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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目： Study on function of type III effectors in *Ralstonia solanacearum*
Title of Dissertation （青枯病菌におけるIII型エフェクターの機能に関する研究）

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Ralstonia solanacearum is a devastating plant pathogen with a global distribution and invades an unusually wide host range, including economically important crops such as tomato, potato, tobacco, and eggplant. Like other gram-negative phytopathogens, *R. solanacearum* possesses a type III secretion system (T3SS) and injects type III effectors (T3Es) directly into the host plant cells. Type III effectors, once internalized in the plant cells, interact with plant substrates either to activate or suppress plant defense systems, resulting in the hypersensitive response (HR) or disease promotion. Bacterial T3Es contribute to disease development, although the mechanisms, by which each T3E functions inside plant cells, are not fully understood. The objective of my study is to investigate the functions of T3Es of *R. solanacearum*.

A well-known family of type III effector, RipG (GALA), consisting of seven genes contains an F-box domain and leucine-rich repeats (LRRs). The F-box domain resembles eukaryotic F-box proteins, which form SCF ubiquitin ligase complex in combination with Skp1 and Cullin1 and control specific protein ubiquitination. RipG effectors are known to interact with Skp1-like protein in *Arabidopsis thaliana* through their F-box domains. Several reports indicate non-eukaryotic F-box proteins act by hijacking host SCF ubiquitin ligase complex for disease development. However, proteins targeted by the complex are still unknown. In my first study, in order to find target proteins by the hijacked E3 ubiquitin ligase, I investigated the proteins that interact with RipG T3Es of *R. solanacearum*.

Yeast two-hybrid (Y2H) screening of *Nicotiana benthamiana* and *Nicotiana tabaccum* cDNA library was conducted to find out plant proteins interacting with the RipG

effectors of *R. solanacearum* OE1-1 strain. In addition to the previously reported Skp1, several chloroplastic proteins were found to interact with the RipG effectors, especially RipG2 and RipG7. Among the identified proteins, I cloned the full lengths of cDNAs of chlorophyll a-b binding protein 13, chaperonin-like RbcX protein, and ribulose bisphosphate carboxylase small chain from *N. benthamiana* mRNA. All three chloroplastic proteins interacted well with RipG2 and RipG7.

To find binding sites for Skp1 and chloroplastic proteins in RipG T3Es of *R. solanacearum*, I deleted F-box domain from RipG2 and F-box domain, LRR, and N-terminal region from RipG7. While Skp1 bound to RipGs through the F-box domain, the chloroplastic proteins I tested did not bind to the F-box domain but bound to either LRR or the N-terminal region. In yeast three-hybrid assay, Skp1 and ribulose bisphosphate carboxylase small chain from *N. benthamiana* simultaneously bind to RipG7. From these results, I hypothesize that chloroplastic proteins could be the targets for ubiquitination via RipG effectors.

In another study, I investigated the involvement of two avirulence genes, *avrA* (*ripAA* in unified nomenclature) and *popP1* (*ripP1* in unified nomenclature), of Japanese *Ralstonia solanacearum* strains in the pathogenicity to tobacco. One virulent strain OE1-1 and four hypersensitive response (HR)-eliciting strains, 8107, MAFF 211471, MAFF 211496, and MAFF 301520, were used. While 8107 and MAFF 211471 contain *popP1*, other three strains do not. When *popP1* of strain 8107 was transferred into the virulent strain OE1-1, the transconjugant strain had significantly reduced virulence but did not become a HR-eliciting strain. I deleted *avrA* and/or *popP1* from the HR-eliciting strains; all deletion mutants still elicited a HR and did not become virulent to tobacco, although leaf lesion appearance was delayed on infection with *avrA* mutants. These results indicate that although *avrA* and *popP1* could be functional as avirulence determinants, other unidentified factors are necessary for full virulence or HR elicitation by Japanese *R. solanacearum* strains.

From my studies, I understood that the functions and modes of action of T3Es are diverse. Further studies and researches are necessary to comprehend the pathogenicity of *R. solanacearum*.