GENETIC DIVERSITY OF *XANTHOMONOUS CITRI* SUBSP. *CITRI*, CAUSAL AGENT OF CITRUS CANKER

FARIMAH ARSHADI

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GENETIC DIVERSITY OF XANTHOMONOUS CITRI SUBSP. CITRI, CAUSAL AGENT OF CITRUS CANKER

By

FARIMAH ARSHADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2013
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DEDICATION

I dedicate this thesis to my beloved mother, for her endless love and support is the reason of this achievement.
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science.

GENETIC DIVERSITY OF XANTHOMONOUS CITRI SUBSP. CITRI, CAUSAL AGENT OF CITRUS CANKER

By

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May 2013

Chairman: Associate Professor Kamaruzaman Sijam, PhD

Faculty: Agriculture

Asiatic citrus bacterial canker disease is one of the most widespread and economically damaging diseases of citrus, affecting nearly all commercial citrus species and cultivars worldwide and is endemic in Malaysia. It is caused by the bacterium Xanthomonas citri subsp. citri, which causes raised lesions often surrounded by hallow on young leaves, fruits and stems of citrus trees. In this study four states of Peninsular Malaysia were surveyed for occurrence of the disease. Canker was observed in all the states surveyed except Pahang state. Specimens were gathered and 25 strains of the bacterium were isolated. Four different diagnostic tests were used to identify the bacterium, including morphological and biochemical characterization, detached-leaf pathogenicity test, conventional PCR using primer set 2/3 and sequencing. All four methods confirmed the isolates to be Xanthomonas citri subsp. citri. After identification, the isolates were subjected to molecular characterization using rep-PCR primers, ERIC and BOX. After combining the data
obtained from each primer pair, similarity coefficients for a pair of isolates were calculated using Dice’s coefficient index and phylogenetic tree was constructed based on UPGMA clustering method. The mean similarity coefficient for isolates was 73% and our tree clearly grouped isolates according to the geographical location, but not the citrus host they were collected from. The tree was separated into two main clusters at 51% similarity, one including the isolates from Terengganu and the other containing the rest of the isolates from Johor and Selangor. Previous studies show that similarity coefficient less than 70% is associated with different strains or pathovars of a bacterium. This high amount of genetic distance refers to a distinct genetic structure and heterogeneity in populations of Terengganu isolates, while isolates from Johor and Selangor seem to be more genetically uniformed and similar. However, more evidence is required to prove the presence of distinct forms a disease in Malaysia.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhikeperluan untuk ijazah Master Sains

KEPELBAGAIAN GENETIKXANTHOMONOUSCITRISUBSP. CITRI,
SEBAB-MUSABABAGENPENYAKITMAWAR LIARLIMAU

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primers, ERIC dan BOX. Selepas menggabungkan data yang diperoleh daripada setiap pasangan primer, pekali persamaan bagi sepasang pencilan dikira dengan menggunakan indeks pekali Dice dan pokok filogenetik telah dibina berdasarkan kaedah clustering UPGMA. Purata pekali persamaan min untuk pencilan adalah 73% dan pokok kami secara jelas mengumpulkan pencilan mengikut lokasi geografi, tetapi bukan tuan rumah sitrus dimana ianya dikumpulkan. Pokok tersebut dipisahkan kepada dua kluster utama pada kadar 51% persamaan, dimana satu pencilan adalah daripada Terengganu dan satu lagi pencilan terdiri daripada pencilan daripada Johor dan Selangor. Kajian terdahulu menunjukkan pekali persamaan kurang dari 70% dan ianya berkaitan dengan strain yang berbeza atau pathovar bakteria. Jumlah jarak genetik yang tinggi adalah merujuk kepada struktur genetik yang jelas dan kepelbagaian genetik dalam populasi pencilan di Terengganu, manakala pencilan daripada Johor dan Selangor secara genetik nya lebih uniform dan serupa. Walau bagaimanapun, lebih banyak bukti diperlukan bagi membuktikan kewujudan bentuk penyakit yang jelas di Malaysia.
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I want to thank Faculty of Agriculture, University Putra Malaysia for providing the necessary fundings to fulfill this research.

Last but not least, I want to thank my family, without whom I could not be able to do this. Thank you for all the generosity, love, support and motivation you have given me to be who I am today. I hope one day I can make you proud.
I certify that an Examination Committee has met on 29th May 2013 to conduct the final examination of Farimah Arshadi on her Master’s thesis titled “Molecular characterization of Xanthomonas citri subsp. citri, causal agent of citrus canker” in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and University Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Master of Science in Plant Pathology.

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DECLARATION

I declare that this thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not currently, submitted for any other degree at University Putra Malaysia or at any other institution.

FARIMAH ARSHADI

Date: 29 May 2013
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