Loss of Virulence in *Ralstonia solanacearum* Strains by Infection with ϕRSM Phages and Utilization of the Avirulent Strains as Biocontrol Agents against Tomato Bacterial Wilt

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Ralstonia solanacearum is a widely distributed soil-borne phytopathogen belonging to the β subdivision of Proteobacteria. It causes lethal bacterial wilt of more than 200 plant species, including tomato. During infection, *R. solanacearum* cells express various virulence and pathogenicity factors resulting in typical wilting symptoms in host plants. ϕRSM1 and ϕRSM3 (ϕRSM phages) are filamentous phages (inoviruses) that infect *R. solanacearum*. Infection by ϕRSM phages causes several cultural and physiological changes to host cells, especially loss of virulence. In this study, we characterized changes related to the virulence in ϕRSM3-infected cells, including (i) reduced twitching motility and reduced amounts of type IV pili (Tfp), (ii) lower levels of β-1,4-endoglucanase (Egl) activity and extracellular polysaccharides (EPS) production, and (iii) reduced expression of certain genes (*egl*, *pehC*, *phcA*, *phcB*, *pilT,* and *hrpB*). The significantly lower levels of *phcA* and *phcB* expression in ϕRSM3-infected cells suggested that functional PhcA was insufficient to activate many virulence genes. Tomato plants injected with ϕRSM3-infected cells of different *R. solanacearum* strains did not show wilting symptoms. The virulence and virulence factors were restored when ϕRSM3-encoded *orf15*, the gene for a putative repressor-like protein was disrupted. Expression levels of *phcA* as well as other virulence-related genes in ϕRSM3-ϕORF15-infected cells were comparable with those in wild type cells, suggesting that *orf15* of ϕRSM3 may repress *phcA* and consequently result in loss of virulence. Causative strains isolated from wilted tomato can be easily converted to be avirulent and be used to prevent expansion of the disease on-site.