UNIVERSITI PUTRA MALAYSIA

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

FARIMAH ARSHADI

FP 2013 23

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

COPYRIGHT

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

FARIMAH ARSHADI

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

Oleh

FARIMAH ARSHADI

Mei 2013

Pengerusi: Professor Madya Kamaruzaman Sijam, PhD

Fakulti: Pertanian

Penyakit Asiatic Citrus Bacterial Canker adalah sejenis penyakit yang merebak dan secara ekonominya merosakkan limau. Ia merupakan penyakit yang menjejaskan hampir kesemua spesies limau komersial dan kultivars di seluruh dunia serta endemik di Malaysia.Penyakit ini yang berpunca daripada bakteria *Xanthomonas citri* subsp. *citri,* menyebabkan luka pada daun muda, buah dan batang pokok limau. Untuk tujuan kajian ini, sampel daripada empat negeri di Semenanjung Malaysia dikumpul dan penyakit ini diperhatikan di semua lokasi kecuali di negeri Pahang. Spesimen telah dikumpulkan dan 25 strain bakteria telah diasingkan sepenuhnya. Empat ujian diagnosis yang berbeza telah digunakan untuk mengenal pasti bakteria termasuk pencirian morfologi dan biokimia, ujian daun berkembar pathogenicity, konvensional PCR menggunakan set primer 2/3 dan penjujukan. Kesemua empat ujian telah mengesahkan pencilan merupakan *Xanthomonas citri* subsp. *citri* dan kesemua pencilan menunjukkan keputusan yang sama. Setelah dikenal pasti,pencilan itu kemudiannya tertakluk kepada pencirian molekul dengan menggunakan rep- PCR

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.

ACKNOWLEDGEMENTS

I'd like to express my deepest gratitute to my supervisory committee chairman, Dr. Kamaruzaman Sijam, for his genorous help and support through out this research.

I would also like to appreciate Dr. Yahya bin Awang, my supervisory committee member, for his sincere supports and helpful suggestions for this work.

My special thank goes to Dr. Hadi Zokayie, Ms. Neda Naderali, Dr. Eisa Nazerian, and all of laboratory's staff and personel for all of their kind help, assistance and inspiration they gave me during my work.

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, whithout whome I could not be able to this. Thank you for all the generousity, 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 2013to conduct the final examination of Farimah Arshadi on her Master'sthesis 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 recomends that the student be awarded the Master of Science in Plant Pathology.

Members of Examination Committee were as follows:

Ganesan Vadamalai, PhD
Faculty of Agriculture
University Putra Malaysia
(Chairman)

Jugah Kadir, PhD
Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Internal Examiner)

Phebe Ding, PhD
Faculty of Agriculture
University Putra Malaysia
(Internal Examiner)

Mohamad Roff bin Mohd. Noor, PhD
Horticulture Research Center
Malaysian Agricultural Research and Development Institute
(External Examiner)

NORITAH OMAR, PhD
Assoc. Professor and Deputy Dean
School of Graduate Studies
University Putra Malaysia

Date:

This thesis was submitted to Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

Kamaruzaman Sijam, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Yahya bin Awang, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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 istitutaion.

FARIMAH ARSHADI

Date: 29 May 2013

TABLE OF CONTENTS