THESIS

IDENTIFICATION OF GENES INVOLVING IN SUGAR UTILIZATION OF *RALSTONIA SOLANACEARUM*

DUANGKHAE KANJANASOPA

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Agricultural Biotechnology)
Graduate School, Kasetsart University
2006

ISBN 974-16-1789-5

Duangkhae Kanjanasopa 2006: Identification of Genes Involving in Sugar Utilization of *Ralstonia solanacearum*. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Niphone Thaveechai, Ph.D. 105 pages. ISBN 974-16-1789-5

The gene coding for sorbitol dehydrogenase (*polS*) from *Ralstonia solanacearum* strain To 264 race 1 biovar 3 was cloned, sequenced, and compared to homologous sequences from other bacteria. The result showed that the sorbitol dehydrogenase gene from this *R. solanacearum* displayed the highest similarity to *polS* of *R. solanacearum* GMI1000 with 99.6% amino acid similarity and least similarity to sequence of *Pseudomonas syringae* pv. *syringae* with 56% amino acid similarity. Phylogenetic analysis of *polS* gene showed the *Burkholderia cepacia* sequence joined as sister to the *R. solanacearum* To 264 pair. Analysis of the deduced amino acid sequence revealed homology to enzymes of the short-chain dehydrogenase/reductase protein family. The eight amino acid residues are conserved in most of these proteins. The motif includes the highly conserved tyrosine (Y) and lysine (K) residues of consensus sequence Y-X-X-X-K, which are essential for catalysis and are located in the active site in C-terminal whereas the glycine (G) residues of the G-X-X-X-G-X-G segment are characteristic of the NAD^+ binding domain in the N-terminal region. The 771 bp of partial *polS* gene from *R. solanacearum* To 264 was subcloned into expression pGEX-2T vector. The partial *polS* ORF encodes a protein consisting of 256 amino acid residues with estimated molecular mass of 27 kDa by SDS-PAGE analysis. Construction of deleted *polS* (Δ*polS*) plasmid namely pKS was generated in order to produce mutation at endogenous *polS* gene of *R. solanacearum* strain To-Ud3 by homologous recombination. The site directed mutagenesis was created by overlapping PCR to eliminate NADP binding and catalytic site. Homologous recombination mutants were screened base upon biochemical property of sorbitol utilization. Subsequently, replacement recombination with Δ*polS* by double crossover was elucidated by PCR. Enzymatic activity of non-oxidizing sorbitol mutant was abolished and unable to oxidize not only sorbitol but also dulcitol whereas other sugars were not affected. The severity of wilt symptom and quantification of EPS production from wild type and mutant did not significantly different when testing *in vitro*. The overall experiments can not clearly elucidate the correlation between sugars metabolism and pathogenicity of *R. solanacearum.*

_____ _____ ____/____/____

Student signature Thesis Advisor's signature

ACKNOWLEDGMENTS

I wish to express my sincere gratitude and profound appreciation to my thesis chairman; Assoc. Prof. Dr. Niphone Thaveechai for his patience, advice, guidance and encouragement throughout the course of my study, and their kindness reviewing this manuscript.

I would like to deeply thank to the member of my committee; Asst. Prof. Dr. Wichai Kositratana, Dr. Orawan Chatchawankanphanich, Center for Agricultural Biotechnology for their valuable comments and suggestion for the completeness of this thesis.

I am particularly grateful to Center for Agricultural Biotechnology and also to the University Development Committion (UDC) Scholarship from Prince of Songkla University, Surat Thani campus for financial support to my study and reproduction of this thesis, respectively and I am grateful to Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bacteriological section, Department of Agriculture for providing facility in this study.

I am deeply grateful to Dr. Srimek Chowpongpang for his guidance, continuous comments and proofreading paper of this study and Mrs. Nattima Kositcharoenkul and Mrs. Piyarat Thammakijjawat for her help and encouragement shown to me during this study and my sincerely thank to Asst. Prof. Dr. Somchai Pornbanlualap Department of Biochemistry, Kasetsart University for proofreading paper of this study. Many thanks should be expressed to my friends at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University for their help, encouragement and friendships during the period of this study.

Finally, I greatly appreciate to my parents for their understanding, encouragement throughout my study.

Duangkhae Kanjanasopa

April 2006