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과제 일련번호: 10

Identification of novel pathogenicity genes by large-scale of insertional mutagenesis using *Agrobacterium tumefaciens*-mediated transformation in *Magnaporthe grisea*

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Magnaporthe grisea is a causal agent of rice blast and considered as a model pathogen for studying plant-microbe interactions. This is due to not only economic significance of rice blast disease worldwide but genetic and molecular tractability of this fungal pathogen. These features include genetic crossing with two different mating types, extensive genetic maps, developments of transformation and gene knock-out technologies. Currently whole genome sequence of strain 70-15 is available in the public database. To decipher fungal pathogenicity factors at genome-wide level in this fungus, we initiated a large-scale insertional mutagenesis using *Agrobacterium tumefaciens*-mediated transformation (ATMT). This project include (1) construction of transformants library (2) development of high throughput phenotype screening and DNA extraction systems, and (3) rescuing flanking sequences of T-DNA insertion from selected transformants. We generated 21,168 transformants and screened for 7 phenotypes as a high throughput manner; conidiation, conidial morphology, conidial germination, appressorium formation, mycelial growth, pigmentation, and pathogenicity. Over 1,000 loss of virulence and several hundreds of transformants including auxotrophs, development-defective and oleate-nonutilizing mutants were obtained from ATMT mutant library. The T-DNA tagged sequences from the selected transformants are being rescued by TAIL-PCR technology and 700 unique loci are identified thus far. To verify phenotype changes by T-DNA insertion, crossing with a wild type and targeted gene knock-out are being applied. Distribution and integration patterns of T-DNA on fungal chromosomes are also analyzed. Furthermore, we developed the ATMT database system to manage all phenomics and genomics data of these transformants.

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