論文の要旨

氏名 **Abdelmonim Ali Ahmad Ebrahim**

論文題目 **Biotechnological application of bacteriophages for controlling citrus canker and citrus bacterial spot**
(カンキツかいよう病バイオコントロールへのファージの有効利用)

Asiatic citrus canker (ACC), which is caused by the phytopathogenic bacterium *Xanthomonas axonopodis* pv. *citri* (*Xac)* , is one of the biggest problems in citrus production worldwide. Given the difficulties in controlling this disease using conventional methods, considerable efforts have been made to find alternative strategies. Recently, biological controlling agents, especially bacteriophages, have been successfully used in several plant diseases and are also promising candidates for control of ACC. However there may be many challenges that are facing and limiting the success of bacteriophages as biological control agent. These include the narrow host range and high specificity, which put phages at a disadvantage against other anti-bacterial materials such as antibiotics. Furthermore, the emergency of resistance mutants is a practical problem, which significantly limits the use of bacteriophages as abiocotrol agent. Other major affecting factors are the environmental effects such as temperature, pH, host physiology and inactivation by UV light. These environmental factors have great negative impact on the ability of phage to infect the target pathogen as well as on the persistence of phages in both phyllosphere and rizosphere. Molecular characterization and studies of the biology and ecology of phages are very important to understand the phage host interaction and to figure out the most effective way of bacteriophage utilization as biological control agents.

The first subject of this study was to make a full characterization of the historical *Xanthomonas* Cp1 and Cp2 phages. Nearly *all Xac* strains isolated from different regions in Japan are lysed by either of phage Cp1 or Cp2; Cp1-sensitive ($Cp1^S$) strains have been observed to be resistant to Cp2 ($Cp2^R$) and *vice versa*. Morphologically, Cp1 belonged to the *Siphoviridae*. Both Cp1 and Cp2 form clear plaques with various *Xac* strains. The infection cycle of Cp1 showed that the latent period was ~ 60 min, followed by a raise period of 20-30 min, giving an entire cycle of 80-90 min with average burst size of ~20 plaque forming unit per infected cell. For Cp2 replication, the infection cycle showed that the latent period was ~90 min, with a 60 min raise period, taking 150-180 min per cycle with burst size of ~100 plaque forming unit per infected cell. Genomic analysis revealed that its genome comprised 43,870-bp dsDNA, with 10-bp 3'-extruding cohesive ends, and contained 48 open reading frames (ORFs). The genomic organization was similar to that of *Xanthomonas* phage phiL7, but it lacked a group-I intron in the DNA polymerase gene. Cp2 resembled morphologically *Escherichia coli* T7-like phages of *Podoviridae*. The 42,963-bp linear dsDNA genome of Cp2 contained terminal repeats. The Cp2 genomic sequence had 40 ORFs, many of which did not show detectable homologs in the current databases. By proteomic analysis, a gene cluster encoding structural proteins corresponding to the Class III module of T7-like phages was identified on the Cp2 genome. Therefore, Cp1 and Cp2 were found to belong to completely different virus groups. In addition, I found that Cp1 and Cp2 used different molecules on the host cell surface as phage receptors and that host-selection of *X. axonopodis* pv. *citri* strains by Cp1 and Cp2 was not determined at the initial stage by binding to receptors. Further investigation was done to examine the fate of the phage DNA after cell attachment to non-host strains. This was conducted by adding the SYBR-gold labeled phages to cells of *Xac* then the movement of SYBR Gold-phage DNA was monitored. The result showed that the DNA of Cp1 and Cp2 was injected into the host cells as well as into the

non-host cells. Thus, further molecular studies are needed to deeply understand the molecular mechanism of host selection by these phages.

My second goal was to find a better solution that may contribute to solving the major challenge associated with the use of phages, mainly instability of phages during treatment of field crop plants. XacF1, which can infect *Xanthomonas axonopodis* pv. *citri* (*Xac*) strains, was isolated and characterized. Electron microscopy showed that XacF1 was a member of the family *Inoviridae* and was about 600 nm long. The genome of XacF1 was 7,325 nucleotides in size, containing 13 predicted ORFs, some of which showed significant homology to Ff-like phage proteins such as ORF1 (pII), ORF2 (pV), ORF6 (pIII), and ORF8 (pVI**).** XacF1 showed a relatively wide host range, infecting seven out of 11 strains tested in this study. Frequently, XacF1 was found to be integrated into the genome of *Xac* strains. This integration occurred at the host *dif* site (*att*B) and was mediated by the host XerC/D recombination system. The *att*P sequence was identical to that of *Xanthomonas* phage Cf1c**.** Preliminary results of the deletion mutant of XacF1 lacking ORF12 (ΔXacF1) showed that XaCF1could not integrated into the host genome and solely replicated as episomic forms. Interestingly, infection by XacF1 phage caused several physiological changes to the bacterial host cells, including lower levels of extracellular polysaccharide production, reduced motility, slower growth rate, and a dramatic reduction in virulence. In particular, the reduction in virulence suggested possible utilization of XacF1 as a biological control agent against citrus canker disease. The *Inovirus* nature of XacF1 establishing a coexisting state with the host cells ensures long-lasting effects of this phage. This is an advantage of filamentous phages to solve the problem of bacteriophages easily inactivated by sunlight UV irradiation and other environmental factors.

This research provides valuable information and new insight into the molecular mechanism of host selection between Cp1 and Cp2; the historical problem in phytopathology as well as the molecular basis of loss of virulence caused by XacF1 infection. They may open a new strategy of phage therapy, solve many of the problems, which are associated with phage application including the weak persistence of phage in the environment, and bring an ecofriendly sustainable way for next-generation agriculture or food production.