別紙２

Results report

１． Title of Research and Development : Genetic and pathogenic diversity of *Pantoea ananatis* strains

２． Principal Investigator : Yuichi Takikawa （Professor, Graduate School of Science and Technology, Shizuoka University）

３． Counterpart Principal investigator : Teresa A. Coutinho （Professor, Microbiology and Plant Pathology, Natural and Agricultural Sciences, University of Pretoria（South Africa））

４． Results of Research and Development:

This research aimed to clarify the genetic and pathogenic diversity of *Pantoea ananatis* and to develop a method identifying the pathogenic strain infecting important crops such as rice, corn and onions, in collaborative work between Japanese and South African researchers.

We had revealed the presence of a new pathogenicity determining genetic locus in *P. ananatis*, which was named as PASVIL (*P. ananatis* specific virulence locus). RT-PCR techniques were applied to determine the operons in PASVIL. More than 3 operons having 16 ORFs could be identified and the putative promoter regions were suggested. Introduction of the entire PASVIL region to a non-pathogenic *P. ananatis* strain lead to acquisition of pathogenicity on tobacco and onions. Using this PASVIL introduction system together with in vitro transposon insertion technique revealed that not the all ORFs in PASVIL were required for pathogenicity but some were dispensable. Construction of a plasmid vector for site-directed mutagenesis enabled us to disrupt any genes and to assay their contributions to pathogenicity. Some PCR primer sets could detect PASVIL and its homologue in *P. ananatis* and *P. agglomerans* strains. These primers were considered as very useful tools for rapid detection of pathogenic strains in *Pantoea* without time-consuming bioassay methods. A PASVIL homologue found in *P. agglomerans* has ca. 80% sequence homology with PASVIL of *P. ananatis*, suggesting that considerable time has passed since the horizontal transfer of PASVIL took place between theses two species.

The Type VI protein secretion system gene sets had been also identified in the genome of P. ananatis strains. The significance of the Type VI secretion systems in plant pathogenesis was assayed by disrupting the genes using site-directed mutagenesis. Our study revealed that the disruption of Type VI secretion system of *P. ananatis* strain isolated from rice in Japan did not abolish its pathogenicity, but significantly reduced its ability to compete with other bacteria when they were co-cultivated. In contrast, the Type VI secretion system of P. ananatis strain isolated from onion in South Africa was shown to be indispensable for pathogenicity. These results indicated that the significance of the Type VI secretion system depends on bacterial strains used and that the system might not serve as an index of plant pathogenicity.

In tobacco plants, expression of some resistance-related genes of plant was assayed after injection of the pathogenic strain of *P. ananatis*. RT-PCR analysis demonstrated that the kind of genes induced was similar to that induced in hypersensitive reaction (HR), but the induction was delayed by ca. 12 hours compared to HR. At the same time, PASVIL of *P. ananatis* was shown to be strongly induced when the bacterium was introduced into tobacco tissues.

Phylogenetic study on *Pantoea* spp. revealed their core genome and specification of each species. Even in *P. ananatis*, there is significant diversity among the strains and the pathogenic strains were shown to be scattered in diverse groups.

In conclusion, we revealed the pathogenicity determinants and diversity of *P. ananatis*, and developed a method to discriminate its pathogenic population.