学位論文の要約

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論文題目　Molecular study on dynamic interaction between *Ralstonia solanacearum* and its phages in bio-control of bacterial wilt disease
(青枯病菌バイオコントロールにおけるファージ宿主間動的相互作用の分子解析)

R. solanacearum causes a vascular wilt disease and has been ranked as the second most important bacterial pathogen. It is one of the most destructive pathogens identified to date because it induces rapid and fatal wilting symptoms in host plants. Bacteriophages have recently been evaluated for controlling a number of pathogenic bacteria and are now commercially available for some diseases. Major challenges of agricultural use of phages arise from the inherent diversity of target bacteria, high probability of resistance development, and weak phage persistence in the plant environment. To maximize the use of bacteriophages in biocontrol on bacterial wilt disease, thorough understanding both of phage ecology and the complex phage-host interactions in various environments is necessary. Genomic information on the phages and their host bacteria (*R. solanacearum* strains) will help in this regard. Recently, various phages infecting *R. solanacearum* were characterized. Five podoviruses of these were distinctively separated into two groups based on the genomic position of the RNA polymerase gene, □T7-type and □KMV-type phages. In this work, I further investigated in their characterization and comparative genomics. I also studied the phage resistance mechanism of *R. solanacearum* through *pilQ* mutation and transposon mutagenesis method.

The genome organization, gene structure, and host range of five podoviruses that infect *Ralstonia solanacearum*, the causative agent of bacterial wilt disease were characterized. The phages fell into two distinctive groups based on the genome position of the RNA polymerase gene (i.e., T7-type and φKMV-type). φKMV group phages subdivided into two subgroups by phylogenetic analysis of the podovirus major genes. One-step growth experiments revealed that φRSB2 (a T7-like phage) lysed host cells more efficiently with a shorter infection cycle (ca. 60 min corresponding to half the doubling time of the host) than φKMV-like phages (with an infection cycle of ca. 180 min). Most phages had wide host-ranges and the phage particles usually did not attach to the resistant strains; when occasionally some did, the phage genome was injected into the resistant strain's cytoplasm, as revealed by fluorescence microscopy with SYBR Gold-labelled phage particles.

PilQ is a member of the secretin family of outer membrane proteins and specifically involved in type IV secretion. Here I examined the effects of *pilQ* mutation in *R. solanacearum* on the host physiology including susceptibility to several phage types (*Inoviridae, Podoviridae* and *Myoviridae*). With three lines of cells, namely wild type, Δ*pilQ* and *pilQ*-complemented cells, the cell surface proteins, twitching motility and sensitivity to phages were compared. SDS-PAGE analysis revealed that the major TFP pilin (PilA) was specifically lost in *pilQ* mutants and was recovered in the complemented cells. Drastically inactivated twitching motility in *pilQ* mutants was recovered to the wild type level in the complemented cells. Several phages of different types including those of *Inoviridae*, *Podoviridae*, and *Myoviridae* that infect wild type cells could not form plaques on *pilQ* mutants but showed infectivity to *pilQ*-complemented cells. These results indicate that PilQ function is generally required for phage infection in *R. solanacearum*.

One of the important problems in using phages for biocontrol is that host bacteria often change to become phage-resistant. Thus far, some bacteriophage-resistance-mechanisms have been already characterized, for example, restriction/modification systems, the CRISPR-Cas system, and abortive infection system. But there may still be unknown mechanisms of phage-resistance; not all bacteria have the CRISPR-Cas system, so that, alternative system are possible. In the previous

research (Fujiwara, 2011), a random-gene-knockout library of strain Ps29 (race 1) was prepared by using an EZ-Tn5<KAN-2> Tnp Transposome kit (Epicentre Biotechnologies), and 20 transposon mutants were isolated that showed resistance to φRSA1 and/or φRSB1 (among 5,184 mutants; approximately 0,3%). Interestingly, some of 20 phage-resistant mutants turned out to be resistant to ϕRSL1, too (Fujiwara, 2011). These data might suggest that there is general phage-resistant system, irrespective of the phage-type. Therefore, in this study the importance gene that involved in those mutations were determined clearly in order to gain comprehensive analysis of phage-resistant system of *R. solanacearum*. In region 3 that consists of two mutants, O25-C1 and O45-E3, the gene involves in phage resistance mechanism was the probable transmembrane protein. While gene involves in phage-resistance mechanism of region 4 was shown to be putative glycosyl transferase for O2-A3 and probable lipopolysaccharide o-antigen ligase transmembrane for O28-C5, which are associated with LPS biosynthesis of Gram-negative bacteria.

Phage–host dynamics may also be important in the re-emerging field of phage therapy. Because the phenomenon of antibiotic resistance has grown into a global public health concern, phage therapy is now being re-evaluated as a means to treat or prevent bacterial infections worldwide. The advantages and disadvantages of phage therapy have already been extensively documented. One of the notable disadvantages is the risk of encountering phage-resistant bacterial pathogens or favoring the emergence of phage-insensitive bacterial strains. Comprehensive reviews concerning interaction of *R. solanacearum* and its phages mechanism have been studied in this study. Five phages isolates from Japan and Thailand have different host range. Three different conditions in the phage-resistant cells were revealed by fluorescence microscopy of cells infected with SYBR-gold labeled phages: (i) low adsorption of phages to the cell, (ii) positive adsorption but no injection of phage DNA into the cells, and (iii) positive adsorption and injection of DNA into the cells. The former two conditions may be explained by the insufficient interaction between phage receptors on the cells and host binding proteins on the virion. The phage-resistant strains displaying a type iii response may drive an unknown abortive infection mechanism. It is important to understand phage-host interaction deeply. In this study, it was shown that *pilQ* mutants of two different strains were converted to be resistant to phages of different families including *Myoviridae*, *Podoviridae* as well as *Inoviridae.* Most of such myoviruses and podoviruses recognize LPS on the cell surface as a receptor and adsorbed normally to the cells of *pilQ* mutants. Therefore, infection processes after cell adsorption were somehow blocked in these pilQ mutants. It is suggested that PilQ involved in phage infection. However, PilQ is one of the most important virulence factors of *R. solanacearum*, so that PilQ-mutants always lose the virulence.