIS1 in melon (*Cucumis melo* L.). NLP1 strongly induced cell death in melon but, in contrast to the effects seen in *N. benthamiana*, mutations in conserved residues in the heptapeptide motif or the putative glycosylinositol phosphorylceramides-binding site of NLP1 did not cancel its cell death-inducing activity in melon. Furthermore, NLP1 lacking the signal peptide caused cell death in melon but not in *N. benthamiana*. Study of the transient expression of NIS1 also revealed that, unlike in *N. benthamiana*, NIS1 did not induce cell death in melon. By contrast, NIS1 suppressed flg22-induced ROS generation in melon, as seen in *N. benthamiana.* These findings indicate distinct cell death-inducing activities of NLP1 and NIS1 in these two plants that *C. orbiculare* infects.

CHAPTER II: Inappropriate expression of putative pectin-degrading enzymes in multiple *Colletotrichum* fungi reduces virulence on their host plants via the activation of plant immunity

NLP1 of *C. orbiculare* is preferentially expressed at the late necrotrophic phase and it was reported that constitutive expression of NLP1 in *C. orbiculare* impairs pathogen infection on cucurbits via activation of the host plant immunity. In this chapter, I investigated whether other secreted proteins preferentially expressed at the late infection phase have similar effects on pathogen virulence when constitutively expressed. Based on the RNA sequencing data of *C. orbiculare*, I found that three putative pectate lyases are preferentially expressed at the late infection phase of the pathogen, therefore I decided to focus on the pectate lyases. The studies revealed that constitutive expression of a pectate lyase CoPL3 in *C. orbiculare* reduced the pathogen virulence toward *N.benthamiana* but not cucumber. I also revealed that constitutive expression of ChPL3, a *C. higginsianum* homolog of CoPL3, in *C. higginsianum*, reduced the pathogen virulence toward multiple brassica plants. Constitutive expression of ChPL3 in *C. trifolii* also reduced the pathogen virulence toward host plant alfalfa. Mutational studies in ChPL3 also suggested that the enzymatic activity of ChLP3 is involved in ChPL3-dependent negative effects on the virulence of *C. higginsianum*. Furthermore, I revealed that constitutive expression of two putative pectate lyases (ChPL53 and ChPL85) and a putative pectin lyase (ChPNL73), showing high homology to ChPL3, strongly reduced the virulence of *C. higginsianum* on a brassica cultivar Komatsuna. These findings revealed that the appropriate expression of multiple secreted proteins including both effectors and cell wall-degrading enzymes is critical for the successful infection of fungal pathogens to avoid the activation of host immune responses.