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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

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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Dissertation Summary

Plant pathogens differ with respect to the kinds of plants that they can attack, to the organs and tissues that they can infect, and to the age of the organ or tissue of the plant on which they can grow. The pathogenicity of pathogens to plants and animals has evolved through an arms race of attack and defense. On interactions between plants and pathogens, Jones and Dangle have demonstrated a simple and elegant view of innate immunity model, the zigzag model. This model proposes that the first line of active plant defense is formed by pattern recognition receptors (PRRs). Pathogen-associated molecular patterns (PAMPs) are recognize by these cell surface PRRs. PAMPs are originally defined as highly conserved molecules within a class of microbes that have an essential function in microbial fitness or survival. PRRs activate an innate immune response upon detection of PAMPs (PAMP-triggered immunity, PTI). Successful pathogens are able to overcome PTI by means of secreted effectors that suppress PTI responses, resulting in induction of effector-triggered susceptibility (ETS). A given effector is specifically recognized by one of the NB-LRR proteins, resulting in effector-triggered immunity (ETI). The ETI is an accelerated and amplified PTI response, resulting in disease resistance and, usually, a hypersensitive

response at the infection site. As a result of selection pressure, pathogen isolates have evolved to obtain novel effectors to suppress the ETI response. In turn, new plant receptors have evolved to recognize either the obvious effectors or the newly acquired effectors, resulting in ETI. This coevolution proceeds, with continuous selection for novel pathogen isolates that overcome ETI and new plant genotypes that resurrect ETI. The immune responses activated in ETI occur quicker, and are more prolonged and robust than those in PTI.

Bacterial plant pathogens have evolved diverse and complex mechanisms for interacting with their respective hosts. The most clearly understood example of such an interaction can be seen in the utilization of the type III secretion system (TTSS) and its associated type III effectors by bacterial plant pathogens. The TTSS is conserved among a wide range of both animal pathogens (e.g. *Escherichia coli*, *Salmonella enterica*, *Shigella spp*., and *Yersinia spp*.) and plant pathogens (e.g. *Pseudomonas syringae*, *Xanthomonas* sp., *Erwinia* sp. and *Ralstonia* sp.) and is required for the full bacterial virulence. Phytopathogenic bacteria deliver dozens of type III effectors per strain into host cells using the TTSS. The type III effectors contribute to pathogen virulence, often by mimicking or inhibiting eukaryotic cellular functions. A *P. syringae* strain mutated in the TTSS is unable to deliver any type III effectors and triggers a faster and stronger transcriptional reprogramming in bean than does the isogenic wild-type strain. The mutant strain, representing the sum of all bacterial PAMPs, induces transcription of essentially the same genes as *flg22*. Hence, the type III effectors from any successful bacterial pathogen dampen PTI sufficiently to allow successful colonization. It is thus thought that type III effectors may be involved in host range determination of the bacteria.

Disease development in plants is thought to be a passive event after an attack by

phytopathogenic bacteria. In plants, the presence of rigid walls surrounding cells limits the possibilities for the microbe to thrive intracellularly. Plant pathogens often produce cell wall degrading enzymes, leading to cell injury and access to nutrients. The pathogenicity of multi-host plant bacteria is thought to depend on non-specific toxins and/or enzymes that affect substances or processes found commonly among plants, similar to those of other non-obligate parasites. The main virulence factor for soft rot bacterium *Pectobacterium carotovorum* which is one of multi-host plant bacteria, is pectate lyase, which degrades components of plant cell walls, leading to rot symptoms on its host plants. Furthermore, the main virulence factor of the wilt bacterium *R. solanacearum,* which is also a multi-host plant bacterium, is exopolysaccharide, which leads to reduced sap flow in xylem vessels and wilt symptoms in infected plants. It is thus thought that virulence of most multi-host plant bacteria on their host plants is dependently on the main virulence factor.

Pseudomonads are widespread Gram-negative bacteria displaying various lifestyles and habitats. *Pseudomonas cichorii* is a gram negative multi-host plant bacterium. It was first isolated on endives (*Cichorium endivia*). *P. cichorii* can infect so many kinds of vegetables, ornamentals, cereals and woody plants, especially economically important plant cultivars including lettuce, eggplant, celery, chrysanthemum, tomato, coffee, and soybean. *P. cichorii* infects distinct organs and causes distinct symptoms according to the host species. Therefore, the common names of plant diseases in Japan reported that the bacterium causes bacterial black spot on sweet basil, bacterial blight on pot marigold and gerbera, bacterial brown spot on bellflower and strawberry, bacterial leaf blight on sweet pepper, white clover, sunflower and okra, bacterial leaf spot on chrysanthemum and tickseed, bacterial rot on melon, endive and garlic, bacterial spot on celery, and bacterial vein blight on cinnamon. Though differences in the symptoms

suggest that *P. cichorii* may have several virulence mechanisms, and the responses of each host plant to infection with the bacterium may differ according to the host plant species, virulence mechnisms of *P. cichorii* on multi-hosts has remained unclear.

In this study, I analyzed virulence diversity of *P. cichorii* strain SPC9018 (SPC9018) on multi-host plants. I genetically and phylogenetically analyzed virulence diversity of SPC9018, and identified a genomic region including the *hrp* genes encoding components of TTSS, aldehyde dehydrogenase gene (*aldH*) and an *N*-acetyltransferase gene (*pat*), which are involved in its virulence diversity. I analyzed involvement of SPC9018 iron acquisition in its virulence diversity. Last I analyzed implication of *pat* in iron acquisition of SPC9018, leading to its virulence diversity. I summarized functional and phylogenetical mechanisms of virulence diversity of SPC9018.

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 14 host species including eggplant, but did not influence SPC9018 virulence on five host species. 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.

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