

# 論文の要旨

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論文題目

## **Molecular biological studies on chemotaxis of *Ralstonia solanacearum* and development of novel method to control bacterial wilt**

(青枯病菌 *Ralstonia solanacearum* の走化性の分子生物学的解析と新規感染防除技術開発)

### **Introduction:**

*Ralstonia solanacearum*, a soil-borne bacterium, is the causal agent of bacterial wilt disease in economically important crops including tomato, potato, tobacco etc. Having been hampered by persistency of *R. solanacearum* to survive for years in wet soil and by a broad diversity of the pathogen strains, the available disease managements remain limited success. Previous studies found that chemotaxis is essential for the early stage of host invasion, virulence and pathogenic fitness of this pathogen, providing us a new target for the disease control. However, the signal compounds attracting this pathogen to host root are unclear. In chemotaxis sensory system, specific chemicals are detected by cell membrane-bound chemoreceptors, called methyl-accepting chemotaxis proteins (MCPs). *R. solanacearum* strains possess more than 20 MCPs. So far only four of these MCPs were characterized, although several other compounds were reported as chemoattractant for *R. solanacearum*. In this study, *R. solanacearum* strain Ps29, a superior motility phenotype strain, has been chosen as chemotactic studied model. And *R. solanacearum* strain MAFF106611, a highly virulent strain in tomato, has been used in virulent studies.

### **Topic 1: Identification and characterization of chemoreceptors mediating positive response to D-malate, an unnatural enantiomer of malate**

*R. solanacearum* Ps29 exhibited attractive response to a wide range of organic compounds. One of interesting attractants is D-malate, an unnatural occurred form and non-metabolizable compound. Since chemotaxis is believed to assist bacteria in efficiently moving toward environments suitable for their growth, it was very curious why this pathogen showed highly chemotactic response to D-malate. Among 22 MCPs strain Ps29 possesses, McpT and McpM were identified as D-malate chemosensors. McpT was newly introduced in this study and serves as a fifth identified MCP in *R. solanacearum*, while McpM was previously reported as L-malate sensor. Investigation of ligand specificity of both MCPs revealed that McpT and McpM are broad-ligand-specificity chemoreceptors. Besides McpT is chemoreceptor distinguishing attractive response toward L-tartrate which has structurally and stereochemically similar to D-malate. These results likely suggested that McpT and McpM are chemoreceptor mediate taxis to naturally occurring compounds (McpT is L-tartrate sensor and McpM is L-malate sensor) which also are able to sense structurally similar chemicals including non-natural occurred enantiomers and non-metabolized compounds such as D-malate and D-tartrate. While McpM was previously reported as essential chemoreceptor facilitating *R. solanacearum* to

tomato root, McpT was not likely involved during the early stage of host invasion in gnotobiotic sand virulence assay. Therefore, McpT-mediated chemotaxis is likely to play a role in their persistence and better survival in soil by allowing the pathogen to find environmental sources of L-tartrate which is their growth and energy substrate.

### **Topic 2: Identification and characterization of chemoreceptor mediating negative response to maleate**

To develop the method to control bacterial wilt disease targeted on motility of this pathogen, one of interesting approaches is using repellent compound. I measured the chemotactic response of *R. solanacearum* Ps29 to various compounds and found that it was repelled by maleate. The movement of bacteria away from maleate was drastic that cell number around the capillary decreased about 60% at 30 seconds exposed with 5 mM maleate and continuously decreased to approximately 70% at one minute. Like most of reported repellents, maleate is harmful to Ps29 by reducing its growth rate. By screening of a complete collection of single-*mcp*-gene deletion mutant of Ps29, McpP was identified as a sole receptor mediating negative chemotaxis to maleate. The *mcpP*-deletion mutant was weakly attracted by maleate, indicating that this bacterium possesses MCPs for both positive and negative chemotaxis to maleate. Interestingly, results from characterization of McpP revealed that this MCP senses citrate and inorganic phosphate as chemoattractant. Seven other *R. solanacearum* strains, including MAFF106611, possessing functional *mcpP* orthologues were not repelled by maleate. Quantitative RT-PCR analysis revealed that these seven *R. solanacearum* strains did not show negative chemotaxis to maleate because of negligible transcription of *mcpP* genes. McpP serves as sixth identified MCP in *R. solanacearum*. This work also expands the list of known chemorepellent in bacteria.

### **Topic 3: Development of a new method to control bacterial wilt**

*R. solanacearum* strain MAFF106611 showed strongly positive chemotactic responses toward both L- and D-malate. MAFF106611 possesses McpM and McpT orthologues with amino acid sequence highly identical to that of Ps29 (99.8% and 100% respectively). From previously report, *mcpM*-deletion mutant, a chemotactic deficient strain toward L-malate, significantly reduced in virulence in tomato. As one of major component in tomato root exudates, L-malate may be one of the signal compounds attracting bacteria to tomato root. Consequently, there is the possibility that soil amended with malate can interfere the pathogen chemotaxis and blind their key signal causing a delay or even suppression the infection.

The experiments were conducted in gnotobiotic sand system of tomato by placing MAFF106611 cell at a certain distance away from the plant. Interestingly, about 60% reduction of infected tomato was observed from both L- and D-malate treatments as well as mix DL-malate treatment. The other treatments such as serine, aspartate and lactate did not show the suppression effect. When directly injected the pathogen into tomato root, same virulent level was observed in both malate and non-treatment condition. These results likely indicated that the inhibition effect of malate treatments may be caused by inefficiently motile to host root at the early stage of host-invasion. To clarify whether chemotaxis is mechanism behind this inhibition,  $\Delta cheA$  mutant, a non-chemotactic strain, was applied to tomato instead of wild-type strain. However, D- and

DL-malate treatments still showed inhibition effect on the disease, while L-malate treatment did not. Results from competitive chemotaxis assay showed that presence of L-malate in the assay buffer abolished MAFF1106611 L-malate response. The presence of D-malate, besides, hugely reduced the L-malate response. Moreover, D-malate was found to suppress *R. solanacearum* twitching motility, which is also required for the pathogen full virulence. Taking together, soil amendment with malate has a great potential to suppress bacterial wilt disease at host-invasion stage, since L- and D-malate can interfere *R. solanacearum* chemotaxis, as well as D-malate can inhibit twitching motility.