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SUMMARY OF DOCTORAL THESIS

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Title: **Molecular mechanisms of pathogenicity of the tomato pathotype of *Alternaria alternata***
（トマトアルターナリア茎枯病菌における病原性発現の分子機構に関する研究）

Successful plant pathogens have evolved various strategies to establish compatible interactions with host plants. Although essential components for fungal pathogenesis are still unknown in many plant pathogenic fungi, certain fungal pathogens, especially the genera *Alternaria* and *Cochliobolus*, are known to produce host-specific toxins (HSTs) as compatibility agents. The tomato pathotype of *A. alternata* produces host-specific AAL-toxin and causes Alternaria stem canker on tomato. In general, targeted gene disruption lead to elimination of toxin production and loss of pathogenicity, which indicates that HSTs are specific pathogenicity factors of plant pathogens. On the other hand, fungal melanin has been implicated as a general pathogenicity factor in some phytopathogenic fungi. In addition, nonspecific toxins such as 6-methylesalicylic acid produced by plant pathogens are also known as virulent factors of pathogens. However, a comprehensive vision of molecular mechanisms of pathogenic strategies of plant pathogens is still unclear. To understand the complex mechanisms of pathogenetic processes, separate approach to both specific and general pathogenesis, which allows the efficient molecular genetic dissection of the processes, has been of great value. This study mainly focuses on the functional analysis of genes for AAL-toxin biosynthesis as specific factors, and of genes for melanin or 6-methylesalicylic acid biosynthesis as general factors. In addition, determination of pathogen development and toxin production in infected plants is of prime importance for pathological evaluation of toxins. Here, *in planta* production of AAL-toxin and localization of the pathogen in infected tomato plants have been examined to evaluate the pathological significance of AAL-toxin during the infection process. A thorough understanding of pathogenic processes may lead to development of new strategies for controlling plant diseases.

1) Functional analysis of the melanin biosynthesis genes *ALM1* and *BRM2-1* in the tomato pathotype of *A. alternata*

The tomato pathotype of *A. alternata* (*A. arborescens*) produces the dark brown or black pigment melanin, which accumulates in the cell walls of hyphae and conidia. Melanin has been implicated as a pathogenicity factor in some phytopathogenic fungi. Here, two genes of the tomato pathotype for melanin biosynthesis, *ALM1* and *BRM2-1*, which encode a polyketide synthetase and a 1,3,8-trihydroxynaphthalene (THN) reductase, respectively, have been cloned and disrupted in the pathogen. The gene-disrupted mutants, *alm1* and *brm2-1,* showed albino and brown phenotypes, respectively. The wild type and mutants caused the same necrotic lesions on the leaves following spore inoculation. These results suggest that melanin is unlikely to play a direct role in pathogenicity in the tomato pathotype *A. alternata*. Scanning electron microscopy revealed that the conidia of the mutants have smooth-walled surface structures in comparison to the wild type. The conidia of those mutants were more sensitive to UV light than those of the wild type, demonstrating that melanin confers UV tolerance.

2) Functional analysis of the 6-methylesalicylic acid synthase gene in the tomato pathotype of *A. alternata*

A polyketide, 6-methylesalicylic acid (6-MSA) is known as a nonspecific toxin of plant pathogenic fungi. The compound is synthesized by a polyketide biosynthetic gene known as the 6-MSA synthase (6-MSAS) gene. The gene is expressed by the tomato pathotype during the end of the logarithmic growth phase. The 6-MSAS gene (*AaMSAS*) was identified by draft genome sequencing date of As-27 strain of the tomato pathotype of *A. alternata.* A highly efficient method for gene disruption (knockout) approach was used to replace the *AaMSAS* in the wild-type strain As-27 with the *hph* marker gene. The result showed that there was no significant difference in the conidial formation, vegetative growth rate and morphology between the wild-type and the *AaMSAS*-deleted mutants, suggesting that the gene is not involved in those phenotypes. The pathogenicity and the toxin production of the mutants were compared with those of the wild-type strain.

3) Functional analysis of the ceramide synthase gene *ALT7*, a homologue of the plant disease resistant gene *Asc1*, in a plant pathogenic fungus *A. alternata*

AAL-toxin of the tomato pathotype of *A. alternata* is a sphinganine-analog mycotoxin which induces apoptotic cell death in tomato cells and mammalian cells by inhibiting ceramide biosynthesis. Insensitivity to the AAL-toxin in resistant tomatoes and other plants is conferred by the *Asc1* gene, a homolog of the yeast ceramide synthase gene *Lag1*. The *ALT7* gene, a putative acyl-CoA-dependent ceramide synthase, was found to be located in the AAL-toxin biosynthetic (*ALT*) gene cluster of the tomato pathotype of *A. alternata*. *ALT7* and *Asc1* have the TLC (TRAM/Lag1/CLN8) domain characteristic of proteins involved in ceramide biosynthesis and are members of the *LASS/Lag* family. To test the hypothesis that *ALT7* and *Asc1*, both of which are *Lag1* ceramide synthase gene homologs, might share a common biological function as toxin tolerance genes, we have cloned and characterized *ALT7*. *ALT7*-deleted mutants were generated to investigate the effects of the deletion on vegetative growth, sporulation, toxin-sensitivity, toxin-production and pathogenicity. The deletion of *ALT7* has no deleterious effect on the toxin-producing pathogen, indicating that the gene does not act as a resistance/self-tolerance factor against the toxin in the toxin biosynthetic gene cluster.

4) Fungal growth and *in planta* distribution of host-specific AAL-toxin in tomato plants infected with the tomato pathotype of *A. alternata*

The tomato pathotype of *A. alternata* causes Alternaria stem canker of tomato by producing a host-specific AAL-toxin. The chemical structure and biological activities of AAL-toxin are analogous to those of the mycotoxin fumonisin. Determination of pathogen development and toxin production in infected tomato plants is of prime importance for pathological evaluation and risk assessment of AAL-toxin. On a resistant cultivar, AAL-toxin and the pathogen were restricted to a small region surrounding only the initial inoculation site. On the other hand, widespread distribution of the toxin and the pathogen were detected in a susceptible cultivar. The pathological significance and contamination risk from the toxin were supported by the results of in planta production of the toxin in diseased tomato plants.