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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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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）

学位論文要約：
Dissertation Summary

Ginger is an important spice crop vegetatively propagated through rhizomes. In vitro propagation is an alternative way for ginger production. *Ralstonia solanacearum*, a bacterial wilt causal, hampers ginger production. The virulence is usually evaluated by artificially infecting *R. solanacearum* cells into conventionally grown ginger plants. Although the research in monocot plants like ginger is limited. This study aimed to investigate the infection mechanism of *R. solanacearum* in ginger using in vitro propagation as an alternative solution of ginger host preparation.

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

Since ginger takes long time to mature and pathogenicity test requires many host plants. Generally, conventionally grown ginger plants are used to evaluate the virulence of *R. solanacearum*. 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. Wilt symptoms such as yellowing of leaves were reproducibly observed when a wild-type strain MAFF 211479 was inoculated with root-dipping method in aseptically regenerated ginger plants. MAFF 301069 isolated from diseased tobacco did not show the symptom on the ginger plants. When the regenerated ginger plants inoculated with MAFF 211479 mutants in *hrp* genes, none of mutants caused wilt symptoms. The ginger plants were inoculated with the mutants using pseudostem and leaf-clipping inoculation methods, the inoculated plants did not show wilt symptoms either. 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.

Complete genome sequences of *Ralstonia solanacearum* strains isolated from infected ginger plants.

In Chapter 1, I present the complete genome sequences of *Ralstonia solanacearum* strains isolated from ginger plants. There are many complete genome sequences of strains infecting Solanaceae plants, and we plan to compare our sequences to these references to find out differences to determine the specific factors to ginger strains. Bacterial type III effectors (T3Es), which are secreted from the type III secretion system (T3SS), are one of the major pathogenic determinants and important factors in determining host specificity. 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. This whole-genome shotgun (WGS) project has been deposited at DDBJ/EMBL/GenBank. The versions described in this paper are the first versions. The read archives have been deposited in DDBJ DRA.

New evaluation method of *Ralstonia solanacearum* infection on ginger using aseptically regenerated ginger plants.

In Chapter 2, I reported a new pathogenicity assay of *R. solanacearum* on aseptically regenerated ginger plants as an alternative solution which is a more natural way for bacterial inoculation. Firstly, I regenerated ginger plants in MS (Murashige-Skoog) agar media with combination concentrations of 6-benzyl adenine (BA) and 1-naphthyl acetic acid (NAA) using shoot tips. First regenerated plants stems were used to multiply the regenerated plants in large numbers. Regenerated stems were cultured in MS agar media supplemented with the combination concentration of BA 4.5 mg/L and NAA 0.5 mg/L. Plants were separated into single stems from the clumps of regenerated plants with 3-4 roots. The individual plant placed into a polypropylene sheet hole was floated in liquid MS media with NAA and cultured in the growth chamber for 4 weeks at 25±1 °C with a 16 h light/8 h dark-light cycles. These regenerated ginger plants were used for *R. solanacearum* infection. Regenerated ginger plant roots were dipped into a bacterial suspension (100 ml) at a concentration of 10^8 cells/ml for 20 min. Inoculated plant roots were washed 4 times with autoclaved DW. Plants were transferred to liquid MS media without growth hormones. 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.

Elucidation of infection mechanism of *Ralstonia solanacearum* on ginger using aseptically regenerated plants

In Chapter 3, I presented elucidation of infection mechanism of *Ralstonia solanacearum* on ginger using aseptically regenerated ginger plants. The pathogenicity is evaluated by infecting conventionally grown ginger plants with *R. solanacearum* cells. In this study, aseptically regenerated ginger plants were infected with four inoculation methods: root-dipping inoculation, leaf-clipping, pseudostem, and leaf infiltration to test the pathogenicity of *R. solanacearum* in ginger. T3SS is a major determinant of the pathogenicity in *R. solanacearum*. Genes for T3SS and T3Es are clustered on the genome as a *hrp* regulon. *R. solanacearum* strains MAFF 211479 used in this study as wild-type, which contains the *hrp* regulon. Both *hrpG* and *hrpB* encode the transcriptional regulator, which regulates the entire *hrp* regulon. *HrpY* and *HrcJ* are components of T3SS. In this

study, deletion mutants in the *hrp* genes of *hrpG*, *hrpB*, *hrpY*, and *hrcJ* of the MAFF 211479 strain infecting ginger were constructed, and the involvement of T3SS on the pathogenicity of *R. solanacearum* ginger strains was investigated. All of these mutants lost pathogenicity on *Nicotiana benthamiana* and eggplants. 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. The regenerated ginger plants inoculated with MAFF 211479 mutants in *hrp* genes were healthy throughout the experiments. When the regenerated ginger plants were inoculated with the mutants using pseudostem and leaf-clipping inoculation methods, the inoculated plants did not show wilt symptoms either. 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.