（第３号様式）(Form No. 3)

学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Studies on virulence diversity of a multi-host plant bacterium, *Pseudomonas cichorii*
（多犯性植物細菌*Pseudomonas cichorii*の病原力の多様性に関する研究）

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Pseudomonas cichorii is a multi-host plant bacterium. *P. cichorii* strain SPC9018 (SPC9018) causes rot symptoms in lettuce, though the bacterium does not produce pectate lyase, which is the most important plant cell wall-degrading enzyme. The development of disease symptoms is closely associated with programmed cell death (PCD) following heterochromatin aggregation and laddering of genomic DNA in SPC9018-infected lettuce cells. SPC9018 also causes necrotic spots on eggplant distinct from the disease symptoms on lettuce. Development of necrotic spot symptoms is observed following PCD in eggplant leaves. SPC9018 harbors the *hrp* genes encoding proteins in the type III secretion system. The *hrp* genes are essential for virulence of this bacterium in eggplant but not lettuce, indicating that the *hrp* genes are implicated in the diversity of SPC9018 virulence. The *aldH* encoding aldehyde dehydrogenase and *pat* encoding *N*-acetyltransferase are located in the flanking region of the *hrp* genes. The objective of this study is to analyze its virulence diversity on multi-host plants.

Phylogenetic study of the *hrp* genes and *pat* based on the nucleotide sequences showed that a pathogenicity island of *P. cichorii* consists of these genes. The *aldH* was though to conserve among genome of pseudomonad. The *aldH*-deleted mutant and *pat*-deleted mutant lost their virulence on eggplant but not lettuce. Inoculation into *Asteraceae* species susceptible to SPC9018 showed that the involvement of *hrp*, *pat* and *aldH* in SPC9018 virulence is independent of each other and has no relationship with the phylogenetic diversity of *Asteraceae* species based on the nucleotide sequences of *ndhF* and *rbcL*. These results suggest that not only the *hrp* genes but also *aldH* and *pat* are

implicated in the diversity of SPC9018 virulence on susceptible host plant species. Therefore, virulence diversity of *P. cichorii* might be established after species diversification of *Asteraceae* plants and be responsible for the virulence of respective species.

From the present results, it was thought that SPC9018 has several virulence mechanisms. I then analyzed the influence of limited iron acquisition on SPC9018 virulence. High performance liquid chromatography and liquid chromatography-mass spectrometry analyses showed that SPC9018 produces pyoverdine. Spectrophotometric assay showed that the total concentration of pyoverdine is negatively correlated with the bacterial density. The *in vitro* growth of SPC9018 with mugineic acid (MA), a phytosiderophore, was lower than that without MA. When $FeCl_3$ was included in the medium, the *in vitro* growth of SPC9018 in the presence of MA was complemented. Application of MA reduced SPC9018 virulence on 15 host species including eggplant, but did not influence SPC9018 virulence on five host species including lettuce. Furthermore, MA application led to loss in SPC9018 virulence on eight host species. The population of SPC9018 in the intercellular spaces of eggplant leaves with MA was significantly lower than that without MA. MA application enhanced expression of pyoverdine production-related *pvd* genes in SPC9018. Furthermore, MA application reduced adhesion activity of SPC9018. These results suggests that limited iron acquisition plays specific role in adhesion of *P. cichorii* to the intercellular spaces and its growth in the intercellular spaces, involved in its virulence on respective host plants.

Interestingly, the *pat*-deleted mutant showed virulence on tested plant species, at levels similar to those of SPC9018 applied with MA. The deletion of *pat* resulted in decreased production of pyoverdine at bacterial densities of less than 1.0×10^8 cfu/ml, leading to decreased siderophore activity and iron acquisition. Furthermore, at a bacterial density of 2.0×10^7 cfu/ml, the *pat* deletion enhanced expression of *pvdL* and *pvdR* to a similar degree as SPC9018 treated with MA. The *pat* is therefore involved in not only pyoverdine-mediated iron acquisition, implicated in SPC9018 virulence on respective host plants.

In conclusion, SPC9018 implicate not only the *hrp* genes but also *aldH* and *pat* in its virulence on respective host species. Iron acquisition by SPC9018, in which *pat* is involved, plays an important role in adhesion ability of this bacterium in the intercellular spaces of infected host plants immediately after invasion, establishing its virulence diversity on the respective host plants.