



UNIVERSITI PUTRA MALAYSIA

**CHARACTERIZATION OF *Pectobacterium carotovorum* AND
P. wasabiae AND THEIR POTENTIAL CONTROL
USING ANTAGONISTIC BACTERIA**

ELHAM GOLKHANDAN

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By

ELHAM GOLKHANDAN

**Thesis submitted to the school of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the degree of Doctor of Philosophy**

June 2014

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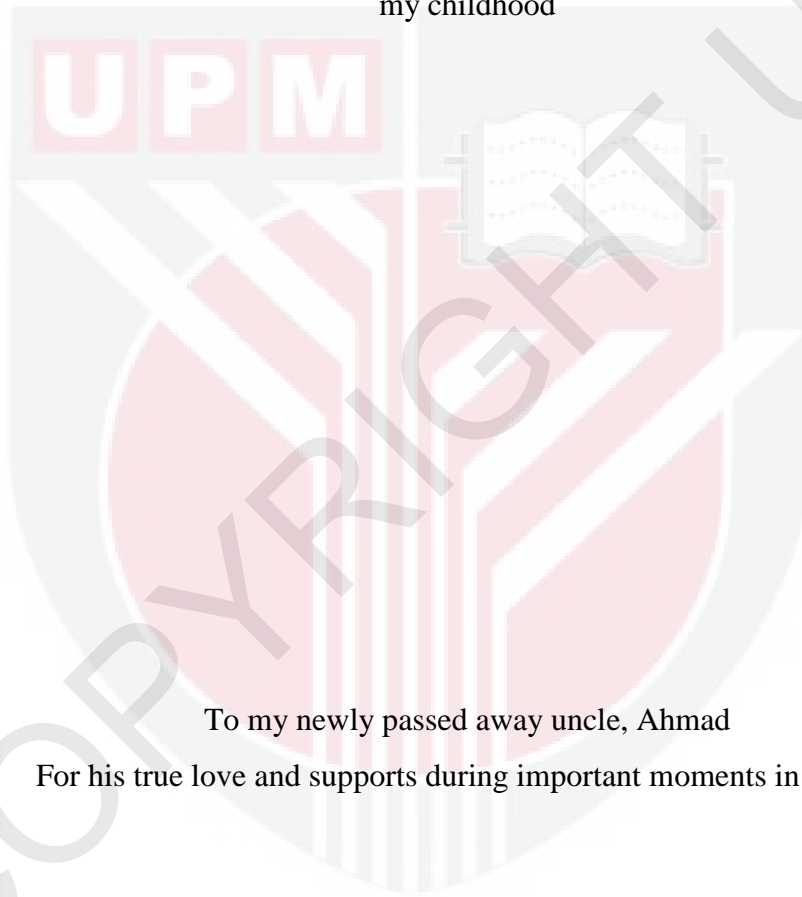
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DEDICATION

To my most beloved parents, Zarrintaj & AliReza

For their true love, understanding, patience, principle guide and encouragement since
my childhood



To my newly passed away uncle, Ahmad
For his true love and supports during important moments in my life

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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P. wasabiae AND THEIR POTENTIAL CONTROL
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By

ELHAM GOLKHANDAN

June 2014

Chair: Associate Professor Kamaruzaman Sijam, PhD

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In August 2011, vegetable crops showing symptoms of maceration and water soaked lesions on their tuber, leaf, and fruit were collected from four major vegetable growing states in Malaysia including Pahang, Johor, Melaka and Selangor. The majority of the causal organisms isolated from infected tissues (52 strains) were identified as *Pectobacterium* spp. based on PCR amplification of the pectate lyase (*pel*) gene and amplification of the 16S-23S rRNA (ITS) with G1 and L1 primers. Physiological and biochemical assays divided Malaysian *Pectobacterium* species into two main groups: *Pectobacterium wasabiae* and *Pectobacterium carotovorum* subsp *carotovorum*. Partial sequence of PCR product from reaction of putative *Pectobacterium* spp. with 16S rRNA confirmed the results obtained from physiological and biochemical assays used for identification of the bacterium. Application of specific primers such as Eca1F/Eca2r, Br1f/L1r, EXPCCF/EXPCCR, and also ITS-PCR following by RFLP by restriction enzyme (*RsaI*) successfully differentiated Malaysian *P. wasabiae* and *P. carotovorum* subsp *carotovorum* isolates from other species and subspecies of *Pectobacterium*. Phylogenetic analysis of Malaysian isolates with housekeeping genes (*mdh*, *gapA*) grouped Malaysian *P. carotovorum* subsp *carotovorum* and *P. wasabiae* in the same cluster with *P. carotovorum* subsp *carotovorum* (Ecc380) and *P. wasabiae* (SCRI488) respectively. In this study, some bacterial strains from the vegetable farm soil showed strong antagonistic activity against *Pectobacterium carotovorum* and *Pectobacterium wasabiae* in vitro on bell peppers, Chinese cabbage, cucumber, and tomato, and their cell-free filtered supernatants showed excellent biocontrol effect in controlling the potato maceration at room temperature. According to analysis of partial nucleotide sequences of 16S rRNA the isolates were identified as *Pseudomonas chlororaphis*, *Pseudomonas fluorescens*, *Burkholderia cepacia*, *Bacillus subtilis*, and *Serratia liquefaciens*. No significant differences in prevention of weight reduction by *Pectobacterium* spp. were observed between potato slices treated with antagonists and

treated with SH. Antibacterial agents isolated from Malaysian soils seem to be promising disinfectants to protect vegetables from soft rotting bacteria, and allow vegetables' consumers and industries to reduce the amount of chemicals such as SH.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

CHARACTERIZATION *Pectobacterium carotovorum* DAN *P. wasabiae* DAN KAWALAN POTENSI MEREKA MENGGUNAKAN BAKTERIA BERMUSUHAN

Oleh

ELHAM GOLKHANDAN

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Pada bulan Ogos 2011, tanaman sayur-sayuran yang menunjukkan tanda-tanda maserasi dan lesi direndam air pada tuber, daun, dan buah telah dikumpulkan daripada empat negeri pengeluar utama sayur-sayuran yang semakin meningkat di Malaysia termasuk Pahang, Johor, Melaka dan Selangor. Majoriti organisma penyebab yang dipencilkan daripada tisu terjangkit (52 strain) telah dikenal pasti sebagai *Pectobacterium* spp. berdasarkan amplifikasi PCR terhadap gen liase pectate (*pel*) dan amplifikasi 16S-23S rRNA (ITS) dengan menggunakan primer G1 dan L1. Ujian fisiologi dan biokimia membahagikan spesies *Pectobacterium* Malaysia kepada dua kumpulan utama: *Pectobacterium wasabiae* dan *Pectobacterium carotovorum* subsp *carotovorum*. Jujukan separa produk PCR dari tindak balas putatif *Pectobacterium* spp. dengan 16S rRNA mengesahkan keputusan yang diperolehi daripada ujian fisiologi dan biokimia digunakan untuk pengesanan bakteria. Penggunaan primer yang spesifik seperti Eca1F/Eca2r, Br1f/L1r, EXPCCF / EXPCCR, dan juga ITS-PCR diikuti dengan RFLP oleh enzim penekatan (*RsaI*) berjaya membezakan pencilan Malaysia *P. wasabiae* dan pencilan *P. carotovorum* subsp *carotovorum* daripada spesies dan subspecies yang berlainan daripada *Pectobacterium*. Analisis pokok filogenetik pencilan Malaysia dengan gen penyelenggara (*mdh*, *gapA*) mengumpulkan *P. carotovorum* subsp *carotovorum* Malaysia dan *P. wasabiae* dalam kelompok yang sama dengan *P. carotovorum* subsp *carotovorum* (Ecc380) dan *P. wasabiae* (SCRI488). Dalam kajian ini, terdapat beberapa strain bakteria dari tanah ladang sayur-sayuran menunjukkan aktiviti antagonis yang kuat terhadap *in vitro Pectobacterium carotovorum* dan *Pectobacterium wasabiae* pada cili kembung, kubis china, timun, dan tomato, dan supernatan bebas sel yang ditapis menunjukkan kesan kawalan biologi yang sangat baik dalam mengawal maserasi kentang pada suhu bilik. Menurut analisis jujukan separa nukleotida 16S rRNA, pencilan telah dikenal pasti

sebagai *Pseudomonas chlororaphis*, *Pseudomonas fluorescens*, *Burkholderia cepacia*, *Bacillus subtilis* dan *Serratia liquefaciens*. Tiada perbezaan yang ketara dalam pengurangan berat oleh *Pectobacterium* spp. diperhatikan di antara hirisan kentang yang dirawat dengan antagonis dan dirawat dengan SH. Agen antibakteria yang dipencilkan daripada tanah di Malaysia seolah-olah menjanjikan disinfektan yang berguna untuk melindungi sayur-sayuran dari bakteria lembut reput, dan membolehkan pengguna dan industri sayur-sayuran untuk mengurangkan jumlah bahan kimia seperti SH.



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I certify that a Thesis Examination Committee has met on 18 June 2014 to conduct the final examination of Elham Golkhandan on her thesis entitled "Characterization of *Pectobacterium carotovorum* and *P. wasabiae* and Their Potential Control using Antagonistic Bacteria" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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