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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Elucidation of infection mechanism of *Ralstonia solanacearum* on ginger
（青枯病菌のショウガへの感染機構の解明）

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Ginger is a spice crop belonging to the *Zingiberaceae* family. In general, ginger is propagated vegetatively through rhizomes. In vitro propagation is an alternative way for ginger production. A plant pathogen, *Ralstonia solanacearum*, causes bacterial wilt disease in ginger. This study aimed to investigate the infection mechanism of *R. solanacearum* in ginger.

Type III effectors (T3Es) are considered host specificity determinants in bacterial plant pathogens. *R. solanacearum* strains isolated from ginger could contain specific T3Es for ginger infection. To identify the ginger-specific T3Es, complete genome sequences of six ginger strains were determined in this study. 69, 64, 65, 69, 72, and 64 of T3Es were detected in MAFF 211471, MAFF 211479, MAFF 211491, MAFF 301560, MAFF 241647, and MAFF 241648, respectively

Pathogenicity test requires many host plants. I have developed a new method to prepare enough ginger plants with in vitro regeneration. Ginger plants were regenerated *in vitro* with a combination of 4.5 mg/L of 6-benzyl adenine (BA) and 0.5 mg/L of 1-naphthalene acid (NAA) in Murashige-Skoog (MS) agar media from shoot tips. The regenerated plants obtained from stem segments were cultured in liquid MS media supplemented with NAA. The regenerated plants were separated individually and placed in the holes of plastic plates for floating in liquid media. The aseptically regenerated ginger plants were used to evaluate the pathogenicity by root dipping inoculation.

Yellowing of leaves of the infected ginger plants reproducibly began to appear within 15 days of post-inoculation (dpi), and ginger plants died at 28 dpi. The newly developed pathogenicity test of *R. solanacearum* using the in vitro regenerated ginger plants is suitable for high-throughput assay screening mutants, which lose pathogenicity.

Type III secretion system (T3SS) is a major determinant of the pathogenicity in *R. solanacearum*, and genes for T3SS are located on the genome as an *hrp* gene cluster. Aseptically regenerated ginger plants were infected with four inoculation methods: root-dipping inoculation, leaf-clipping, pseudostem, and leaf infiltration to evaluate the pathogenicity on ginger. In root-dipping inoculation, wilt symptoms such as yellowing of leaves were reproducibly observed when a wild-type strain MAFF 211479 was inoculated. MAFF 301069 isolated from diseased tobacco did not show the symptom on the ginger plants. Four hrp mutants were constructed using *hrpG*, *hrpB*, *hrpY*, and *hrcJ* genes, which lost pathogenicity on *Nicotiana benthamiana* and eggplants. These mutants inoculated the regenerated ginger plants with root-dipping, leaf-clipping, and pseudostem inoculation methods. None of the mutants caused wilt symptoms. The mutant cells proliferated less efficiently than the wild type in the inoculated ginger plants. Based on these results, we conclude that the aseptically regenerated ginger plants could be used to elucidate the infection mechanism of *R. solanacearum* in ginger.