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Erwinia amylovora HrpN 단백질에 대한 돌연변이의 영향

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Effect of mutations on *Erwinia amylovora* HrpN protein

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Objectives

Fire blight caused by *Erwinia amylovora* is a devastating disease of rosaceous plants such as apple and pear. HrpN protein produced by *Erwinia amylovora* is composed of 403 amino acids and the major virulence factor of fire blight. The N-terminal half is known to be essential for interacting with HIPM of apple. Several mutant HrpN proteins carrying mutations in the N-terminal half were created. The effect of those mutations were tested on immature pear fruit to find out the region responsible for pathogenecity.

Methods

Electroporation. Electroporation was performed at 2.5 kV in a 0.2 cm cuvette with MicroPulser^{TM} Electroporation Apparatus from Bio-Rad Laboratories (Hercules, CA) as suggested by the manufacturer. The electroporated cells were plated on an LB agar plate containing 15 μg/ml gentamycin. Transformed cells were obtained after 2 days.

Preparation of genomic DNA and PCR. The primers used for PCR were *glmS* up (5'-CTGCGCTAACTAACCGGATACTTCCATTCTG-3') and *glmS* down (5'-GATGTAGACCAGCCGCGCAACCTG-3'). Conditions for PCR were as suggested by the manufacturer and the PCR product was analyzed by agarose gel electrophoresis.

Virulence assay. Each pear half was inoculated with *E. amylovora* Ea273 Δ*hrpN* carrying a mutated version of *hrpN* gene or an empty vector. *E. amylovora* Ea273 wild type strain was used as a positive control and buffer without any bacteria was used as a negative control. The inoculated pears

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were incubated at 26℃ and picture was taken at every 24 hrs after inoculation.

Enumeration of bacterial cell count. Approximately 10 μl of ooze was taken from each pear at 8 days after inoculation. Ooze was weighed and diluted serially by assuming one mg equals one μl. Diluted ooze was plated on a selective agar plate and incubated at 26℃ until colonies can be counted.

Results

Co-transformation of donor and helper plasmid. The donor plasmid used was pUC18R6K-mini-Tn7T-Gm carrying a copy of wild type or mutant *hrpN* gene. The helper plasmid used was pTns3. The transposition was verified by a PCR using *glmS* up and *glmS* down primers. All the transformants have the inserts of correct length at the correct site.

Pathogenicity test with immature pear fruit halves. Each mutant strain was tested in six replicas and incubated for eight days. Ooze began to be visible about 2 days after inoculation and necrosis was visible about 4 days after inoculation (Fig. 1). Both ooze and necrosis was visible for mutants 1, 2, 3, 4, 7 and wild type at 8 days after inoculation. Only ooze was visible for mutants 5, 6 and empty vector. None of ooze or necrosis was visible for the pear inoculated only with buffer except one pear. Ooze was produced at one pear inoculated only with buffer and this might be due to cross-contamination from the ooze from a neighboring pear. Since production of ooze but not necrosis was observed on the pears inoculated with the mutant strain carrying an empty vector and the host strain for this mutant is *E. amylovora* Ea273 *ΔhrpN*, it is obvious that intact HrpN protein is necessary not for production of ooze but for necrosis. One might conclude that the mutation in the *hrpN* gene of the mutants 5 and 6 knocked out the ability to cause necrosis. Further study would be necessary to clear out this aspect.

Bacterial cell count in the ooze. Ooze was taken from the pears inoculated with mutants 4, 5, 6, empty vector and wild type *E. amylovora* 273 and plated on the selective agars after serial dilutions. The results are shown in Table 1. The cell count was similar with one another for mutants 4, 5, 6 and empty vector, but it was much lower for the wild type strain. Since progress of pathogenecity was faster for the wild type strain than the others, the wild type cells might be in the death phase when the ooze was taken for analysis.