

Studies on virulence-related effectors and transcription factors preferentially expressed at the pre-invasion stage in *Colletotrichum orbiculare*

(ウリ類炭疽病菌の侵入前に優先的に発現する病原性関連エフェクターおよび転写因子の研究)

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Summary

Chapter I. Studies on virulence-related effectors preferentially expressed at the pre-invasion stage in *Colletotrichum orbiculare*

Pathogens manipulate effectors to interfere with various defense signaling pathways for successful infection. However, only a small portion of fungal effectors have been characterized for virulence functions, possibly due to the functional redundancy of effectors in virulence. To establish a compatible interaction leading to proliferation, it is plausible that *C. orbiculare* expresses key virulence-related effectors at a stage when the pathogen has not yet developed invasive hyphae (called the pre-invasion stage) thereby suppressing non-host resistance. To identify *C. orbiculare* effectors that have a role in the pre-invasion stage, I first selected 8 putative secreted protein genes that are highly expressed in *C. orbiculare* at 1 dpi on *Nicotiana benthamiana*, which is considered to be the typical pre-invasion stage. I performed a functional screening of eight candidates via virulence enhancement assay, as a result, the transient expression of SIB1 and SIB2 (Suppression of Immunity in *N. benthamiana*) significantly enhanced lesion development of *C. orbiculare* on *N. benthamiana*. Further studies on the effector SIB1 showed that the generation of reactive oxygen species triggered by two different PAMPs (pathogen-associated molecular patterns), chitin and flg22, were commonly suppressed when SIB1 was transiently expressed in *N. benthamiana*. In addition to that, transient expression of SIB1 suppressed cell death triggered by INF1 which is a well-known oomycete PAMP elicitor that can induce the hypersensitive response in *N. benthamiana* leaves. However, the *SIB1* knockout mutant of *C. orbiculare* showed the wild-type virulence on both cucumber and *N. benthamiana*, indicating that *SIB1* is dispensable for virulence of *C. orbiculare*. This phenotype is possibly due to the functional redundancy of effectors in pathogen virulence. Taking these results together, I here identified one effector called *SIB1* that is involved in the suppression of multiple PAMP-triggered immunities.

Chapter II. Studies on virulence-related transcription factors preferentially expressed at the pre-invasion stage in *Colletotrichum orbiculare*

Colletotrichum fungi secrete a large number of effectors at each different infection stage for successful infection. However, the regulatory mechanism of this stage-specific expression of effectors remains largely unknown. For the timely expression of these effectors for host invasion, host recognition and subsequent signaling are likely important. Then it is plausible that, as a next step, transcription factors play crucial roles in the effectors' expression. Here I focused on transcription factors highly induced at the pre-invasion stage in *C. orbiculare* on host plants to identify the virulence-related transcription factors of *C. orbiculare* that mainly control effector expression. The knockout analysis showed that the disruption of *TFV1* (transcription factor for virulence 1) reduced the virulence of *C. orbiculare* against cucurbitaceous plants, including cucumber and melon, but not *N. benthamiana*. Additional deletion of *TVL1* (*TFV1* like 1), which is the closest homolog of *TFV1* in *C. orbiculare*, enhanced the reduction of lesion development on cucurbitaceous plants. To investigate the regulatory role of *TFV1* and *TVL1*, comparative RNA sequencing analyses between the wild-type strain and each mutant were performed. The results showed that virulence effector genes (*EPC1*, *EPC2*, *EPC3*, and *EPC4*) are commonly down-regulated in both *tfv1Δ* mutant and *tfv1Δ tvl1Δ* mutant. Furthermore, there is no significant difference in the expression of *EPC1*, *EPC2*, *EPC3*, and *EPC4* between single and double knockout mutants, indicating that *TFV1* encodes a transcriptional factor critical for the stage-specific *EPCs* expression. Apart from that, there are 39 putative secreted protein genes specifically downregulated in the *tfv1Δ tvl1Δ* mutant but not in the *tfv1Δ* mutant, suggesting that *TVL1* contributes to the *C. orbiculare* virulence probably by controlling the expression of uncharacterized effectors. In conclusion, these results suggest that *TFV1* and *TVL1* act as two regulators controlling the virulence of *C. orbiculare* on cucurbitaceous plants through the regulation of virulence effector gene expression.