

(05-1-15)

Isolation and characterization of virulence-deficient mutants *Xanthomonas oryzae* pv. *oryzae* by transposon mutagenesis

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Objectives

This study was to construct a mutant pool of *Xanthomonas oryzae* pv. *oryzae* (KACC10331) by transposon mutagenesis. Virulence-deficient mutants of Xoo were to isolate and characterize

Materials and Methods

1. Materials: *Xanthomonas oryzae* pv. *oryzae* strain- KACC10331 (K-1 race), Transposon mutagenesis: Electroporation using Transposome
2. Methods: Tn5 mutants of Xoo were inoculated in susceptible rice variety, Milyang 23.

Results and Discussion

Xanthomonas oryzae pv. *oryzae* (KACC10331) is causal agent of bacterial leaf blight on rice. Recently, whole genome sequence of the Xoo strain was determined and the functional studies of Xoo have been done on basis of the sequencing information. In this study, transposon was randomly introduced into the genome to identify virulence genes in *X. oryzae* pv. *oryzae* genome (KACC10331). Transposon mutant clones of Xoo were selected from media containing Kanamycin and inoculated on rice variety, Milyang 23 which is susceptible to Xoo for investigating their pathogenicity. Of primary screened 24,540 Xoo-mutant strains, 542 mutant strains showed intermediate or non-pathogenic feature. Furthermore, ideal non-pathogenic mutant strains were finally selected by second and third pathogenic screening. Insertion copy numbers of transposon in the mutant genomes were analyzed by Southern blot analysis. The transposon insertion sequences in the mutant genomes were determined by direct sequencing based on Tailing-PCR technique.